Investigating Non-Equilibrium Phenomena in Active Matter Systems

by

Joshua Paul Steimel

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Signature redacted

Author .................................................................

Department of Materials Science and Engineering

May 11, 2017

Signature redacted

Certified by ...........................................................

Alfredo Alexander-Katz

Associate Professor of Materials Science and Engineering

Thesis Supervisor

Signature redacted

Accepted by ...........................................................

Donald Sadoway

Chair, Departmental Committee on Graduate Students
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Abstract

Active matter systems have very recently received a great deal of interest due to their rich emergent non-equilibrium behavior. Some of the most vital and ubiquitous biological systems and processes are active matter systems including reproduction, wound healing, dynamical adaptation, chemotaxis, and cell differentiation. Active matter systems span multiple length scales from meter to nanometer and can vary depending on the shape of the active agent, mode of motility, and environment. However, active matter systems are unified in that they are all composed of active units or particles that continuously convert ambient, stored, or chemical energy locally into motion and exhibit emergent non-equilibrium collective dynamical or phase behavior. Active matter systems have been studied extensively in the biological context, as well as in simulation and theory. However, there are relatively few artificial or synthetic experimental model soft active matter systems that can effectively mimic the rich emergent behavior exhibited by many active matter systems. Such model experimental systems are crucial not only to confirm the exotic behavior predicted by theoretical and simulation systems, but to study and investigate the underlying physical phenomenon which may contribute to or even drive some emergent phenomenon. These model systems are crucial to help determine what behavior is due to purely physical phenomenon and what behavior requires some type of biological or biochemical stimuli.

In this thesis, I will develop several artificial experimental model active matter systems that are able to effectively mimic and reproduce some of the rich emergent non-equilibrium behavior exhibited by several active matter systems or processes, like chemotaxis, in order to uncover the underlying physical phenomenon that govern this emergent behavior. I will start by designing an extremely simple active matter system composed of a single active unit and then build up in complexity by adding many active components, changing the mode of motility, and including passive components which may or may not be fixed. I will show in this thesis that this emergent behavior is guided by fundamental physical phenomenon like friction and the mechanical properties of the environment. The thesis divides this study into two Parts.

In Part I, I will develop an artificial soft active matter system that is able to effectively perform chemotaxis in a non-equilibrium manner by leveraging the concept of effective friction. The active component in this system will be magnetic particles that are coated with a biological ligand or receptor and placed on a substrate with the corresponding ligand or receptor. A rotating magnetic field will be applied and the magnetic particle will proceed to rotate with the applied field and convert some of that rotational energy into translational energy due to the effective friction induced by the breaking of reversible bonds between the surface of the particle and the substrate. I will then create gradients in the density of such binding sites and by placing the magnetic particle on a stochastic, random walk the differences in effective friction will lead to directed motion or drift reminiscent of chemotaxis. I will show that this concept of sensing based on effective friction induced by a binding interaction is general and scales with the affinity of the interaction being investigated.
(i.e. protein-lipid, metal ion, electrostatic, antigen-antibody, or hydrophobic interactions). In Part II, I will build up in complexity and develop an artificial soft active matter system consisting of two active units embedded in a dense passive matrix in order to mimic the emergent behavior of many biological systems composed of both active and passive components. In this system, an ultra-long range attractive interaction emerges due to a combination of activity and the mechanical properties of the dense passive media. The range of the interaction can be tuned by changing the level of activity, the actuation protocol, the mode of motility, the composition of the dense passive monolayer, and the concentration of active units. Alternatively, if the passive components are fixed to the substrate, the active components undergo a disorder induced delocalization and exhibit super-diffusive transport properties. On the basis of these results, I propose several guidelines to developing novel artificial soft active matter systems which bear future investigation.

The findings in this thesis represent a comprehensive study of the exotic emergent non-equilibrium behavior exhibited by many active matter systems by developing novel artificial experimental soft model active matter systems. These novel model experimental systems revealed some underlying fundamental physical phenomenon that contribute to some of the non-equilibrium behavior observed in the biological system of interest. These results may generalize not only to other simulation or theoretical active matter systems but potentially to biological systems as well. This work will be essential not only in guiding the design of future artificial experimental soft active matter systems, but can also be extended towards designing hybrid artificial-biological soft active matter systems.

Thesis Supervisor: Alfredo Alexander-Katz

Title: Associate Professor of Materials Science and Engineering
Acknowledgments

The information presented in this thesis represents over five years of research and experimentation that would not have been possible without the outpouring support from many family members, faculty, colleagues, and friends.

I was fortunate enough to join the AAK group as many of the old guard: Charles, Hsieh, YJ, and Reid, were finishing up their PhD work. They were all very welcoming when I joined the group as an undergraduate, and even in the dingy confines of the Building 12 basement, it was always a very lively and fun working environment. I will especially remember the summer group basketball games, karaoke, and late night Cantab shenanigans. I also would like to thank all the current AAK group members: Yi, Karim, Mukarram, Ric, Shayna, Emi, and Shahrzad as well. The group seems to be in very capable hands.

I have also had the privilege to work with amazing colleagues and collaborators both at MIT and beyond. I would like thank Juan Aragones for his diligent simulation and theory work on my many different experimental systems. I am indebted to Naser Qureshi and Guillermo Mendoza for their help in building the 3D Helmholtz coil apparatus. This was a project that had plagued me for nearly a year and in the span of just a month or two, we were able to build a truly amazing piece of equipment. I learned so much and had a ton of fun as well. I also would like to acknowledge David Bono and Mike Tarkanian at MIT for their constant support. I bothered them both the past nine years as an MIT undergraduate and graduate student. Yet, in asking for their help to build new apparatuses or getting trained on a new piece of equipment, they were always available and willing to help. I think they are the most underappreciated and underutilized resources DMSE has to offer. I would also like to thank our adopted lab manager, Ty, now that we are in NE-46.

I’ve also had the privilege to teach during my time at MIT, in particular 3.014. I’ve been a TA for this class four times and I just want to thank the other 3.014 instructors: Linn Hobbs, Donald Sadoway, Lionel Kimerling, Andreas Karatsolis, Meri Treska, Hilary Sheldon, Ike, James, and especially Geetha Berera. 3.014 was one of my favorite classes during my undergraduate studies and Geetha wrote a comment on her evaluation of my 3.014 poster saying that it was “graduate student caliber work.” This comment gave me the confidence that I could not only succeed in Course 3, but signified to me that grad school might be a possible route I could pursue, something I previously thought to be impossible. I loved teaching 3.014 and interacting with the students, especially during office hours or during my final review sessions. It was particularly gratifying to finish my 3.014 tenure this Fall 2016, teaching what has been my favorite group of students during my time at MIT. This group was extremely enthusiastic, energetic, respectful, hardworking, and receptive to teaching. No group exemplified these qualities more than Group 4, particularly Judy Wang.

I want to thank my committee members, Darrel Irvine and Niels Holten-Andersen. Darrell was my undergraduate thermo professor and I haven’t stopped bugging him with questions since. Also Niels has been a steady and constant source for support and advice both professionally and personally. Finally, I want to especially thank my advisor, Alfredo Alexander-Katz. I wouldn’t have
pursued my PhD had it not been for Alfredo. When I started to make progress on my undergraduate thesis, he took the time to talk to me about the possibility of graduate school and continuing my work within the group, and really guided me through the entire process. Then, during my first year of graduate school, I began struggling with the academic course work and was considering leaving MIT. It was Alfredo, who was about to begin the intense period of his tenure process, who took the time to go over difficult concepts and through practice exams. I know that there aren’t many professors at MIT, or anywhere for that matter, that would do this for a graduate student just two months into their tenure, but Alfredo did. His actions are a testament to his character and the type of person that he is. I’m eternally grateful for his support during that particular time in my life. I hope there comes a time where I can repay him for that.

During my tenure at MIT, I was very fortunate to be actively involved in both intramural sports and MIT’s football team. I had a blast playing MIT football as an undergraduate with Kyle, Billy, Johnny, Wilmer, Sean, Pete, Kresz, Darby, Doug, Dave, Coach Po, and Coach Adams. As a graduate student, I loved playing intramural flag football, basketball, ultimate frisbee, and softball with Reid, Rodger, Gibson, Brendan, Heidlberg, Campion, Grindy, and Lucas. However, what I enjoyed most was playing summer and fall slow pitch softball both at MIT and in various HUB, SBS, and BSSC leagues all around Boston. I want to thank all the members of the ShadowMasks, Squeeze the Juice, One Bad Inning, Hazmats, Biohazards, Sunflowers, Jesse and The Rippers, and Mudville teams. This past summer was a very memorable one as our DMSE team, the Hazmats, finally won the MIT Summer Softball Championship for the first time. The team has been playing since the early 1970s and previously had a 0-7 record in championship games. So, I just want to thank Kevin Spencer, Lee, Nelson, Luke, Jake, Schaefer, Dave, Karrie, Tim, Beth, Tom, and Abe. I would not want to play softball with anybody else!

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here so I'll wrap up by saying that if I've achieved any measure of success, it's due solely to your love, support, guidance, teaching, and parenting. Your selflessness, sacrifice, integrity, and kindness is an inspiration and I can honestly say without exaggeration that I am fortunate to have the best parents anyone can hope to ask for. I've given my absolute best in everything I've done at MIT and I'm very proud of the work that I've done, but the thing that I'm most proud of is being your son.
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CHAPTER 1
INTRODUCTION

Living organisms and systems constantly convert available, ambient, or stored energy into motion [1]. This motion or activity continually pushes these systems out of thermal equilibrium [2, 3, 4]. This intrinsic non-equilibrium nature allows these systems to display exotic behavior not observed or accessible when in thermal equilibrium [5, 6, 7, 8, 9]. In recent years, active matter systems have received a tremendous amount of interest as active matter systems are ubiquitous in nature and perform vital biological processes. Chemotaxis, wound healing, reproduction, dynamical adaptation, and cell differentiation are just some of the processes performed by active matter systems [10, 11, 6, 12, 13, 14, 15, 16]. Active matter systems are traditionally defined as being composed of active units or agents that continually convert ambient, stored, biological, or chemical energy locally into motion [17]. There is an extremely large parameter space to explore in these systems as active matter systems vary in the mode of motility, shape of the active agent, density of active agent, and the environment or medium; and to that end there has been a prodigious amount of simulation and theoretical work done to explore the emergent behavior exhibited in such systems. However, there is still a lack of artificial experimental model systems that can effectively mimic the emergent non-equilibrium behavior exhibited by active matter systems. Developing such model systems is crucial not only to better understand the exotic and emergent behavior exhibited by many active matter systems, but to distinguish which behavior is due to fundamental physical phenomenon and what behavior requires some type of biological or biochemical stimuli.

In this thesis, I will design a novel robust experimental artificial model active matter system that utilizes magnetically driven particles as the active unit or agent. A 3D magnetic coil apparatus will apply an external rotating magnetic field to induce several different modes of motility such as walkers, rollers, and tops. The mode of motility, the frequency of the rotating field, and the magnitude of the magnetic field can be changed instantaneously using a custom written MatLab code that sends a signal directly to the coils. This apparatus will be used in conjunction with custom fabricated microfluidic devices that will contain the active matter which can be modified to change the environment surrounding the active components. I will start by demonstrating that this artificial model system is capable of reproducing and mimicking chemotaxis. By functionalizing the active components with a biological ligand and the surface with the corresponding receptor, we will leverage the effective friction induced by binding to achieve directed motion. I will also demonstrate that the effective friction induced is related to the binding affinity of the interaction. Therefore, the resulting chemotactic response will change depending on the functionalization of the active components or frequency of rotation of the magnetic fields; giving us insight into the role friction can play in chemotaxis. I will then increase in complexity and investigate the non-equilibrium behavior exhibited by active matter systems composed of many active units in complex passive media. The emergent non-equilibrium behavior will depend on the mechanical properties of the
passive medium, the order of the passive medium, the activity of the active particles, and the mode of motility. This thesis presents a fundamental physical understanding of the principles contributing to emergent non-equilibrium behavior observed in many biological systems. Such insight will be crucial for future applications which can integrate some of the artificial soft active matter system components into the biological system of interest in order to push the biological system of interest to perform behavior previously inaccessible in the purely biological system.

1.1 Non-equilibrium steady states in biological active matter systems

Typically, one is concerned with finding the state of a system in thermodynamic equilibrium. However, life is constantly operating out of thermodynamic equilibrium and it is the inherent non-equilibrium nature of such systems that enables them to exhibit such exotic and remarkable emergent behavior. Within the realm of non-equilibrium systems, there is a specific subset of systems that have recently garnered a great deal of interest in recent years. This set of systems is referred to as active matter systems. The distinguishing feature of active matter systems is that they are composed of active particles or units that continuously convert ambient, stored, or chemical energy locally into motion\[17\]. Although it should be noted that recently this definition has been extended to include systems where the energy required for activity or motility is applied externally as well. It is the active nature of these systems which continuously pushes this system out of thermodynamic equilibrium and which, under the right conditions, can lead to emergent non-equilibrium steady states or collective motion.

This interest in active matter systems is well founded as active matter systems are ubiquitous in nature and span multiple length scales. From macroscopic examples, meter length scale, of schools of aquatic organisms like rays or fish (and my personal favorite example being a crowd moshing during a heavy metal concert), to the micrometer length scale with swarms of bacteria or sheets of epithelial cells migrating toward a wound, to sub-micrometer length scales where bundles of biological filaments like microtubules associate with kinesin motors to exhibit complex collective motion, active matter systems are able to exhibit emergent non-equilibrium dynamics as seen in Fig. 1-1. [18, 19, 20, 21, 22, 23, 24, 25, 9, 25, 26, 27, 28, 29, 30, 31].

The ability of these non-equilibrium active matter systems to exhibit such emergent dynamical rearrangement and phase separation is crucial in order to perform vital biological functions, particularly at the micrometer and sub-micrometer length scales. During tissue development and wound healing, dense crowds of cells must often move collectively through tissue or the extracellular matrix (ECM) [6, 30, 14]. During sea urchin reproduction, when the density of spermatozoa is large enough, the spermatozoa have been found to phase separate into arrays of vortices [12]. During the process of chemotaxis, if the concentration of chemotactant is high enough, the cells performing chemotaxis will collectively move and phase separate to form bacterial biofilms [10, 7]. Even at the sub-micrometer length scales, complex dynamical adaptations of kinesin motors and microtubules
is required for cells to move on a substrate [9]. A myriad of biological systems exhibit nonequilibrium steady states, dynamical reorganization, or collective motion ranging from swarming, flocking, spiraling, laning, and vortexing, some of which can be seen in Fig. 1-2 [27, 32, 33, 34, 35, 19, 36, 37, 38].

What is clear in all of these systems is that a combination of the mode of activity, shape of the active agent, environment, and a number of other factors lead to these effective emergent interactions. However, it is extremely difficult to understand the origin or the fundamental physical forces that contribute to driving such emergent behavior in these biological systems. Most of these systems are extremely complicated and it is difficult to differentiate what behavior is due to biological stimuli and what behavior is purely or somewhat due to physical phenomenon. To this effect, active matter systems have become attractive systems for simulation and theoretical studies in order to solve this problem.

1.2 Modeling attempts to capture physical forces driving emergent behavior

Active matter systems are extremely attractive systems for theoretical and simulation studies due to the extremely large parameter space that can be explored and the relatively few experimental artificial model active matter systems that can effectively mimic the emergent behavior observed in biological systems. While it is unfeasible and ill advised to review all the theoretical and simulation active matter systems, several seminal theoretical models must be mentioned in order to survey
the work done in the field of active matter and to identify gaps in the current research which will be addressed in this thesis. Perhaps the first and arguably the most important simulation and theoretical study of active matter systems was conducted by Vicsek. Vicsek was the first to attempt to explain this emergent non-equilibrium behavior, particularly the flocking behavior of birds, using simple physical rules [40]. The Vicsek model is simple and minimal but sufficient to reproduce and explain this flocking behavior. The three key assumptions made are: i) the active units in the system can be treated as self-propelled particles (SPP) that move at a constant velocity, ii) the SPP will assume the average orientation or alignment of its neighboring particles, iii) this alignment competes with some degree of random perturbation added into the system[40]. These were the only assumptions made in the Vicsek model. The only other parameters that are varied in the model are the density or number, $N$, of SPPs and the amount or amplitude of noise in the system. The position of the particles is described by

$$x_i(t+1) = x_i(t) + v_i(t)\Delta t$$  \hspace{1cm} (1.1)$$

where the velocity of the particles is an absolute value whose direction is given by the following expression

$$\theta(t+1) = <\theta(t)>_r + \Delta\theta$$  \hspace{1cm} (1.2)$$

where $<\theta(t)>_r$ is the direction of the particle velocity within a certain radius, $r$, and $\Delta\theta$ is the noise [40]. Vicsek showed that at small densities and low noise the SPP tended to form flocks, at high density and high noise the system is disordered with some correlation, and at large densities and small noise all the particles spontaneously move in some selected direction [40].
physical model was able to effectively mimic this emergent non-equilibrium steady state or non-equilibrium phase transition and resultant collective motion behavior. Moreover, it illustrates that this flocking behavior observed in biological systems can be explained, in this instance, by purely physical phenomenon.

This model has been extended and developed to study active matter systems that exhibit different modes of activity, shape of active agent, environment, and confinement. Yet, despite these vastly different systems, they all have two fundamental unifying features: i) they all exhibit some type of emergent interaction or collective motion behavior and ii) these systems are typically densely packed with a high concentration of active (and for a very few cases inactive components) units. Several of these simulation systems can be seen in Fig. 1-3.

Figure 1-3: A) Vlahovskas self-rotating non-aligning particles spinning clockwise, counter-clockwise, and with no rotation or activity. B) Glotzers oppositely rotating spinner system. C) Reichhardt's ratchet active matter system. D) Granicks Janus active colloid system. E) Ramaswamys active granular matter system. Reproduced from [41, 42, 43, 44, 45].

Reichhardt has extensively examined the effects of environment and confinement particularly in ratchet systems with SPP and the resultant directed motion that emerges in these systems [43, 46, 47, 48, 49, 50]. Glotzer investigated a different mode of motility with rotating spinner particles instead of the traditional SPP [45]. In a binary mixture of spinners rotating either clockwise or counter-clockwise, phase separation occurs at high enough densities and activity [45]. Moreover, different emergent behavior can be induced when the shape of the spinner or rotor is changed. Vlahovska’s spinner model shows similar phase separation however, by embedding the co-rotating and counter-rotating spinners in an environment filled with inactive or passive particles [41]. Here, instead of a binary phase separation between co-rotating and counter-rotating spinners as was observed in the Glotzer system, a ternary non-equilibrium steady state emerged where co-rotating, counter-rotating, and passive particles segregated in this non-equilibrium system.
[41]. There have also been some simulations models that have been realized experimentally as well. Granick’s phoretic Janus particles show random isotropic motion (some correlation), chain formation, swarming, and clustering behavior depending on the difference in dipole moments between the Janus hemispheres and the dipolar repulsive force between two spheres at contact [42]. Ramaswamy, using polar rods, showed a transition from disordered to ordered flocking behavior in a confined geometry on a vibrating granular bed [44].

Yet, despite this wealth of research conducted on simulation and theoretical active matter systems (as well as some of the aforementioned biological active matter systems), realizing many of these systems in a synthetic experimental system has remained a tremendous challenge. There are relatively few experimental artificial or synthetic soft active matter systems that are able to effectively reproduce or mimic the emergent non-equilibrium behavior observed in biological active matter systems. In recent years, however, there has been progress in developing experimental model systems to confirm the exotic behavior reported in many of these theoretical and simulation studies. Yet, developing a novel, versatile, and biologically relevant artificial soft active matter systems still remain a challenge.

1.3 Experimental design motifs of experimental artificial soft active matter systems

When developing experimental artificial soft active matter systems, the most natural starting point is the development of the active unit or units. To this end, there has been an extensive amount of work done on developing artificial microswimmers or SPP. There are a multitude of different design architectures that have been utilized to develop active units, some of which are driven externally and others which are driven locally. The designs range from artificial flagella swimmers whose flagella moves via actuation of a magnetic field, electrically driven micro-machined robotic swimmers, laser-heated metal capped particles, and particles driven by an externally applied electric field or magnetic field [42, 51, 52, 53, 54]. Other swimmers or SPP locally convert energy into motion, usually via the catalysis of some type of reaction. This type of SPP is usually a phoretic swimmer, light activated colloid, Janus particle, Quincke particle, Pt-Au rod, and even platinum loaded stomatocyte [42, 55, 56, 57, 58]. While it is clear that these designs are numerous and varied there are several design motifs that are consistently utilized when developing active units for experimental artificial soft active matter systems, which can be seen in Fig. 1-4.

These designs can be classified as i) phoretic/catalytic/light activated or Janus particles, ii) biological motor, iii) optical tweezers, iv) vibrated granular beds, v) Quincke particles, and vi) magnetically driven particles.

The first classification is the most broad and encompassing. It is also perhaps the closest to the traditional definition of active matter systems; that being a system composed of active units that \textit{locally} convert available energy into motion. These types of particles intrinsically break the forward-backward symmetry due to their asymmetric surfaces. Take for example one of the first phoretic
Figure 1-4: A) Magnetically driven active particles. B) Quincke rotating active particles. C) Phoretic Pt-Au active rods. D) Microtubule filament bundles and kinesin motors. E) Janus particles driven by external electric field. F) Light activated active colloids. Reproduced from [54, 55, 9, 42, 56, 57].

Janus particles, the Pt-Au rod. These rods utilize hydrogen peroxide, H₂O₂, as fuel. This fuel is electrochemically decomposed, creating a gradient in H⁺ ions and thus an electric field which points from Pt to Au [59]. The rod itself has an inherent negative charge [59]. Therefore, this positively charged electrical double layer surrounding the rod experiences a force due to the generated electric field [59]. A fluid flow then develops, flowing from Pt to Au, causing the rod to swim [59]. This process is termed swimming via self-electrophoreses [59]. This swimming mechanism is ideal in serving as an artificial swimmer to be used in artificial active matter systems as they are capable of autonomous motion without the application of an external field [59]. However, there are some key limitations to this type of active unit. One of which is that the fuel, hydrogen peroxide, is being continuously consumed and decomposed and therefore the particles cannot maintain such autonomous motion indefinitely without specialized experimental considerations. The other issue is that if such a system is to be utilized to mimic a biological system of interest, the necessity of a hydrogen peroxide environment is a limiting factor. Although it should be noted that there has been work done recently in exploring active catalytic particles that utilize glucose as reaction fuel instead of hydrogen peroxide.

Perhaps the most successful attempt at developing model experimental active matter systems has been integrating biological components into synthetic in-vitro systems. Dogic’s system consisting of microtubule bundles and kinesin motor clusters is a prime example of such a system. Bundles of microtubules form via the addition of polyethylene glycol (PEG) depletants [9]. Biotinylated kinesin were formed into motor clusters by binding to tetrameric streptavidin ligands that can bind and move along the microtubule filaments [9]. This binding induces sliding of the microtubules depending on the polarity of the microtubule pair (i.e. no sliding force is generated for the same polarity whereas a sliding force is generated for opposite polarity) [9]. The introduction of adeno-
sine triphosphate (ATP) induces the motility of kinesin cluster and subsequent microtubule sliding leading to emergent streaming 2D nematic nonequilibrium steady states [9]. Similar emergent phenomenon has been seen in systems which utilize bacteria in-vitro under confinement (ratchets or other topological defects), bacteria interacting with colloids, and anisotropic bacteria of differing lengths. These systems are very effective at reproducing some of the emergent phenomenon observed in biological systems, particularly because they use many of the biological components of the system under investigation. However, this methodology has some flaws in developing novel model experimental active matter systems. One of the first that comes to mind is again that fuel is utilized to induce motility whether that be ATP, oxygen, etc., and eventually that fuel runs out or dips below a threshold concentration where activity is then affected. There are clever ways to engineer a system to supply the system with fuel, however, this is not always a feasible solution. Another problem is that utilizing so many biological components can defeat the purpose of developing model synthetic active matter systems as it makes it difficult to extract what behavior is due to fundamental driving forces and what behavior is due to biology. Finally, many of these systems are not as versatile as one would like and it is difficult, or in some cases impossible, to change the mode of activity exhibited in many of these system.

Optical tweezers are a relatively new, yet very attractive method to investigate non-equilibrium emergent interactions. This technique has been established previously to measure the viscoelastic behavior of complex fluids as well as measuring the mechanical properties of cells. However, recently this has emerged as an attractive technique, particularly to the physics community, to measure emergent attractive interactions in non-equilibrium active matter systems. This technique allows for precise control over single active agents. The mode of motility or motion can be manipulated precisely and the force acting on the active agent can be quantified as well. The drawbacks of this technique is the complexity of the experimental set-up and the difficulty of changing system parameters on the fly during experimental runs. On the other side of the spectrum an exceedingly simple experimental design to induce motility, a vibrating granular bed, has proven to be an exceptional synthetic model system that can reproduce emergent flocking and directed motion observed in many biological systems of interest [44]. Ramaswamy’s vibrating granular bed allows one to change activity induced on the fly. It is relatively simple to change the shape of the active agent and amount of activity. Increasing the concentration of active agents is also fairly simple. Additionally, with minimal modifications one can investigate confinement effects in this system as well [44]. The one drawback to this system is that it is more a study in granular active matter rather than biological active matter. In this system, there are no hydrodynamic interactions present and in many active matter systems hydrodynamic interactions play a critical role in many different types of emergent behavior.

Two of the most prominent and promising designs for synthetic active units are Quincke particles and magnetically actuated ferromagnetic or superparamagnetic particles. The Quincke particles are the ideal active agent. Quincke particles become motile upon application of an externally applied electric field large enough so that charge accumulation at the surface of the particle induces a
dipole moment opposite to the direction of the applied electric field [55]. The resultant electrostatic torque, causes the particle to rotate and if the particle is in contact with a substrate with a non-zero coefficient of friction some of that rotational motion will be converted into translational motion [55]. These particles are non-interacting and traverse in random directions but under certain confinement, activity, and concentration conditions can exhibit emergent flocking or swarming behavior [55]. For a physicist this is the ideal synthetic active matter system as there is no fuel requirement limitations as in the other phoretic particle systems. However, the one limitation to this system is the ability to investigate the behavior of these particles in complex media or see how different sized particles interact. In order to exhibit Quincke, rotation the solution of the system is calibrated to the type and size of particles utilized. Also, the solution in this system, hexadecane, is not ideal to extend the study to in-vitro biological conditions [55]. Ferromagnetic or super-paramagnetic particles are made active via the actuation of an externally applied magnetic field. Depending on the configuration of the magnetic coils, the particles can exhibit virtually any type of motility and a 3D magnetic coil apparatus could allow one to change the mode of motility on the fly during an experiment [54]. Additionally, these particles are motile in biologically relevant environments and one can easily functionalize the surface of the particles to better mimic biological conditions. Parameters in these systems are easily tunable from the activity of the particles via the externally applied magnetic field and there is no dependence on activity with the solution in which the particles are embedded [54]. Also, because the magnetic field is externally applied, there is no fear of fuel being consumed to induce activity. One drawback for this design is the ever present magnetic interactions between ferromagnetic particles. This can be avoided by using superparamagnetic particles, but at a cost of activity (unless one compensates by using much larger magnetic fields to obtain similar torques exerted on the particles)[54]. This magnetic dipole-dipole interaction is particularly troublesome for active matter studies as typically one would like to work with non-interacting particles. There is also additional and sometimes exhaustive testing that must be conducted to ensure the observed emergent behavior is due to some driving force other than the magnetic dipole-dipole interaction. This can be done via simulations or experiments, however both are preferential.

1.4 Developing artificial soft active matter systems: from single active particle to many active units in complex media

The aim of this thesis is to develop novel, robust synthetic soft active matter systems. We will start with a relatively simple system with a single active unit to understand the role that friction plays in the biological process of chemotaxis. We will then increase in complexity and look at the emergent behavior of active units embedded in complex passive media. Again, here we will start simple and explore emergent two body interactions and investigate the effect of the mechanics of the passive complex media, activity, actuation protocol, composition of the complex passive media, and mode of activity on the range and strength of any emergent attractive interactions between active units. Then, we will investigate emergent interactions in complex passive media with a large
concentration of active units and illustrate that the growth rate of active clusters is optimized at a particular cluster size which minimizes the drag force exerted on the cluster by the medium and maximizes the stress exerted on the passive medium. Finally, we will illustrate that active agents in a complex, disordered environment of fixed obstacles or posts exhibit super-diffusive transport behavior. We will investigate the effect an externally applied force, gravitaxis, has on this disorder induced transport. To this end, the thesis will be organized as follows:

- Chapter 2 describes the development of a magnetically actuated tribotactic system that leverages the concept of friction to develop a synthetic soft active matter system that performs chemotaxis.

- Chapter 3 elaborates on the effect of friction between magnetically actuated rollers and both soft and hard substrates, including the development of a rolling parameter to characterize this effective friction interaction.

- Chapter 4 describes the development of a hybrid active-inactive soft active matter systems and investigates the role that the mechanical properties of the medium, activity, mode of activity, actuation protocol, and the composition of the passive media has on the strength and range of an emergent attractive interaction between active units.

- Chapter 5 describes Lattice-Boltzmann simulations that confirms the origin and nature of this non-equilibrium emergent interaction and subsequent phase separation in the absence of magnetic interactions. We confirm this interaction is due to the inherent non-equilibrium nature of this system.

- Chapter 6 describes the aggregation behavior of many active units embedded in dense passive media and finds an optical cluster growth rate at a particular cluster size in such systems.

- Chapter 7 describes disordered induced transport phenomenon observed when active agents are placed in a disordered post filled environment.

- Chapter 8 summarizes the findings of this thesis and describes open questions and possible directions for future applications.
Part I

Active Tribological Probes
CHAPTER 2
SYNTHETIC CHEMOTAXIS: MAGNETICALLY ACTUATED TRIBOTACTIC MICROSCOPIC WALKERS

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The primary aim of this thesis is to develop artificial experimental model soft active matter systems that are able to effectively mimic the emergent non-equilibrium behavior observed in many vital and ubiquitous biological systems and processes. To accomplish this, we started with the most simple active matter system that we could develop; a simple, single active unit (specifically a sphere) in a fluid in contact with a substrate. The biological system that we sought to mimic was not nearly as simple. It was the ubiquitous biological process of chemotaxis and up to this point, there had been many attempts to create a synthetic system able to perform chemotaxis in a non-equilibrium fashion but none had succeeded. Chemotaxis is a vital biological process that is crucial to many biological functions like reproduction, wound healing, and cell differentiation just to name a few [60, 61, 62, 63, 64, 65, 66]. Chemotaxis is the ability of a cell to detect and move along a concentration gradient and cells are able to detect or sense minute changes in these concentration gradients [67, 68, 69, 70]. We imagined that we could design a microscopic scenario where chemical and frictional gradients were essentially equivalent and we could leverage this concept of friction between the active agent and the substrate to obtain emergent direction motion, i.e. chemotaxis. Except in this case, since chemical and frictional gradient were equivalent, we called it triobotaxis.

In this Chapter, we will describe the design of a novel soft active matter system that is able to perform chemotaxis. The active units are composed of two superparamagnetic particles that are made motile upon actuation of a rotating magnetic field. These particles are coated with streptavidin ligands and placed on a substrate with a gradient in the density of the corresponding ligand, biotin. Upon actuation of the rotating magnetic field, the magnetic moments of the superparamagnetic particles will align with the applied field and begin to rotate. This rotational motion will be converted into translational motion due to the effective friction between the particles and the substrate and thus begin to translate or walk, hence walkers. If the walkers encounter a biotin binding site, the effective friction between the walkers and the substrate is increased and more of the rotational motion is converted into translational motion and the walker translates more. In the absence of such a binding site, the effective friction is reduced and the walker translates less
somewhat analogous to a tire translating more efficiently on an asphalt road rather than an icy road. The walkers are able to effectively sense differences in the concentration of biotin binding sites in a nonequilibrium fashion. And since here the density of binding sites is intimately related to effective friction, we are simultaneously sensing a chemical and frictional gradient. Moreover, when the walkers are actuated in a stochastic manner in one dimension, they exhibit chemotactic drift (or tribotactic drift) towards regions with a higher density of biotin binding sites and thus regions with a higher coefficient of friction. We will investigate the existence of and quantify the strength of tribotactic traps. These tribotactic traps are equivalent to chemotactic cells becoming trapped in their own chemoattractant and forming biofilms. We will evaluate the stiffness of this trap by drawing an analogy to the probability distribution of a harmonic oscillator. Finally, we will develop a master-equation-based model of the stochastic motion exhibited by the walkers. By deriving a Fokker-Planck equation via a truncated Kramers-Moyal expansion of the original master equation, it is possible to solve the time evolution of the probability distribution of the position of the walkers.

2.1 Ubiquitous nature of chemotaxis

The basis of life is the continual search for chemical energy and the subsequent conversion of that chemical energy into motion. This basic necessity for survival requires that organisms must have some facility that allows them to detect and respond to changes in the environment. A number of mechanisms have evolved by which organisms sense their environment and respond to signals they detect. The typical response involves movement toward favorable environmental signals and away from potentially toxic environments. This movement involves a process called kinesis, which is a change in the frequency of turning by the organism in a particular environment [69]. Organisms may also utilize a movement response called taxis, which is a directed movement toward or away from a particular environment [69, 68, 70]. An organism's response may involve a combination of these two processes.

One such response is the chemotactic process, where a cell is able to detect the concentration gradient of a particular chemical signal in an environment and then moves accordingly along that gradient [69, 68, 70]. Numerous types of biological cells exhibit this process, which only serves to exponentially increase the possible applications if one could synthetically mimic chemotaxis. Bacterial E.coli perform run and tumble chemotaxis in order to move toward regions that are rich in nutrients or to avoid regions of high toxicity [69, 68, 70]. Spermatozoa swimming is directed towards an unfertilized egg based on a concentration gradient of sperm activity and attracting factor (SAAF) and Ca^{2+} ion which modulates flagellar beating as well as swimming direction [65, 66]. Additionally, neutrophils, a type of white blood cell involved in combating infections can detect chemical signals, specifically cytokines, which are produced by pathogens in a wound [60, 61, 62, 63, 64]. They migrate toward this smell in order to kill the pathogen via phagocytosis (ingestion), the release of soluble anti-microbials, or the generation of neutrophil extracellular traps.
Some of these chemotactic processes can be seen in Fig. 2-1.

Figure 2-1: A) Bacterial E.coli run and tumble chemotaxis. B) Spermatozoa chemotaxis in presence of SAAF and Ca$^{2+}$. C) Neutrophils chemotaxis towards pathogens in a wound. Reproduced from [71, 72, 65].

There have been many attempts to develop a synthetic system that is able to perform chemotaxis in a non-equilibrium fashion. To this end, there has been a tremendous effort in designing artificial microswimmers or propulsion mechanisms that can mimic a biological organism’s mode of propulsion, particularly flagella beating/rotation and ciliary beating. Motifs range from artificial flagella swimmers that are made active via the actuation of an externally applied magnetic field, phoretic swimmers that catalyze a reaction on one side of the swimmer causing a pressure differential and subsequent propulsion, and electrical micro-machined real robic swimmers [42, 51, 52, 53, 54, 42, 55, 56, 57, 58]. Interestingly, despite the tremendous experimental and theoretical effort in the last several years in designing artificial active systems that can perform chemotaxis in an intrinsic way [73, 74, 75], the design of a chemotactic artificial active system that detects biological molecules remains a challenge.
2.2 Tribotactic system: leveraging friction to achieve directed motion

To develop an artificial soft active matter system capable of mimicking the biological process of chemotaxis, we wanted to leverage the concept of effective friction between an object and a substrate. In doing so, we would be effectively performing tribotaxis in order to achieve the emergent directed motion cells exhibit during the chemotactic response. Tribotaxis is the process by which an active object, biotic or abiotic, detects differences in the effective local friction coefficient and moves to regions of higher or lower friction according to a given protocol. The local friction coefficient between an object and a surface is dictated by the effective interactions between both. If these interactions are of directional nature, the friction coefficient is anisotropic. A prominent example of this is the skin of many animals that feels rough when stroked in one direction, yet soft in the other. The origin of this asymmetry is the directionality and ordering of hair or scales sticking out from the surface at a slanted angle, which helps in modulating the effective friction between the skin (or scales) of the animal and the surrounding fluid\[76, 77\]. These types of materials are thus important in many other processes, such as in regulating the flow of complex fluids\[78\], in controlling the motion of cells\[79\], or even in skiing up a mountain!

While the motifs that give rise to these asymmetric friction coefficients are rather large, one can envision an alternative microscopic scenario. For example, one can think of exchanging the mechanical texture by a chemical texture in which friction is dominated by the strength and spatial density of reversible bonds between an object and a substrate\[80, 81\]. Moreover, gradients on the spatial density of such ligands would produce anisotropic friction coefficients in addition to a concentration gradient in the density of such ligands. Live cells naturally detect surface ligand gradients and move accordingly\[82\]. This is one of the most important clues for locomotion since cells are constantly encountering surfaces in our bodies. Mimicking this behavior using biological ligand-receptor pairs can potentially allow one to walk on and sense different conditions in the vast amounts of interfaces in tissues and organs. In addition, chemically-based tribotaxis can be used to locally sense friction in purely synthetic environments.

Here, we have developed a tribotactic system able to detect gradients in friction due to gradients in the density of biological receptors, thus effectively performing chemotaxis\[83, 84, 85\]. The system is a magnetically assembled colloidal doublet (a microwalker) which converts rotational motion into translational motion due to enhanced friction near a surface. Clearly, for weak friction scenarios the doublet primarily slips and translates slowly, while for higher friction coefficients the doublet will walk faster in a hinge-type fashion as seen in Fig. 2-2. The effective friction for this system can be tuned by functionalizing the surface of the magnetic beads and the substrate with complementary ligand-receptor pairs (Fig. 2-2). Thus, by controlling the spatial density of ligands on the surface it is possible to create gradients in the friction coefficient. From non-equilibrium mechanics, it is known that if a particle is stochastically driven on such gradients, its motion resembles that of biased random walk \[86\]. To create such stochastic walks, we utilize a particular protocol that can
be best described in a 2-part scheme per random step. In the first part of the step, so-called the move, we rotate the microwalker by applying a torque in a given direction for a fixed time $\tau$. In the second part, so-called the relax step, we allow the doublet to lie flat for a period of time $\tau_{\text{relax}}$, which increases the probability of forming bonds with the substrate. We repeat this protocol for the total number of steps of the walk, and randomize the direction of the torque per step according to a 1D random walk. Clearly, over the course of multiple steps following the protocol previously described, the microwalker will drift towards regions of higher ligand density where friction is higher, eventually becoming trapped in such regions. And since here friction is intimately related to the density of binding sites, this process of friction directed motion can also be understood as a type of chemotaxis-like behavior[83, 84, 85].

![Diagram](image)

Figure 2-2: A) A superparamagnetic doublet, or microwalker, spontaneously self-assembles upon actuation of a magnetic field, B. To rotate the magnetic doublet on the substrate, we couple the doublet to a rotating magnetic field. The microwalker is actuated for a time period $\tau$, and is then allowed to relax for a certain period $\tau_{\text{relax}}$; this constitutes one random walk step. The motion of the center of mass of the tribotactic walkers is in general denoted by $\delta$. In particular, the walkers tend to perform larger displacements $\delta_2$ when moving towards regions of higher friction, and display smaller steps towards regions of lower friction $\delta_1$. Hence, the microwalker will drift toward regions of higher friction, effectively migrating toward areas with a higher ligand density. B) The effective friction (or interaction) between the beads and the substrate is controlled by functionalizing both surfaces with complementary ligand-receptor pairs. In this work, the beads are functionalized with streptavidin and the substrate with biotin. The beads, 3 $\mu$m in diameter, are further functionalized with mPEG-biotin to mask and reduce the effective binding strength between the walker and the substrate. Free avidin is also used to block available binding sites on the substrate.

As mentioned above, our tribotactic system is composed of two superparamagnetic beads coated with streptavidin and a biotinylated substrate. To modulate the surface and reduce the binding strength of the biotin-streptavidin bond, we decorate such beads with biotinylated PEG, leaving only a few active sites on the surface. To passivate the surface of the walkers, the super-paramagnetic streptavidin coated particles 2.8 $\mu$m in diameter and provided by Solulink (MagnaLink$^{TM}$ Streptavidin Magnetic Beads) were diluted 50x in an aqueous solution. mPEG-
biotin (MW 5000), provided by Nanocs, were diluted to a concentration of 100 mg/mL in an aqueous solution and then added to the solution of super-paramagnetic beads. The concentration of magnetic beads and mPEG-biotin was such that each of the streptavidin proteins were, theoretically, coated with one PEG molecule. The solution was left to react for 24 hrs at room temperature before use. The solution of super-paramagnetic particles was then diluted 30x in water prior to insertion into the channel and subsequently sealed to prevent evaporation. The density and distribution of biotin available sites on the substrate can also be screened using free avidin (see Fig. 2-2).

To realize this model, the synthetic tribotactic system requires that: i) the biotinylated substrate must exhibit an anisotropic coefficient of friction or similarly there must be a gradient in the concentration of free or available biotin ligands and ii) the walkers must be driven by an apparatus that can produce a rotating magnetic field that exerts enough torque to break the biotin-streptavidin binding interaction.

2.3 Driving walker motion using magnetic coil apparatus

The experimental apparatus, seen Fig. 2-3, consisted of a compound binocular light microscope, provided by OMAX, mounted with two coils that were centered on the sample. A wave generator ran two sinusoidal signals (one per coil), phase shifted by 90 degrees, to obtain a homogeneous rotating magnetic field. The signal from the wave generator was passed through an amplifier before reaching the coils. A 300W amplifier (150W/channel) ran 5 Amps through the more than 100 turns of wire to obtain a field strength of approximately 10 mT. The magnetic field can be described by the following equation

\[
B(x) = \frac{INR^3\mu_o}{(R^2 + (d_x - x)^2)^{\frac{3}{2}}} + \frac{INR^3\mu_o}{(R^2 + (d_x + x)^2)^{\frac{3}{2}}}
\]  

(2.1)

where I is the current passed through the coils in Amperes, \(N\) is the number of turns of wire, R is the radius of the coils, \(\mu_o\) is the magnetic constant, and \(d_x\) is the distance between the two coils centered on the x-axis. The applied magnetic field induces a magnetic dipole moment on each of the beads which can be calculated using the relationship below

\[
m = \frac{V_c\Delta\chi}{\mu_0}B
\]  

(2.2)

where \(\mu_0\) is the magnetic permittivity, \(V_c\) is the effective magnetic volume of the superparamagnetic particle, \(B\) is the applied magnetic field strength, and \(\Delta\chi\) is the magnetic susceptibility difference between the particle and medium. The torque that is applied to these particles is simply given by

\[
\tau_B = m \times B
\]  

(2.3)

where \(m\) and \(B\) have been previously defined. As previously mentioned, this torque produces rotational motion and this motion is converted into translational motion due to the effective friction
between the object and the substrate. This motion is opposed by the Stokes drag force, which is imparted in this instance by an aqueous medium, given by the equation

\[ F_d = 6\pi \zeta r \nu \]  

where \( \zeta \) is the viscosity of the fluid, \( r \) is the particle radius, and \( \nu \) is the particle velocity. This field strength is large enough to maintain alignment between the magnetic moment of the walkers and the magnetic fields, as well as being able to break the reduced biotin-streptavidin interaction. It should be noted that without passivation, the walkers are unable to walk on the substrate. The signal from the amplifier was routed to an oscilloscope, HP (Hewlett-Packard) 5460A, in order to get a precise measurement of the frequency and the voltage at the output. The frequency utilized throughout these experiments was 1 Hz. Data acquisition was accomplished via a CCD camera that had been mounted on the microscope. The camera was connected to a computer for visualization, video capture, and subsequent analysis.

![Diagram of experimental setup](image)

Figure 2-3: A) A light microscope was mounted with two coils to produce a rotating magnetic field. A wave generator ran two sinusoidal signals phase shifted by 90 degrees through an amplifier to generate a field strength of 10mT. An oscilloscope was used to monitor the frequency and a CCD camera mounted on the microscope was used to capture videos of the walker motion. B) Schematic of resultant magnetic field, reproduced from [87].

To analyze the motion and velocity of the particles we used HyperCam 2, a screen capture program, to take live videos of the walkers in motion, as seen in Fig. 2-4. The videos were loaded
into Tracker Video Analysis and Modeling Tool version 4.72 and then analyzed using a user defined square area to find the contrast between the image background and the walkers. The centroid of the walker was then calculated and tracked through the remaining frames. The file output gave the position of the particle in pixels at each time step. The pixel units were then converted into micrometers and the output file was then imported into Mathematica for subsequent analysis.

This experimental apparatus makes the walkers active in this soft active matter system. However, to perform chemotaxis and achieve directed motion, the substrate must exhibit an anisotropic coefficient of friction and gradients in the density of biotin binding sites.

2.4 Detecting gradients in the coefficient of friction

The gradients in friction were created by drying a concentrated droplet of free avidin ligands which were left to evaporate on a biotinylated substrate, provided by Xenopore, with a biotin binding density of $\sim 10 \times 10^{12}$ sites cm$^{-2}$ (see Fig. 2-5). An 8mm×8mm square was marked on the back of the biotin slide. A 50μL drop of a 1 mg/mL concentrated droplet of avidin, provided by Sigma-Aldrich, was placed on the biotin slide at the center of the square and left to evaporate overnight. The capillarity flows induced by the differential evaporation rates across the droplet carried most of the avidin ligands to the edge, thereby producing a coffee-ring like pattern along the perimeter of the droplet [88]. Interestingly, a robust annular deposition pattern of biotin binding sites also develops around the center of the droplet. The typical length between regions that display larger friction coefficients is on the order of 1 mm, as shown in Fig. 2-5. Such phenomenon has been observed in other systems as well, such as polymer deposition [89]. Note that the particular concentric annular pattern obtained varies with droplet size and concentration of avidin, yet is highly reproducible for the same conditions. The avidin residue was cleaned off with water and two pieces of 3M double sided tape were placed at the edge of the square and a glass coverslip was placed atop the pieces.
of tape. The solution of super-paramagnetic beads and mPEG-biotin was then inserted into the channel and taken up by capillary forces. The edges of the coverslip were sealed with wax to prevent evaporation. The slide was then placed on the sample stage to be magnetically actuated. It should be noted that the particle concentration was sparse enough so that there were several doublets, on the order of ten (and occasionally some longer chains). The rest of the particles were sparsely distributed, single particles found throughout the 8mm² area. Occasionally, there was a preformed doublet close enough to the area of interest so that the experiment could begin once the magnetic field was initiated. If there was not a preformed doublet but instead two isolated single particles in close enough proximity, on the order of the tens of μm, we actuated the magnetic field back and forth until a doublet formed. This typically lasted for 2 min.

![Image](image.png)

Figure 2-5: A) A water droplet containing avidin is placed on the biotinylated substrate, and left to evaporate for 24hrs. B) Schematic of the gradient in the density of unblocked biotin binding sites based on the frictional landscape obtained by measuring the local walking velocities. C) Average velocity profile (μm /s) of the microwalkers as a function of the distance to the center of the droplet in μm. The origin (x = 0 μm) corresponds to the center of the avidin droplet. The horizontal red line denotes the velocity of the walkers on a fully passivated biotin surface, measured in an independent experiment. The colored dots denote the initial positions from which the random walks in Fig. 2-8 were launched. The frequency of rotation is set to 1Hz.

With the magnetic coil apparatus driving the walker motion and the substrate with an anisotropic coefficient of friction created, we can test the experimental model system to see if these walkers can not only detect differences in effective friction but move along those gradients as well. The resulting frictional landscape exhibits areas of high friction separated by low friction valleys. This can be seen in Fig. 2-5, where we plot the velocity of translation of the doublet vs. the distance to the center of the droplet. In this case we continuously allow the microwalkers to move, effectively setting τ → ∞. However, in reality the walkers were moved to the center or origin of the sample, (center of the evaporated avidin droplet or 0 μm) and then allowed to walk until traversing approximately 75 μm. While continuously traversing this relatively large distance, the walkers exhibit continuous movement with little to no sticking. To ensure there was no hysteresis depending on
the direction of rotation, we measured the velocity for the walker going away from the origin of the sample as well as towards the center of the droplet. Both measurements coincided, which confirms the system is reversible. This was repeated four more times and then the walker was moved to the next section of the sample and this procedure was repeated across the sample region of interest. This entire procedure was then reproduced two more times using new substrates and materials to ensure reproducibility as seen in Fig. 2-6.

Figure 2-6: The tribotactic walker velocity profile across the sample for three different droplets with average velocity (red).

Note that higher velocities correspond to higher effective friction. In particular, we find the velocity at \( x \approx 0 \, \mu\text{m} \) and \( x \approx 800 \, \mu\text{m} \) to be approximately 30-40 \% larger than at the lowest point, corresponding to \( x = 450 \, \mu\text{m} \). The velocity displayed in this latter region corresponded to a biotin substrate that was completely coated with avidin ligands (horizontal red dashed line), which was confirmed by an independent measurement on a surface with no free biotin (see Fig. 2-7). Assuming a simple Stokes drag scenario, the variations in velocity imply a difference in effective friction forces between both regions of less than 60 fN. Thus, our microwalkers are extremely sensitive to minute variations in the friction coefficient. This is an extremely small force compared to the streptavidin-biotin bond and we speculate that we are forming very tense bonds with extremely low affinity. This is due to the presence of the dense PEG brush. In fact, in this system we can modulate the effective friction between the walkers and the substrate via passivation of the surface of the walkers or the biotinylated glass substrate. As seen in Fig. 2-7, when we completely coated the biotinylated glass substrate with free avidin ligands, which was accomplished by increasing the concentration of free avidin ligands in the droplet and not allowing evaporation to occur, the velocity profile is flat. There are no available biotin binding sites and the effective friction is reduced and the walkers essentially
sense a homogeneous frictional environment. Similarly, once can increase the passivation on the surface of the walkers to decrease the effective friction as well. Instead of coating the walkers so there was only one biotin-PEG per streptavidin molecule, the solution was saturated with biotin-PEG molecules so that there were $50\times$ the number of biotin-PEG molecules per streptavidin binding sites. As described above, the same procedure to map the velocity profile across the substrate was followed and can be seen in Fig. 2-7. As anticipated, the effective friction is reduced but it is of interest that using this passivation technique we are still able to sense the differences in effective friction across the substrate, albeit the differences have been reduced drastically. Even though the concentration of PEG is high enough to coat the walkers $50\times$ over, due to crowding of the PEG chains, it is feasible that there are still a number of free streptavidin binding sites which is why the walkers can still sense the friction and concentration gradient.

![Figure 2-7: A) Tribotactic walker velocity profile across the sample on a circular patch, comparable to the droplet size, homogenously coated in avidin. B) Velocity profile of walker on coffee-stain droplet completely saturated with biotin-PEG (blue) compared with a walker coated with one biotin-PEG per streptavidin binding site (red).](image)

2.5 Tribotactic drift along gradients in the coefficient of friction and density of biotin binding sites

In the previous section, we have demonstrated that our synthetic experimental model soft active matter system is able to detect gradients in the coefficient of friction along a substrate or equivalently a gradient in the density of biotin binding sites. However, the process of chemotaxis involves not only the ability to detect gradients in concentration, but also the ability to move along these gradients. In testing this system to see if it is truly able to perform chemotaxis, the walkers when actuated in a stochastic fashion should be able to detect gradients in concentration and migrate towards regions with a higher density of biotin and higher effective friction. The tribotactic nature of our microwalkers was probed by actuating them with a rotating magnetic field where the
direction of rotation was varied in a stochastic fashion, as in a one-dimensional random walk. The
total number of steps in each walk was set to 300. The list of random steps was generated in such
a manner that the total number of right and left steps was approximately equal. By doing this, we
avoid the potential drift of the random walk due to limited sampling. We allowed the system to
relax for $T_{\text{relax}} = 10$ s in between steps, which provides enough time for the doublet to lie parallel to
the substrate. The period of actuation, $\tau$, was set to 2 s. The trajectories for several independent
random walks originating from 0, 300, 450, 600, and 800 $\mu$m are shown in Fig. 2-8A.

Figure 2-8: A) Random-walk trajectories for doublets initially placed at approximately 0, 300,
450, 600, and 800 $\mu$m from the origin. The color of the walk corresponds to the highlighted
position in Fig. 2-5. A drift towards regions with a higher friction coefficient is observed. Walkers
initially placed in regions with the maximum friction coefficient became trapped. B) Detailed
trajectories for walkers initially placed at 600 $\mu$m. The solid, dashed, and dotted lines corresponds
to a linear fit to the experimental trajectories (slope 0.28 $\mu$m/s), the simulated trajectories, and
the theoretical drift velocity ($u = (\delta_2(x) - \delta_1(x))/\tau$) respectively. Inset: probability distribution of
the displacement in each direction. $\delta_1(x)$ corresponds to the mean displacement in the direction
of lower friction, while $\delta_2(x)$ denotes the average displacement toward higher friction areas. C)
Trajectories for walkers initially placed at 300 $\mu$m. $\delta_1(x)$ and $\delta_2(x)$ are defined as in part B. The
different lines correspond, as in part B, to the linear fits to the experimental (solid line), simulated
(dashed line), and theoretical (dotted line) results. The slope of the linear fit to the experimental
data is 0.32 $\mu$m/s.
As is clear from this plot, the doublets initially positioned at \( x = 0 \) and \( x = 800 \) \( \mu \)m become trapped in these regions with larger friction coefficient. Walkers initially positioned at regions with a lower coefficient of friction, like \( x = 300 \) and \( x = 600 \) \( \mu \)m, drift along the gradient in the direction of stronger friction. The region with the lowest friction is at an unstable point and the microwalkers can go either way. This is clearly observed for the walkers initially positioned around \( x = 450 \) \( \mu \)m, which drifted in both directions (see green trajectories in Fig. 2-8). For microwalkers starting at \( x = 300 \) \( \mu \)m and \( x = 600 \) \( \mu \)m, we computed the drift velocity from the slope of a linear fit to their trajectories as seen in Fig. 2-8B and C. The drift was estimated to be \( u = 0.32 \) \( \mu \)m/s \( (x = 300 \) \( \mu \)m) and \( u = 0.28 \) \( \mu \)m/s \( (x = 600 \) \( \mu \)m). Notice, however, that the drift velocity of these tribotactic microwalkers can be tuned by modifying the parameters of the system such as the actuation protocol or the size of the walkers. Furthermore, our approach is extremely versatile because the beads can be functionalized with a multitude of biologically relevant motifs or can even be multiplexed, meaning they can have combinations of motifs to explore cooperative effects or multiple gradients. Thus, such tribotactic microwalkers can be used as sensitive chemotactic probes in biological environments. This overcomes a present challenge that current approaches face in creating artificial chemotactic systems because they typically rely on particles that consume or catalyze non-biological fuels in order to achieve a chemotactic response\[90, 91, 75\]. Notice that the origin of the tribotactic response in our system is due to the frictional landscape of the environment and not on the presence of a non-biological fuel source. Interestingly, this system where the viscous drag and the forces are both relatively small and weak, yields tribotactic drifts on the right order of magnitude compared to biological systems.

To further understand our tribotactic system, we analyzed the distances traveled by the walkers at each step for both directions. A probability distribution of such displacements is shown in the insets of Fig. 2-8 for the doublets positioned at \( x = 600 \) \( \mu \)m and \( x = 300 \) \( \mu \)m, respectively. From these distributions it is clear that the tribotactic walkers exhibit, on average, asymmetric displacements depending on the direction of motion. The average displacement toward regions with weaker friction is denoted by \( \delta_1(x) \) and toward regions with stronger friction is denoted as \( \delta_2(x) \), and their corresponding standard deviations are \( \sigma_1(x) \) and \( \sigma_2(x) \) (see insets Fig. 2-8). It is well known that such asymmetric distributions lead to directed motion (drift). Additionally, to gain a more fundamental understanding of tribotactic drift, we took a deeper look at the behavior of walkers initially placed at 300 \( \mu \)m. The distributions towards and away from the high friction regions are presented in Fig. 2-9.

Again, it is clear that directed motion is achieved by walkers breaking the symmetry of the system by traveling further on average per step when moving towards a region with a stronger friction coefficient (\( \delta_2 \)), than towards low friction regimes (\( \delta_1 \)). The three random walk trajectories of 300 steps were then segmented into 50 step increments to compute the average displacements in these segments across the sample distance as seen in Fig. 2-9B. The distance traveled is consistently larger when moving towards high regimes of friction across the sample (red symbols) and it is independent on the position of the walker across the sample. This is an incredible result as it shows
Figure 2-9: A) Probability distribution of the displacement at each step for the 3 random walk trajectories of the walkers initially placed at 300 μm. The mean is given by δ₂ when walking towards a region with stronger friction (left side, red solid line and bars). The mean is given by δ₁ when walking away from a region with stronger friction (left side, blue solid line and bars). B) Distance traveled towards (red symbols) and away (blue symbols) from regions of strong frictions as a function of the distance from the center of the droplet. C) True time traveled during the 2 seconds random walk actuation time as a function of the distance from the center of the droplet. The red symbols denote time traveled when moving towards regions of strong friction while blue symbols represent time traveled when moving away from regions of strong friction. D) Velocity of the walkers as a function of the position on the sample when walking towards (red symbols) and away (blue symbols) from regions with strong friction coefficients. Green symbols and the black dashed line correspond to the velocity of the microwalkers when the rotating magnetic field is continuously actuated for approximately a minute.

The sensitivity of these walkers when detecting differences in effective friction and the distinction between tribotaxis and chemotaxis, as typically chemotaxis directed motion is due to differences in effective velocities across the sample. It should be noted that while conducting the experiments it was observed that occasionally the walkers would stick to the surface and not exhibit coherent walking motion for the entire 2 s actuation period. To investigate the correlation between the time the walkers were traveling and the effective distance traversed, we computed the true time moving towards regions with high and low friction coefficients by segmenting the trajectories into 50 step segments. The time that the walker travels clearly decreases as the walker moves into regions with a stronger friction coefficient, which was expected and confirmed by experimental observation. The fluctuations in the true time moving either toward or away from a region with higher friction is not related to the fluctuations in the distance traversed. We also computed the average velocity of the walkers when walking in each direction across the sample, as seen in Fig.
As expected, the velocity of the walkers towards high friction regions is larger than towards low friction regions. Interestingly, the velocity in both directions was significantly larger than the velocity of the walkers when it was continuously actuated (green symbols). In light of these results, we denote two frictional regimes: i) one static friction regime at short actuation periods and ii) a dynamical friction regime at long actuation periods. The origin of these two regimes is due to the fact that after each actuation period we allowed the walker to relax for $\tau_{\text{relax}} = 10$ s. This relaxation time is compatible with the formation of the bonds between the walkers and the substrate. Therefore, at short actuation periods the walker feels higher chemical friction than at longer actuation periods. In the dynamical regime, the hydrodynamic friction exerted by the walker on the wall almost compensates for this difference in the actuation periods studied here.

It is clear that this tribotactic system can not only detect gradients in the coefficient of friction (or gradients in the density of biotin binding sites) but also move along those gradients. Directed motion is achieved by the ability of walkers to locally detect differences in the density of biotin binding sites which leads to an asymmetric displacement of walkers. The displacements towards regions with a high density of binding sites are on average larger than displacements towards regions with a lower density of binding sites. This breaking of the system symmetry leads to directed motion and tribotactic drift. However, there was a particularly interesting finding that was mentioned previously and that was the observation of walkers that were initially positioned in high friction regimes remaining trapped in those regions for the remainder of the random walk protocol. This type of behavior was highly reminiscent of bacteria performing chemotaxis and then getting stuck in a region with a high concentration of chemoattractant. In fact, this process has been found to aid in the formation of biofilms. We sought to further investigate if these were truly traps and if they were traps, we wanted to measure the strength of these traps.

### 2.6 Tribotactic traps

As described above, the regions of highest friction appear to act as tribotactic traps. To measure the relative strength and existence of these traps with respect to the spatial step size of the random walks, we performed longer stochastic sequences of 500 steps starting at the center of the tribotactic trap ($x = 0 \mu m$). The effective spatial step size was varied by changing the actuation period, $\tau$, of the rotating magnetic field from 2, 5, 10, and 20 s. The resulting trajectories were analyzed and the corresponding probability distributions are presented in Fig. 2-10. The solid lines in Fig. 2-10A represent the numerical solutions based on Eq. (2.25) of our tribotactic model (to be discussed in the following section), including the directional dependence of the drift term due to the trap. We also simulated many more repetitions of such random walks to get better statistics (to be discussed in the following section). As expected, the increase in $\tau$ translates to a broadening of the steady-state distributions. In light of the Gaussian-like shape of the steady state distribution, we evaluated the stiffness of the trap $\kappa$ using the standard deviation, $\sigma$, of such distribution from Fig. 2-10A. From statistical mechanics, we know that the probability distribution of a harmonic oscillator can be
written as $P(x) \propto \exp\left(-\frac{1}{2}\kappa(T)x^2\right)$, where $\kappa = 1/\sigma^2$. In Fig. 2-10B, we can see that $\kappa \propto 1/\tau^2$; thus $\tau$ plays the role of a square temperature by analogy to the simple harmonic well. To corroborate the results from the steady-state distributions, we also calculated the root-mean-square displacement (RMSD) for the trajectories. As expected, the RMSD for both simulations and experiments exhibit a plateau at long times for all the different $\tau$. Such behavior is characteristic of a confined random walker and directly corroborates the presence of a tribotactic trap.

This concept of varying the actuation time was also interesting as another parameter, aside from passivation protocol, that could be utilized to tune the tribotactic drift behavior of the walkers. In
particular, we were interested in two tunable parameters: i) the actuation period, $T$, and ii) the frequency of rotation, $\omega$. Walkers were again positioned at 300\textmu m relative to the sample origin and then actuated on a random walk consisting of 300 steps using different actuation periods. The trajectories were traced for the different actuation periods, $T$, and were plotted as seen in Fig. 2-11A. The walkers exhibit tribotactic drift towards the region with a high coefficient of friction and then become trapped in this region, independently of $\tau$. However, the effect of increasing frequency yields a much different result. Walkers were again positioned at 300\textmu m and then actuated at a rotational frequency, $\omega$ of 5Hz, using a random walk protocol of 56 steps. As seen in Fig. 2-11B, the walkers no longer exhibit tribotactic drift. As the frequency of rotation increases, the amount of time the streptavidin ligands can bind to the biotin decreases dramatically. Clearly, for this system at 5Hz, the effective friction decreases and the walkers can no longer detect the concentration gradient of biotin binding sites. This is something that will be explored in Chapter 3.

![Figure 2-11: A) Experimental trajectories of the tribotactic walkers, initially placed at approximately 300 \textmu m, using different actuation periods: 2s (violet), 5s (blue), 10s (red), and 20s (green). B) Experimental trajectories of the tribotactic walkers, initially placed at approximately 300 \textmu m actuated at a rotational frequency, $\omega$, of 5Hz.](image)

### 2.7 Theoretical/analytical tribotaxis model: random walk with asymmetric displacements

We have experimentally developed a tribotactic system that exhibits chemotactic directed drift due to asymmetric displacements depending on the direction of motion. It is well known that such asymmetric distributions lead to directed motion or drift. In order to observe this, one can develop a master-equation based model of the stochastic motion exhibited by the doublets that incorporates such distributions of microscopic displacements. By deriving a Fokker-Planck equation via a truncated Kramers-Moyal expansion of the original Master Equation [92], it is possible to solve the time evolution of the probability distribution of the position of the walkers. This model equation has been previously used to describe chemotaxis [93, 94]. Note that the actuation protocol
is that of a random walk, and thus has no memory.

\[ P_3(x_1, t_1 | x_3, t_3) = \int_{-\infty}^{+\infty} dx P_1(x_1, t_1 | x_2, t_2) P_2(x_2, t_2 | x_3, t_3) \]  

\[ P_3(x_1, t_1 | x_3, t_3) = \int_{-\infty}^{+\infty} dx P_1(x_1, t_1 | x_2, t_2) P_2(x_2, t_2 | x_3, t_3) \]  

\[ P(x_0 | x, t + \tau) = \int_{-\infty}^{+\infty} d\delta_1 P(x_0 | x - \delta_1, t) \Pi(x - \delta_1 | x, \tau) + \int_{-\infty}^{+\infty} d\delta_2 P(x_0 | x + \delta_2, t) \Pi(x + \delta_2 | x, \tau) \]
\( P(x_0|x, t + \tau) \approx P(x_0|x, t) + \frac{\partial P(x_0|x, t)}{\partial t} \tau \) (2.8)

Substituting into Eq.(2.7), we arrive at the Master Equation of a Markov process which is the differential version of the Chapman-Kolmogorov equation:

\[
\frac{\partial P(x_0|x, t)}{\partial t} \tau = -P(x_0|x, t) + \int_{-\infty}^{+\infty} d\delta_1 P(x_0|x-\delta_1, t)\Pi(x-\delta_1|x, \tau) + \int_{-\infty}^{+\infty} d\delta_2 P(x_0|x+\delta_2, t)\Pi(x+\delta_2|x, \tau)
\]

(2.9)

Now, we perform a Taylor expansion around \( x - \delta_1 \) and \( x + \delta_2 \) of the two integral terms of Eq.(2.9):

\[
\frac{\partial P(x_0|x, t)}{\partial t} \tau = -P(x_0|x, t) + \sum_{n=0}^{\infty} \frac{(-1)^n}{n!} \frac{\partial^n}{\partial x^n} \left[ P(x_0|x, t) \int_{-\infty}^{+\infty} d\delta_1 \delta_1^n \Pi(x|x-\delta_1, \tau) \right] + \sum_{n=0}^{\infty} \frac{1}{n!} \frac{\partial^n}{\partial x^n} \left[ P(x_0|x, t) \int_{-\infty}^{+\infty} d\delta_2 \delta_2^n \Pi(x|x+\delta_2, \tau) \right]
\]

(2.10)

The zeroth order terms of the Taylor expansion around \( x - \delta_1 \) and \( x + \delta_2 \) cancel the first term in the right hand of Eq.(2.9):

\[
\frac{\partial P(x_0|x, t)}{\partial t} \tau = \sum_{n=1}^{\infty} \frac{(-1)^n}{n!} \frac{\partial^n}{\partial x^n} \left[ P(x_0|x, t) \int_{-\infty}^{+\infty} d\delta_1 \delta_1^n \Pi(x|x-\delta_1, \tau) \right] + \sum_{n=1}^{\infty} \frac{1}{n!} \frac{\partial^n}{\partial x^n} \left[ P(x_0|x, t) \int_{-\infty}^{+\infty} d\delta_2 \delta_2^n \Pi(x|x+\delta_2, \tau) \right]
\]

(2.11)

This is the Kramers-Moyal expansion of the Eq.(2.6). Truncating the expansion in second order we obtain the Fokker-Planck equation,

\[
\frac{\partial P(x,t)}{\partial t} = -\frac{\partial}{\partial x}[u(x)P(x,t)] + \frac{1}{2} \frac{\partial^2}{\partial x^2}[D(x)P(x,t)]
\]

(2.12)

where \( u(x) \) is the drift velocity and \( D(x) \) the diffusion coefficient. We assume \( \Pi(x|x-\delta_1) \) and \( \Pi(x|x+\delta_2) \) to be Gaussian. Then, \( u(x) \) and \( D(x) \) are given by the rate of growth of the mean and of the standard deviation. Taking into account that,
we can rewrite these coefficients in terms of the means and standard deviations of the random walk displacements:

\[ u(x) = \frac{\delta_2(x) - \delta_1(x)}{\tau} \]  
\[ D(x) = \frac{\delta_1(x)^2 + \delta_2(x)^2 + \sigma_1(x)^2 + \sigma_2(x)^2}{\tau} \]

The means and standard deviations obtained from the experimental data fittings are tabulated for tribotactic walkers initially placed at 0 \( \mu m \), the center of the tribotactic traps. If we assume \( u \) and \( D \) not to depend on the position (the average displacement is independent of the position as can be seen in Fig. 2-9), then:

\[
\frac{\partial P(x, t)}{\partial t} = -u \frac{\partial P(x, t)}{\partial x} + D \frac{\partial^2 P(x, t)}{\partial x^2}
\]

This is the \textit{advection-diffusion} equation. In our case, the drift term does not appear because of any bias due to a preferred direction or memory of the random path, but because of the asymmetry in the displacements in each direction. Notice that when both displacements, \( \delta_1 \) and \( \delta_2 \), become equal, the drift term goes to zero and the diffusion equation is recovered. Using \( P(x, 0) = \delta(x) \) and according to the Green’s function, one arrives at the following solution [95], which is a Gaussian distribution:

\[
P(x, t) = \frac{1}{\sqrt{4\pi Dt}} \exp\left(\frac{-(x - ut)^2}{4Dt}\right)
\]

The time-dependent statistics of this process are the mean location \( \langle x \rangle \), the mean square displacement \( \langle x^2 \rangle \), and the standard deviation \( \sigma^2 \) which can be obtained as [86]:

\[
\langle x \rangle = \int_{-\infty}^{\infty} x P(x, t) dx = ut
\]

\[
\langle x^2 \rangle = \int_{-\infty}^{\infty} x^2 P(x, t) dx = u^2 t^2 + 2Dt
\]

\[
\sigma^2 = \int_{-\infty}^{\infty} (x - \langle x \rangle)^2 P(x, t) dx = 2Dt
\]
In the case of tribotactic traps, the random walk displacements are no longer independent of position. To model these tribotactic traps, we assume the magnitude of the displacements to be independent on the position, yet the direction of the drift term is position dependent. This is backed by experimental results showing that the average displacement is independent but dependent on the direction (i.e. toward regions of high friction or away from regions of high friction). This can be mathematically represented by a function such as the \( \tanh \). Therefore, in the presence of a tribotactic trap (0 or 800 \( \mu m \)) the diffusion equation becomes:

\[
\frac{\partial P(x, t)}{\partial t} = -u \cdot \tanh(x) \frac{\partial}{\partial x} [P(x, t)] + D \frac{\partial^2 P(x, t)}{\partial x^2}
\]

(2.24)

The analytical solution of this equation is complex; therefore, we have solved it numerically using the experimental results for the drift velocities and diffusion coefficients as input parameters. The time evolution of the probability distribution for the shortest actuation period (\( \tau \)) is presented in Fig. 2-13A. At longer times, the solution to Eq. (2.24) reaches a steady-state solution. The evolution of the standard deviation with the number of iterations is presented in Fig. 2-13B.

Figure 2-13: A) Time evolution of the probability distribution of the positions of a tribotatic walker in a tribotactic trap (Eq. (2.24)) for \( \tau = 2s \). Probability distributions are plotted every 20 steps of a total of 10000. B) Evolution of the standard-deviation of the probability distributions for \( \tau = 2s \).

In summation, the final equation that describes the temporal evolution of the probability density is given by:

\[
\frac{\partial P(x, t)}{\partial t} = -\frac{\partial}{\partial x} [u(x)P(x, t)] + \frac{1}{2} \frac{\partial^2}{\partial x^2} [D(x)P(x, t)]
\]

(2.25)

where \( u(x) \) and \( D(x) \) are the drift velocity and diffusion coefficient of the walkers, respectively. These coefficients have a microscopic origin and their functional form can be written in terms of the displacements \( \delta_1(x) \) and \( \delta_2(x) \) as \( u(x) = (\delta_2(x) - \delta_1(x))/\tau \) and \( D(x) = (\delta_1(x)^2 + \delta_2(x)^2)/\tau \). The first term is responsible for tribotactic motion and represents the drift.
due to the asymmetry in displacements, while the second term corresponds to diffusive motion. Assuming the magnitude of the displacements to be independent on the position see Fig. 2-9, i.e. constant value of $u$, the solution to Eq. (2.25) is a normal distribution whose average drifts with time at a velocity $u$ and the width of the distribution grows as $t^{1/2}$.

### 2.8 Simulating tribotactic drift and tribotactic traps

Such growth can be better seen from simulated random walks with displacements distributed according to those found experimentally. To develop our simulation, we generated a list of 300 random numbers that correspond to either a step towards regions of high friction or away from regions of high friction. The randomly generated lists were required to have the same number of steps toward and away from the regions of high friction in order to be consistent with the experimental protocol. We generated approximately 5000 lists of 300 random walk steps. Then, the distances traveled at each time step were generated by using the probability distributions of the distance traveled obtained from the experimental trajectories (see Fig. 2-8). The probability distributions of the distances traveled were obtained as follows: positive distances traveled are defined in reference to the sample coordinate system, i.e. for walkers initially placed at $300 \, \mu m$ moving toward $0 \, \mu m$ are considered negative displacements and toward $450 \, \mu m$ are positive displacements. Here, we can clearly see the tribotactic walkers travel greater distances when moving toward regions with stronger coefficients of friction, as denoted by $\delta_2$, than when moving toward regions with lower coefficients of friction, denoted by $\delta_1$. This is in agreement with our previously developed theory concerning a continuous gradient in the strength of friction across the sample. The simulated trajectories are presented in Fig. 2-14.

As anticipated, the trajectories span a larger width than what was observed experimentally. However, the average drift velocity for the trajectories (denoted by the dashed black line) is remarkably similar to what was obtained experimentally (solid line) and analytically (dotted line). The drift velocities obtained from the slope of the simulated trajectories are $u = 0.28 \mu m/s$ and $u = 0.31 \mu m/s$ for the tribotactic walkers initially positioned at 600 and 300 $\mu$, respectively. The drift velocities from such simulated experiments are in excellent agreement with the theoretical and experimental values as can be seen in Fig. 2-8.

Similar simulations can be executed to estimate the strength of the tribotactic traps and supplement the data that was gathered experimentally. The simulation method is very similar but with several key distinctions. Here, we generated 5000 lists of 500 random steps that corresponded to a step either toward or away from regions with a strong friction coefficient. Again, we imposed that the total number of steps either toward or away from regions with a strong friction coefficient, which again are denoted by $\delta_2$ or $\delta_1$ respectively, must be equal. The parameters of these distributions are given in Table I (these parameters were also inserted into the analytical model as well). Once these lists were generated, we utilized the experimental trajectories and resultant distributions (seen in Fig. 2-15) to generate the simulation trajectories utilizing the same procedure.
Figure 2-14: A) Simulated trajectories for walkers initially placed at 300 μm. The solid line corresponds to a linear fit to the experimental trajectories (slope 0.32 μm / s), the dashed line to a fit of simulated trajectories, and the dotted line has the slope obtained from the theoretical model \( u = (\delta_2 - \delta_1) / \tau \). Inset: probability distribution of the displacement at each step in each direction. The \( \delta_1 \) value corresponds to the mean displacement opposite to the gradient in friction, while \( \delta_2 \) denotes displacement along the gradient, as explained in the analytical model. B) Simulated trajectories for walkers initially placed at 600 μm. The lines correspond to the same calculations as in part A. In this case, the slope of the linear fit is 0.28 μm / s).

that was just described to generate the simulated tribotactic drift simulation trajectories. Ten of these trajectories are presented in Fig. 2-15. Additionally, using the ten simulated trajectories we computed the root-mean-squared displacement (RMSD) for the different actuation times, \( \tau \). These results are presented in Fig. 2-15. The tribotactic walkers clearly become trapped as evidenced from the RMSD plateau.

Table 2.1: Mean distances and standard deviations of the displacements distributions towards high friction regions, \( \delta_2 \) and \( \sigma_2 \), and away from high friction regions, \( \delta_1 \) and \( \sigma_1 \), for different actuation periods of microwalkers initially placed at tribotactic traps (0 μm).

<table>
<thead>
<tr>
<th>Actuation period / s</th>
<th>( \delta_1 ) / μm</th>
<th>( \delta_2 ) / μm</th>
<th>( \sigma_1 ) / μm²</th>
<th>( \sigma_2 ) / μm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2.5</td>
<td>2.9</td>
<td>0.67</td>
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<tr>
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<td>5.9</td>
<td>0.75</td>
<td>0.75</td>
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<tr>
<td>20</td>
<td>26</td>
<td>26.7</td>
<td>2.50</td>
<td>2.40</td>
</tr>
</tbody>
</table>

2.9 Conclusions

In this Chapter, we have demonstrated tribotaxis using a superparamagnetic doublet functionalized with biological ligands, specifically biotin and streptavidin. The microwalker exploits friction to sense ligand concentration gradients and migrates toward the higher density regions simultaneously,
Figure 2-15: A) Probability distributions of the displacements of the tribotactic walkers moving in the trap (x = 0 m) toward regions of high friction (upper panel), the mean is given by \( \delta_2 \), and away from regions of high friction (bottom panel), the mean is given by \( \delta_1 \). The random walks consist of 500 steps with actuation periods of 2s (purple), 5s (blue), 10s (red), and 20s (green). Data available in Table 2.1. B) Simulated random walk trajectories for the different actuation periods: 2s (purple), 5s (blue), 10s (red), and 20s (green). C) Root mean squared displacement (RMSD) calculated from the simulated trajectories for actuation periods of 2s (purple), 5s (blue), 10s (red), and 20s (green). The RMSDs show the characteristic behavior of a confined random walker.

Eventually becoming trapped in such regions. To avoid trapping, we have shown that increasing the actuation time yields a softening of the trap and can in principle lead to escape, similar in spirit to the way bacteria escape from their own chemoattractant[96]. Thus, the frictional landscapes felt by these tribotactic microwalkers can be tuned, making this system ideal to measure differences in friction coefficients in complex environments or to find areas with relatively higher density of ligands without a priori knowledge. Furthermore, given the similarities between cells and the aforementioned microwalkers in terms of size and the environment in which they move, we believe that nature may already be using friction as an ultra-sensitive probe for differences in the density of ligands which ultimately play a determining role in biological processes.

These results may have important implications, specifically in regards to artificial and natural locomotion as interfaces are a prominent motif in nature. There are ample opportunities utilize this concept: biomedical or novel ligand assay development, the study effective hydrodynamic interactions between arbitrary surfaces, and even the transport of fluid at the nanoscale in order to
understand how cilia moves mucus out of our lungs and dictates embryo development. However, the biomedical and assay development applications are of particular interest. This tribotactic system could be utilized in conjunction with MRI imaging techniques as a new novel diagnostic imaging tool. The MRI would essentially function to stochastically actuate the walkers. The walkers can be functionalized with a biological ligand of interest that binds specifically to a tumor or some interface of interest to health-care professionals. Even more interesting is the potential of this technique to measure the effective friction between biological ligand-receptor interactions. This system is sensitive to forces on the order of 10 fN. This is particularly relevant, as more and more in biology crucial interactions are not necessarily interactions that have a high binding affinity or $K_d$, but instead are interactions that are very weak and very fast. This is particularly true in the process of blood clotting and the role that Von Willebrand factor (VWF) plays in this process [97]. Traditional techniques utilized to attempt to measure the strength and dynamics of such interactions like enzyme linked immunosorben assay (ELISA) and fluorescence resonance energy transfer (FRET) are unable to measure these very weak interactions. Our new, novel tribotactic walkers are very easily adaptable to measure a myriad of interactions. These walkers can be easily functionalized using biotin-streptavidin chemistry, are high throughput, and do not require large amounts of ligands, unlike surface plasmon resonance techniques (SPR).

To further investigate this concept of effective friction between different biological interactions and assess the viability of this system to be utilized in the aforementioned applications, there are several key open questions that must be addressed and technical/experimental limitations that must be overcome. We have already seen evidence that the passivation procedure, actuation protocol, and frequency of rotation can affect the effective friction between the walkers and the substrate. However, these are just a few of the parameters that could potentially modulate the effective friction, the binding affinity, and the kinetics of the interaction of interest. How does temperature, pH, strength of the magnetic field, mechanical properties of the substrate, size of the walker, density of binding sites, and many other factors can affect the effective friction? There are also technical limitations. For example, in this study the walkers had to be passivated to reduce the biotin-streptavidin interaction because the strength of the magnetic field was not large enough to break this interaction in the absence of passivation. Additionally, if one would eventually like to move from in-vitro to in-vivo or work in three dimensions a new apparatus must be designed.
CHAPTER 3
ACTIVE TRIBOLOGICAL PROBES

Work in this chapter was published in:


In the previous Chapter, we developed a novel experimental artificial soft active tribotactic system that was able to mimic the biological process of chemotaxis. The tribotactic walkers were able to detect differences in effective friction along a substrate and since in this system effective friction scales with the density or concentration of biotin binding sites, the walkers were able to detect concentration gradients as well. Not only were these walkers able to detect a gradient in concentration but they were able to move along those gradients, migrating towards regions with a high density of binding sites and eventually becoming trapped in those regions. We also found that effective friction and thus the frictional environment felt by the walkers can be tuned via the actuation period, actuation protocol, and the rotational frequency of the applied magnetic field. As such, this system is ideal to measure differences in effective friction in complex environments and in finding areas with higher density of ligands without a priori knowledge. This experimental tribotactic active matter system clearly elucidates the key role that friction can play in contributing to or even guiding the biological process of chemotaxis. To better understand how these fundamental physical principles can contribute to vital biological processes, further investigation must be conducted using this tribotactic system to measure effective frictional interactions beyond that which was studied in the previous Chapter.

However, to accomplish this aim there are several technical and experimental limitations that must be overcome and a number of essential open questions that must be addressed in order to study the full spectrum of effective friction that arises from biological interactions. As for technical and experimental limitations, we mentioned in the previous Chapter that if the microwalkers were not passivated, the applied torque was not large enough to break the biotin-streptavidin bonds. This presents an immediate and pressing challenge. If we are to investigate the effective friction due to biological ligand interactions, we need a system that can measure the full spectrum of interactions in terms of binding affinity and kinetics. Therefore, we need to be able to measure the biotin-streptavidin interaction, $K_d \approx 10^{-15}$M [98], without passivating either the substrate or the surface of the walkers. It should be noted that while the tribotactic walkers were unable to measure high affinity interactions, they showed a remarkable ability to sense forces as low as 10fN.

In terms of open questions in the previous Chapter, we illustrated that the effective friction
between the walker and the biotin coated substrate can be tuned and modulated by changing the actuation period, the rotational frequency of the magnetic field, passivation procedure, and even the actuation protocol. These are just a few of the factors that can influence the effective friction interaction. There are many more factors such as temperature, pH, ligand or protein concentration on the surface of the particle or on the substrate, binding affinity of the biological interaction of interest, binding kinetics of the biological interaction of interest, and strength of the magnetic field.

In this Chapter we aim to investigate the fundamental relationship between biological ligand binding affinity and kinetics, and the resultant effective friction induced between an object and a substrate in order to better understand the role friction plays in vital biological processes. To do this we must redesign our active tribotactic system. Specifically, we must redesign our active tribotactic walkers into active tribological probes. These new active units are no longer chains of superparamagnetic particles that walk along a substrate but rather a single ferromagnetic particle that rolls across a substrate, hence rollers. Additionally, we must also redesign our magnetic coil apparatus in order to increase the strength of the magnetic field, reduce coil overheating during high duty cycles, and achieve 3D motion. This new system allows for a large enough torque to be exerted so that even unpassivated biotin-streptavidin interactions can be broken. The ferromagnetic rollers have a much higher magnetic moment than the superparamagnetic chains and the new 3D magnetic coil apparatus can achieve higher magnetic field strengths. We will measure a myriad of different biological interactions of varying binding affinity and kinetics from electrostatic, hydrophobic, antibody-antigen, metal-ion, and biotin-streptavidin interactions. We will obtain effective friction measurements for all these interactions and compare them using a rolling parameter to obtain an apples-to-apples comparison. The effective friction scales with the binding affinity which scales inversely with $K_d$. The binding kinetics can be deduced by measuring the effective friction as a function of the rotational frequency of the rotating magnetic field, $\omega$. While most of the interactions probed show a complete loss of binding above 5Hz (biotin-streptavidin being the surprising exception), we believe that by examining the nature of the effective friction vs. frequency decay we can deduce some information about the binding kinetics. Additionally, we also show how the effective friction can also be modulated via the concentration of ligands on both the substrate and on the surface of the rollers.

We investigated weak lipid-protein interactions on a soft substrate, specifically supported lipid bilayers (SLB). Most lipid-protein interactions are very weak, with affinities in the 1mM-1µM range so they are difficult to characterize using standard techniques (i.e. SPR, ELISA, etc.) [99, 100, 101, 102]. We studied the interactions of many proteins with lipid membranes containing phosphatidylinositol phosphates (PIP) and two previously unexplored proteins Auxilin 1 (PTEN) and Auxilin 2 (GAK). These proteins play a role in clathrin-mediated endocytosis and it is believed that PTEN preferentially binds to PI3P and GAK to PI4P but as of yet no one has been able to verify this quantitatively in terms of binding affinity [103]. However, we were able to detect these weak protein-lipid interactions and confirmed that in fact, PTEN binds preferentially to PI3P and GAK binds preferentially to PI4P. Moreover, the effective friction and rolling parameters serve to
support the understanding that these are very weak interactions (i.e. \(K_d\) is larger than 1\(\mu\)M). These results are significant as they illustrate the ability of this system to detect differences in effective friction due to biological interactions is ubiquitous and can be utilized in the future as a ligand binding assay tool to probe interactions with unknown affinities or binding kinetics.

### 3.1 Novel technique to determine effective interaction between biological interactions

Friction is fundamental at the cellular level because it is at the core of many locomotion modes utilized by cells to navigate the complex and often crowded cellular environments. And interfaces are a prominent motif, specifically interfaces decorated with an assortment of ligands, adhesion receptors, sugars, integrins, focal adhesion sites, and chemical signals [104, 105, 106, 107, 108, 109, 110, 111, 112]. Understanding to what extent friction plays in biological processes like chemotaxis or haptotaxis is crucial [113, 114]. Typically, the first step in doing so is by simplifying the complex biological system and investigating the binding affinity and kinetics of any receptors or biological ligands of interest. To be more specific, it is becoming more crucial to be able to detect and quantify weak and fast biological interactions. More and more it appears that many vital biological processes are determined by interactions that are both weak, \(K_d = 1\text{mM-1}\mu\text{M}\), and fast. A prime example can be observed in the blood clotting process and VWF [97]. Blood clotting occurs within blood vessels where shear flow rates are extremely high. Intuitively, one might imagine that in order to bind to platelets and form a clot these interactions must be extremely strong to overcome these other opposing forces [97]. However, this is not the case. In fact, the interactions are fairly weak but extremely fast [97], so it is important to be able to detect the full range of biological interaction affinities and kinetics. To accomplish this many techniques have been established to measure and quantify these properties. A few of these techniques are highlighted in Fig. 3-1.

Perhaps the most widely utilized technique is enzyme-linked immunosorbent assay (ELISA). There are a number of different types of ELISA measurements but the essential principles of the technique can be best described as follows [115, 120, 121, 122, 123, 124]. The antigen of interest is deposited at various concentrations, typically on a 96 well plate. A primary antibody specific to that antigen is added to the well. This primary antibody is typically functionalized with some type of enzyme or fluorescent molecule at a non-bonding position on the antibody [115, 120, 121, 122, 123, 124]. The primary antibody binds to the antigen and after some time another solution or chemical is added to induce some change in the enzyme or fluorescent marker attached to the primary antibody, typically in the form of some type of color change or fluorescent signal [115, 120, 121, 122, 123, 124]. The signal is detected, quantified, and plotted as a function of antigen concentration [115, 120, 121, 122, 123, 124]. This technique is very well established within the biological field. However, there are some limitations to the technique. One of the key limitations is that ELISA can only detect relatively strong interactions and cannot measure or detect weak interactions. The technique is also relatively expensive and requires large amounts of proteins and

Fluorescence polarization (FP) is a somewhat similar technique that measures changes in the rotational speed of fluorescently labeled ligands when they bind to a protein [125, 126, 116, 127]. Polarized light is utilized to excite the ligand or small molecule, and light of the same orientation is emitted [125, 126, 116, 127]. However, this small molecule is fluctuating and rotating so the orientation of the emitted light is changing as it hits the detector [125, 126, 116, 127]. This reduces the amount of polarized light reaching the detector. Once the protein or receptor binds to the small molecule, the rotational fluctuations decrease which results in a larger polarization signal [125, 126, 116, 127]. The technique is relatively simple and only requires one fluorescent labeling step, but has an important limitation. Similar to ELISA, it can only detect high affinity interaction and fails to detect weak biological interactions.

Fluorescence resonance energy transfer (FRET) works a bit differently than ELISA or FP. Both the ligand and the receptor of interest are fluorescently labeled, one of which is labeled with a donor and the other is labeled with an acceptor. The donor is excited by a specific wavelength of light and will emit a certain color if that ligand is unbound to the receptor, typically if the distance is larger than 10 nm [117, 128, 129, 130, 131, 132]. If the donor and acceptor are bound, energy is transferred from the donor to the acceptor and the acceptor becomes excited as well [117, 128, 129, 130, 131, 132]. This results in an overlap in the absorption spectrum and a new color is emitted [117, 128, 129, 130, 131, 132]. FRET is a useful technique to observe if a protein ligand pair is interacting. However, it is difficult to obtain quantitative binding affinity measurements from this technique.

Another technique utilized to measure binding interactions is atomic force microscopy (AFM).
The concept of this technique is incredibly simple. A substrate is coated with the ligand or protein of interest and the tip of a probe is coated with the corresponding ligand or protein [118, 133, 134, 135, 136, 137]. The tip of the probe is brought close to the surface so the ligand on the probe will interact with the receptor on the substrate. The deflection of the probe is recorded and from this the interaction force can be deduced [118, 133, 134, 135, 136, 137]. Performing these experiments, especially to measure weak biological interactions, is incredibly difficult however. The preparation of the tips and substrate require expertise, the technique is inherently very low throughput, and very expensive as well.

Surface plasmon resonance (SPR) is an extremely promising technique to measure interaction affinity and kinetics. SPR detects binding interaction by measuring changes in surface plasmon waves due to the adsorption of ligands to a substrate. When the ligand binds to a substrate, the refractive index changes locally which changes the surface plasmon radiation [119, 138, 139, 140]. This technique is label free and is one of the few techniques able to measure relatively weak biological interactions. However, this technique requires a tremendous amount of proteins or ligands that must be continuously flown over the substrate.

We aim to develop a novel synthetic experimental soft active matter system that can probe interactions with binding affinities ranging from $10^{-5}$ M to $10^{-15}$ M. This method will be high throughput, cheap, simple to use, and easily adaptable. This method can be utilized on both hard and soft substrates and does not require large amounts of protein.

### 3.2 Walkers to rollers and designing 3D magnetic coil apparatus

To achieve this aim and develop a synthetic experimental soft active matter system that can effectively serve as a novel high throughput ligand binding assay, we cannot use the active units from the tribotactic walker system or the magnetic coil apparatus because the applied torque was not large enough to break the full spectrum of biological interactions we tend to probe. Additionally, the coils in that set-up became extremely hot during high duty cycle experiments. There are two parameters that can be tuned to increase the torque applied to the system: i) magnetic field strength and ii) magnetic moment. We have leveraged both parameters to develop our novel active tribological probe system.

The magnetic coil apparatus described in the previous Chapter was able to generate a magnetic field strength of 10 mT as described by equation 2.1. While this apparatus was sufficient for the tribotactic walker, there are some limitations. The first is that this apparatus can only produce one type of motility (rolling or walking) in 1D. This can prove problematic for future studies where we may want to investigate other active systems that exhibit different modes of motility, or if we want to investigate walkers in a 3D system like a functionalized collagen matrix, or even an in-vitro cellular system. Another limitation of this apparatus was the problem of the coils heating up during high duty cycle experimental runs. This was not an issue for the tribotactic microwalker experiments due to the actuation protocol utilized, specifically the $\tau_{relax}$ period where the field was turned off.
However, when the field is actuated continuously for more than twenty minutes the coils begin to get extremely hot, over 150°C. In fact, the coils became so hot that the plastic supports structure on which the coils were mounted started to creep and even melt. This coil heating also presents a problem for using this apparatus to measure biological interactions because the binding affinity and kinetics is dependent on temperature. If the temperature is changing within the sample stage during the experiments, we cannot make any definitive statements or comparisons of the effective friction and binding affinity due to the lack of temperature control in the system.

With these issues in mind, we developed a new magnetic coil apparatus to drive the motion of active units. This new apparatus is composed of three pairs of coils. The coils are secured in a structure composed of aluminum (non-magnetic) T-slots and set screws prevent the possibility of the coils falling out of the structure. This entire structure is placed on an optical breadboard, provided by ThorLabs, 24 in x 18 in x 2.2 inches with 1/4 in - 20 taps. The overall structure is secured in place by 4 set screws placed around the square base of the T-slot structure. To eliminate any vibrations, particularly from the nearby centrifuge, this optical breadboard is placed on top of 4 sorbothane feet, 45 mm in diameter and provided by ThorLabs. The coils have an inner diameter of 7 cm and an outer diameter of 13 cm as seen in Fig. 3-2.

![Diagram](image)

Figure 3-2: Three pairs of Helmholtz coils were mounted on an aluminum T-slot assembly. Two sinusoidal signals are generated in Matlab, passed through a DAQ, amplifier, and then to the Helmholtz coils. A Thor Labs x-y-z stage was controlled using a Matlab code and an Arduino. Visualization is accomplished using a lens tube, 10X objective, and CCD camera. A 10 mT field was utilized for all experiments unless otherwise noted.

The thickness of the coils is approximately 4 cm. The distance between each pair of coils is approximately 18 cm. The coils consist of approximately 500 turns of gauge 19 copper wire which is then sealed and coated with a ceramic resin. The magnetic coils are encased in a ceramic coating to reduce overheating during high duty cycles. To send signals to coils, we utilized a National Instruments X Series USB-6353 data acquisition device (DAQ). The device has 32 analog input channels and four analog output channels in the -10 to 10V range. There are 48 digital channels
as well. Using a session based interface, we generate the signal of interest in MatLab which can then be sent independently to the three pairs of coils. Each pair of coils has a dedicated analog output channel ao0, ao1, and ao2 for the X, Y, and Z coils respectively. The signal is generated in MatLab and sent to the DAQ. The signal is also passed through a 300W Crown DC30A amplifier (150W/channel) before being sent through each pair of coils. The amplifier can run up to 7 Amps through the 500 turns of wire in each pair of coils. Magnetic fields strengths up to 50 mT can be reached utilizing this apparatus which is large enough to ensure alignment of the rotational frequency of the particles with the frequency, \( \omega \), of the magnetic field. The signal from the amplifier was routed to a Keysight DSOX2004A Oscilloscope to measure the frequency and the voltage as seen in Fig. 3-3. Data acquisition was accomplished via a CMOS camera mounted on a C-mount DIN objective tube assembly. The camera was connected to a computer for visualization, video capture, and subsequent analysis.

![Image of apparatus and controls](image)

Figure 3-3: A) 3D magnetic coil apparatus and motorized stage. B) Crown amplifiers, one amplifier per coil pair. C) External DC power supply for motorized stage. D) National Instruments DAQ and Arduino controller. E) Keysight DSOX2004A Oscilloscope. Three pairs of Helmholtz coils were mounted on an aluminum T-slot assembly.

This sample stage in this 3D magnetic coil apparatus is also controlled by a custom written MatLab and DAQ script. The sample holder was made from aluminum and attached to a 1 in XYZ translation stage with 1/4 in - 20 taps, provided by Thor Labs. Each of the three adjuster screws was then connected to stepper motors using a nylon adapter. The stepper motors are utilized to
drive the XYZ stage. The stepper motors are powered by an external DC power supply, seen in Fig. 3-3, which operates at 12V. The motors are first connected to an Arduino controller. The X, Y, and Z direction stage controls are connected to the port0/line8:12, port0/line13:17, and port0/line0:4 digital output channels, respectively. The signals are generated in MatLab which controls the stepper motor rotation and thus the stage translation. This apparatus expands our technical and experimental capabilities as we can now run extremely long experiments (continuously generate a signal for more than 12 hours), achieve field strengths that were previously inaccessible, can vary mode of motility and dimensionality of motility on the fly, and the automated stage can be integrated with tracking protocols to perform extremely long (order of days) tribotactic experiments.

However, we found that increasing the magnetic field strength alone would not break the unpas-sivated biotin-streptavidin interaction. The superparamagnetic walkers were also a bit problematic to work with for other reasons. One pragmatic problem was the issue of finding or even worse trying to form doublets of microwalkers. This process is laborious and cannot be well controlled without using some type of particle exclusion technique based on size. There are also some fundamental physical challenges when it comes to modeling the motion of the tribotactic walkers. As the walker turns and walks on the substrate, the force exerted is not uniform throughout the entire rotation cycle due to the weight of the two superparamagnetic particles. To overcome these obstacles, we decided to not only increase the magnetic moment of the new active tribological probes but also change the design of the active unit. These new active tribological probes are ferromagnetic particles, 9μm in diameter and provided by Spherotech, composed of a core of polystyrene and an outer shell composed of CrO₂ and polystyrene as seen in Fig. 3-4A. These probes are no longer doublets that walk across the substrate but instead are single particles that roll across a substrate which is shown schematically in Fig. 3-4B. Due to the nature of the superparamagnetic particles, investigating the motion of a single particle is impossible as they only form a magnetic dipole moment when coupled to another superparamagnetic particle. This active tribological probe design is much simpler and easier to investigate experimentally. The large size of these particles will also offer easier optical observation. It should be noted that even though these particles are 3× as large as the superparamagnetic particles used in tribotaxis, the effective footprint (contact of the particle with the substrate) is smaller.

### 3.3 Quantifying effective friction: the rolling parameter

With these new active tribological probes, we needed to test if they could truly investigate the full spectrum of biological interaction strengths of interest, $10^{-5} - 10^{-15}$ M. However, before doing so we needed a measurement of effective friction that could be used as an apples-to-apples comparison. In the previous Chapter, we measured the velocity of the tribotactic walkers. However, the velocity can be a misleading measurement. The velocity of the walkers will change depending on the frequency of rotation of the magnetic field and the size of particles even if the effective friction between the
Figure 3-4: A) Single ferromagnetic active tribological probe or roller that is 9μm in diameter and is composed of polystyrene and CrO₂. B) Rolling motion of active tribological probe. The displacement, δ, can be seen for a given actuation period, τ, and rotational frequency of the magnetic field, ω.

Object and the substrate is the same. We also mentioned in the previous Chapter two different frictional regimes which emerge depending on the actuation time: the static and dynamical friction regimes. Therefore, we need to develop some parameter that takes into account factors like the size of the particle, the frequency of rotation of the magnetic field, and the actuation period. To parametrize the effective friction, we developed our own dimensionless parameter which we refer to as the rolling parameter which is defined as:

\[ \zeta = \frac{\delta}{\pi D \omega \tau} \]  

(3.1)

which is the ratio of the observed displacement, δ, over the maximum theoretical displacement of sphere rolling a surface where D is the diameter of the particle, \( \omega \) is the frequency of the rotating magnetic field, and \( \tau \) is the actuation period as seen in Fig. 3-4. This is a dimensionless parameter that varies from 0-1. A rolling parameter of 0 corresponds to a scenario where the roller is perfectly slipping and there is no effective friction between the roller and the substrate. This scenario is never realized experimentally because in reality there will always be some contribution of effective friction due to hydrodynamic friction. A rolling parameter of 1 corresponds to a scenario where the roller is perfectly rolling or translating and the effective friction between the substrate and roller is extremely large. Between these two extreme scenarios the roller exhibits some period of time sticking to the substrate and slipping on the substrate. We refer to this type of motion as stick-slip.

To test if these active tribological probes can also detect differences in effective friction on a substrate coated with biological ligands and to ensure that this ability to detect differences in effective friction is ubiquitous and not unique to the tribotactic system, we measure the rolling
parameter of the roller on a biotinylated substrate. The ferromagnetic particles are coated with streptavidin ligands and 100μL of stock solution is diluted into 5mL of a 0.15M solution of PBS (7.4 pH) to simulate biological conditions. 10μL of this solution is diluted again into 400μL of the same 0.15M solution of PBS. These dilution steps are taken in order to reduce the number of particles that will eventually be inserted into the microfluidic channel. If there are too many rollers, there are likely to be many dimers and longer chains of particles that cannot be utilized to measure effective friction. Having many rollers on the substrate also increases the likelihood that two rollers may run into one another during the experiment. This makes the analysis extremely difficult as one must disregard displacement measurements after dimer formation. Ideally, on a 1 mm x 1 mm sample, 10-30 rollers are enough to obtain sufficient statistics and limit dimer formation. The final diluted solution is then inserted into a microfluidic channel that is built using a similar protocol that was discussed in the previous Chapter but with some key distinctions. Two pieces of double sided 3M tape are cut into 22 mm x 3 mm strips and placed on the biotinylated glass substrate to form channels of approximately the same dimensions and then a glass cover slip is placed on top. The solution is inserted into the channel and then sealed with epoxy to prevent evaporation and evaporative flows which can affect the value of the rolling parameter. To actuate the rollers we send two sinusoidal signals through the Y-Z coils to produce 1D walker motion, just as in the tribotactic system. However, here the rollers are actuated at 1 Hz for a period of 5 s in one rotational direction (i.e. clockwise or counter-clockwise), the field is turned off for a period of 5 s, actuated in the other rotational direction for a period of 5 s, and then the field is turned off for another period of 5 s. This protocol is repeated until the total time of the experiment reaches 3-6 minutes which is long enough to obtain enough statistics so that the effective friction measurements are statistically significant.

The rolling parameter was measured for the streptavidin-biotin interaction as a function of avidin concentration deposited on the biotinylated glass substrate. Avidin solutions at different concentrations were inserted into the channels and droplets of avidin served as reservoirs at the edge of the channel to prevent evaporation during deposition. The avidin was left in the channel for two hours to allow enough time for binding to the substrate. After two hours, the solution was removed using a kimwipe and the capillary forces of the channel. The channel was also flushed with a PBS buffer (0.15M at 7.4 pH) to remove any excess, unbound free avidin in the channel. The diluted solution of rollers was then inserted in to the channel, sealed with epoxy, and the actuation protocol previously described was initiated. The rolling parameter as a function of avidin concentration (which is inversely related to the number of available biotin binding sites) can be seen in Fig. 3-5.

At large concentrations of avidin ligands, the rolling parameter saturates at a value of 0.1. This is a predominantly slipping scenario and is close to the rolling parameter limit where only hydrodynamic friction contributes, which occurs at a rolling parameter of 0.08 for a hard substrate as will be discussed later. As avidin concentration decreases, we observe a sigmoidal increase in the rolling parameter (the inflection point occurs when the number of binding sites on the slide is
Figure 3-5: Rolling parameter as a function of avidin concentration. The rolling parameter is close to one at low avidin concentrations and high density of available biotin binding sites, which is expected for the biotin-streptavidin interaction, $K_d \approx 10^{-15}\text{M}$. At higher avidin concentrations, the rolling parameter drops precipitously until reaching the hydrodynamic limit value of approximately 0.1. The concentration region enclosed in the red dashed circle indicates the approximate avidin concentration utilized in the tribotaxis experiments.

approximately 5 times the number of free avidin ligands deposited) until the value plateaus at a rolling parameter of approximately 0.95. This value is very close to the perfect rolling value which intuitively makes sense due the high affinity of the biotin-streptavidin bond, the strongest biological non-covalent bond as it takes approximately 100pN to break a single bond. This confirms that this active tribological probe can detect extremely strong biological interactions. Interestingly, as seen in the inset in Fig. 3-5, in the tribotactic system we were in the concentration range indicated by the dashed red circle. It was extremely fortuitous that we were in this range. If the avidin concentration we utilized was slightly larger, we would most likely have not have been able to detect differences in effective friction because at a larger concentration the effective friction is almost completely flat. If the avidin concentration was lower, we would not have been able to break the higher density of binding interactions. This was an excellent example that in research sometimes it is better to be lucky than good.

To ensure these new tribological probes could still detect gradients in the concentration of a specific ligand and gradients in the coefficient of friction, we performed the tribotaxis experiments again with new active units. The same protocol was followed as described in the previous Chapter. The resulting trajectories of the rollers can be seen in Fig. 3-6. The rollers clearly seem to show drift towards the regions previously determined to exhibit a larger coefficient of friction. What is also apparent, however, is that the amount of drift or equivalently the drift velocity is much lower.
for the rollers than walkers. This is surprising as the rollers are not passivated so one would expect that the effective friction should be larger and thus the drift velocity should increase. However, even though the rollers are 9μm in diameter, the footprint of the roller is much smaller than the walkers. The footprint of the rollers can be estimated to be several hundred nm whereas the footprint of the walkers is 6μm which is much larger. Thus, the walkers can sense environments with much larger effective frictional differences which explains the reduced drift.

Figure 3-6: Tribotactic motion of roller. The tribotactic drift is reduced due to the smaller footprint of the rollers compared to the walkers.

The rollers and the new 3D magnetic coil apparatus seem to be an effective system for sensing differences in effective frictional differences. We have also shown through passivation of the substrate that we can detect both very strong and much weaker biotin-streptavidin interactions and thus, both stronger and weaker effective friction. However, thus far we have only investigated the biotin-streptavidin interaction. For this apparatus to be an effective ligand assay technique, it needs to be versatile and able to detect a myriad of interactions with differences in binding affinities.

3.4 Tribological interactions on a hard substrate

To accomplish this task, we must be able to easily functionalize the substrate and the surface of the rollers with whatever ligand, protein, receptor, or molecule of interest. Fortunately, one of the benefits of this novel technique is that this is easily accomplished by simply biotinylating whatever ligand or receptor of interest and then sticking it to the surface of the beads via biotin-streptavidin binding interaction. The ligands can also be attached to the avidin coated substrate via a biotin-avidin interaction, a schematic of which can be seen in Fig. 3-7. This is typically the method that
we utilize to functionalize the rollers and the substrate. However, it should be noted that if it is difficult to biotinylate a ligand of interest, other methods can be utilized to bind the ligand to the substrate or to the rollers. Polyhistidine tags, GST tags, and cysteine functionalized ligands can all be used to attach the molecule of interest.

The first general classification of interactions that we were interested in measuring were: i) hydrophobic interactions, ii) electrostatic interactions, iii) metal ion interactions, and iv) antibody-antigen interactions as seen in Fig. 3-8. The hydrophobic interaction that we investigated was biotin-PEG-alkyne, specifically alkyne-alkyne interactions. The biotin-PEG-alkyne, 5000MW and provided by Nanocs, was diluted in an aqueous solution. A similar protocol was followed to coat the rollers and the substrate as described in the previous Chapter. Enough biotin-PEG-alkyne was added to coat the rollers and the substrate 50×. The ferromagnetic rollers particles, ~ 9μm in diameter, bind to 0.16nmole of biotin per mg of ferromagnetic particles. The ferromagnetic particle stock solution we used is 1.0% w/v. We took 100μL of this solution, diluted it into 5mL of water or PBS buffer pH 7.4, and then extracted 1mL from this solution to be functionalized. At this point, there are approximately 0.2mg of particles that must be functionalized. To know how many molecules of biotin-PEG-alkyne is required to functionalize the particles, one only needs to perform some simple stoichiometric calculations to calculate how much solution of biotin-PEG-alkyne is required to coat the particles. The biotin-PEG-alkyne is added to the solution of particles, vortexed for several minutes and left at room temperature for 2-4 hours to bind before being placed in the refrigerator overnight prior to use. The same calculation must be performed to coat the

Figure 3-7: Ferromagnetic roller and substrate functionalized with a myriad of ligands and the corresponding receptors of interest.
substrate. Here the substrate is SuperAvidin, provided by Arrayit, which is a glass substrate of dimensions $25\text{mm} \times 76\text{mm}$ that contains $1.1 \times 10^{10}$ avidin molecules per mm$^2$. The dimensions of each microfluidic channel is approximately $22\text{mm} \times 3\text{mm}$. To calculate the number of binding sites one must simply multiply the dimensions of the channel by the density of binding sites. After several stoichiometric calculations, the amount of biotin-PEG-alkyne required to coat the substrate $50\times$ can be deduced. This solution is inserted into the channel and two droplets are placed at the edge of the channel to serve as reservoirs which prevent evaporation. The solution is left to bind for two hours after which the solution is washed out of the channel, typically with water, PBS buffer, or whatever buffer in which the beads are incubated. This wash procedure is repeated several times to ensure any unbound ligands are washed away and removed from the substrate. A kimwipe in conjunction with capillary forces from the microfluidic channel is used to flush the channel. Once the channel has been sufficiently washed, the solution of functionalized rollers that has been diluted to the same concentration as previously described for the biotin-streptavidin interaction is inserted into the channel and sealed with epoxy. The sample is magnetized prior to insertion into the magnetic apparatus using a neodymium magnet and then inserted into the apparatus sample stage. Again, the same actuation protocol was followed as previously described for measuring the rolling parameter for the biotin-streptavidin interaction.

Figure 3-8: Schematic of functionalization scheme utilized to measure rolling parameter for PEG-Alkyne - PEG-Alkyne hydrophobic interactions, PEG-NH$_2$ - PEG-COOH electrostatic interactions, Histidine metal ion interactions, and IgG Protein A antibody-antigen interactions.
A similar procedure was followed for investigating electrostatic interactions where biotin-PEG-COOH and biotin-PEG-NH₂, 5000MW and provided by Creative PEGWorks, were the ligands of interest. Biotin-PEG-NH₂ coated the substrate and biotin-PEG-COOH coated the surface of the beads. Again here the buffer solution utilized was 0.15M PBS (pH 7.4) and the rest of the procedure was consistent with what was previously described. The procedure to functionalize the substrate and surfaces with antibodies-antigen as well as metal-ion coordination interactions are also similar, but have several key details differentiating them from the above procedure. The antibody-antigen interactions investigated were Protein A (antigen), obtained from Vector Labs. Interactions with Biotin-SP-conjugated ChromPure Chicken IgY whole molecule, Biotin-SP-conjugated ChromPure Rabbit IgG whole molecule, and Biotin-SP-conjugated ChromPure Human IgM whole molecule which were all obtained from Jackson ImmunoResearch Laboratories. The antibodies were provided in a lyophilized state and were rehydrated to a concentration of 2mg/mL in an aqueous solution. Protein A was provided in solution and did not require any rehydration steps. Appropriate amounts of Protein A were utilized to coat the surface of the rollers. IgG, IgM, and IgY was utilized to coat the avidin substrate. It should be noted that after the overnight roller functionalization with Protein A, the solution was diluted in a PBS buffer 0.15M at a pH of 7.4. That buffer was also utilized to wash any remaining IgG, IgM, or IgY off of the avidin substrate. We also ran experiments investigating the interaction of monoclonal anti-IgG antibodies and polyclonal anti-IgG antibodies. Specifically, Biotin Mouse Monoclonal Anti-Rabbit IgG Whole Molecule and Biotin Anti-Rabbit IgG Whole Molecule (Polyclonal), both provided by Sigma Aldrich, interacting with Biotin Rabbit IgG Whole Molecule, also provided by Sigma Aldrich. These antibodies were provided in solution and an appropriate amount was added to coat the rollers. Once functionalized, they were also diluted in the same PBS buffer previously mentioned. The metal-ion coordination interaction utilizes a slightly different functionalization procedure. Amine functionalized beads and substrates were coated with histidine. The metal-ion salts, Cu, Zn, Co, and Ni were hydrated with H₂O. The functionalized beads were diluted in a 10mM bicine buffer, pH 8.5, and enough metal ions to form an ion coordination bond with each histidine. The remaining procedure was followed as previously described.

The rolling parameter was measured for all the aforementioned interactions as seen in Fig. 3-9. The error bars in Fig. 3-9 represent the standard error in the measurements, meaning the standard deviation of each rolling parameter measurement divided by the square root of the number of measurements. This data can also be visualized in Fig. 3-10. Here, each point is the average rolling parameter measured for each roller. The dark black line represents the mean rolling parameter for all of the rollers and the dashed grey lines represent the 95% confidence interval for the dataset. Fig. 3-9 and Fig. 3-10 illustrate the remarkable ability of this technique in distinguishing the effective friction measured for different interactions. It should be noted that the control measurement is simply the rolling parameter of a streptavidin coated bead on an avidin coated substrate. As can be seen, the rolling parameter is ≈ 0.08 which is extremely low and close to the limiting scenario of no effective friction between the roller and the substrate. There should be no interaction between
streptavidin and avidin so we believe that this rolling parameter value corresponds to the effective friction due to purely hydrodynamic friction. In other words, this value should be subtracted from all measurements to truly measure the effective interaction due to the binding interaction. The next interactions of interest are the hydrophobic, polymer-polymer, and electrostatic interactions. There is a clear, noticeable, and statistically significant difference in the rolling parameter for the hydrophobic alkyne interactions and the electrostatic interactions. This seems to be generally consistent with known values for the strength of hydrophobic vs. electrostatic interactions. What is interesting and may require further investigation is that rolling parameter for PEG interacting with itself is comparable to the electrostatic NH2-COOH interaction. Yet, for this classification of interactions (which are very weak) the rolling parameters seem to be in the predominantly slipping regime as anticipated. However, it is still distinguishable from the purely hydrodynamic friction case.

![Graph](image.jpg)

**Figure 3-9:** The rolling parameter appears to scale with the interaction affinity and the rollers can effectively detect differences in effective friction due to different interaction affinities. The rollers can also distinguish between binding affinity within the same class of binding interaction (i.e. antigen-antibody interactions or metal-ion interactions).

The next class of interactions, antigen-antibody interactions, show some striking behavior in terms of both the rolling parameter and the distribution of the rolling parameter. Finding consistent
Figure 3-10: Each point represents the average rolling parameter measure for a single roller for the duration of the experiment. The solid black line represents the average rolling parameter for all the roller measurements. The dashed grey lines represent the 95% confidence interval.

and reproducible quantitative information about the binding affinity, $K_d$, of IgG variants to Protein A is extremely difficult; however, qualitative comparisons are extremely consistent. For example, Rabbit-IgG affinity for Protein A is classified as high, Human-IgM affinity for Protein A is classified as weak, and Chicken-IgY affinity for Protein A is classified as no interaction [141]. Looking at Fig. 3-9, we see that the rolling parameter appears to scale with these qualitative assessments of binding affinities. What is particularly remarkable is that the rolling parameter measured for Chicken IgY is approximately 0.09 which is very close to the hydrodynamic friction limit, indicating that there is almost no interaction with Protein A.

Interestingly, the polyclonal Anti Rabbit-IgG antibody exhibits a larger rolling parameter than the mouse monoclonal Anti Rabbit-IgG antibody. This result must be due to the difference between the nature of polyclonal and monoclonal antibodies. Monoclonal antibodies can only bind to a single specific site or epitope, and will only recognize a particular protein structure. Polyclonal antibodies,
on the other hand, may bind to multiple areas or epitopes of the antibody (it is also tolerant of small changes in protein structure). Therefore, one potential explanation is that in the monoclonal scenario there is only one specific site or epitome where binding can occur. Perhaps there are certain orientations of IgG antibodies or IgG either on the substrate or on the bead where this site is inaccessible. This will reduce the effective friction compared to the polyclonal scenario where there are multiple epitomes. Thus, the probability for binding increases as a function of the number of epitopes on the IgG molecule. However, this is a very large increase in the rolling parameter, approximately two fold, to be attributed only to an increase in the number of potential binding sites. Another factor that could be at play is that the binding affinity of these other epitopes could be much larger, smaller, or faster than the monoclonal epitome. A change in the nature of the binding interaction in conjunction with the increase in the number of binding sites would be a more logical explanation for the two fold increase in the rolling parameter. This hypothesis is supported by rolling parameter distribution of polyclonal, monoclonal, and biotin-streptavidin interactions as seen in Fig. 3-11.

![Graphs showing rolling parameter distributions](image)

Figure 3-11: A) The biotin-streptavidin rolling parameter distribution is very narrow when compared to the more larger deviations observed for monoclonal (B) and particularly polyclonal (C) binding interactions.

The distribution of rolling parameter measurements for the polyclonal antibodies is extremely disperse with measurement ranging from 0.1 to 0.8. This is much larger in comparison to the
monoclonal distribution. Although it should be noted here that the monoclonal distribution also exhibits a large spread in rolling parameter measurements when compared to the biotin-streptavidin distribution as well. This large deviation in the distribution in the rolling parameters observed in the polyclonal distribution seems to support the hypothesis that the increase in the number of epitopes, changes in the binding affinity in the multiple epitopes, or the change in the binding kinetics at different epitopes, could explain this large distribution in the rolling parameter. In order to confirm this hypothesis, one would have to somehow map the number of epitopes, location, binding affinity, and kinetics of the epitopes. This is beyond the scope of this thesis and our experimental capabilities.

The histidine metal ion coordination bonds are also extremely interesting due to their large rolling parameter. These interactions are extremely fast so one would not necessarily expect the affinity to be so large, but these interactions seem to combine high affinity with fast binding kinetics. These metal-ion interactions are clearly distinguishable from electrostatic, hydrophobic, antibody-antigen interactions, and biotin-streptavidin interactions as well. However, this ligand assay binding technique can also distinguish between the affinities due to the metal ion used in the coordination interaction. It has been previously established in literature that histidine affinity for Cu, Ni, Co, and Zn rank as follows Cu > Ni > Co ≈ Zn [142]. And as we see in Fig. 3-10, the rolling parameter matches with this ranking qualitatively.

It is clear that the rolling parameter is related to the binding affinity of the interactions being investigated. In fact, theoretically the rolling parameter should scale proportionally to the logarithm of the \( K_{off} \) and linear on the \( K_{on} \). It is this last scaling relationship that brings us to the question of investigating the binding kinetics of such interactions. To investigate the binding kinetics of interactions, one must be able to change the time the ligand can interact with the receptor of interest. This novel ligand binding assay has a very simple way to do this which is via modulating the rotational frequency of the magnetic field. We have investigated the effect of frequency on the rolling parameter for the antibody-antigen interactions, biotin-streptavidin, and the control case as seen in Fig. 3-12.

Interestingly it seems that regardless of the difference in the measured rolling parameter, the antibody-antigen interactions appear to lose all effective friction or binding interaction once the frequency is increased to 5Hz. This would seem to indicate that all these interactions exhibit similar \( K_{on} \) rates. Not surprisingly, the control measurement shows no change in effective friction as a function of the rotational frequency, \( \omega \), of the magnetic field indicating the hydrodynamic friction does not depend on the rotational frequency of the magnetic field. Although it is interesting that most interactions show a small, but noticeable increase in the rolling parameter as the rotational frequency is decreased. What is very surprising and a bit counter-intuitive is that the biotin-streptavidin retains binding interaction or information even at 10Hz and possibly at even higher frequencies. This is a very interesting result as one would anticipate that due to the high \( K_d \) value of biotin-streptavidin \((10^{-15}M)\), the interaction might be slow. However, these measurements seem to indicate that this interaction is fast as well as strong. It should be noted that there has been
some work in theory and simulation to investigate the potential of two modes of biotin-streptavidin interactions. The primary interaction depends on a tight configurational pocket that yields the extremely high binding affinity. However, some hypothesize that there might be another binding mechanism that is faster and does not require such a specific binding site or pocket. More work needs to be done, both experimentally and in theory, to obtain quantitative measurements concerning binding kinetics. Specifically, the $K_{on}$ may be deduced from the decay of the rolling parameter as a function of the rotational frequency of the magnetic field.

In this binding assay the other parameter that can be modulated is the strength of the applied magnetic field. The magnetic field was varied for the interaction of Rabbit IgG with Protein A. One might imagine that the rolling parameter should decrease as the magnetic field decreases as it should become more difficult to break the bonds with the substrate. However, the rolling parameter only decreases very slightly and there appears to still be some binding interaction detected even at 0.1 mT as seen in Fig. 3-13. This type of measurement could be useful as a way to estimate the
force required to break the bonds formed with the substrate or even to estimate $K_{off}$ values. By measuring the rolling parameter as the magnetic field is slowly reduced one can find the point at which the field, and thereby the torque, is no longer large enough to break all the bonds formed at the footprint of the roller. One can estimate the footprint of the rollers and the density of ligands on the surface of the rollers and on the substrate. However, this is still an open question and more experimental and simulation work must be done to get quantitative values from such a measurement.

We have shown the effectiveness of this novel ligand binding assay to measure the effective friction induced by many different types of interactions. This technique is able to measure and distinguish effective friction from a wide range of interactions from relatively weak hydrophobic and electrostatic interaction, to stronger antibody-antigen interactions, to much stronger metal ion coordination interactions and the biotin-streptavidin interaction. This technique is not only able to distinguish between these general interactions, but can detect differences in effective interactions within each class of interaction as well. However, thus far we have only investigated this technique on hard substrates and with known and well characterized interactions. To truly test if this technique is general and can detect differences in effective friction due to binding affinity, we must test this technique on interactions that cannot be measured using traditional techniques.
3.5 Clathrin mediated endocytosis: lipid protein interactions on soft substrates

3.5.1 Clathrin mediated endocytosis

To test the effectiveness of this novel active tribological probe system we set out to measure weak lipid protein interactions that up to this point have been difficult to measure in-vitro using traditional ligand binding assay techniques. Lipid-protein interactions play an important role in multiple processes inside and outside of the cell. In many circumstances, the interactions between lipids and proteins are very weak with affinities above 1μM, yet they tend to be rather specific for a particular lipid-protein pair. The weak affinity of important lipid-protein interactions implies a short lifetime of the bonds as well equilibrium constants in the order of tens of μMs which makes studying such interactions with traditional methods difficult. Many of such lipid-protein interactions are related to lipid signaling and membrane sculpting [143, 144, 145, 146, 147, 103]. An important example is kinases and phosphatases which can phosphorylate or dephosphorylate phosphatidylinositol lipids (PIP) that serve as signaling agents in multiple cell processes and pathways [143, 144, 146, 147, 103].

It is believed that such interactions need to be weak, yet specific, because these protein enzymes must catalyze their reactions in multiple substrates within a short amount of time [103]. Otherwise, their catalytic function would be very inefficient [103]. In other cases, such as in endocytosis, the overall process is short lived on the order of 100s of milliseconds to several seconds which imposes a bound on the lifetime of the protein-lipid interactions occurring in endocytosis [103, 148, 149, 150]. Furthermore, the complete process includes multiple steps where proteins on the lipid membranes must exchange, implying that their lifetime must be shorter than the time it takes to complete the process. While these are only a few examples it is believed that weak interactions between proteins or between lipids and proteins are at the core of a myriad of processes that drive the most dynamic aspects of cellular and tissue function.

We were particularly interested in Auxilin 1 (PTEN) and Auxilin 2 (GAK) interactions with phosphoinositides which play a key role in clathrin mediated endocytosis. There are many endocytic proteins which modulate lipid coat assembly and membrane deformation that interact in-vitro with different phosphoinositides. The endocytic clathrin adaptor AP2 binds directly to PI(4,5)P2 and the depletion of this lipid will block the association of cytosolic AP2 with the plasma membrane, thus preventing coated pits initiations [143, 144]. Although other endocytic proteins such as dynamin, epsin, eps15 and auxilin bind phosphoinositide in-vitro, their low affinity makes the lipid interaction difficult to establish [145, 146, 147, 103]. The lipid binding domain of Auxilin PTEN-like domain was characterized in two different studies, but the results were not comparable and difficult to interpret. Therefore, more characterization was required using in vivo and in vitro models. Recently, the Kirchhausen group has generated new live-cell imaging probes which sense the phosphoinositide environment during clathrin coated pit formation, maturation, and disassembly in-vivo. By replacing the PTEN-like domain of EGFP-Auxilin 1 with other domains of defined phosphoinositide specificity, the recruitment of the modified Auxilin 1 to coated vesicles
Figure 3-14: Recruitment of clathrin, Auxilin 1, Auxilin 2 and lipid sensor (PI3P, PI4P and PI3,4P2) during clathrin mediated endocytosis. The X axis represents time and Y axis represents the fluorescence intensity.

proceeds but it now depends on the phosphoinositide composition of the underlying membrane. The recruitment of these probes was followed using SUM159 cell line expressing the clathrin light chain A tagged with TagRFP imaged by total internal reflection fluorescence (TIRF) microscopy. By tracking the fluorescence intensity of each probe (see Fig. 3-14), one can map the distribution of the phosphoinositide during the clathrin mediated endocytosis process. The PI3P sensors mimic the pattern of Auxilin 1 and Auxilin 2 association, absent from assembling coated pits and appearing in a burst immediately following scission. The PI4P sensor had a different recruitment pattern. It accumulated at a low but steady rate along with clathrin and AP2, and then like Auxilin 1 and Auxilin 2, appeared in an acute burst at the time of coat disassembly. The maximum PI3P sensor peak is 1s before Auxilin 1, followed by the burst of PI4P sensor and finally the burst of Auxilin 2 as seen in Fig. 3-15. Such observation indicates that Auxilin 1 is recruited to membrane rich in PI3P, while the recruitment of Auxilin 2 occurs with membrane rich in PI4P. Interestingly, PI34P2 recruits Auxilin2 and remains associated with the vesicular carrier even after uncoating has ended.

3.5.2 Measuring protein-lipid interaction with tribological probes

Here, we study the interactions of several proteins with PIP lipids using our active tribological probes on a supported lipid membrane as seen schematically in Fig. 3-16. The beads are rolled at a constant frequency, ω, given by the rotational frequency of the magnetic field. The field is applied using the 3D magnetic coil apparatus described in previous sections. Using this assay, we were able to study such weak interactions under conditions similar to those occurring in biological settings. For example, the low concentration of PIP in a PC membrane. Also, this rolling method only uses very small quantities of reagents, and in particular protein, something that can be very useful
when it is difficult or expensive to obtain large quantities of the protein in question. Again, the premise of the technique is that friction between a rotating bead and a surface is dependent on the interaction strength between the protein with which the beads are functionalized and the substrate (see Fig. 3-16). If the rolling parameter is 1, the bead does not slip and implies a strong interaction between the protein and the substrate. As the bead rolls, the bonds between the proteins and the lipids on the substrate lead to an effective friction force onto which the bead \textit{grabs} the substrate (as depicted schematically in Fig. 3-16). Eventually, the protein-lipid pair must break but newer ones are formed from the front of the contact zone. If the lipid-protein interactions are weaker, the bonds do not last as long and thus the effective friction force on the beads is reduced leading to a rolling parameter below 1. The limiting case of no interactions yields experimentally a finite but very small rolling parameter. The origin of the friction in this case is hydrodynamic and is always present. This corresponds to the base line.

Comparisons between different rolling parameters from the same beads on different substrates illustrates that we able to discern very weak yet specific interactions. This is due to the logarithmic sensing capabilities of this assay since the effective friction in these systems is believed to scale proportional to the logarithm of the $K_{off}$, and linear on the $K_{on}$ of the particular interaction under question. To test this technique, we first studied the canonical interaction between Biotin and Streptavidin, as well as other known proteins that interact with different PIP lipids with a varying degree of affinity. In particular, we studied DrrA$_{WT}$, DrrA$_{K568A}$, PH-$\delta$, and 2XYFVE interacting with PC, PIP, PI3P, PI4P, PI34P$_2$, and PI45P$_2$. The lipids chosen were selected on the basis of the presence and position of the phosphate group on the inositol ring as well as the degree of overall charge.

Figure 3-15: Difference between maximum peak of Aux1, Aux2, PI3P and PI4P sensors.
To do this, streptavidin coated ferromagnetic particles, 9 µm in diameter and provided by Spherotech, were diluted in an aqueous solution from a 4mL 1.0% w/v stock. 100µL of the stock solution was diluted into 5mL of water. 1mL was extracted from the diluted solution and the desired amount of biotinylated protein was added to the solution to coat the streptavidin coated ferromagnetic particles. To prepare and biotinylate the proteins, residues 40-400 of Auxilin 1 (B. taurus; hereafter referred to as PTEN or Auxilin 1) were inserted into a pRSET-based vector also encoding a N-terminal His6-tag followed by a TEV cleavage sequence. A DNA construct encoding residues 400-766 of GAK (H. sapiens; hereafter referred to as Auxilin 2 or GAK) as an N-terminal His6-tagged, small ubiquitin-like modifier (SUMO)-fusion was generated by PCR, followed by ligation-independent cloning into a non-commercial vector based on the pET series that features a T7 promoter for inducible bacterial expression. The PH domain of PLC was purified and cleaved from GST following published procedures. PTEN-Auxilin 1, WT and K568A DrrA,
GST-FYVE, and PTEN-GAK, were expressed in BL21 E coli. Cells were lysed using sonication. Lysates were clarified by centrifugation, and the supernatant was applied to nickel-NTA resin. Proteins were eluted with imidazole. His$_6$-tags and any fusion protein moieties were not removed for lipid-binding studies, with the exception of PTEN-Auxilin 1 and the PH domain of PLC$_3$ which were treated with TEV protease for tag removal. Proteins were dialyzed into bicarbonate buffer and biotinylated using EZ-Link Sulfo-NHS-LC-Biotin (ThermoFisher) following the manufacturers protocol. For some preparations, an HABA/avidin assay (ThermoFisher) was used to quantify the extent of biotinylation (3-4 biotin/protein). To ensure that all the sites were completely coated with protein, the amount of protein added was enough to coat the beads 50×. The bead and protein solution was then left to react for one hour at room temperature and then stored at 4°C overnight.

Microfluidic channels were created as the support for the lipid bilayer. Two pieces of doubled sided tape, provided by 3M, were placed on glass slides, provided by Fisher Scientific. A cover slip, provided by VWR, was placed on top of the double sided tape creating a channel approximately 22mm×5mm. Once the channel was created a solution of NaOH was pipetted into the channel and left to sit for approximately 5 minutes. The solution was then flushed with a lipid buffer solution (20mM Heps, 150mM NaCL), pH 4.8, by pipetting the solution in from one side of the channel and sucking it out from the other end with a kimwipe. The channel was washed several times to remove a NaOH residue.

The lipids were formed by 90 mol: mol of DOPC and 10 mol: mol of the specific phosphoinositide, solubilized in chloroform/methanol/water (20:9:1 v/v/v) and thoroughly mixed in a glass tube for a total amount of 0.3mg. Lipids were adhered along the sides of the glass tube under a stream of nitrogen gas by gently rotating the tube until the solvent had evaporated. Residual traces of solvent were then evaporated under vacuum overnight. 300 L of lipid buffer (Heps 20mM, NaCl 150mM pH 4.8 or pH 6.8; acidic pH was used to improve the homogeneity of the distribution of PIs in the bilayer) was added to the dried lipid, and the tube was allowed to incubate at room temperature for 45-60 mins. This incubation allows the lipids to gradually peel off the glass surface and swell. Multiple vigorous vortexing cycles were applied, forming vesicles with heterogeneous size. Small unilamellar vesicles at 1mg/mL were formed by 31 extrusion cycles using polycarbonate membranes of 50nm pore size (Avanti Polar Lipids). This stock solution was diluted in 250μL of the 4.8 pH buffer solution. The diluted solution of lipids was then inserted into the channel allowed to form for 30 minutes. The channel was monitored throughout to ensure no evaporation took place which can disrupt the lipid bilayer formation. After this 30 minute period the channel was washed again several times with a 6.8 pH buffer solution, making sure no evaporation occurred in the channel. 10μL of the desired functionalized bead solution, described in the section above, was diluted into 800μL of the 6.8 pH buffer. This diluted solution of beads was then inserted into the channel and the channel was sealed at both ends with epoxy to prevent evaporation. The channel was then placed on top of neodymium magnet to magnetize the ferromagnetic particles and then placed on the slide holder within the experimental apparatus.
3.5.3 Rolling parameter scales with known lipid-protein binding affinity

As can be seen from Fig. 3-17, our results confirm previous studies which had shown that these set of proteins had specific interactions with a particular PIP [151, 152, 153, 154, 155, 156]. Moreover, the relative affinities of the interactions are in agreement with the obtained rolling parameters, as the $K_d$ of DrsAWT-PI4P is approximately 5nM, 2XFYVE-PI3P is approximately 100nM, and PH-δ-PI34P₂ is approximately 1μM [151, 152, 153, 154, 155, 156].

![Graphical Image]

**Figure 3-17:** A) Kymographs of rollers functionalized with DrsAWT, DrsAK568A, PH-δ, and 2XFYVE rolling on supported lipid bilayers (SLB) containing 10% PI4P, PI4P, PI3P, and PI45P₂, respectively. The oscillations in motion are large for DrsAWT which is to be expected due its high affinity for PI4P but the oscillations become much smaller and the translation diminishes drastically once a point mutation is introduced, DrsAK568A. Similarly, the amplitude of oscillations decrease from 2XFYVE to PH-δ as expected. B) The rolling parameter is plotted for different biological interactions with SLBs. Each point is the average rolling parameter measured for a roller. There are 34 measurements per roller. The solid black line indicates the average rolling parameter of the entire data set. The dashed grey lines represent the 95% confidence interval of each dataset. The SLB contains 10% of each ligand. The rolling parameter correlates well with the known binding affinity of these interactions and is specific to the ligand phosphorylation.

A graphical image showing the differences on the back and forth rolling of at least ten different beads on multiple substrates is shown in Fig. 3-17. The frequency of rolling is set to 1 Hz unless otherwise noted. In the top kymograph, we show the rolling of DrsAWT coated beads on membranes containing 10% of PI4P. As can be seen from the amplitude of oscillation, the rolling parameter in this case is high which is expected as DrsAWT exhibits a high affinity for PI4P. In fact, the rolling parameter measured is close to that observed for that of a streptavidin coated ferromagnetic particle.
rolling on a supported lipid bilayer (SLB) containing 10% PI4P. In principle, one would expect to obtain a rolling parameter of 1 for this ligand-receptor pair because it is one of the strongest bonds in biology. We believe this discrepancy is due to the fact that the beads might be stripping lipids from the membrane rather than breaking the bonds formed with the biotinylated lipids. This can also explain why at longer times the interaction is dramatically reduced. By introducing a point mutation into the DrrA<sub>WT</sub> protein, we can see this strong affinity for PI4P is lost in the mutated DrrA<sub>K568A</sub> protein as the oscillations are now much smaller. The same can be seen for 2XFYVE and PH-δ on SLB containing 10% PI3P and PI45P<sub>2</sub>, respectively. The oscillations are smaller for PH-δ which is expected as the affinity for PI45P<sub>2</sub> is lower than the affinity of 2XFYVE for PI3P [151, 152, 153, 154, 155, 156].

A convenient way to quantify the relative strength of the interaction previously mentioned is to plot the average rolling parameter and 95% confidence interval for each system. This is shown in Fig. 3-17. As mentioned above, the biotin-streptavidin case has the highest rolling parameter, but once streptavidin beads are coated with free biotin ligands in solution the rolling

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Figure 3-18: A) Rolling parameter of DrrA<sub>WT</sub> on SLB with 10% PC, PI, PI3P, PI4P, PI34P<sub>2</sub>, and PI45P<sub>2</sub>. DrrA<sub>WT</sub> shows extremely high affinity for PI4P compared to other SLB functionalization. B) Rolling parameter for 2XFYVE shows preferential affinity for PI3P. C) Rolling parameter for PH-δ shows preferential affinity for PI45P<sub>2</sub>.

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parameter becomes comparable to the baseline case where the streptavidin coated particles roll on a PC SLB. The interaction affinity of DrrA<sub>WT</sub> for PI4P, 2XFYVE for PI3P, and PH-δ for PI45P<sub>2</sub> have been previously reported and to be approximately 5nM, 100nM, and 1μM, respectively [151, 152, 153, 154, 155, 156]. As we see in Fig. 3-17, the rolling parameter seems to match well qualitatively with those literature values and confirms that this new technique can resolve these previously investigated interactions. These measured rolling parameters are also much larger than when rolled on the PI SLB, indicating the specificity of these interactions. We further confirm that this roller bead binding assay technique can confirm the sterospecificity of these known interactions as seen in Fig. 3-18. Here, we see that DrrA<sub>WT</sub> is specific to PI4P, 2XFYVE to PI3P, and PH-δ to PI45P<sub>2</sub> when compared to other functionalized SLBs.

### 3.5.4 PTEN preferentially binds to PI3P and GAK to PI4P

Figure 3-19: A) Rolling parameter measurements show Auxilin 1<sub>PTEN</sub> affinity for PI3P when compared to other PIPs. This correlates well with biological observations. B) Rolling parameter measurements show Auxilin 2<sub>GAK</sub> affinity for PI4P and a newly discovered interaction with PI34P<sub>2</sub>. Again, this result correlates well to biological observations.

As a further test, we decided to study the interaction between PIPs and two proteins Auxilin 1 and Auxilin 2 that are required in the endocytotic pathway. Auxilin 1 has a PTEN-like domain and is expected to interact with PI3P. PTEN is a diphosphatase protein involved in lipid signaling and apoptosis, and it appears in a mutated form in many different cancers [143, 144, 145, 146, 147, 103]. Auxilin 2 is another protein that is more prevalent in the brain and believed to be very important for brain function in synaptic joints [143, 144, 145, 146, 147, 103]. In this case, it is believed to be specific for PI4P. As can be seen from the rolling parameters we obtained and shown in Fig. 3-19, we indeed confirm that Auxilin 1 and Auxilin 2 are specific for PI3P and PI4P, respectively. In the case of GAK, we also found an unexplored interaction that has a higher affinity than GAK-PI4P, and this interaction is with PI34P<sub>2</sub>. Here, we speculate that such interaction is stabilized cooperatively by charge. This is in a good agreement with the biological data where PI34P<sub>2</sub> sensor began to
recruit with Auxilin 2 and remains associated with the vesicular carrier even after uncoating had ended as seen in Fig. 3-14. To finalize, we have compiled a heat map, as seen in Fig. 3-20, showing the relative strengths of the Auxilin lipid complexes. One can see that they are in general very weak, but specific to different kinds of lipids.

![Heat map](image)

**Figure 3-20:** This heat map shows the average rolling parameter for each protein-ligand interaction. Black squares indicate interactions that were not measured.

In summary, we have introduced a new technique to study weak protein-lipid interactions. We have applied this assay to study previously known interactions as well as new unexplored protein-lipid interactions. Our findings show unequivocally that Auxilin 1 (PTEN) and Auxilin 2 (GAK) display very weak interactions, comparable to the case of PH, but have specificity to different PIP lipids. Our results also show that it is possible to study in a scalable fashion the other weak interaction using the method here introduced, and we expect it to become an important tool in the discovery and characterization of weakly interacting complexes in biology.

### 3.6 Conclusions

In this section, we have developed a new and novel artificial experimental active matter system that can be utilized in conjunction with the tribotactic system to further investigate and probe the fundamental role that friction may play in guiding vital biological processes like chemotaxis and cellular locomotion in general. Using ferromagnetic rollers and a novel 3D magnetic coil
apparatus, we can now apply enough torque to allow us to probe high affinity interactions like biotin-streptavidin which takes approximately 100 pN to break a single bond. We investigated many different classifications of interactions on a hard avidin coated glass substrate using rollers or active tribological probes. In so doing, we were able to distinguish between hydrophobic, electrostatic, antigen-antibody, metal ion coordination interactions, and of course the biotin-streptavidin interaction by measuring the rolling parameter, a dimensionless number that allows us to quantify the effective friction which is related to the affinity of the interaction of interest. This technique can also distinguish differences in effective friction and thus, affinity within each broader classification (i.e. different antigen-antibody interactions and metal ion coordination interactions).

This new ligand binding assay was also utilized to detect differences in binding affinity of protein-lipid interactions that previously could not be quantified by traditional ligand binding assay techniques. Protein-lipid interactions constitute a very important class of biological interactions critical for multiple cell and tissue functions. It is believed that most lipid-protein interactions are very weak, with affinities in the 1mM-1μM range. We investigated the interactions of multiple proteins with lipid membranes containing signaling lipids known as PIP at concentrations comparable to biological conditions. We demonstrated that this assay can measure ultra-weak interactions and can measure the interactions close to the biological lipid concentration regime. In particular, we have studied the interaction of DrtAWT, DrtAΔK568A, PH-δ, and 2XFYVE as well as previously unexplored proteins such as Auxilin 1 (PTEN) and Auxilin 2 (GAK) against a wide palette of PIP. Our results confirm that each of these proteins interact specifically with a PIP partner. In the case of Auxilin 1 and Auxilin 2, both critical proteins in the clathrin endocytotic pathway, we found they interact more weakly than any of the previously studied proteins. Yet, they have specific interactions with PI3P and PI4P, respectively. We have also found a new unknown medium-high affinity interaction between GAK with PI34P2. Our work, thus, provides a direct route to measure and catalog protein lipid interactions which are important in many processes such as signaling and membrane sculpting. Furthermore, this method can be extended in a straightforward way to study other interactions such as ligand-receptor or antibody-antigen.

While this technique is extremely powerful and shows tremendous promise as a new, cheap, high throughput, simple method to measure and quantify interactions that could not be captured or quantified using traditional techniques like ELISA or SPR, there are still several open questions and work that must be done. The most pressing question by far is to dedicate more time and resources into developing theory or conducting simulations that can give us insight into what experiments to conduct that will allow us to obtain quantitative measurements of $K_d$, $K_{on}$, and $K_{off}$. This is essential because while the rolling parameter can be utilized to distinguish between unknown interaction affinities, one must first establish a baseline measurement by which all further measurements will be compared. One can imagine that the rolling parameter measured for the baseline can change depending on the substrate so this can be problematic for comparing different measurements relative to a baseline, even if you subtract the baseline measurement. It is essential to be able to get quantitative numbers from this ligand binding assay for it to be of use as a tool to
further guide us in investigating the binding affinity and kinetics of interactions. The throughput of this ligand binding assay can also easily be increased using 96 well plates instead of the custom microfluidic devices previously described in conjunction with individual CMOS cameras for each well.

Getting these quantitative measurements is also crucial in order to reverse engineer and design rollers that can exploit advantageous components of different binding interactions. For example, if one wanted to design a roller with high affinity and fast binding kinetics in order to roll as fast as possible for a particular application, it would be very difficult. There are very few, if any, interactions that exhibit this behavior. However, by multiplexing; that is by functionalizing the roller and/or the substrate with a motif of two or more interactions that combine both high binding affinity with another interaction with fast binding kinetics, one can tune rolling behavior of the active tribological probes. Another interesting question that was beyond the scope of this thesis is if the roller can perform durotaxis, which is directed motion or migration on a substrate, or within the ECM, towards regions with greater stiffness. Additionally, we have yet to fully utilize the 3D magnetic coil apparatus to drive 3D motion of the active tribological probes in a 3D collagen matrix or even within the gut of transparent C.elegan worms. Thus, there is still much work to be done with this system.

Thus far, we have only investigated active matter systems with a single active unit. However, most active matter systems are very dense and composed of many active units, typically in complex or confined environments. In the next Part and Chapter, we will increase in complexity the design of our experimental artificial active matter systems to investigate emergent interactions, nonequilibrium steady states, and collective motion of active units in complex environments.
Part II

Emergent Non-Equilibrium Phenomenon in Complex Passive Media
CHAPTER 4
EMERGENT INTERACTIONS IN COMPLEX PASSIVE MEDIA

Work in this chapter was published in:

Vital, yet ubiquitous processes such as reproduction, migration, wound healing, and cell differentiation are all inherently non-equilibrium processes [10, 11, 6, 12, 13, 14, 15, 16]. The systems that perform these processes are additionally unique as they are composed of both actively driven components and passive components. In this Chapter, we will present a novel artificial hybrid active-passive model soft active matter system which mimics some of the non-equilibrium phase behavior of such biological processes. In this experimental soft active matter system, the active components are ferromagnetic colloidal particles embedded within a dense monolayer of inactive or passive (non-motile) polystyrene colloidal particles. Motility is induced via actuation of an external magnetic field in the plane of the ferromagnetic particles which rotates around the axis normal to said plane which causes the active units to spin, hence spinners. Both experimental and theoretical studies show a force reversal between the spinners in the presence and absence of a dense passive colloidal monolayer. In a dilute suspension of purely active particles, there is no interaction felt between the spinners. However, when the dilute suspension of spinners is inserted into a dense passive colloidal monolayer, an ultra-long range attractive interaction between the spinners emerges. This effective attractive interaction leads to non-equilibrium phase separation between the active and passive components in this system and arises due to the elastic nature of the dense passive monolayer. The motility of the spinners stresses the passive monolayer between the spinners, compressing and shearing this passive interface which we denote as the bridge. After some characteristic time period, enough stress has built up in the bridge and the bridge stochastically yields. The spinners move closer together and after enough yielding events, the spinners aggregate.

The range and strength of this emergent attractive interaction can be modulated by several factors: i) mechanical properties of the passive medium, ii) activity of the spinners, iii) actuation protocol, iv) composition of the passive monolayer, and v) mode of motility. The attractive interaction emerges and the range increases as the mechanical properties of the passive media transitions from behaving more solid-like than fluid-like (i.e. when the ratio of the storage to loss modulus becomes greater than one). The range of the attractive interaction increases as the activity of the spinners increases. There is a critical activity below which the passive medium is able to dissi-
pate the energy faster than the rate of energy input. The spinners are unable to stress or yield the passive monolayer at this activity level. Above this critical activity, the system is unable to dissipate energy fast enough so energy can only be released via cooperative passive particle motions (i.e. yielding events). There is also a minimum time scale of continuous activity required to yield the passive monolayer, on the order of 10s for a rotational frequency of 5Hz. The emergent interaction strength and range can be reduced by alloying the passive monolayer, which decreases the probability of inducing yielding events in the passive monolayer. Additionally, the formation of spinner dimers can be prevented utilizing a new mode of activity and active units, tops. This model experimental active matter system represents a simple, yet versatile system, that can be used to model a number of biological process and provide an explanation as to the origin of this non-equilibrium phase separation in active-passive systems which can apply to many biological and theoretical systems.

4.1 Nonequilibrium active matter systems

Active matter systems have received much interest due to their emergent, non-equilibrium phase and collective dynamical behavior. This interest is well founded as some of the most ubiquitous and important biological systems or processes can exhibit such emergent non-equilibrium behavior. Perhaps, this behavior is most recognizable in macroscopic examples ranging from schools of aquatic organisms like rays or fish [157, 24, 158, 159], herds of livestock [160], flocks of birds [37, 161, 38], and even a mosh pit at a heavy metal concert [25]; all of which exhibit collective dynamical behavior. Active matter systems are additionally unique in that such phenomena is not restricted to a single length scale but instead spans multiple length scales from meter to nanometer. It is at these smaller length scales where it is clear that such dynamical adaptation and phase separation is necessary to perform many vital biological processes. Dense crowds of cells move collectively through tissue during development and in many of the immune response processes (i.e. wound healing) [15].

Sea urchin sperm cells have been found to phase separate and organize into arrays of vortices when the density of spermatozoa is large enough [12]. In fact, a myriad of biological systems and experimental systems with biological components, have reported swarming [162, 163, 164, 165, 166, 34], flocking [36, 161, 38, 25, 14, 167], spiraling [168, 34], and many other non-equilibrium steady states [9, 169]. It is clear that in all these systems, a combination of the activity, shape of the active agents, and the environment lead to effective out-of-equilibrium interactions that determine their non-equilibrium steady states. These active matter systems have been particularly attractive for theoretical studies due to the large parameter space that can be explored, and the fact that there are relatively few artificial systems that can mimic the emergent behavior observed in these active matter systems. Simulations have been able to capture a wide range of collective dynamical behavior and spontaneous phase separation in these non-equilibrium systems [4, 170, 171, 172, 173, 167, 4].

While most experimental and simulation studies have focused on systems composed of purely active components [17], here we investigate a hybrid system composed of active and passive com-
ponents. This hybrid active-passive system serves as an excellent model for collective motion in complex, crowded biological environments where active units must move through or past non-motile cells or tissues. Such a model system could potentially help to explain collective motion and phase separation observed in bacterial biofilms [7], cells migrating through tissue interfaces, cells moving collectively within tissues, or in cell sheets [174, 175, 30, 14, 16, 176, 15]. In the past, there have been very few studies investigating hybrid active-passive systems and almost all of them are simulation or theoretical studies. Interestingly, these studies have reported non-equilibrium phase segregation in systems of motile and non-motile rods [172], passive spheres and active rods [173], active and passive agents [176, 167], and active and passive hard spheres [4]. Attempts to observe similar behavior in experimental artificial model systems has proven to be a more difficult task, presumably due to the fact that other interactions are present in such systems compared to the relatively simple models that theory and simulation have considered. Despite this, there are many biological examples, as well as the aforementioned simulation and theoretical results, which show an emergent attraction between active agents in mixtures of active and passive populations and subsequent phase separation. Nevertheless, the origin of this non-equilibrium phase segregation is not well understood. Here, we clearly show that two active spinning particles in a dense monolayer of passive colloids attract, and such attraction can be felt at extremely long distances. This range is much longer than the magnetic dipole-dipole interaction, tunable via the activity, and kinetics of the system. The origin of such long-range attraction is due to the elasticity of the monolayer. Our results, provide the much needed understanding on the emergent interactions in synthetic mixed active systems, and can help distinguish what biological interactions are due to purely physical phenomena and which interactions require presumably physical and biological/biochemical stimuli.

4.2 Hybrid active-inactive spinner system

These hybrid systems are composed of a mixture of active and passive particles in water. The active particles are ferromagnetic colloids composed of a polystyrene core and an outer shell composed of CrO₂ and polystyrene. The passive particles are purely polystyrene. We use a dilute solution of active particles, \( \approx 2\mu g/ml \), to study two body interactions between active particles. This concentration is kept fixed, unless otherwise noted. We impart activity to the ferromagnetic particles by rotating an external magnetic field around the axis, perpendicular to the monolayer plane. This causes the active particles to spin in place, henceforth referred to as spinners. The experiments are conducted in small channels, \((22\text{mm (L)} \times 3\text{mm (W)} \times 300 \mu \text{m (H)})\), which are made between a slide and a cover slip. After the particle solution is injected into the channel, the channel is sealed with epoxy. Before applying the rotating magnetic field, the particles are allowed to sediment for approximately 10 min. This allows for the formation of a dense colloidal monolayer on the substrate, as shown in Fig. 4-1.

To drive spinner motility we initially developed the experimental apparatus seen in Fig. 4-2. The apparatus consisted of two pairs of coils attached to a rectangular wooden box. The
Figure 4-1: A mixture of passive polystyrene particles (blue spheres) and active ferromagnetic particles (red spheres) is mixed and inserted into a small channel that is then sealed with epoxy. Visualization is accomplished using a microscope in bright field mode. Active particles are rotated at an angular frequency, $\omega$, in the x-y plane in a dense monolayer of passive particles. The active and inactive particles are both 5 $\mu$m in diameter.

A wooden box was mounted on a modified compound binocular light microscope (condenser lens, iris diaphragm, stage, and condenser focus all removed) provided by OMAX. Another OMAX microscope was modified so that it would act as a 3D mobile stage. The specimen clip was removed and a rectangular piece of nylon, approximately 300mm x 30mm, was screwed into the mechanical stage. The sample was mounted onto this piece of nylon (later the nylon was replaced by a wooden piece of similar dimensions). The box was then mounted so that the coils were centered on the sample holder. Two sinusoidal signals, phase shifted by 90 degrees, were generated by an external program (Audacity and/or MatLab) and passed through each pair of coils (one signal per pair of coils). A 300W amplifier (150W/channel) ran 2.5 Amps through more than 100 turns of wire (in each coil) to obtain a field strength of approximately 5mT.

The magnitude of the field can be described by the following equation,

$$B(x, y) = \frac{INR^3\mu_o}{(R^2 + (d_x - x)^2)^{\frac{3}{2}}} + \frac{INR^3\mu_o}{(R^2 + (d_x + x)^2)^{\frac{3}{2}}} + \frac{INR^3\mu_o}{(R^2 + (d_y - y)^2)^{\frac{3}{2}}} + \frac{INR^3\mu_o}{(R^2 + (d_y + y)^2)^{\frac{3}{2}}} \quad (4.1)$$

where $I$ is the current passed through the coils in Amperes, $N$ is the number of turns of wire, $R$ is
Figure 4-2: A) Schematic of experimental set-up. A light microscope was mounted with four coils to produce an in plane rotating magnetic field. A wave generator ran two sinusoidal signals, phase shifted by 90 degrees, through an amplifier to generate a field strength of 5mT. An oscilloscope was used to monitor the frequency and a CCD camera mounted on the microscope was used to capture videos of the walker motion. B) Image of the experimental apparatus. C) The sample was placed at the center of the coils where the field is the most homogeneous. The solution of passive and active colloids in a surfactant solution was inserted into a microfluidic channel and sealed.

The magnetic field was largest at the center of each of the four coils. Clearly, across the entire apparatus the magnetic field is not homogeneous. But if we take a closer look at the area of interest where the sample was placed, the field was essentially homogeneous with a field strength of approximately 5mT. The signal from the amplifier was routed to an oscilloscope, HP (Hewlett-Packard) H4601A, in order to get a precise measure of the frequency and the voltage at the output. Data acquisition was accomplished via a CCD camera that had been mounted on the microscope. The camera was connected to a computer for visualization, video capture, and subsequent analysis. We also conducted experiments utilizing the 3D magnetic coil apparatus described in the previous chapter as well. However, the preliminary work was conducted using the apparatus seen in Fig. 4-2. Therefore, with the design of the system in place and the apparatus to drive spinner motility, we set off to investigate emergent attraction between active units in a dense, complex, and passive environment.
Figure 4-3: A) The magnetic field strength was plotted across the entire experimental apparatus. The magnetic field was largest at the center of each of the four coils. B) The magnetic field appears to be essentially homogeneous in the area of interest.

4.3 Emergent ultra-long-range spinner interaction in dense passive media

To study pair-wise interactions between spinners, we control both the density of passive particles and the activity of the spinners; the latter of which is regulated by changing the frequency of rotation of the magnetic field, \( \omega \), which is shown schematically in Fig. 4-1. The magnitude of the field is maintained constant at 5mT which is large enough to ensure alignment of the rotational frequency of the ferromagnetic particles with the rotational frequency of the field. As a reference system, we investigated the interaction between two spinners in the absence of a passive medium (i.e. no passive particles). To create this system, the stock solution of 10mg/mL was diluted to a concentration of approximately 2 \( \mu g/\text{ml} \). The solution was vortexed for several minutes and then 10\( \mu \text{L} \) of this solution was inserted into a microfluidic channel and allowed to sediment for 5-10 minutes. The channel was sealed with epoxy, taken to a neodymium magnet (provided by KJ Magnetics Inc) with a maximum field of 52MG Oe, to be magnetized prior to insertion into the sample holder. We identified isolated pairs of spinners, seen in Fig. 4-4, separated by different initial distances and magnetically actuated them by rotating the magnetic field at an angular frequency \( \omega \), of 5 Hz.

Next, we tracked the time evolution of the distance between the identified spinner pairs, \( R \). The trajectories, normalized by the diameter of the particles \( D \), are shown in Fig. 4-5. We observed that spinners initially separated by distances larger than \( R/D > 4 \) do not interact and thus, the distance between them remains almost constant (as shown by the red trajectory and the experimental snapshots in Fig. 4-5A). These trajectories exhibit minor fluctuations, most likely due to the fluid flows generated by other spinners in the channel. When spinners were initially positioned closer than \( R/D \approx 4 \), they attracted very rapidly and formed a dimer (as seen in the green trajectory of Fig. 4-5A). This attraction is due to the magnetic dipole-dipole interaction,
Figure 4-4: A) Two spinners in an extremely dilute, purely active system actuated at a frequency of 5 Hz. B) Two spinners in a passive medium, $\phi_A \approx 0.7$, actuated at a frequency of 5 Hz.

which becomes quite small above $R/D > 4$, as the force decays as $1/R^4$.

Figure 4-5: A) Time evolution of the normalized distance $R/D$ between two active spinning particles in the absence of a passive colloidal monolayer. B) Time evolution of the normalized distance between two spinners in a dense colloidal monolayer of $\phi_A \sim 0.7$. In both cases, the frequency of rotation is $\omega = 5$ Hz. The snapshots correspond to selected configurations along the trajectories at the time indicated by the arrows.

The interaction between spinners embedded in a dense passive media is of a different nature. The ferromagnetic particles were diluted to a concentration of 10 $\mu$g/mL. 10$\mu$L of this solution was mixed with 40$\mu$L of polystyrene particles at a concentration of 10 mg/mL (approximately 5$\mu$m in diameter which was provided by Phosphorex). This solution was vortexed for several minutes and then 10$\mu$L were inserted into the microfluidic channel. (This concentration corresponds to an extremely small average density of the order of 1 active particle per 4000 passive particles. In this limit, it is almost impossible to image all the active neighbors that might affect the interactions observed.) The slide was placed atop a neodymium magnet to magnetize the spinners and then moved to the sample holder. After several minutes, to allow for sedimentation, the packing fraction
was determined to be approximately 0.7. We observed that spinners embedded within a dense monolayer of passive polystyrene particles at an area fraction of $\phi_A \sim 0.7$, attract. However, the range of the interaction is dramatically increased, and spinners initially separated up to $17D$ apart are still able to attract. Interestingly, the trajectories for spinners which exhibit an attractive interaction are characterized by two distinct regimes: i) at smaller distances, 4D or less, the slope of the trajectory is extremely steep as the dominant attractive force in this regime is controlled by the magnetic dipole-dipole interaction, ii) at distances larger than 4D, the slope is much smaller and close to 0 for certain periods of time which indicates that the dominant attractive interaction in this regime is of a different nature than a magnetic dipole-dipole interaction. In fact, within the time frame of the experiments the force is both stochastic and attractive.

Figure 4-6: Secondary flow in the $xz$ plane generated by a spinner rotating about the $z$-axis in bulk (A) and close to a wall (B)

To gain a deeper insight into the nature of the interaction between spinners embedded in passive mediums, we carried out numerical simulations using a coarse-grained model of this system. Specifically, we performed Lattice-Boltzmann (LB) simulations for a fluid of density, $\rho=1$, and kinematic viscosity, $\nu=1/6$, coupled to a Molecular Dynamics (MD) simulation for the colloidal particles [177]. We explicitly solved the fluid field generated by the spinners by means of the fluctuating Lattice-Boltzmann equation [178] with $k_BT=0.00002$. We then obtained the forces exerted by the fluid on the colloidal particles, represented by hard solid objects [179], and enforcing no-slip boundary conditions at their surfaces we integrated the time evolution of the system. For this model, which neglects the dipole-dipole interaction between ferromagnetic particles, we observed that spinners suspended in a viscous fluid repel each other, whereas spinners embedded in passive monolayers attract each other [180]. In the simulations, we only observed the stochastic attractive regime (ii), indicating that the monolayer of passive particles is responsible for the attraction, and that the strong short range attraction is due to the magnetic dipole-dipole interaction present in the experiments.

Regarding the fluid flows, one spinner suspended in a viscous fluid generates a rotational flow
around the axis of rotation, (i.e. $z$). At a Reynolds number (Re) zero this is the only contribution to the fluid field. However, at small but finite Re, inertial effects produce the so-called secondary flows [181]. As a consequence, the fluid is pulled in towards the poles and expelled from the equator (as shown in Fig. 4-6). These secondary flows cause one spinner to repel other spinners or neighboring passive particles, and thus the formation of the corona [180].

4.4 Emergent interaction mediated by mechanical properties of passive media

To understand the attractive interaction between spinners in a passive monolayer, we need to understand how the activity of the spinners modifies the passive monolayer. Under the actuation of the rotating magnetic field, the spinners rotate in place and generate a rotational flow around the axis of rotation and secondary flows as previously mentioned. In the purely active system, these flows produce small perturbations on the positions of neighboring spinners (Fig. 4-7), which may lead to a fast attraction between two spinners separated by distances of about 4D due to the strong magnetic dipole-dipole interaction.

![Graph showing the distance between the spinners normalized by the diameter for actuated frequencies of 0.5, 1, 3, and 5 Hz. There was clearly no attractive force felt between the spinners, unless the spinners were initially positioned a distance of approximately 4D or less.](image.png)

Figure 4-7: The distance between the spinners normalized by the diameter was plotted for spinners actuated at frequencies of 0.5, 1, 3, and 5 Hz. There was clearly no attractive force felt between the spinners, unless the spinners were initially positioned a distance of approximately 4D or less.

In the presence of a passive monolayer, the rotational flows generated by the spinners rotates the surrounding passive particles. Therefore, spinners embedded in a dense passive medium rotate the
first shell of passive particles around them as a consequence of the momentum transferred through the fluid. In addition, the secondary flows generated by the spinners push the first shell of passive particles away from the spinners, creating a region which is devoid of passive particles as shown in Fig. 4-8A (e.g. \( \phi_A \approx 0.7 \)).

Figure 4-8: A) Experimental snapshots of the active spinners in passive mediums with area fractions \( \phi_A \approx 0.3 \), 0.5, and 0.7, and their corresponding distance trajectories. The color of the trajectories correspond to experimental snapshots and passive monolayer area fractions with \( \phi_A \approx 0.3 \) red, \( \phi_A \approx 0.5 \) blue, and \( \phi_A \approx 0.7 \) green. B) The range of the interaction as a function of the area fraction of passive monolayer computed for actuation periods of 5 min. The different symbols represent: i) no attraction (blue symbols) and ii) bound (red symbols). The threshold committor probability is represented by black stars and fitted by the dashed black line. Square symbols indicate experiments using ferromagnetic polystyrene active particles and polystyrene passive particles, both 5\( \mu \)m in diameter. Triangular symbols indicate experiments using ferromagnetic polystyrene active particles, 5\( \mu \)m in diameter, and electrically polarized silica passive particles, 3\( \mu \)m in diameter. The ratio of \( G' \) to \( G'' \) was calculated, at experimental times of about 5 minutes, for various packing fractions of \( \phi_A \). The dashed line indicates a value of one which delineates mediums which behave elastically and viscous.

We denote this region as the corona as seen in Fig. 4-9. The creation of the corona is similar to shear banding phenomena [182] and implies an effective hydrodynamic repulsive interaction between the spinners and the passive particles that leads to a weak, but noticeable compression of the monolayer. The size and angular velocity of the corona depends on both the passive monolayer area fraction and the rotational frequency of the magnetic field (see Fig. 4-10 and 4-11) where the size of the corona was calculated after 1 minute of actuation at the different frequencies. The rotational frequency of the passive particles at the edge of the corona was calculated by tracking...
Figure 4-9: Different regions in the experimental system, specifically the bridge (green), bulk (blue), spinner (yellow), and the first shell of passive particles which mark the edge of the corona (red).

Figure 4-10: The average distance of the first shell of particles from the spinner as a function of frequency, effectively a measure of the size of the corona.

Upon actuation of a magnetic field, the spinners begin to degrade or erode the passive monolayer that initially separates the pair of spinners. We refer to this region of passive particles between the spinners as the bridge. The degradation or erosion of the bridge is a slow, stochastic, but nevertheless steady process resulting in an effective attractive interaction between the spinners. The fact that the bridge is degraded to a greater extent than the other regions of the monolayer is related to the activity-induced elastic stresses imposed on the monolayer. To prove directly the importance of elasticity in this system, we measure the range of the attractive interaction between spinners in passive monolayers with area fractions ranging from $\phi_A = 0$ to 0.8. We have previously described the procedure for preparing a passive monolayer with $\phi_A \approx 0$ and $\phi_A \approx 0.7$. To achieve a packing fraction of $\phi_A \approx 0.5$, 8μL of passive particles (at a concentration of 10 mg/mL) were mixed with 10μL of a solution of ferromagnetic particles at a concentration of 2μg/mL. A packing fraction of $\phi_A \approx 0.3$ was achieved by mixing 5μL of passive particles, at a concentration of 5 mg/mL, with
10μL of ferromagnetic particles at a concentration of approximately 0.62 μg/mL. The slide was then placed again on the neodymium magnetic and moved to the sample holder and left for several minutes for sedimentation. Two spinners were then isolated and the magnetic field was actuated for approximately five minutes at 5 Hz or until the two spinners formed a dimer. The distance between particles, R, was measured as a function of time to measure the attractive force between spinners. The long range attractive interaction between spinners in passive monolayers is lost at $\phi_A \approx 0.3$, and it appears at $\phi_A \approx 0.5$, yet the strength is not nearly as large as for the case of higher area fractions as shown in Fig. 4-8A. The smaller the $\phi_A$, the longer the time for the two spinners to form a doublet (bound state).

From the experiments at different monolayer area fractions, we built a range diagram (presented in Fig. 4-8B) together with the ratio between the storage modulus, $G'$, and the loss modulus, $G''$. These mechanical parameters are obtained from very long experimental times (5 minutes) using passive microrheology of the purely passive monolayers at different area fractions. Passive monolayers of $\phi_A \approx 0.3, 0.5, 0.7$, and a silica monolayer with an applied voltage of 2.5 were created using the protocol previously described. Additionally, a monolayer with a packing fraction of $\phi_A \approx 0.9$ was created by following the same protocol for $\phi_A \approx 0.7$ with the exception that the solution was inserted into a channel that was 5 pieces of double sided tape high instead of the usual 3 pieces. To characterize the visco-elastic nature of these monolayers, the passive particles were tracked for a period of approximately 6 minutes. A MatLab program was then used to track the particles, calculate the mean square displacement, and fit the data with an exponential function as seen in Fig. 4-12.

This fit function was then used to calculate the complex modulus by feeding the fit function data into a MatLab function that fits this data with a second-order polynomial function from which the first and second time derivative are computed and from that, the complex modulus. The storage modulus (elastic) $G'$ and loss modulus (viscous) $G''$ have been plotted for different
The complex modulus measurements illustrate that for polystyrene monolayers at $\phi_A \geq 0.5$, the monolayer behaves elastically for all frequencies or times. Yet for $\phi_A \approx 0.3$, the monolayer behaves elastically at very short times but then transitions to a more viscous medium at longer times. The ITO silica bead monolayer elastically behavior is observed for all frequencies as well.

The range of spinner interactions, $R_i/D$, corresponds to the initial distance between a pair of spinners and the results are cataloged as: i) no attraction or ii) bound. The state i) of no attraction (or no dimer formation) implies that there is no observable attraction found within the experimental time scale (in and all future discussions set to 5 min per spinner pair). State ii) is the bound state, and it means that the spinners have come to a bound state within the experimental time protocol. We observe that only the larger area fractions, $\phi_A \geq 0.5$, increase the range of the interaction with respect to the reference system (i.e. spinners in the absence of passive particles, $\phi_A = 0$) as shown in Fig. 4-8B. The long range attraction in this system emerges when the ratio between the storage and loss modulus, $G'/G''$, becomes larger than 1 which implies a solid-like character of the monolayer as shown in the bottom panel of Fig. 4-8B. Thus, the long range interaction between spinners is mediated by the activity-induced elastic stresses imposed on the monolayer. In fact, we observe even longer ranged interactions between spinners if embedded in monolayers of passive particles interacting through a strong repulsive potential (see the red triangles in Fig. 4-8B). We achieve
Figure 4-13: Storage and loss modulus as a function of frequency for $\phi_A \approx 0.3, 0.5, 0.7, 0.9$, and the ITO silica monolayer $\phi_A \approx 0.3$. The storage modulus $G'$ is the darker line and the loss modulus $G''$ is the lighter line.

This repulsive interaction between the passive particles in the monolayer by using silica beads that are polarized by an externally applied potential. This system contains 5\(\mu\)m active particles and 3\(\mu\)m silica passive particles with a strong repulsive potential once a voltage is established between two slides. To establish this voltage we used two ITO slides, 50 \(\Omega/\), and create a channel by placing two pieces of double sided tape on one ITO slide (the ITO coated side) and placed the other slide on top (ITO coated side down). We inserted the solution of active ferromagnetic particles (same concentration as previously described) and passive silica particles (at a concentration of 40 \(\mu\)g/mL) into the channel and sealed it with epoxy. We then placed alligator clips on two ITO slides and applied a voltage of 2.5V which polarizes the silica particles, creating a strong repulsive potential between particles which compose the colloidal monolayer as can be seen in Fig. 4-14.

The voltage was maintained at 2.5V and spinners were again isolated. The magnetic field was actuated at an angular frequency of 5 Hz. These passive matrices present a more pronounced solid-like character which allows the spinners to interact at distances up to 20D apart at much smaller area fractions, $\phi \approx 0.3$. We study other systems composed of mixtures of spheres of different sizes at 5Hz to ensure the attraction between spinners is indeed ubiquitous and not unique to the particular system previously described. In particular, we dope a passive monolayer, $\phi_A = 0.7 \pm 0.1$, composed of 5\(\mu\)m passive polystyrene particles with a very dilute concentration of 9\(\mu\)m ferromagnetic polystyrene particles. We doped a solution of 5\(\mu\)m passive particles, at a concentration of
Figure 4-14: Schematic of ITO microfluidic device and experimental snapshots of silica monolayer at applied 0 and 2.5V.

8 mg/mL, with 9μm ferromagnetic particles at a concentration of 5μg/mL and inserted the solution into the channel. We should expect to see the same long range attraction and perhaps, even more so as the larger active particles have a larger magnetic content and should be able to stress the elastic medium more than 5μm active particles (due to the larger magnetic content and thus torque). And indeed, attraction is still observed up to distances of 18D. A monolayer, φA = 0.7 ± 0.1, of 3μm silica particles was doped with a small concentration of 5μm ferromagnetic polystyrene particles by inserting a dilute suspension of active particles, 3 μg/mL, with 3μm silica particles at a concentration of 8 mg/mL. The range for this system is up to 20D, normalized by the diameter of the passive particles. This range can be observed in Fig. 4-15 and 4-16.

To provide a more quantitative analysis about the range of the attractive interaction between spinners embedded in passive mediums, we perform a committor-like analysis of the trajectories presented in Fig.4-8B. Committor analyses have been utilized to identify transition states in protein folding, vesicle fusion, and gold nanoparticle insertion [183, 184, 185, 186]. A similar analysis can be utilized in this hybrid active-passive system to analyze the transition between the i) no attraction and ii) bound states, and thus, identify the threshold of this emergent spinner-spinner interaction. As a consequence of the mechanical properties of the passive matrix, an average interaction potential between active particles emerges, similar to how a depletion force or potential emerges between two colloids upon introduction of depletants. Thus, in this system we can define two stable energy basins: i) no attraction and ii) bound, as seen in the inset in Fig.4-17. We can compute the
committor probability, $P$, between these two states. The committor probability is assigned a value of 0 for state i) and a value of 1 for state ii), with intermediate values occurring between these two basins. To calculate the committor probability as a function of the inter-spinner distance, all spinner trajectories for passive monolayer area fractions $\phi_A \approx 0$, $\phi_A \approx 0.3$, $\phi_A \approx 0.5$, and $\phi_A \approx 0.7$ were binned in time intervals of 5, 10, and 20 seconds. If the inter-spinner distance decreases between time intervals, we then assign a committor probability of $P = 1$ at that inter-spinner distance. If the distance increases between time intervals, the committor probability at that inter-spinner distance is assigned a value of $P = 0$. This procedure is repeated for all the trajectories represented in Fig.4-8 to calculate the average committor probability as a function of
inter-spinner distance. The committor probability for spinners in a passive monolayer area fraction of $\phi_A \approx 0.5$ as calculated for the three different time intervals is shown in Fig. 4-17. We define the committor threshold, or equivalently the interaction threshold, as the inter-spinner distance at which the committor probability is $P \approx 0.5$. This inter-spinner distance corresponds to the point where the spinner pair is equally likely to move into state i) no attraction or state ii) bound, as indicated by black star symbols in Fig. 4-8. This committor threshold would be analogous to transition states of activated processes.

![Figure 4-17](image)

Figure 4-17: Committor probability as a function of normalized spinner distance. Spinner trajectories were binned in time intervals of 5 (blue), 10 (purple), and 20 (green) seconds and the committor probability was calculated. The dashed red line represents the committor probability threshold. This occurs when the committor probability is $P \approx 0.5$. The spinner pair at this point is equally likely to be in state i) no attraction or ii) bound. This distance at which this occurs is represented by the black star symbols in the range diagrams in Fig. 4-8. The committor threshold for spinners actuated at an angular frequency of 5Hz in a polystyrene passive monolayer with a packing fraction of $\phi_A \approx 0.5$ is approximately 11 R/D.

### 4.5 Spinner attraction mediated by spinner activity and actuation protocol

The elastic stresses, as well as the kinetics of the monolayer, also depend on the frequency of rotation. Therefore, we study the spinner-spinner interaction as a function of the angular frequency of the spinners in monolayers of $\phi_A = 0.7 \pm 0.1$ doped with a very dilute concentration of spinners. We identify pairs of spinners at initial distances ranging from 3 to 40D and then actuate the magnetic field for a period of 5 min at frequencies of $\omega = 0.5, 1.0, 3.0$ and 5.0 Hz. For comparison,
Figure 4-18: A) Range of the interaction $(R_i/D)$ in the pure active system as a function of the angular frequency of the spinners. The spinners show essentially no long range attractive behavior. Spinners only form dimers when they are initially placed closer than $R_i/D < 4D$, where magnetic interactions dominate. B) Range of the interaction $(R_i/D)$ between spinners embedded in a dense colloidal monolayer of $\phi_A = 0.7 \pm 0.1$ for different angular frequencies. The spinners show extremely long range attraction at distances up to $17D$ as frequency increases. The diamond symbols indicate experiments using active ferromagnetic particles, 9$\mu$m in diameter, and were used in a polystyrene passive medium, 5$\mu$m in diameter. The circle symbols indicate experiments using active ferromagnetic particles, 5$\mu$m in diameter, and a passive monolayer composed of silica particles, 3$\mu$m in diameter.

this is also done in the absence of a passive medium to show that there is no long range interaction between spinners in this case, regardless of the frequency at which the magnetic field is actuated. The range diagram for both of these scenarios is shown in Fig. 4-18. In the case of no passive particles, the system has a threshold interaction range on the order of 4D. In contrast, in the case where a dense passive monolayer is present, we find a much longer range of attraction. Interestingly, the range rapidly decreases below a frequency of 1Hz. At this point, the spinners are unable to stress the monolayer (or form a corona see Fig. 4-10), and we believe that the rate at which the system can dissipate energy is faster than the rate of energy input. As the frequency is increased, part of the energy is stored in deformations of the monolayer that can only be released by cooperative particle motions, such as yielding events [180].

In the previous sections, we have shown that the activity of the spinners and the elasticity of the monolayer determine the range of the interaction between spinners. The activity of the spinners produces the rotation of the neighboring particles which in turn increase the mobility of the surrounding particles. However, the mobility of the passive particles in the monolayer follows activated dynamics [187] which have a characteristic time scale associated. To detect the minimum time scale associated with the activity necessary to observe the emergent long-range interaction, we modify the non-equilibrium actuation protocol. In particular, we use a protocol in which the spinner rotation, clockwise or counter-clockwise, is alternated every period of time $\Delta t$ until the 5 min actuation time is reached. The angular frequency is set to 5 Hz for both spinner rotational
Figure 4-19: Range diagram for an alternating protocol in which the spinners are rotated in alternating directions, first for a period of time $\Delta t$ in a counterclockwise direction and afterwards in a clockwise direction for another $\Delta t$. This process is repeated for 5min. Notice the lack of long range attraction until the spinners are rotated continuously in the same direction for more than $\Delta t = 10s$. The area fraction of the monolayer is $\phi_A = 0.7 \pm 0.1$ for all experiments.

directions. We observe no attraction between spinners for loading times, $\Delta t$, lower than 10s, as shown in Fig. 4-19. This implies that there is indeed a characteristic monolayer rearrangement time scale under stress and that the level of stress must be constant on the monolayer before it yields. These yielding events can be easily detected by measuring the velocity of the passive particles. The mobility of the passive particles located in the bridge is low during the initial loading process, whereas when the system yields, groups of passive particles move; this becomes apparent from the areas of high mobility in contour maps of the spatial velocity of the passive particles as shown in Fig. 4-20.

In this time series we do not use the alternating protocol, but it is evident that the system undergoes repeated cycles of high and low mobility in the area between both spinners. The characteristic time between these events is also in the range of 10-20s. In addition, the area fraction in the bridge decreases momentarily when the system yields as shown in Fig. 4-20. The time evolution of the density of passive particles in the bridge increases and decreases in agreement with periods of loading and yielding, respectively. The dynamics of attraction seems to be highly correlated with this temporal evolution of the area fraction. In particular, one can clearly see that around $t = 55s$, the density rises when the relative motion between the spinners becomes slower. It is important to highlight that all the time scales associated with the microscopic rearrangement of the monolayer are consistent with the 10s minimum time scale found for attraction. In particular, it is clear that the shear stresses in the bridge region are higher because of the imposed flow conditions (i.e. the direction of the flow reverses on opposite sides of the bridge). In the other regions, the stresses de-
Figure 4-20: A) Spatial velocity contour map of the passive particles averaged over 10s intervals. The positions of the spinners are marked by blue dots. It is clear that the passive particles just outside of the corona (i.e. the first shell of passive particles around the spinners, as well as the spinners) have the largest mobility. The bridge region appears to alternate between periods where the mobility of the particles in the bridge are enhanced (corresponding to yielding events in the bridge) and periods where the particles appear somewhat arrested, (corresponding to the compression of the bridge). B) Time evolution of the distance between spinners normalized by the diameter of the spinners (red line) and the area fraction of the passive particles located in the bridge (blue line). The black dotted lines indicate the time at which a line of passive particles was removed from the bridge. The fluctuation in the density of the passive particles in the bridge corresponds to periodic compression of the bridge (where the density increases) and shearing and yielding of the bridge (when the packing fraction decreases). C) Time to contact, $t_c$, at different initial distances for rotational frequencies of 3 and 5 Hz.

cay more slowly. While in equilibrium one would naturally see an equilibration of the system, here the energy to move the particles is coming mostly from the activity of the spinners. By imploring a quasi-equilibrium approximation, this would imply a locally higher free energy per passive particle as now one needs to include elastic terms in the free energy. Moreover, taking a look at longer time scales, this system clearly follows activated dynamics. To show this, we evaluate the average time required for spinners to bind or time to contact ($t_c$) as a function of initial separation distance for two different frequencies, 3 and 5 Hz, as shown in Fig. 4-20. The average time to contact decreases with the separation distance between the spinners. However, the slope of that curve increases with frequency or similarly with the activity of the spinners. As the frequency increases, the amount of stress exerted on the system by the spinners increases. The mobility of the passive particles increases as well and the time required for the spinners to bind decreases. Thus, the spinners’
activity controls both the strength as well as the kinetics of this emergent interaction.

4.6 Spinner attraction reduced by alloying the passive media and dimer formation prevented by inducing top motility

It is clear that this emergent long range interaction depends on the ability to stress the passive monolayer and the range depends on the ability to yield the bridge of passive particles. Specifically, the range of the interaction depends on how difficult or easy it is to induce yielding events. To test this hypothesis, we created a passive monolayer composed of a majority of 5μm polystyrene particles doped with 10% and 25% by area fraction of 3μm and 8μm polystyrene particles in order to ensure that the area fraction of $\phi_A \approx 0.7$ (to ensure the passive medium still behaves solid-like). The same procedure and concentration of spinner was utilized as previously described and the rotational frequency of the magnetic field was 5Hz. However, it should be noted that the spinners were actuated for a period of 10 minutes. The inter-spinner distance was tracked as a function of time and can be seen in Fig. 4-21. We also developed a range diagram for the spinners in this new complex passive media which can be seen in Fig. 4-22, as well as the associated committor analysis in Fig. 4-23.

![Figure 4-21](image)

Figure 4-21: A) Inter-spinner distance trajectory for spinner actuated at 5 Hz for ten minutes in a dense passive media doped with 10% of 3μm and 8μm dopant particles. B) Inter-spinner distance trajectory for spinner actuated at 5 Hz for ten minutes in a dense passive media doped with 25% of 3μm and 8μm dopant particles.

What is immediately interesting is that the range of spinner attraction is not dependent on the size of the dopant at either 10% or 25% concentrations. One might imagine that the larger 8μm particle, which is almost twice as large as the 5μm passive particle, might be more difficult to move and induce yielding events. With that same line of reasoning, one might expect that it should be easier for the 3μm dopant particles to yield, which would lead to spinner dimer formation and...
attraction. However, it is clear in Fig. 4-22 that this is not the case as there appears to be just as many dimes forming or not forming in each scenario. What is also clearly noticeable is that the range of the interaction is essentially unaffected at a 10% dopant concentration and is consistent with the 0% dopant committor threshold. However, the range of interaction decreases quite dramatically,
by approximately 5D, when the dopant concentration is increased to 25%. This result is very interesting as it draws a direct analogy with the mechanical behavior that is generally observed in metallic alloy materials, compared to their pure element counter-parts. Generally speaking, as you alloy a material, the strength of the alloy and its resistance to deformation mechanisms (i.e. dislocation motion) is greater than that of its pure counterpart. As you introduce substitutional or interstitial alloy elements into the pure lattice, local compressive or tensile stresses are generated depending on the size of the alloying element. This explains the decrease in the range of interaction as the dopant concentration increases. This new alloyed passive monolayer is more resistant to yielding (via dislocation glide). Thus, it is more difficult for the spinners to erode the bridge and the range of attractive interactions are reduced. This also explains how the range is not dependent on the size of the dopant particle as there is some amount of pre-stress built into the monolayer, regardless of the size, (albeit the stress will be compressive for larger dopants and tensile for smaller dopants) so the resistance to monolayer yielding exists regardless of the size.

The magnetic dipole-dipole attractive range can be reduced, and even eliminated, while preserving this emergent long range attractive interaction by inducing a different mode of motility which we refer to as tops. The top motility is derived from its namesake and is accomplished by using the 3D magnetic coil apparatus. Two sinusoidal signals phase shifted by 90 degrees are sent to the X and Y axis coils with an amplitude of 1. Additionally, a continuous signal with an amplitude of 0.6 was sent to the Z axis coils in order for the resulting field to produce a top-like motion in the active ferromagnetic particles. This amplitude value for the Z axis coils is the lowest value which ensures that the ferromagnetic particles will not form a dimer due to magnetic dipole-dipole interactions. Below this value of 0.6, the tops will still repel up to a point but can eventually form a dimer. We did not choose a larger value because as the amplitude grows in the Z direction, the torque that is applied to the passive monolayer decreases when compared to the spinner case. In fact, we attempted to compensate for this by increasing the rotational frequency of the field in the X and Y
axis to 10Hz for the following series of tests. A dilute concentration of tops, the same concentration as previously described for the spinners embedded in a passive monolayer with a packing fraction of $\phi_A \approx 0.7$ (5μm polystyrene particles), was embedded in a passive monolayer composed of 5μ polystyrene particles with a packing fraction of $\phi_A \approx 0.7$.

![Graph showing committor analysis](image)

Figure 4-25: Committor analysis of tops for binning times of 5, 10, and 20s. Interestingly, the tops repel at distances smaller than approximately 4D but then attract up to distances of 12D.

The tops were actuated for a period of ten minutes and the inter-spinner distance evolution in time can be seen in Fig. 4-24, as well as the committor analysis in Fig. 4-25. Interestingly, the committor analysis shows several distinct regimes for the tops. At distances less than 4D, the tops repel from one another. This is due to the nature in which the ferromagnetic particles are actuated. In the spinners system, it is easy for the magnetic moments of spinners to align and form a dimer. However, the addition of the Z component of the magnetic field to the tops frustrates the alignment of the magnetic moment of two tops and actually causes them to repel at short distances. At distances greater than 4D, the tops will again attract as they still stress and erode the bridge due to the X and Y components of the motion. In fact, the committor threshold range is comparable to the spinner emergent attraction. Then, as anticipated, at larger distances the tops no longer attract as they are unable to stress and erode the bridge. The ability to tune the aggregation behavior and emergent attractive interaction between active units, by changing the composition of the passive monolayer or changing the mode of motility, offers one leverage to investigate vastly different phenomenon particularly when the concentration of active units is increased.

### 4.7 Conclusions

Particle-particle interactions determine the state of a system. Control over the range of such interactions, as well as their magnitude, has been an active area of research for decades due to the fundamental challenges it poses in science and technology. Very recently, effective interactions between active particles have gathered much attention as they can lead to out-of-equilibrium co-
operative states such as flocking. Inspired by nature, where active living cells coexist with lifeless objects and structures, we study the effective interactions that appear in systems composed of active and passive mixtures of colloids. We have developed an artificial non-equilibrium hybrid active passive matter system where two dimensional colloidal monolayers are composed primarily of passive (inactive) colloids and a very small fraction of active (spinning) ferromagnetic colloids. An ultra-long-range attractive interaction between the active components, spinners, emerges when the spinners are actuated in these dense passive mediums. This attractive interaction between spinners depends on the monolayer area fraction and the angular frequency of the rotating magnetic field. The area fraction of the monolayer determines the mechanical properties of the passive matrix, whereas the angular frequency controls the activity of the spinners. We find that the long-range attractive interaction between spinners is mediated by the mechanical properties of the medium and the ability of the active agents to erode the passive medium. This interaction emerges when the passive medium behaves elastically and disappears when the medium behaves as a viscous fluid. In the presence of a solid-like media, the activity of the spinners increases the stress on the system, thereby increasing the range of the interaction between them. We also find a characteristic minimum actuation time scale below which the spinners are unable to stress the monolayer, as the system dissipates energy faster than the rate of energy input. For actuation periods longer than this characteristic time period, the medium is not able to dissipate energy fast enough and the monolayer dissipates the stored energy via yielding events.

The range of the interaction can also be tuned by changing the compositional makeup of the passive 2D colloidal monolayer and by changing the mode of motility. By doping a passive colloidal monolayer with increasing amounts of smaller or larger dopant colloids, one can effectively alloy the passive monolayer. This alloyed passive monolayer is more resistant to the movement of dislocations or other deformation mechanisms when compared to the pure single component passive monolayer. Thus, it is more difficult to yield the alloyed passive monolayer. The emergent attractive interaction is dependent on the ability of the spinners to erode and yield the bridge. If it is more difficult, or the probability of inducing yielding events is reduced, the interaction range of the spinners will decrease. Additionally, if one changes the mode of motility, the formation of dimers at distances less than $4D$ can be eliminated. By inducing a top mode of motility instead of spinner motility, this addition of the $Z$ component in the tops case makes it more difficult (and depending on the amplitude of the $Z$ component impossible) for the ferromagnetic particle magnetic moments to align and form a dimer. However, the tops can still yield the passive monolayer, attract at distances greater than $4D$, and exhibit a similar threshold interaction range as the spinners in similar passive monolayer area fractions. These tunable parameters can be leveraged to investigate and observe different emergent interactions or collective dynamical behavior.

These results have far reaching implications: i) activity in a dense monolayer yields ultra-long range interactions due to elastic stresses. This physical mechanism could be used for communication in synthetic or biological systems without the need for chemical sensing [30], ii) this elastically induced interaction can be modulated and can be applied to many different systems to control...
their out of equilibrium states, iii) this particular phenomenon could offer insights into wear and erosion at the microscopic scale, as the attraction is due to the erosion of the bridge that occurs through avalanches of passive particles that reconfigure the monolayer. Such a mechanism might potentially be important for some biological systems, and can be harnessed for newer developments in synthetic active soft materials.
CHAPTER 5
FORCE REVERSAL BETWEEN ACTIVE SPINNING PARTICLES IN DENSE PASSIVE MEDIA

Work in this chapter was published in:


In the previous Chapter, we developed a hybrid active-inactive experimental soft active matter system which exhibited an emergent ultra-long range interaction between active units when activity was induced. We found the strength and range of this interaction was dependent on the mechanical properties of the passive monolayer, the compositional makeup of the passive monolayer, the activity of the spinners, the actuation protocol, and the mode of activity utilized. Initially, there was a concern that perhaps this long-range interaction could be in part due to the magnetic dipole-dipole interaction between the spinners. We believe this cannot be the case because the magnetic dipole-dipole force decays as $1/R^4$ and the range of this emergent interaction was as large as $20D$ or equivalently $100\mu m$. Additionally, it was also clearly apparent from the trajectories of the inter-spinner distance that there were two regimes with two distinct slopes, which differentiated from magnetic dipole-dipole interaction and the emergent activity induced interaction. However, we are still interested in showing that this emergent attractive interaction is general and depends on the parameters previously described and not on the magnetic nature of our spinners.

To prove this experimentally is possible but very difficult. One could utilize an optical tweezer set-up to induce activity and measure the attractive interaction. Since these active particles do not interact, this could prove the ubiquitous nature of the interaction. Phoretic or Quincke particles could also be utilized as these particles are non-interacting as well, but then the extension of the system to biological systems of interest would be severely limited. It should also be noted that developing these systems were beyond the scope of our experimental expertise. However, another way to verify that this emergent interaction is not due to magnetic dipole-dipole interactions is via simulation and theory. To gain a deeper insight into the nature of this non-equilibrium emergent long range interaction in the absence of magnetic interactions, we carried out numerical simulations using hybrid molecular dynamic simulations of the colloidal particles coupled to a Lattice-Boltzmann fluid.

The self-organization of active particles is governed by their dynamic effective interactions. Such interactions are controlled by the medium in which such active agents reside. Here, we study
the interactions between active agents in a dense non-active medium. Our system consists of non-magnetic spinning (active) particles embedded in a dense monolayer of passive (non-active) particles. We demonstrate that the presence of the passive monolayer dramatically alters the properties of the system and results in a reversal of the forces between active spinning particles from repulsive to attractive. The origin of such reversal is due to the coupling between the active stresses and elasticity of the passive monolayer. This discovery provides a new mechanism for the interaction between active agents in complex and structured media, opening up new opportunities to tune the interaction range and directionality via the mechanical properties of the medium.

5.1 Active spinning particles in complex passive media

Life occurs out of equilibrium. Living organisms are continuously generating and consuming energy to achieve self-generated motion. In addition, equilibrium conditions are rarely found in nature or industrial material processing. Thus, active systems have attracted much attention in recent years. These systems exhibit exotic behaviors not possible under equilibrium constraints such as emergent collective motion \cite{188, 189}, pattern formation \cite{190, 191, 192}, or even phase segregation in the absence of attractive interactions \cite{193, 194, 195, 196}. The most studied active agents are those which convert some sort of energy into rotational motion. Such systems resemble how bacteria swim and are known as self-propelled agents \cite{193, 194, 197, 198}. Energy conversion into rotational motion is also common in nature; important examples are the motor adenosine triphosphate synthase \cite{199}, certain cilia \cite{200}, the vortex array formation of sperm cells \cite{190}, and the dancing Volvox \cite{201}. Furthermore, experiments and several numerical and theoretical studies have focused on this type of active system in viscous media \cite{202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212}.

In addition to the type of activity, the medium can have a tremendous influence on the effective interaction between different particles which can be particularly important for active systems. Mixtures of active and passive particles can be used as a model system where active particles are embedded in a complex passive system. This scenario is prevalent in many biological systems or processes such as, bacterial biofilms where live and dead bacteria phase segregate \cite{213, 214}, cell migration through tissues \cite{215, 216}, and sperm swimming through the viscoelastic cervical mucus \cite{217}. Although these systems are ubiquitous, very few studies have investigated these hybrid active-passive matter systems \cite{189, 218, 219, 220, 221, 222, 223}. McCandlish et al. reported phase segregation of active rods in the presence of passive rods where they point to a dynamical instability as the origin of activity-induced phase segregation. This instability originates from the differential parallel and transversal diffusion coefficients originating from the anisotropy of the rods \cite{218}. Ni et al. focused on the behavior of a passive particle suspension in a glassy state doped with active agents. They observed that the presence of active particles shift the glass transition toward higher packing fractions \cite{219} and promote the crystallization of hard-sphere glasses \cite{224}. Stenhammar et al. showed that mixtures of self-propelled and passive particles phase separate into a dense and
a dilute phase [221], between which the interfacial tension is negative [225]. However, despite all these efforts, the origin of the emergent interactions between active agents in mixtures with passive agents remains unclear.

5.2 Lattice-Boltzmann simulation spinner system

To shed light on the emergent interactions that govern the self-organization of non-Brownian active rotating particles (henceforth referred to as spinners) in systems composed of mixtures of active and passive particles, we use both experiments and simulations. We focus on the behavior of pairs of co-rotating and counter-rotating spinners suspended in a viscous fluid or embedded in dense monolayers of passive particles. Importantly, we show a force reversal between spinners as the concentration of passive particles increases above a critical threshold. In particular, we observe that in a viscous fluid at small but finite Reynold numbers (Re), the fluid flows generated by co-rotating spinners produce a repulsion between spinners (Fig. 5-1A). Whereas, for counter-rotating spinners, the resulting forces are attractive (5-1B). By contrast, two co-rotating spinners in a dense passive monolayer attract each other (5-1C), whereas counter-rotating spinners repel (5-1D). We demonstrate that this force reversal is induced by the change in the mechanical properties of the matrix from a viscous medium, if suspended in the fluid, to a solid-like viscoelastic medium in the presence of passive particles. We anticipate that this mechanical attraction between co-rotating spinners is responsible for the phase separation between active and passive particles in macroscopic systems.

Our experimental system is composed of spherical ferromagnetic particles of diameter $\sigma$, coupled to an external rotating magnetic field of frequency $\omega$, as described in the previous Chapter. For the experimental conditions, the rotational frequency of the particles always coincides with the rotational frequency of the field. Spinners suspended in an incompressible fluid ($\nabla \cdot u = 0$) of viscosity $\eta$ and density $\rho$ generate fluid flows that can be described by the Navier-Stokes equation,

$$Re \, (u \cdot \nabla) = -\nabla p + \nabla^2 u + f$$

(5.1)

where we have assumed no-slip boundary conditions at the particle surface $u = U + \omega \times r$. $U$ being the translational velocity of the particle, $\omega$ the angular velocity, and $r$ the vector pointing from the center of mass of the particle to the surface of the particle. In Eq. (5.1), we have chosen a translating reference frame at the center of the spinner (i.e. such as the flow is steady) and scaled the velocities and lengths by $\omega \sigma$ and $\sigma$, respectively. $u$ corresponds to the fluid velocity field, $Re$ is the Reynolds number ($Re = \omega \sigma^2 \rho / \eta$), $p$ is the pressure, and $f$ is the force density exerted by the particle on the fluid. Therefore, in the absence of any other particle and in the limit of $Re = 0$ (i.e. where the left hand terms in Eq. (5.1) are 0), a rotating spherical particle generates a velocity field given by [226]
Figure 5-1: A) Fluid flows generated by two co-rotating spinners in a viscous fluid medium at finite Re results in spinner-spinner repulsion, as shown by the black arrows which indicate the direction of forces exerted by the medium on the spinners. The tangential components ($\vec{t}$) come from the fluid flows generated by the neighboring spinner, whereas the normal components ($\vec{n}$) come from secondary flows. The resultant force generates trajectories where both spinners rotate around their center of mass while moving apart. B) Fluid flows generated by two counter-rotating spinners at finite Re result in attraction. C) Two co-rotating (red spheres) spinners rotating at frequencies $\omega$ in a dense monolayer of passive particles (blue spheres) attract. The effective forces exerted on the spinners by the passive medium are represented by black arrows. D) Two counter-rotating spinners in a dense monolayer of passive particles repel (forces in black).

$$u(r) = \frac{\tau}{8\pi \eta r^3} \hat{\phi} \times \mathbf{r} \tag{5.2}$$

where $\mathbf{r}$ is the position of the fluid from the center of the particle, $\tau$ is the torque acting on the particle, and $\frac{\tau}{8\pi \eta r^3}$ corresponds to the angular rotational frequency ($\omega$) of the spinner which is constant in our system. This rotating field decays as $1/r^2$ from the center of the spinner as shown in Figs. 5-2, 5-3, and 5-4.

A spinner suspended in a viscous fluid and rotating around the z-axis at a frequency $\omega$ generates a rotating fluid flow which velocity field is given by Eq. (5.2) at $\text{Re} = 0$. In spherical polar coordinates ($r, \theta, \phi$), the azimuthal fluid velocity decays as $\frac{1}{r^2}$ as shown in Fig. 5-2A. This fluid velocity contribution is the only contribution at $\text{Re} = 0$ and is independent of the Re. However, at small but finite Re an additional polar contribution to the fluid velocity appears originated by inertial effects, so-called secondary flow, and which therefore depends on the Re, as demonstrated in Fig. 5-2B. This described scenario corresponds to spinners suspended on a fluid and far from a wall.
The presence of a wall modifies such a scenario as the fluid velocity (azimuthal and polar) decays faster than in the absence of walls \((\frac{1}{\sqrt{r}})\) as shown in Fig. 5-3A and B. This type of confinement effect has been previously described [227].

The presence of a wall breaks the symmetry of the secondary flows generated by the spinner rotation and also reduces the magnitude of the secondary flow, which translates into a depletion of the inertial effects for spinners close to a wall. In Fig. 5-4, the profiles of the azimuthal and polar fluid velocities are presented as a function of the channel height for a spinner rotating at \(\text{Re}=0.6\). The azimuthal fluid velocity component is scarcely affected by the channel size. On the contrary,
the strength of the secondary flow is significantly reduced by narrowing the channel which results in a shorter repulsion distance for smaller channels.

Figure 5-4: A) Profile of the azimuthal velocity in the equatorial plane of a spinner at Re = 0.6 in a channel of height 30Ax (black circles), 50Ax (red squares), and 100Ax (blue diamonds). The black solid line corresponds to the solution of the Eq. (2) in the main text. B) Profile of the polar velocity in the equatorial plane of spinners at Re = 0.6 in a channel of height 30Ax (black circles), 50Ax (red squares), and 100Ax (blue diamonds).

In these simulations, the simulation box was discretized in three dimensional grids with resolution \( N_x \times N_y \times N_z = 220 \times 220 \times 200 \) and periodic boundary conditions. For the simulations in the absence of walls or \( N_x \times N_y \times N_z = 220 \times 220 \times 30 \) bounded in the z direction by no-slip walls and periodic boundary conditions in the x and y directions for the simulations in the presence of a channel. The rest of the simulations were carried out utilizing the following simulation parameters unless otherwise noted. The simulation box is discretized in three-dimensional grids with resolution \( N_x \times N_y \times N_z = 214 \times 214 \times 30 \), bounded in the z direction by no-slip walls and periodic boundary conditions in the x and y directions. The Lattice-Boltzmann (LB) fluid is described by the fluctuating Lattice-Boltzmann equation [178] which properly describes the dissipative and fluctuating hydrodynamic interactions. We implement the discrete 19-velocity model (D3Q19). The LB fluid parameters are density \( \rho = 1 \), kinematic viscosity \( \nu = 1/6 \), and temperature \( k_B T = 2 \cdot 10^{-5} \). For simplicity, we set the grid spacing \( \Delta x \) and the LB time step \( \Delta t \) equal to unity. Interactions between the LB fluid and the particles are described by the bounce-back rule [228] and enforcing no-slip boundary conditions at the surface of the particles. Specifically, we implement the ALD method [229] where particles are treated as real solid objects. Therefore, we do not take into account lubrication forces. However, we found that including lubrication forces only serves to shift the behavior observed here towards smaller Re numbers (i.e. smaller rotational frequencies). In our simulation model, colloidal particles are considered as hard-spheres [179] of diameter \( \sigma = 12\Delta x \). Thus, we are only considering excluded volume interactions. The spinner activity is generated by imposing an external torque, \( \tau \), about the z-axis. To form the monolayer, we also include a gravity force \( F_G = 0.005 \). Since we are interested in the hydrodynamic interactions that occur between
spinners and not their magnetic interaction, we do not include dipole-dipole interactions in our simulation model to more clearly determine the origin of the effective interactions. The typical simulation length is of about $0.2\tau_B$, where $\tau_B$ is the characteristic Brownian time.

5.3 Co-rotating spinners repel and counter-rotating spinners attract in an incompressible fluid

First, we study the effective interaction between two spinners in a dilute system of co-rotating spinners in the absence of passive particles. In this system, the interaction between spinners is controlled by the fluid flows generated by the rotation of the spinners and the magnetic dipole-dipole interaction between them. Experimentally, we observe that spinners initially positioned further than four particle diameters do not feel either the fluid flow created by other spinners or the permanent dipole of the other spinners. Therefore, they rotate in place without experiencing any translation as shown in Fig. 5-5.

![Figure 5-5: Time evolution of the distance between two co-rotating, $r_{ij}$, in the experiments at 5 Hz ($Re = 1.25 \times 10^{-3}$) (blue lines) and 50 Hz ($Re = 1.25 \times 10^{-2}$) (blue circles), in our simulation model (green lines) at $Re = 0.84$ and between two counter-rotating spinners (red lines) using simulations at $Re = 0.84$. The dashed line at $4\sigma$ points out the experimental interaction threshold between spinners suspended in an incompressible fluid. The spinners are on the bottom wall of a channel of height $h = 30 \Delta x$.]

By contrast, spinners closer than $4\sigma$ attract due to the magnetic dipole-dipole interaction, thereby forming a doublet and rotating around its center of mass. To get a deeper insight into...
the behavior of the system, we also perform hybrid molecular dynamic simulations of the spinners sedimented onto a wall within a channel of height $h = 30 \Delta x$ and coupled to a Lattice-Boltzmann fluid [177]. These simulations, which lack the dipole-dipole interaction, show that co-rotating spinners closer than $3\sigma$ experience a hydrodynamic repulsion while rotating around the center of mass of the repulsive pair. We hypothesize that in our experiments, the hydrodynamic repulsion is hidden by the strong dipole-dipole interaction between ferromagnetic particles. To test this, we increase the rotation frequency of the applied magnetic field up to 50 Hz. At this frequency, the hydrodynamic repulsion overcomes the dipole-dipole attraction and spinners separate up to $3\sigma$ (blue circles in Fig. 5-5). The fact that the hydrodynamic repulsion increases with Re proves the inertial nature of the interaction [202] and is in good agreement with previous observations on millimeter-sized rotating magnetic disks adsorbed at the air-water interface [202, 203]. Although our experimental setup does not provide us with control over the direction of rotation of individual spinners, our simulations allow us to explore the case of spinners rotating on opposite directions. In this case, we find that two counter-rotating spinners closer than $3\sigma$ attract each other until the separation distance between them becomes about $1.15\sigma$ (see Fig. 5-6) and then simultaneously translates as a doublet in the direction orthogonal to the vector joining both centers [230].

![Figure 5-6: A) Distance between counter-rotating spinners as a function of Re. B) Translational velocity of the center of mass of the counter-rotating pair along the direction orthogonal to the vector joining both centers at $Re=0.18$ (black lines), 0.46 (red lines), and 0.84 (blue lines).](image)

When suspended in a viscous fluid, counter-rotating spinners separated by $2\sigma$ or less show an effective attraction, thereby forming a doublet that moves along the direction orthogonal to the vector joining both centers [230]. The equilibrium distance between both spinners is independent on Re as shown in Fig. 5-6A. However, the strength of the interaction given by the slope of the
attraction trajectory and the translational velocity of the doublet does, as seen in Fig. 5-6B. This illustrates that the interaction between counter-rotating spinners suspended in a viscous fluid is of an inertial nature.

Inertial contributions to the fluid velocity field, left hand terms in Eq. (5.1), are the origin of the repulsion between co-rotating spinners [231, 204] and the attraction between counter-rotating spinners. At a finite Re, inertial terms generate additional forces on the particles due to the momentum of the fluid. These type of forces, known as lift forces, originate from the relative translation of a rotating particle with respect to the fluid [232], known as Magnus forces, or by the translation of the rotating particle with a shear flow [233]. Both lift forces depend on the translational velocity of the rotating particle. Under these conditions, the fluid velocity profile generated by a rotating sphere, Eq. (5.2), needs to be corrected to include these inertial terms which generate a so-called secondary flow. Perturbation methods have been used to calculate the secondary flow around a rotating sphere due to small inertial effects [181]. These studies have shown that the secondary flow produces no correction in the azimuthal part of the fluid velocity profile (Eq. (5.2)). However, because of the centrifugal force effect, the fluid is pulled in toward the poles and expelled from the equator which generates a secondary flow on the zx-plane. The presence of a second rotating sphere breaks the symmetry of the secondary flow. Around the equator of the spheres the fluid velocity between the spinners decreases for co-rotating spinners and increases for counter-rotating spinners as shown in Fig. 5-7.

We compute the forces exerted by the fluid on co- and counter-rotating spinner pairs along trajectories of repulsion and attraction as shown in Fig. 5-7C and D, respectively. For two co-rotating spinners, hydrodynamic forces generate a net repulsion between them while a hydrodynamic attraction is generated for the case of counter-rotating spinners. The fluid flows generated by two co-rotating spinners cause the spinners to rotate around their center of mass. This translation of the spinners generates a lift force that results in a repulsion of co-rotating spinners [204, 205]. Previous studies have shown that both co-rotating and counter-rotating spinners repel each other as a consequence of the lift forces [231]. However, in the presence of a channel the hydrodynamic attraction between two counter-rotating spinners overcomes the lift force, resulting in an effective attraction [212]. As discussed below, the equilibrium distance between counter-rotating spinners does not depend on the Re number (Fig. 5-6); however, it does on the channel height (Fig. 5-8). Decreasing the height of the channel leads to a reduction of the hydrodynamic repulsion between co-rotating spinners due to the reduction of the lift forces exerted on the spinners. The presence of a channel weakens the strength of the secondary flow, such that it is reduced by shortening the channel height. In the case of counter-rotating spinners, this depletion of the lift forces leads to the switching of the interaction from repulsive to attractive for channel heights smaller than h=50. Thus, the confinement of the counter-rotating pair reduces the strength of the lift forces, making the hydrodynamic attraction dominant. Similarly, the confinement of co-rotating spinners pairs results in a shorter repulsion distance (Fig. 5-8) because of the reduction of the lift force magnitude [212].
Figure 5-7: Polar flow fields generated by two co-rotating spinners (A) and by two counter-rotating spinners (B) that have sedimented onto a wall within a channel of $h = 30 \Delta x$. Hydrodynamic forces acting on each spinner as a function of the distance between them for two co- (C) and counter-rotating spinners (D). Solid lines are fits to the data with the function $a_0/x^3$.

5.4 Force reversal mediated by mechanical properties of passive media: co-rotating spinners attract and counter-rotating spinners repel in dense passive media

The behavior of spinners embedded in monolayers of passive particles is completely different. At finite Re, the fluid flow generated by a spinner repels neighboring passive particles and generates the rotation of the first shell of particles around it. The distance of this first shell of passive particles with respect to the spinner depends on the area fraction of the monolayer, $\phi_A$, and the angular rotational frequency of the spinner, $\omega$. Therefore, the spinner produces a local increase of the mobility of neighboring passive particles and compresses the monolayer. When more than one spinner are present in the monolayer, we observe that two co-rotating spinners attract each other. This behavior is opposite to that observed in the absence of passive particles as shown in both experiments and simulations in Fig. 5-9. The experimental trajectories of two co-rotating spinners in a monolayer with an area fraction of $\phi_A = 0.7 \pm 0.1$ show two well differentiated regimes. If the distance between the spinners is smaller than $4\sigma$, the slope of the trajectory is sharp. This indicates that the attraction between spinners in this regime is governed by the strong magnetic dipole-dipole interaction. At distances larger than $4\sigma$, the slope is small, which indicates that the
attraction between spinners must be of a different nature. By contrast, in the simulations, the trajectories exhibit a single regime of slow attraction due to the lack of dipole-dipole interactions in our model. Furthermore, once the spinners squeeze out all the passive particles initially positioned between them, they remain as a doublet at a distance of about $2\sigma$ for monolayers of $\phi_A = 0.8$. We can only study co-rotating spinners in our experiments, a limitation absent in our simulations. Thus, using simulations we find that two counter-rotating spinners repel each other at a distance of about $5\sigma$ within a monolayer of $\phi_A = 0.8$. Therefore, we also observe a reversal of the interaction force between two counter-rotating spinners with respect to the pure viscous media in the presence of a passive matrix.

To investigate the nature of the interaction between two spinners in the presence of the passive matrix, we define four different regions in the system and label the particles within these regions accordingly. These four regions are: i) the first shell of particles around the spinners, named as corona, ii) the region between the two spinners, referred as bridge, iii) the region besides the spinners on the opposite side of the bridge, denoted as the surroundings, and iv) the bulk, as illustrated in Figs. 5-10A and B. Particles located in the corona rotate coherently around the spinners and collide against neighboring particles, transferring their momentum. These particles rarely escape from this region and the number of particles in the corona remains almost constant until the coronas of the two spinners starts to collide with each other. Therefore, we count these particles as a part of the spinner for every calculation (green shade region in Figs. 5-10A and B). The bridge and the surroundings are very dynamic; particles in these regions are in continuous motion. The stresses
Figure 5-9: Time evolution of the distance between two co-rotating spinners in the experiments at $Re = 1.25 \times 10^{-3}$ (blue lines), in our simulation model at $Re = 0.84$ (green lines), and between two counter-rotating spinners (red lines) at $Re = 0.84$ using simulations. In the simulations, the monolayer area fraction is $\phi_A = 0.8$, whereas in the experiments is of about $\phi_A = 0.7 \pm 0.1$. The dashed line at 4σ points out the experimental interaction threshold between spinners suspended in an incompressible fluid. The spinners are on the bottom wall of a channel of height $h = 30 \Delta x$.

generated by the spinners through the corona are released in these regions. In order to relax the stresses, they need to yield.

To study the evolution of the passive particles in the bridge, surroundings, and bulk decoupled from the spinners rearrangement, we perform numerical simulations freezing the distance between the spinners at different values. These constrained systems are just able to relax the stress coming from the activity of the spinners through the displacement of passive particles and thus, they never reach a steady state. However, the initial time evolution of these systems allows us to study the process of loading and yielding of the monolayer without the relaxation of the system through the displacement of the spinners. In Figs. 5-10C and D, the initial time evolution of the particle area fraction in the different defined regions are presented for co-rotating and counter-rotating spinners separated 6σ and 4σ, respectively. We observe that for co-rotating spinners, the $\phi_A$ of particles in the bridge is significantly reduced as compared to the $\phi_A$ in the surroundings and bulk as shown in Figs. 5-10C and 5-11.

The compression and shear stresses produced by co-rotating spinners in the bridge, through the corona, result in a density reduction within this region as it is constantly yielding due to shear stresses induced by the spinners. Thus, the higher mobility of passive particles initially located in this region allows them to migrate to less stressed regions. On the contrary, for counter-rotating spinners, the density of passive particles in both the bridge and surroundings is similar as presented
Figure 5-10: A) and B) Illustration showing the forces on the system and the different regions we define in the system: i) The corona (green shade) which includes the spinner (red sphere) and the particles around it (grey spheres), ii) the bridge, particles located between the spinners (yellow spheres), iv) the surroundings, particles located besides the spinners on the opposite side of the bridge (purple spheres), and iv) the bulk (blue spheres). C) Time evolution of the particle area fraction of the bridge (black line), the surroundings (red line), and the bulk (blue line) for co-rotating spinners separated by \(6\sigma\). D) Time evolution of the particle area fraction of the bridge (black line), the surroundings (red line), and the bulk (blue line) for counter-rotating spinners separated by \(6\sigma\). The position of the spinners is frozen.

in Figs. 5-11A and B. Figs. 5-11A and B are the counterparts of Figs. 5-10C and D in which the position of the spinners is left free to evolve. The compression and shear stresses produced by the spinners in the bridge, through the corona, result in a \(\phi_A\) reduction within this region as it is constantly yielding due to shear stresses induced by the spinners. Thus, the higher mobility of passive particles initially located in this region allows them to migrate to less stressed regions. On the contrary, for counter-rotating spinners, the \(\phi_A\) of passive particles in the bridge is significantly increased compared to the bulk and surroundings as shown in Figs. 5-10D and 5-11. Therefore, the mechanism by which the passive matrix mediates the interaction between spinners is related to the type of stresses that the spinners exert on their surroundings. Co-rotating spinners compress and shear the bridge as schematically illustrated in Fig. 5-10A. To alleviate the stress, the system prefers to yield by transporting particles from the bridge into the other regions. This occurs through avalanches and single particle hopping, as will be shown later. Clearly, this migration reduces the density on the bridge. This imbalance repositions the spinners closer to each other, thereby restoring the temporal mechanical equilibrium. This process is continuously occurring which slowly degrades the bridge until the active particles are able to come together.
Figure 5-11: A) Time evolution of the distance between two co-rotating spinners embedded in a passive monolayer of $\phi = 0.8$ using numerical simulations at Re = 0.84. Inset: time evolution of the particle area fraction of the bridge (black line), the surroundings (red line), and the bulk (blue line). B) Time evolution of the distance between two counter-rotating spinners embedded in a passive monolayer of $\phi = 0.8$ using numerical simulations at Re = 0.84. Inset: time evolution of the particle area fraction of the bridge (black line), the surroundings (red line), and the bulk (blue line).

5.5 Spinner annealing of passive monolayer

During this process the monolayer is annealed, inducing the defects to migrate and concentrate around the spinners as depicted in Fig. 5-12.

Figure 5-12: Average order, quantified by $Q_6$, of two passive monolayers at $\phi = 0.8$: i) in absence of spinners (red line) and ii) in the presence of two spinners (black line) at Re = 0.84.

To probe the annealing of the passive monolayer induced by spinner activity, we quantify the
degree of order by calculating for each passive particle the 2D local bond-orientational order parameter $Q_6$. Bond-orientational parameters were introduced to measure the local structure around a particle [234, 235]. Bonds are defined as the vector joining a pair of neighboring particles, $r_{ij}$, where we define neighboring particles as those which are at a shorter distance than the first minimum of the RDF. Once we have identified the neighbors of each particle, we define a $(2l + 1)$ dimensional complex vector for each particle with the components,

$$q_{lm}(i) = \frac{1}{N_b(i)} \sum_{j=1}^{N_b(i)} Y_{lm}(r_{ij})$$

(5.3)

where $N_b(i)$ corresponds to the number of neighboring particles of particle $i$. $Y_{lm}(r_{ij})$ are spherical harmonics evaluated for the direction of the bond, determined by the azimuthal angle $\phi_{ij}$. To make the order parameters invariant with respect to rotations of the reference frame, the second-order invariants are defined as

$$q_l(i) = \left( \frac{4\pi}{2l + 1} \sum_{m=-l}^{l} |q_{lm}(i)|^2 \right)^{1/2}$$

(5.4)

The global order parameter is obtained by averaging over all bonds in the system:

$$Q_l = \left( \frac{1}{N_b} \sum_{j=1}^{N_b} e^{i\phi_{ij}} \right)$$

(5.5)

The bond-orientational order parameter $Q_6$, averaged over all bonds, is used to monitor the global structural changes of the system as shown in Fig. 5-12. In a fully disordered state, i.e. fluid, we will observe vanishing values of the bond order parameters. By contrast, the more ordered the system, the higher the value of the global bond order parameter. Therefore, the presence of the spinners promotes the ordering of the passive monolayer.

We discard the migration and coalescence of defects as the driving force for the co-rotating spinners attraction exhibited in dense passive monolayers by performing simulations in which the initial configuration of the monolayer was a perfect hexagonal close packing lattice (hcp). We monitored the time evolution of the distance between two co-rotating spinners in a perfect hcp lattice and observed that they come close together, even in the absence of defects, as a result of the effective attraction induced by the passive monolayer as shown in Fig. 5-13. By contrast, counter-rotating spinners produce compression and dilation stresses in the bridge. Both spinners move passive particles into the bridge, which increases the pressure in this region. This pushes both spinners away, thereby resulting in a repulsion between counter-rotating spinners.
Figure 5-13: Time evolution of the distance between two co-rotating spinners at Re = 0.84 using as initial configuration a perfect hexagonal lattice at $\phi = 0.8$.

Figure 5-14: A) Work done by the springs as a function of the separation distance between two co-rotating spinners. The black circles represent the average PMF over five independent initial configurations of the spinner within the monolayer (red squares, green diamonds, blue up triangles, orange left triangles and violet down triangles). The magenta crosses represent the PMF in the case of spinners suspended in a viscous fluid (i.e. in the absence of passive particles). B) PMF as a function of the separation distance between two counter-rotating spinners. The black circles represent the average work over three independent initial configurations of the spinner within the monolayer (red squares, green diamonds, blue up triangles). The magenta crosses represent the PMF in the case of spinners in a viscous fluid.

5.6 Calculating the potential of mean force between spinner pairs

To further investigate this emergent interaction between spinners embedded in passive matrixes, we estimate the mean spinner-spinner interaction potential in monolayers of $\phi_A = 0.8$ as shown in Fig.
Figure 5-15: Simulated time evolution of the distance between two co-rotating (A) and two counter-rotating (B) spinners at $Re = 0.84$ for spinners initially positioned at different distances. The monolayer area fraction is $\phi = 0.8$.

5-14. By means of the application of harmonic springs, $F = k(r - r_0)$, to restrain the separation distance between the spinner pair at $r_0$, we calculate a potential of mean force (PMF) between co- and counter-rotating spinners as presented in Figs. 5-14A and B, respectively. The mean interaction potential (black circles) is obtained by averaging over 5 independent initial configurations at each separation distance $r_0$. The emergent interaction between two co-rotating spinners within a passive monolayer is repulsive at distances smaller than $2\sigma$. At larger distances, shallow attractive minima are separated by small energy barriers as shown in Fig.5-14A. The local energy input coming from the spinners' activity is enough to overcome these small energy barriers. The presence of these energy barriers at distances larger than $2\sigma$ agree with the observation that the loading of the bridge plays a key role for this interaction. Moreover, the shallow minima would then correspond with the state of the system after a yielding event. In the case of counter-rotating spinners, the interaction is repulsive with a strong repulsive peak at $1.5\sigma$. It also presents a small energy minimum at $3\sigma$ as can be seen in Fig. 5-14B. This shallow energy minimum explains that along the repulsive trajectories, the spinners spend long times at this distance as it can be seen in Fig. 5-15B.

We also use this methodology to evaluate the interaction potential between co- and counter-rotating spinners in the absence of passive particles, represented by the magenta crosses in Figs. 5-14A and B, respectively. In agreement with our previous calculations, Fig. 5-14 clearly shows that the change in the mechanical properties of the media produces not only a reversal of the forces acting on the spinners but also significantly increases the range of the interaction. Interestingly, the PMF at the different separation distances strongly depends on the initial configuration of the monolayer as shown in Fig. 5-14. The average standard deviation between the different initial configurations is about 10%. Therefore, in spite of the general trend captured by these mean spinner-spinner interaction potentials, this interaction is of a stochastic nature. Thus, the strength and range of the interaction between spinners in passive matrices is determined by the instantaneous and previous
configurations of the monolayers (i.e. memory effects). Another proof of this is shown in Fig. 5-16 where we compute the attractive force between the spinners for 100 configurations selected from the unconstrained trajectory presented in Fig. 5-9. The spinners were initially positioned 6σ apart as shown in Fig. 5-16A.

![Figure 5-16: A) Trajectory of attraction between two co-rotating spinners embedded in passive monolayer of φ = 0.8. The red squares correspond to the selected configurations used to compute the force using harmonic springs. B) Spring forces as a function of the separation distance between co-rotating spinners. The black circles represents the average force over five independent initial configurations, while the small red squares correspond to the spring forces measured for the selected configurations taken from the trajectory. The big red squares represent the average over the selected configurations as a function of the distance.](image)

The different configurations present significantly different values of the force as can be seen in Fig.5-16B. However, the average force as a function of the separation distance between the spinners is in good agreement with the one estimated by averaging over five independent configurations at each separation distance. Again, we evaluated the interaction potential between a co- and counter-rotating spinner pair embedded in a passive monolayers of φ_A = 0.8 by restraining the distance between them by means of harmonic potentials, F = k(τ - r_0), at different separation distances r_0. From a disordered monolayer of φ_A = 0.8, we randomly selected a pair of particles separated by a distances r_0 and labeled them as spinners. We chose five independent spinner pairs at each separation distance r_0 in the range of 1σ to 6σ at intervals of 0.5σ. We allowed the system to relax and then sampled over 8000 configurations and calculated the spring force as a function of the distance displacements. Then, by performing the Riemann integral of that curve, we computed the potential of mean force (PMF), W, at each separation distance r_0. The mean spinner-spinner interaction potential was then obtained by averaging over the five independent configurations at each separation distance as shown in Fig. 5-14. Therefore, if the PMF is greater than 0, the interaction due to the media between the spinners is repulsive. If it is negative, the spinner-spinner interaction is attractive.

The force exerted on the spinners by the media or spinner-spinner interaction potential strongly
depends on the instantaneous configuration of the monolayer. This is intimately related with the fact that the spinners need to load the bridge before it yields which results in the approaching of co-rotating spinners. The amount of stress needed to load the bridge above that threshold obviously depends on the bridge/monolayer configuration. To prove this, we compute the spring forces as a function of the separation distance between co-rotating spinners for 100 configurations taken from the simulation trajectory of co-rotating spinners initially separated by a distance of 6σ presented in Fig. 5-9 as shown in Fig. 5-16A. One can clearly see that the forces acting on the spinners, and thus, the force exerted by the spring on the spinners strongly depends on the configuration of the monolayer. Interestingly, by averaging the spring forces as a function of the separation distance between the spinners (red line in Fig. 5-16B), we observe that the forces acting on the spinners follow a similar trend as the ones computed by averaging over five independent initial configurations of the monolayer (black line in Fig. 5-16B) as shown in Fig. 5-16B. Although we cannot predict the exact interaction between two spinners separated by a certain distance because the exact strength of the interaction depends on the instantaneous configuration of the system and not on the separation distance, we can confidently say that the interaction between two co-rotating spinners is attractive.

Figure 5-17: A) Experimental and B) Simulation trajectories of the time evolution of the distance between co-rotating spinners in passive monolayers of about φA =0.7 and φA = 0.8, respectively.

As previously mentioned, the emergent spinner-spinner interaction in the presence of a passive matrix is of a stochastic nature. To show this more clearly, we have computed the work done by co-rotating spinners on the fluid, with which they are in contact, along some experimental and simulation trajectories that exhibit spinner-spinner attraction such as the ones shown in Fig. 5-17. Note that the spinners are typically only in contact with the fluid and they transmit all the stresses through it. From those trajectories, we computed the approaching velocity of the spinners (U) along their reaction coordinate which is the line connecting them. Assuming a Stoke's scenario, the work can be calculated by multiplying the force exerted by spinner translation on the surrounding fluid (F = γU). From the force vs distance curve, we computed the work done by the spinners on
the fluid, or vice versa the work done by the fluid on the spinners. This only depends on the sign of the forces. As can be seen in Fig. 5-17, different trajectories of co-rotating spinners initially positioned at similar distances show different amounts of dissipation (work done by the spinners on the fluid). This proves the stochastic nature of this interaction and reflects its dependence on the monolayer configuration. In fact, if an emergent interaction potential between the spinners embedded in passive monolayers did in fact exist, then this interaction would be deterministic and the work done by the spinners on the surrounding fluid would be the same for all the trajectories starting at point A and ending up at point B. The non-equilibrium nature of this system makes that the spinner-spinner interaction within passive monolayers an activated process and thus, stochastic. As already explained, the spinners need to build up stresses on the bridge before it yields, which obviously depends on the instantaneous configuration of the bridge/monolayer. We would like to point out that the direction of the interaction is not determined for a given distance. For example, in Fig. 5-14A at \( r_{ij} = 4.5\sigma \), the interaction can be either attractive or repulsive depending on the configuration (i.e. seed). Thus, for any spinner pair, it is not possible to predict the exact local evolution but only that they will attract or repel over the length of the experiment.

5.7 Quantifying mechanical properties of the passive monolayer

We have also found in both experiments and simulations that the aggregation process of co-rotating spinners embedded in a monolayer is governed by the elasticity of the medium and the ability of the spinners to increase the elastic energy of the system. This can be directly confirmed by measuring the storage and loss moduli of the system in the absence of active particles, and observing at what particle area fraction the attractive interaction between co-rotating spinners is lost. We measured the time evolution of the mean square displacement, \( \text{MSD} \), of the passive particles in monolayers at different area packing fractions. The \( \text{MSD} \) contains information about the mechanical properties of the material; in pure viscous materials the \( \text{MSD} \) varies linearly with time, where the slope of the curve is determined by the diffusion coefficient, \( D \). However, in solid-like materials, the \( \text{MSD} \) reaches a characteristic plateau which is related to the elastic energy of the system [236, 237, 238]. We observe that for high packing fractions, the \( \text{MSD} \) deviates from the linear behavior (see Fig. 5-18) which indicates the solid-like character of the hard-sphere monolayer at packing fractions above 0.6 [239]. By contrast, in the absence of passive particles, the system behaves as a viscous fluid.

From the time dependent mean square displacement, \( \langle \Delta r^2(t) \rangle \), we obtain \( G^*(\omega) \) from a frequency dependent form of the Stokes-Einstein equation. Although this relationship breaks for glass, in which hopping occurs [240], we have checked that this type of event does not take place during the simulated trajectories from which we computed the \( \text{MSD} \). Hence, we can assume that each passive particle of the monolayer feels an isotropic, incompressible continuum and the viscoelastic spectrum of the medium. Therefore, computing the \( \text{MSD} \) of the probe particle captures the average displacement of all the particles in the system. The complex shear modulus is then
computed using an algebraic form of the generalized Stokes-Einstein equation [241, 242, 243],

\[
G^*(\omega) = \frac{k_B T}{\pi a \langle \Delta r^2(1/\omega) \rangle \Gamma \left[ 1 + (\partial \ln \langle \Delta r^2(1/\omega) \rangle / \partial (1/\omega)) \right] \left[ 1 + (\partial^2 \ln \langle \Delta r^2(1/\omega) \rangle / \partial (1/\omega)^2) \right]} (5.6)
\]

where \( a \) is the particle radii, \( \Gamma \) is the gamma function, and \( \omega \) is the frequency. First, the MSD data is fitted to a second-order polynomial function from which the first and second time derivatives of the MSD are computed, and then \( G^*(\omega) \) is computed following Eq. 5.6. The storage and loss moduli are obtained by fitting \( G^*(\omega) \) to a power-law and solving the following equations,

\[
G'(\omega) = G^*(\omega) \left( \frac{1}{1 + \beta(\omega)} \right) \cos \left[ \frac{\pi}{2} \alpha(\omega) - \beta(\omega) \alpha(\omega) \left( \frac{\pi}{2} - 1 \right) \right] (5.7)
\]

\[
G''(\omega) = G^*(\omega) \left( \frac{1}{1 + \beta(\omega)} \right) \sin \left[ \frac{\pi}{2} \alpha(\omega) - \beta(\omega) \left( 1 - \alpha(\omega) \right) \left( \frac{\pi}{2} - 1 \right) \right] (5.8)
\]

where \( \alpha = (\partial \ln \langle \Delta r^2(1/\omega) \rangle / \partial \omega) \) and \( \beta = (\partial^2 \ln \langle \Delta r^2(1/\omega) \rangle / \partial \omega^2) \).

The computed storage and loss moduli for different packing fractions are presented in Fig. 5-19. These results show that below packing fractions of about 0.7, the elastic response of the system drops below the viscous response. Hence, the system behaves as a viscous material. Whereas for packing fractions above 0.7, the system behaves as a solid-like material. According to the mechanical properties of the passive monolayer, the elastic response of the system dominates at long times for monolayers of \( \phi_A = 0.8 \) and 0.7 as depicted in Fig. 5-19A and B. For the latter, elastic and viscous responses are almost equivalent at long times.
Figure 5-19: Frequency-dependent storage modulus (red circles), $G'(\omega)$, and loss modulus (blue squares), $G''(\omega)$, for passive monolayers at area fractions of $\phi = 0.8$ (A), $\phi = 0.7$ (B), $\phi = 0.6$ (C) and $\phi = 0.5$ (D).

On the contrary, for area fractions of 0.6 and 0.5, the viscous response dominates for the entire frequency range (Figs. 5-19C and D). This demonstrates that an elastic response of the passive media is a necessary condition for co-rotating spinners to interact. As previously mentioned, this elasticity-mediated attraction between active spinning particles is inherently stochastic. In Fig. 5-20, the initial distance between two co-rotating spinners is represented against the distance reached after a long run. For passive monolayers at $\phi_A = 0.8$ and 0.7, spinners initially separated up to $6\sigma$ are attracted to each other up to a distance of about $2\sigma$ and $2.5\sigma$, respectively. However, for particle area fractions smaller than 0.7, passive-mediated interactions are no longer effective and only the hydrodynamic repulsion is observed.

5.8 Yielding mechanisms of passive monolayer

As previously mentioned, this elasticity-mediated attraction between active spinning particles is inherently stochastic. Thus, the initial configuration of the system determines the length of interaction and the time required for spinners to aggregate as shown by the different experimental and simulation trajectories in Fig. 5-9. Hence, one can imagine a rough and dynamic energy
landscape in which the spinners must traverse to form a dimer, and where multiple paths exist when moving from state to state as schematically illustrated in Fig. 5-21A. Erosion of the bridge, which happens when passive particles are removed from it, occurs through three main mechanisms: i) single particle removal from the bridge to the corona and then to the bulk as shown in Fig. 5-21B, ii) multiple particle removal where two or three particles in the bridge, due to the shear stresses, are moved into the bulk or intermittently moved to the corona and then ejected into the bulk as seen in Fig. 5-21C, and iii) avalanches where entire lines of particles in the bridge are pushed into the bulk by the shear stresses exerted by the spinners as depicted in Fig. 5-21D. We use the name avalanche to invoke the instantaneous and dramatic nature of the particle removal process. Whereas the bridge erosion produced by the two first mechanisms is slow and depends on stochastic collisions, the removal of particles in avalanche happens almost instantaneously. In fact, it has been previously shown that the devitrification of hard-sphere glasses is mediated by large rearrangements of particles, so-called avalanches [244]. This exciting new interaction opens up new possibilities to study the mechanism of elasticity-mediated interaction between active particles in these systems governed by glassy dynamics.
Figure 5-21: A) A sketch illustrating the rough energy landscape the spinners have to move through to form a dimer. Three different mechanisms by which the passive particles are squeezed out from the bridge by the stress imparted by the spinners: B) A single passive particle from the bridge jumps into the corona of one of the spinners and is released in the surroundings or the bulk. C) Multiple particle removal mechanism: two or three passive particles are taken from the bridge and moved to the bulk or surroundings. D) Avalanche mechanism: an entire shell of particles is removed from the bridge. The shaded regions indicate the bridge.

5.9 Conclusions

In summary, the forces exerted by an incompressible fluid at small, but finite Re on a pair of non-Brownian active rotating particles depends on the relative sense of rotation of each particle. The result being a repulsive force for co-rotating spinners and an attractive force for counter-rotating spinners when confined in a channel. The presence of a dense passive matrix modifies the mechanical properties of the system from a viscous media to a viscoelastic material. In this latter case, the interaction between spinners becomes controlled by elastic effects which act in the opposite direction of the inertial effects [245, 246, 247]. Hence, the switch between inertial and elastic stresses induces a reversal of the interaction between spinners, resulting in an attraction of co-rotating spinners and a repulsion of counter-rotating spinners. In fact, the structure of the passive dense medium cannot be treated at the mean field level. For example, assuming a pure viscous scenario where the passive matrix would be an homogeneous continuum with a higher viscosity than the system in the absence of passive particles, the stress would be dissipated by the viscous media. One would solely observe the repulsion between co-rotating spinners, however the strength of the secondary flows would be smaller due to the viscosity increment.

Therefore, the change of the mechanical properties of the matrix from a viscous material to a solid-like material is responsible for the force reversal. Furthermore, the interaction between spinners in dense monolayers of passive particles is stochastic in nature; it depends on the configuration of the passive monolayer. Thus, it is the instantaneous configuration of the monolayer that determines the strength and range of the interaction, and the dynamics of attraction is intimately
related to the timescale for the rearrangement of the monolayer. This can be better understood by looking at the oscillation between periods of well-defined distances and periods of fast attraction along the trajectory between two co-rotating spinners, Figs. 5-9 and 5-15A. The level of stress applied to the bridge by the spinners must reach a configuration dependent threshold value, and when this level is reached, the system yields by removing entire groups of particles from the bridge as depicted in Fig. 5-21. Then, the spinners approach each other and start stress-loading the bridge again. Therefore, this effective interaction mediated by the passive medium cannot be seen as a position dependent interaction potential \( U(r_{ij}) \). Interestingly, the dynamic trajectories of the distance between the spinners initially positioned at different distances show an almost linear regime of attraction as seen in Figs. 5-9 and 5-13. This means that the spinners approach each other on average at a constant speed. Assuming a Stokes' scenario, \( F = 6\pi \mu \sigma / 2U \), for the translation of the spinners through the monolayer along their attractive trajectory, the strength would be a constant and independent on the distance between them. Moreover, this elasticity-mediated interaction is of a very long range. For example, we observe in our simulations that spinners separated up to 6 particle diameters still interact, whereas the hydrodynamic interaction reach only 3 particle diameters. Remarkably, our experimental measurements show that the interaction threshold shifts even at longer distances. Spinners separated by up to 17\( \sigma \) attract [248] while the dipole-dipole interaction reach 4\( \sigma \) (see Figs. 5-5 and 5-9). Our results resemble other elastic media, such as lipid membranes, where elasticity-mediated forces between transmembrane embedded proteins show logarithmic decays [249, 250]. However, the origin of the stresses in our system are different.

We have shown that the interaction between active spinning particles depends on the properties of the medium and the dynamics of the active particles. Therefore, this cooperative interaction between the mechanical and dynamical properties of the system offers a variety of possibilities to tune this type of interaction between active agents. Remarkably, we have also observed that the spinners produce an annealing of the passive matrix structure (Fig. 5-12) in agreement with previous simulation results in hybrid passive-active systems, but with different type of active particles [219]. We anticipate that this mechanical attractive force between co-rotating spinners is responsible for the phase separation observed in systems with higher concentrations of spinners. Moreover, in ternary hybrid active-passive systems composed of mixtures of spinners rotating either clockwise or counter clockwise, we observe phase segregation in three different phases: one composed of the passive particles, one of co-rotating spinners, and one of counter-rotating spinners [208, 180]. In principle, this elasticity-induced interaction between active spinning particles is general for other active agents such as self-propelled particles.

This study opens up new and exciting routes to control the range and direction of the interaction between active units in passive and structured environments. This interaction between active spinning particles in passive matrices is different from the emergent interactions observed between passive objects within active fluids. Those effective interactions mediated by active matter between passive objects depend on the mobility of the passive objects [251, 252] and their shape [253]. Therefore, it would be very interesting to investigate these effects in the opposite scenario between
active particles in passive matrixes. More so, this type of interaction could play an important role in overcoming diffusive limitations that active biological molecules encounter when interacting within dense viscoelastic materials, such as the highly viscoelastic nucleus of the cell [254] or cells in extracellular polysaccharide matrix secreted by biofilm forming bacteria [255].
CHAPTER 6

AGGREGATION DYNAMICS OF SPINNERS IN DENSE PASSIVE MEDIA

Work in this chapter was published in:


In the previous Chapter, we confirmed the ubiquitous nature of the emergent ultra-long-range attractive interactions between spinning active particles embedded in a dense passive monolayer. This interaction depends on the mechanical properties of the passive medium and the mode of spinning, specifically the rotational direction. This interaction is not due or dependent on magnetic dipole-dipole interactions, which was confirmed by Lattice-Boltzmann simulations of spinners with no magnetic dipole-dipole interactions. We also observed a force reversal when the rotational nature of the particles was changed. In a purely viscous medium, counter-rotating spinners will attract while co-rotating spinners will repel. When the same spinners were embedded in a dense passive medium, the counter-rotating spinners repel while the co-rotating spinners attract. These simulations not only allowed us to confirm the nature of this emergent interaction but also allowed us to investigate modes of motility that we are currently unable to probe experimentally.

Up to this point, we have investigated emergent interactions in non-equilibrium soft active matter systems between two active units. In biology, simulation, and theory, the non-equilibrium steady states or collective motion exhibited by active matter systems typically only appear in systems that are highly concentrated with active units. Now that we understand the origin of this emergent interaction between two active units, we can increase the complexity of our hybrid active-inactive soft matter system and investigate emergent non-equilibrium steady states that may appear when the concentration of active units is increased. Here, we study the aggregation and dynamical behavior of active rotating particles, spinners, embedded in 2D passive colloidal monolayers. Using both experiments and simulations, we observe aggregation of active particles or spinners whose behavior resembles classical 2D coarsening. The aggregation behavior and spinner attraction depends on the mechanical properties of the passive monolayer and the activity of spinners. Spinner aggregation only occurs when the passive monolayer behaves elastically and when the spinner activity exceeds a minimum activity threshold. Interestingly, for the spinner concentrations investigated here, the spinner concentration does not seem to change the dynamics of the aggregation behavior. There is also a characteristic cluster size at which the dynamics of spinner aggregation was maximized, as the drag through the passive monolayer was minimized and the stress applied on the passive medium was maximized. We also show that a ternary mixture of passive particles, co-rotating, and
counter-rotating spinners also aggregates into clusters of co-rotating and counter-rotating spinners, respectively.

### 6.1 Spinners aggregate in clusters when embedded in dense passive media

Attractive interactions between particles in a homogeneous mixture induces the formation of clusters. These clusters grow in time until the mixture separates into two distinct phases. The dynamics of phase separation in binary mixtures is fairly well characterized [256] and depends on the dimensionality, thermodynamic conditions, and type of cluster growth. Often, these attractive interactions are induced by direct chemical interactions. Alternatively, they can also be induced by electromagnetic, phoretic [257], collisions [258] or hydrodynamic forces [259, 260, 201]. Non-equilibrium interactions can also promote particle aggregation. Because of the out-of-equilibrium nature of active matter systems, they are excellent candidates to study novel mechanisms of particle aggregation and subsequent phase separation.

Active matter systems are composed of active agents that consume energy from their environment and convert it into motion or mechanical forces. The most prominent examples of these active systems are living organisms which exhibit striking emergent non-equilibrium behavior such as swarming, lining, vortexes, etc. [162, 163, 164, 165, 166, 34, 36, 161, 38, 25, 14, 167, 168, 34, 9, 169]. Synthetic active systems that are able to mimic and reproduce some of the emergent behavior exhibited by living organisms can be used as model systems to study the underlying physical principles which govern their behavior. The activity of the active components can convert energy locally into motion, as in living systems, or induced via an externally applied field or stimuli. Some examples include magnetic or electric fields, light-catalyzed chemical reactions, vibrating granular beds and optical tweezers. Importantly, it is this activity which perpetually drives these systems out-of-equilibrium. However, most biological systems and processes are not composed of purely active components. In biological systems, the active or motile components (i.e. cells) are often surrounded by inmobile, passive, or even abiotic interfaces. Investigating emergent non-equilibrium behavior in such an artificial model system, composed of active and passive components, can potentially help distinguish what biological interactions can be attributed to purely physical phenomena and which interactions require presumably physical and biological/biochemical stimuli.

Here, we utilize a model active matter system that is composed of both passive and active components to study the aggregation of active particles. The active particles rotate in place (henceforth referred to as spinners) and are embedded in a dense monolayer of passive particles as schematically shown in Fig. 6-1. In this system, active particles exhibit a long-range attractive interaction [261, 262] which emerge from the non-equilibrium nature of the system and is mediated by the mechanical properties of the passive medium. We observe that spinners embedded in a dense passive monolayer tend to aggregate forming clusters which grow in time as schematically shown in Fig. 6-1. By means of experiments and numerical simulations, we demonstrate that this spin-
ner aggregation is driven by the non-equilibrium attractive interaction induced by the mechanical properties of the passive matrix. Moreover, we show that the spinner aggregation process follows dynamics that resemble a coalescence process. The dynamics of the aggregation process depends on the mechanical properties of the monolayer as well as the activity of the spinners. This type of non-equilibrium attractive interaction opens the door to controlling the state of the system via control of the mechanical properties of the medium, activity of the spinners, and the density of spinners.

![Figure 6-1: A) Co-rotating spinners randomly distributed within a monolayer of passive particles of \( \phi_A = 0.8 \), which under the action of the magnetic field, rotate around the axis perpendicular to the monolayer plane (i.e. z-axis). B) Spinner clusters form due to attraction between active particles.](image)

Our synthetic model system is composed of active spinning particles and passive particles. The spinners are superparamagnetic polymer-based magnetite particles purchased from Bangs Laboratories while the passive particles are composed of polystyrene purchased from Phosphorex; both, active and passive, are 3 \( \mu \)m in diameter. We use a concentrated solution of spinners, \( \approx 4 \mu g/mL \), and passive particles, \( \approx 0.8mg/mL \), to study the aggregation and dynamical behavior of the spinners. The spinners are made active by externally applying a magnetic field which rotates around the axis perpendicular to the plane of the monolayer. The solution of spinners and passive particles is inserted into a channel, \((22\text{mm} \times 3\text{mm} \times 300\mu\text{m})\), fabricated using a glass slide, spacer, and cover slip. Once the solution is inserted into the channel, it is sealed with epoxy and allowed to sediment for ten minutes to form a dense monolayer before being magnetically actuated. The strength of the magnetic field is 5 mT which is large enough to maintain alignment of the superparamagnetic particles with the rotational frequency of the field. The magnetic field is actuated at an angular frequency, \( \omega \), of 5 Hz for approximately ten minutes. This rotational frequency
corresponds to Re = 1.25×10^{-6}. We switch the magnetic field rotational sense every two minutes.

In addition, we carry out numerical simulations of this system. In particular, our coarse grained model consists of pseudo-hard sphere particles [179], N=324. Suspended on a fluid of density $\rho = 1$ and kinetic viscosity $\nu = 1/6$, it is modeled using the Lattice-Boltzman method. We use the fluctuating Lattice-Boltzmann equation [177] with $k_B T = 2×10^{-5}$ and the solver D3Q19. We discretized the simulation box in a three dimensional grid of $N_x \times N_y \times N_z = 101 \times 101 \times 20$ bounded in the z direction by no-slip walls and periodic boundary conditions on the x and y directions. We set the grid spacing, $\Delta x$ and time step, $\Delta t$, equal to unity. We apply the bounce-back rule [228] to describe the interaction between the solid particles and the fluid. The particles are treated as real solid objects [229] of diameter $\sigma = 4\Delta x$. The particles settle on the bottom wall of the channel, forming a monolayer under the action of a gravitational force, $F_G = 0.005$. The activity is achieved by imposing a constant torque which in general corresponds to Re = 0.72, unless otherwise noticed.

We studied the behavior of active rotating particles within monolayers of passive particles. We prepared a dense monolayer composed of passive polystyrene particles, particle area fraction $\phi_A \approx 0.7$, and doped it with a small particle fraction (about 0.5%) of active superparamagnetic particles. Upon actuation of the magnetic field we observe that spinners aggregate, forming nearly circular actively rotating clusters whose average radius, $\langle R_{\text{cluster}} \rangle$, grows with time as shown in Fig. 6-2. These clusters of spinners can be seen growing over time in the experimental snapshots in the top panel, where the spinners are the darker spots in the experimental snapshots. This behavior is different than that observed in a system of purely active ferromagnetic and superparamagnetic particles [263, 264, 265, 266]. At similar particle area fractions and frequencies in the purely active system, the aggregation of particles is dominated by the magnetic dipole-dipole interaction. We observed the formation of small chains or random aggregates [265] whose size grows slightly with time. Therefore, the passive monolayer is not only modifying the shape of the aggregates, but also increases the range of the interaction. In fact, in pure equilibrium arguments one would expect the monolayer to strongly reduce the aggregation because of the high viscosity of the monolayer.

We carry out numerical simulations of this system to study the role of the passive matrix in the spinner aggregation process and consider whether magnetic dipole-dipole interactions play a major role in the spinner aggregation process, particularly at large cluster sizes. Therefore, our coarse grained model neglects the dipole-dipole interactions to isolate the effect of the passive matrix in the spinner aggregation process. In agreement with the experimental results, we observe that spinners aggregate forming circular clusters when embedded in dense monolayers of passive particles of $\phi_A = 0.8$. We calculate the time evolution of the area of the active clusters, $A(t)$, for the experimental and simulation trajectories as shown in Fig. 6-3. We again observe that the size of the clusters grows with time. Hence, the presence of the passive monolayer promotes the aggregation of the spinners, even in the absence of magnetic dipole-dipole interactions. Additionally, we observe in the simulations that the time evolution of the domain length, $A(t)$, presents important fluctuations due to the absence of dipole-dipole interactions. When two clusters collide, the clusters split in pieces. As the newly merged cluster is re-configuring, the size of the clusters fluctuates.
Figure 6-2: Average spinner cluster radius, \(< R_{\text{cluster}} >\), as a function of time. The top panel includes several experimental snapshots which clearly shows the average size of the spinner clusters growing over time. The spinners correspond to the darker spots.

\[ \begin{align*}
\text{A)} &: \text{Log - log scale for the spinner cluster size (area), } A(t), \text{ as a function of time in a passive monolayer of } \phi_A = 0.8 \text{ for four different spinner concentrations (in percent): 1.8 (black circles), 4.94 (red squares), 9.88 (green diamonds) and 20.1 (blue triangles) in simulations.}
\text{B)} &: \text{Log - log scale for the spinner cluster length from experiments as a function of time in a passive monolayer of } \phi_A \approx 0.7.
\end{align*} \]
We have recently shown that an attractive interaction emerges between two co-rotating particles or spinners, if embedded in dense passive monolayers [261, 262]. This emergent attractive interaction, and the subsequent non-equilibrium phase separation, is thus mediated by the elasticity of the medium and the ability of the spinners to stress that medium. Under the actuation of the rotating magnetic field, the spinners rotate around the axis perpendicular to the substrate generating a rotational fluid flow [231, 267, 202]. This causes the surrounding passive particles to rotate, due to the momentum transferred through the fluid in which the particles are suspended. Additionally, at small but finite Re, the spinners' rotational motion produces a so-called secondary flow due to the fluid inertia which pushes away the nearest shell of passive particles, effectively compressing the passive monolayer. Thus, two co-rotating spinners apply compressive and shear stresses on the passive particles located in between the spinners, referred to as the bridge. This produces a stochastic, but steady degradation of the bridge which allows the spinners to approach, resulting in an attractive interaction [261]. Moreover, depending on the mechanical properties of the passive monolayer, this attractive interaction between active rotating particles may be of a very long-range nature [262].

6.2 Aggregation dynamics as a function of concentration and activity

![Figure 6-4: A) Log - log scale for the number of clusters as a function of time as obtained from the experiments at $\phi_A \approx 0.7$. The two sets of data corresponds to two independent experiments. B) Log - log scale for the number of clusters as a function of time as obtained from the simulation model for four different spinner concentrations (in percent) at $\phi_A = 0.8$: 1.8 (black circles), 4.94 (red squares), 9.88 (green diamonds) and 20.1 (blue triangles).]

The solid-like character of the passive monolayer induces an attractive interaction between the active particles, resulting in the aggregation of the spinners embedded in passive matrixes. However, we do not observe the complete phase separation of the system into passive and active domains for the actuation time period investigated. Instead, spinners aggregate forming clusters

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which grow with time embedded within the passive matrix. The dynamics of this aggregation process resembles a spinodal decomposition process in which active clusters coalesce. From the experimental trajectories, we compute the time evolution of the number of active clusters, $N(t)$, as shown in Fig. 6-4. We observe two different dynamical regimes within the experimental time scale. At short time scales (less than 100s), the clusters exhibit an initial regime of slow cluster growth or an almost constant number of clusters. This is followed by another regime (after 100s) where the spinners aggregate at a much faster rate. As can be seen in Fig. 6-4, the scaling of the number of clusters with time in this regime is characterized by an exponent of $\approx -0.7$. We also analyze the dynamic scaling of the aggregation of spinners in our simulation model. In this case, we also observe two dynamical regimes: i) an initial slow decrease in the number of clusters (i.e. growth of the cluster sizes), ii) a regime with a dynamic scaling of exponent $\approx -0.5$ as shown in Fig. 6-4. Moreover, this dynamic scaling seems independent on the spinner concentration. The $t^{1/2}$ dynamical scaling of the cluster growth has been observed in simulations conducted by Vicsek and several distinct purely active systems, although the origin of this scaling behavior is still unclear [268, 269]. However, we believe that the origin of this scaling behavior is similar to that observed in traditional 2D coarsening [270].

We hypothesize that the difference between the exponents of the dynamic scaling observed in experiments and simulations is due to the magnetic interaction between clusters of spinners, which increases the strength of the spinner-spinner attraction at short distances. Additionally, this also helps to stabilize spinner clusters. If we also assume a dynamic scaling factor for the slower initial regime in the spinner aggregation process, we obtain for experiments and simulations exponents, $\approx 0.29(2)$ and $\approx 0.04(5)$, respectively. In this first aggregation regime, the differences between the experimental and simulation dynamic scaling exponents are much greater than for the second regime. This behavior is in agreement with our hypothesis that the effect of the magnetic dipole-dipole interactions significantly increases the spinner aggregation dynamics. At the beginning of the aggregation process, the active clusters are small and the dipoles of the particles are more easily aligned which results in higher magnetization of the clusters. In contrast, as the size of the clusters increases, some of the dipoles of the particles are frustrated by the cluster structure which results in a smaller magnetization of the clusters as their size increases. Therefore, the magnetic interaction is more relevant between smaller clusters than between bigger clusters. It should also be noted that as the cluster size increases the rotational angular frequency within a cluster becomes more inhomogeneous due to the friction between the particles. Therefore, the particles at the edge of the clusters rotate faster than at the center.

The mechanical properties of the passive media determines the interaction between the spinners and thus, the dynamics of the spinner aggregation. The mechanical properties of the monolayer can be calculated by measuring the mean square displacement (MSD) of the particles in the monolayer in the absence of active particles, specifically the storage and loss modulus, $G'$ and $G''$, respectively [237, 243]. In simulations, we observe that monolayers of hard-sphere particles at area fractions $\phi_A > 0.7$ respond as viscoelastic materials, behaving as a viscous system at low frequen-
Figure 6-5: A) Log-log scale of the time evolution of the number of active clusters at Re = 0.72 and a spinner concentration (in percent) of 4.94 within passive monolayers of $\phi_A = 0.8$ (blue squares), 0.7 (red circles) and 0.5 (green diamonds). B) Log-log scale of the time evolution of the number of active clusters at Re = 0.72 and a spinner concentration of 9.88 within passive monolayers of $\phi_A = 0.8$ (blue squares), 0.7 (red circles) and 0.5 (green diamonds).

cies and as a solid-like material at high frequencies [261]. However, for $\phi_A < 0.7$, the monolayer behaves as a viscous material over the entire frequency range. To study the effect of the mechanical properties of the monolayer on the dynamics of the spinner aggregation, we investigate by means of our simulation model, spinners embedded in passive monolayers at different particle area fractions $\phi_A = 0.5$, 0.7, and 0.8. As it can be seen in Fig. 6-5, spinners in monolayers of particle area fraction of $\phi_A = 0.8$ and 0.7 follow similar scaling laws. However, at an area fraction of $\phi_A = 0.5$, the spinners do not aggregate within the simulation time scale as shown by the green diamonds in Fig. 6-5. The small amount of spinner aggregation observed in Fig. 6-5 is due to spinners being initially positioned together or close enough so that the removal of a single passive row of particles was required. It should also be noted that for a more dilute concentration of spinners, no aggregation is observed in the simulation timescale (data not shown). In addition, at these particle area fractions, the monolayer is unable to maintain spinners within a cluster and thus the number of clusters exhibit large fluctuations. Hence, the presence of a passive monolayer that behaves as a solid-like material induces an attractive interaction between the active rotating particles which results in aggregation of spinners. Interestingly, the dynamics of the spinner aggregation seems to be independent of the storage modulus, $G'$, which is higher for a monolayer of $\phi_A = 0.8$ than for a monolayer of $\phi_A = 0.7$. This might be due to the canceling of two competing effects. On the one hand, the effective interaction grows with $G'$. Yet on the other hand, the motion of the medium is controlled by $\eta$, which also grows.

For spinners in a passive monolayer with a packing fraction $\phi_A > 0.7$, the mechanics of the passive monolayer also plays an important role in keeping the cluster of active particles together. We have previously reported that for a system composed of purely active particles, the spinners will repel due to the secondary flows generated by the spinners [261, 212]. The spinners within
the cluster should then repel, but the passive monolayer exerts a force on the spinners that serves to stabilize the active cluster. This is evident from the stochastic fluctuations shown in the cluster size time evolution, as shown in Fig. 6-3 and 6-5, which corresponds to clusters breaking and reforming during the aggregation process. The size of the fluctuations increases with the spinners' concentration, due to the bigger size of the clusters.

Aside from the mechanical properties of the monolayer, the other requisite for spinner attraction is the ability to stress the passive monolayer. Therefore, we also explore the effect of the spinners' activity on the aggregation dynamics by applying different rotational frequencies, Re = 0.1, 0.72 and 3.58, to spinners embedded in passive matrixes of \( \phi_A = 0.8 \). In agreement with our previous observations for the spinner-spinner interaction in passive environments [261, 262], we observe there exists a minimum threshold of loading stress, or spinner activity, for the spinner attractive interaction to be important. Spinners rotating at Re smaller than 0.1 do not aggregate. At these activities, the stress applied to the passive monolayer is not large enough to promote the occurrence of yielding events which ultimately results in spinner aggregation [261]. On the contrary, spinners rotating at Re \( \geq 0.72 \) do aggregate and the higher the rotational frequency, the faster the evolution of the system as shown in Fig. 6-6. Interestingly, the dynamic scaling exponent of the spinner aggregation seems to be independent of the rotational frequency. However, the range of the initial dynamical regime which probably corresponds to the fastest growing unstable composition mode, shifts towards shorter times. The spinner-spinner attraction in passive matrixes follows activated dynamics [262]. The monolayer region, in between the two spinners (i.e. the bridge), needs to be loaded before it yields and has an associated time scale. This time scale depends on the mechanical

Figure 6-6: Aggregation dynamics of spinners within passive monolayers of \( \phi_A = 0.8 \) at two different rotational frequencies Re = 0.72 (black and red symbols) and 3.58 (blue and green symbols). Spinner concentrations (in percent): 4.94 (circles and diamonds) and 9.88 (squares and triangles).
properties and configuration of the monolayer. If the stress applied by the spinners overcomes this time scale, the passive particle mobility increases. This results in yielding events [261] which lead to the erosion or degradation of the bridge. Therefore, the higher the rotational frequency of the spinners (i.e., Re), the shorter the time required to stress the bridge. Consequently, as the frequency of the spinners increases, the faster the growth of the clusters at shorter time scales. The differences observed between the experiment and simulations on the Re comes from the approximations made in our simulation model. In our simulations, the momentum transfer between the spinner and neighboring particles comes exclusively from the fluid while in the experiment, friction and collision between particles may play an important role in transferring momentum.

### 6.3 Optimal spinner cluster size for aggregate growth

![Figure 6-7: A) Average approaching velocity of the active clusters as a function of the cluster size (i.e., radius of the cluster). B) Range of the attractive interaction between active clusters as a function of the cluster size. Error bars correspond to the standard deviation of the trials.](image)

We further investigate the microscopic details of the spinner aggregation process by tracking the active clusters over time, noting when the clusters collide, initial separation distances between clusters which merge, and the velocity at which clusters approach as shown in Fig. 6-7. In Fig. 6-7, the velocity at which spinner clusters approach as a function of cluster size is presented. We observe that there is a maximum velocity associated with a cluster size of approximately 45μm. This behavior of cluster velocity, as a function of size, reveals two competing effects involved in the mobility of the clusters and therefore their aggregation. The effect which opposes spinner aggregation is the effective drag, which opposes the movement of the clusters through the monolayer and the drag increases with cluster size. Meanwhile, the stress that the spinner cluster can exert on the monolayer increases with the size of the cluster. This increases the frequency of the yielding events, resulting in the degradation of the bridge and spinner aggregation. In addition, we observe that the range of the attractive interaction between clusters, \( R_i \), increases with the cluster size as shown in Fig. 6-7B. The individual spinner clusters were tracked and as the clusters collide and
form bigger clusters, the initial distance between colliding clusters was calculated and plotted as a function of the average of the two colliding clusters. As discussed above, the stress exerted on the monolayer increases with the cluster size. Therefore, this increase of the stress on the monolayer produces higher mobility of the passive particles of the monolayer which results in longer ranged interactions.

6.4 Ternary mixtures and aggregation reversal in top system

![Graph showing aggregation dynamics](figure)

Figure 6-8: Aggregation dynamics of spinners rotating clockwise (black circles) at a concentration (in percent) of 1.54, and clockwise (red squares) at a concentration of 1.54 within passive monolayers of $\phi_A = 0.8$.

We also explored the behavior of a ternary mixture in which a passive monolayer is doped with an equal mixture of spinners that are rotating in opposite senses, clockwise and counter-clockwise. In our previous work, we demonstrated that while two co-rotating spinners embedded in a passive matrix experience an attractive interaction, counter-rotating spinners exhibit a repulsive interaction in dense passive environments [261]. Spinners in dense passive monolayers tend to form clusters of co-rotating particles and thus, we observed the formation of three different phases: i) passive particles, ii) spinners rotating clockwise, and iii) spinners rotating counter-clockwise, as shown in Fig. 6-8. This is the result of the attractive interaction between spinners rotating in the same direction, and the repulsive interaction between spinners rotating in opposite directions.

In previous chapters, we have shown that the formation of spinner dimers can be reduced or eliminated by changing the mode of motility from spinning to a top-like motion. The addition of this Z component to the applied magnetic field frustrates alignment of the magnetic moments of the ferromagnetic particles. One can leverage this to tune and change the aggregation behavior observed
in this non-equilibrium active soft matter system. 100μL of the stock solution of ferromagnetic particles, 5μm in diameter, was diluted into 5mL of water and 10μL of this solution was extracted and diluted further in 100μL of water. 10μL of this solution was inserted into 40μL of passive polystyrene particles, provided by Phosphorex and 5μm in diameter. The solution was vortexed and inserted into the microfluidic channel. After several minutes, to allow for sedimentation, the sample was placed in the 3D magnetic coil apparatus and actuated at a rotational frequency of 10Hz and Z magnitude of 1, for approximately one hour.

A) B) C) D) E)

Figure 6-9: Experimental snapshots of tops actuated for approximately one hour every ten minutes from A)-E), respectively.

As seen from the series of snapshots in Fig. 6-9, the emergent non-equilibrium behavior is markedly different from that observed in Fig. 6-2. The tops do not show aggregation or a coarsening type behavior. Instead, they tend to form transient 

loose

clusters of active particles that do not snap together due to magnetic dipole-dipole interactions. There is no marked difference in cluster size from the initial configuration to that observed after 50 minutes of continuous actuation. It should be noted that the tops will attract (as shown extensively in previous Chapters) and form clusters. However, due to the lack of magnetic dipole-dipole interactions, these cluster can be easily broken and then the tops must go through the process of attraction again.

6.5 Conclusions

We investigated the aggregation dynamical behavior of active rotating particles embedded in a passive monolayer. We demonstrated that the non-equilibrium attractive interaction between spinners within dense passive matrixes [261, 262] results in their aggregation. This aggregation resembled a 2D coarsening [270] which has also been described for other pure active systems [268, 269]. Al-
though, the system size we can reach does not allow us to unambiguously determine the dynamic scaling exponent of the spinner aggregation process. We explored the effect of the particle area fraction of the monolayer, spinner concentration, and spinner activity on the aggregation behavior. We observed that the monolayer must behave as a solid, $\phi_A > 0.7$, in order to observe spinner aggregation. Additionally, for the spinners to stress the monolayer and thus produce yielding events that result into the attraction of spinners, there is a minimum activity threshold of $Re > 0.1$ in simulations. Interestingly, the aggregation dynamics seem to be independent of the spinner concentration. We also studied the microscopic details of the cluster aggregation. We observed that spinner clusters move faster as the size increases to reach a velocity maximum at around $R_{\text{cluster}} = 45 \, \mu m$. Finally, we showed that a ternary mixture of passive particles, co-rotating, and counter-rotating spinners results in the formation of clusters of spinners with the same sense of rotation. This is due to the fact that co-rotating spinners within a dense passive monolayer attract while counter-rotating spinners repel.
CHAPTER 7
TRANSPORT IN DISORDERED MEDIA

Work in this chapter was published in:

In previous Chapters, we developed a tribotactic and active tribological probe active matter system which consisted of a single active component to investigate the role of friction in vital biological processes like chemotaxis. We increased the complexity by developing a hybrid active-inactive system where we studied emergent interactions between two active particles embedded in a dense passive colloidal monolayer. We uncovered the role that activity and the mechanical properties of the environment can play in determining the emergent behavior exhibited by active matter systems. Investigating such interactions in an environment with passive components is crucial in order to better understand biological systems where the active components, cells, are constantly surrounded by passive components like tissue and the ECM. In the previous Chapter, we again increased the complexity of this hybrid active-inactive system by investigating emergent interactions or non-equilibrium steady states between many active units.

Transport of active or driven particles plays a crucial role in a myriad of processes, ranging from biological systems to quantum phenomena. Here, we study the transport of active spinning particles on a substrate that contains fixed obstacles. We demonstrate with experiments and simulations that, contrary to intuition, active spinning matter exhibits a disorder-induced delocalization transition dependent on the local order of the obstacles on the substrate. For the regimes studied, we always found anomalous super-diffusive transport. These results shed light on the effect of hydrodynamic boundary conditions and optimal transport processes in active matter in disordered environments, which are ubiquitous in nature.

7.1 Motility in disordered environments: spinners in post and obstacle filled environments

Transport of particles is of critical importance in a multitude of fields, from electrons in solids to classical particles in fluid environments [271, 272, 273, 274, 275]. Except for a handful of systems [276, 277], it is believed that a disordered environment in the form of impurities or obstacles in a material will inhibit transport [278, 279] and under some circumstances lead to localization. Such phenomena has been directly seen in transport of light in disordered photonic crystals [280, 281,
282, 283], for example. Here, we ask the question if the same principle applies for active soft materials where there is a continuous input of energy at the particle level. This is an important question because many vital biological processes depend on the active transport of molecules inside cells and organisms, from molecular motors to cellular transport. Furthermore, the organization of such environments is not perfectly ordered and understanding the effect of disorder on the motion of active matter can shed light on the principles behind transport in living systems. In particular, it is important to know whether disorder leads to the inhibition of transport and localization, or enhances transport. The former scenario might prove catastrophic for living organisms, and in fact, one would expect living systems to be robust against disorder.

This notion of transport or motility in disordered or crowded environments has been a focus for many theoretical and simulation active matter systems, as well as some artificial experimental model systems. Such systems typically study the behavior of active matter systems, specifically active units, under some type of confinement or interacting with pillars, posts, ratchets, or other objects in the environment which can be seen in Fig. 7-1 [43, 284, 285, 286].

![Figure 7-1:](image)

Figure 7-1: A) C.elegans are swimming in PDMS pillar environment. B) Quincke particles are interacting with fixed posts. C) Active rods are interacting with passive particles. D) Active particles are interacting with ratchets. Reproduced from [43, 287, 284, 285, 286].

In particular, Reichhardt has extensively study the dynamical behavior of active units in environments with ratchets as well as obstacles or post filled environments [43]. There have also been
several other simulation studies that show how such posts can serve as nucleation sites in active systems for active crystal growth. There have been several experimental studies as well to investigate this concept of motility on the influence of environment, specifically C.elegans moving in a PDMS pillar environment and Quincke particle swarm movement through randomly positioned, fixed colloidal obstacles [284, 286]. Even more interesting was the fact that C.elegans seemed to transverse more quickly and efficiently as the density of PDMS pillars increased (up to a certain density) due to the hydrodynamic coupling between the C.elegans motion and the pillar [284]. In this respect, we demonstrate that transport of actively rotating systems undergo a disorder-induced delocalization transition that leads to anomalous super diffusive transport.

Figure 7-2: A) Segment of simulation trajectory in a perfectly ordered post lattice. The spinner becomes localized and trapped. B) Segment of simulation trajectory in disordered lattice with noise in the post lattice, $\Delta r = 0.3$. The spinners become delocalized and translate across the system.

Our active system consists of micron-sized ferromagnetic spinning particles in a 2D array of fixed non-magnetic particles (obstacles) as shown in Fig. 7-2. This array of fixed particles is the ordered or disordered substrate. We selected this system because in the absence of obstacles, the spinning particles do not exhibit any motion beyond Brownian transport, a rather slow mechanism at this length scale. The activity of our particles is conveyed by an in-plane rotating magnetic field. We point out that having an actively rotating particle does not cover all the possible activity mechanisms. Some cells and organisms do display this mode of activity but more importantly, assemblies of active agents tend to exhibit mesoscale vorticity [190, 201]. Thus in principle, our work can probe how such assemblies navigate through a disordered medium, as could be in the interior of a cell. On a more general note, this system naturally incorporates a torque on the active particle apart from a weak repulsion from the obstacles. Such systems have received wide attention in the quantum world due to their unusual, yet important properties. We believe that studying the classical counterpart, as we do here, may also lead to new discoveries with potentially important implications in our understanding of the living world.

The active particles in this system are ferromagnetic particles, 5$\mu$m in diameter, provided by Spherotech and composed of a core of polystyrene, and an outer shell composed of CrO$_2$ and
polystyrene. The immobile fixed obstacles in this system are composed of silica particles, 3μm in diameter, provided by Bangs Laboratories. A dilute solution, ≈ 2μg/mL, of spinners and obstacles, ≈ 4μg/mL, in an aqueous solution was inserted into small channels (22mm (L) × 3mm (W) × 300μm (H)) that were created using two pieces of double sided tape and two ITO coated glass slides. The channel was then sealed with epoxy and allowed to sediment, a schematic of which can be seen in Fig. 7-3.

![Schematic of active spinning ferromagnetic particles (yellow) which exhibit Brownian diffusion in the absence of fixed obstacles (blue) and translate when embedded in an environment with disordered fixed obstacles. B) Schematic of ITO coated glass slides and microfluidic channel. A voltage is applied to polarize the silica particles, fixed obstacles.](image)

Figure 7-3: A) Schematic of active spinning ferromagnetic particles (yellow) which exhibit Brownian diffusion in the absence of fixed obstacles (blue) and translate when embedded in an environment with disordered fixed obstacles. B) Schematic of ITO coated glass slides and microfluidic channel. A voltage is applied to polarize the silica particles, fixed obstacles.

After the channel was sealed with epoxy, the silica particles sedimented randomly to the bottom of the ITO substrate and tended to agglomerate into clusters. To create an environment that was patterned with random fixed obstacles, alligator clips were attached to the top and bottom of the ITO slides. The alligator clips were then connected to an external DC power supply. When the power supply was turned on, a potential was established in the channel and the silica particles became polarized. As the voltage increases, the silica particles become more polarized which induces a strong repulsive potential between the silica particles, causing the silica particles to repel until reaching a desired equilibrium distance depending on the voltage applied. In this study, a voltage of 2-3V was utilized to obtain the desired fixed obstacle-obstacle distance. The silica particles are still somewhat Brownian at this point so they cannot function as fixed obstacles. Instead,
they essentially act as a passive medium with a strong repulsive potential [262]. To stick the silica particles to the substrate, the potential is flipped and the particles become stuck to the ITO substrate. The applied voltage here is large enough to overcome the thermodynamic barrier for electrolysis of the aqueous solution. This induces an electrochemical reaction that fixes the silica particles to the ITO coated glass slides as seen in Fig. 7-4.

![Figure 7-4](image)

**Figure 7-4:** A) Schematic of ITO coated glass slides and microfluidic channel. A voltage is applied to polarize the silica particles, fixed obstacles. B) Passive silica particles are initially randomly clustered until an externally applied field of 2-3V polarizes the particles, forming a much more uniform and homogenous post filled environment.

The spinners in this solution, however, are not polarized to the extent of the silica particles due to the difference in their refractive index. Thus, the ferromagnetic particles are relatively unaffected by the applied electric field and do not become stuck to the ITO substrate. Videos of spinners in a disordered fixed obstacle filled environment were recorded via a CMOS camera. The videos were then converted from .wmv to .avi using ArcSoft Media Converter 8. The .avi videos are then converted into a series of .jpeg images using OGG Video Converter.

In this system, the active particles were rotated using a rotating homogeneous magnetic field at different frequencies: 1, 5, and 20 Hz with the 3D magnetic coil apparatus. Thus, the activity is modified by more than an order of magnitude. The protocol for actuation of the spinners consists
of an actuation period of 1, 5, or 20Hz in one rotational direction for 180s followed by actuation in the opposite rotational sense for another 180s. The arrangement of the obstacles was for the most part disordered, although some regions displayed more order.

7.2 Spinners become delocalized in disordered environment

Figure 7-5: A-C) The spinners are surrounded by an ordered post-obstacle array and the spinners remain trapped and localized in the array for the duration of the actuation period. The spinners are actuated at 1Hz (A), 5Hz (B), and 20Hz (C). D-F) When the spinner is surrounded by a more disordered array of obstacles, the spinner becomes delocalized and translates across the environment, independent of frequency. Again, the spinners are actuated at 1Hz (D), 5hz (E), and 20Hz (F).

In Fig. 7-5A-C, we show trajectories in regions of higher symmetry where the particles become trapped. Interestingly, the spinning particles moved rapidly across the substrate when they encounter a region with more disorder/less symmetry, relative from the center of the active particle. This is shown in Fig. 7-5 D-F. It is important to mention that the particles do not touch the obstacle due to a weak repulsive force. This disorder induced translation occurs due to the breaking of the rotational symmetry of the spinners. In a disordered environment, the rotational symmetry is broken by the random position of the posts. In a highly ordered post environment, the effective rotational symmetry of the spinner remains intact as effective contributions from each post cancels out. Furthermore, we point out that the trajectories become more random as the frequency increases.

To probe this system in further depth, we also performed Brownian Dynamics (BD) simulations in a simplified model that has been parametrized from a combination of experiments and Lattice-Boltzmann simulations. The equation of motion for this system is given by,

$$\partial_t \mathbf{r}_i = \beta D \mathbf{F}_{ij} + \sqrt{2D} \mathbf{A}(t)$$  \hspace{1cm} (7.1)
where $\beta = 1/(k_B T)$ is the inverse thermal energy, $r_i(t)$ is the position of the active particle at time $t$, and $D = k_B T/m_i \gamma_i$ corresponds to the diffusion coefficient where $\gamma_i$ is the particle viscosity coefficient. The total conservative force acting on the active particle $i$, $F_{ij}$, is a function of the distance between the active particle $i$ and the $j$th obstacle, $r_{ij}$, and can be written as:

$$F_{ij} = \Omega \left[ \frac{(\eta - 1)}{100} \left( \frac{\sigma}{r} \right)^3 \hat{r} + \eta \left( \frac{\sigma}{r} \right)^3 \hat{\omega} \times \hat{r} \right]$$

(7.2)

The radial contribution corresponds to an inertial repulsion force that arises between a spinning particle and an obstacle that scales as $\frac{1}{r^2}$. The tangential force (or torque) on a spinning particle around a fixed obstacle also scales as $\frac{\eta}{r}$ and arises from a response to the flow stemming from a spinning particle. The best fit parameters were $\eta = 0.98$, and $\Omega = 5, 10$, and $50$ for $1$Hz, $5$Hz, and $20$Hz, respectively. The last term of Eq. 7.1 is the thermal white noise term for which we use a variance of $1$, also in units of $kT$. This value reproduced the noise in the experimental trajectories at different frequencies. We also ran shorter fully explicit simulations using LB that confirm the BD simulations, albeit in a different activity regime. Furthermore, the LB simulations allow us to confirm directly the results and discard experimental artifacts. The disordered lattices were obtained by displacing the positions of the obstacles in an ordered lattice by adding a random vector from a normal distribution to the original position. The amount of disorder was controlled by changing the variance of the distribution, which we denote as $\Delta r$.

\[
\begin{array}{ccc}
\Delta r = 0.0 & \Delta r = 0.1 & \Delta r = 0.2 \\
\end{array}
\]

\[
\begin{array}{ccc}
\Delta r = 0.3 & \Delta r = 0.4 \\
\end{array}
\]

Figure 7-6: Spinners are localized in a perfectly ordered lattice, $\Delta r = 0$, but with the addition of noise in the post lattice the spinners become highly delocalized and translate across the lattice. $\tau$ is the simulation time.

In Fig. 7-6, we show different BD trajectories as a function of disorder. As can be clearly seen, particles are more localized in the perfectly ordered lattice while they become increasingly delocalized as disorder is increased. For these particular parameters we found that the particles are
always localized in the ordered substrates, yet disorder induces transport of the particles. These results are robust for both square and hexagonal lattices.

### 7.3 Super-diffusive behavior in a disordered obstacle filled environment

- **A)** Experimental trajectories of spinners actuated at 1 (blue), 5 (red), and 20 Hz (green) in the absence of posts.
- **B)** MSD of spinners in the absence of posts actuated at 1, 5, and 20 Hz.

Figure 7-7: A) Experimental trajectories of spinners actuated at 1 (blue), 5 (red), and 20 Hz (green) in the absence of posts. B) MSD of spinners in the absence of posts actuated at 1, 5, and 20 Hz.

Here, we demonstrate that the slope of the logarithm of the mean square displacement divided by the area per posts \(\frac{\text{MSD}}{A_p}\) vs the logarithm of the dimensionless time \((\tilde{t})\) is super diffusive for all frequencies investigated, where \(A_p\) is the area per post and \(\tilde{t}\) is the dimensionless time (i.e. the time multiplied by the rotational frequency). A crucial measurement of random transport is the MSD, and in particular the slope of the Log (MSD) vs. Log (time), or \(\alpha\). In a purely diffusive system, the slope is 1 (which is the case for spinners in a Newtonian fluid see Fig. 7-7) while in a ballistic regime the slope is 2. In Fig. 7-8A and 7-9A, we present the average MSD for multiple trajectories from both experiments and simulations. The individual MSD calculations and several selected trajectories can be seen in 7-10.

In both experiments and simulations the slopes are larger than 1, meaning the transport is super-diffusive when there is disorder in the post filled environment. We are able to determine the position of the posts in the simulation spinner system, something that is not able to be done experimentally without fabricating the posts using some type of lithographic technique, and the spinners are clearly sub-diffusive when there is no noise in the lattice. However, once a small amount of noise is introduced the spinners exhibit super-diffusive behavior in the intermediate dimensionless time regime. Specifically, the regime of interest occurs when the dimensionless MSD values exceed the plateau value exhibited by spinners in the perfect lattice (that value corresponds to spinners unable to escape a square lattice). In the experimental scenario, we note that the
average slope decreases as the activity increases. This implies that the fastest transport occurs at an optimal activity and does not scale linearly with activity which is easily observed when rescaling to dimensionless time. We can understand this by observing that as the activity increases, the effective interaction radius of the particle is larger and thus averages more the disorder of the surrounding obstacles. Interestingly, in the simulations the spinners appear to become purely diffusive in the long time limit.

To further elucidate the relationship between the order of posts and this emergent super-diffusive behavior, we quantified the transport behavior as a function of the local post order. In Fig. 7-11, we plotted the power law coefficient, $\alpha$, locally as a function of disorder. The $\alpha$ values were obtained by linearly fitting the logarithm of the MSD at short intervals of time from the trajectories in the experiments.

The experimental trajectories were segmented into ten second intervals for the six minute trajectory. This time interval was chosen somewhat arbitrarily. However, it was long enough for the spinners to translate but not so long where analyzing the average order relative to the spinner would be meaningless. The order of the lattice was calculated using the local bond orientational order parameter previously defined [288],

Figure 7-8: The average MSD/$A_p$ from many experimental trajectories for spinners actuated at 1, 5, and 20Hz. The slopes, $\alpha$, are all larger than 1 and the slope does not scale with the rotational frequency of the magnetic field. The values of $\alpha$ are approximately 1.7, 1.6, and 1.4 for 1, 5, and 20Hz respectively.
Figure 7-9: Simulation spinner transport behavior for $\Omega = 5$, 10, and 50 which corresponds to 1, 5, and 20Hz, respectively. The spinners are sub-diffusive and trapped in a perfect lattice, $\Delta r = 0.0$ (blue). However, once noise is introduced in the lattice, super diffusive behavior is observed at intermediate dimensionless times, $\tilde{\tau}$, for noise values $\Delta r = 0.1$ (red), 0.2 (green), 0.3 (yellow), and 0.4 (orange).

Figure 7-10: All experimental MDS used to calculated the average MSD for 1, 5, and 20Hz.

$$\Phi_6 = \left\langle \frac{1}{N_b} \sum_{n=1}^{N_b} e^{i\theta_{mn}} \right\rangle$$

(7.3)

where $\theta_{mn}$ is the angle between a fixed axis, either x or y in this case, and the bond that
joins the m-th particles (i.e. the spinner) and the neighboring n-th particle. $N_b$ is the number of particle-neighbor bonds. This value is calculated at each time step along the trajectory. A value of 0 is a completely disordered system and a value of 1 denotes perfect order ref. The local bond orientational order parameter was calculated from the reference frame of the spinner. The number of neighbors can be calculated from the density of the posts as traditionally defined by the effective hydrodynamic radius of the spinner and/or via manual inspection. This procedure can be visualized schematically in Fig. 7-12. The trajectory is segmented into smaller time intervals as shown by the different colors along the trajectory. The local order is calculated at each time step along the trajectory. One such step is shown, the black dot, and the number of neighbors inside the search radius are illustrated by the orange colored posts. The bond orientational order parameter is calculated from the angles between the posts and the spinner.

As can be seen, the value of $\alpha$ depends on the order of the obstacle filled environment and becomes either diffusive or even sub-diffusive once the local environment becomes highly ordered. As the local environment becomes more disordered, the spinner exhibits increasingly super-diffusive behavior up to a certain value of disorder. This result indicates the role that environment, particularly the arrangement or order of the environment, can play in determining transport properties of active colloids which can potentially be extended to cellular transport as well.
Figure 7-12: The trajectory is segmented into smaller time segments, as indicated by the different colors along the trajectory. The local bond orientational order parameter is calculated by calculating the angles between the location of the spinner (black dot) and the neighbors within a pre-defined search radius (orange posts).

7.4 Effect of externally applied force on spinner transport in disordered environments

This concept of disorder induced transport and the ability of active spinning colloids to exhibit super-diffusive behavior and even approach the ballistic regime, inspired us to ask the question of what would happen to the transport properties if one was able to add an additional external force to the spinning active units. In this system, the symmetry is broken by the presence of obstacles or posts, in particular disordered posts or obstacles which interact with the spinners through hydrodynamic interactions. We have previously seen in Fig. 7-7 that in the absence of fixed obstacles, the spinner cannot interact with the environment hydrodynamically and simply exhibits diffusive behavior. However, if the spinner is surrounded by an ordered post environment, the spinners become trapped as the effective hydrodynamic repulsion from each post effectively cancels out. As a result, the spinner is unable to translate unless some thermal fluctuation or noise in the system perturbs the position of the spinner.

This constraint or necessity to have a disordered post environment to induce spinner transport can be overcome via the addition of another external force. This is a commonly utilized technique in many simulation systems as the addition of another external driving force can be easily applied.
Doing this experimentally is usually much more difficult to accomplish as one typically must apply an external electric field or permanent magnetic field. However, we take a much more simple approach by utilizing gravitaxis. In previous Chapters, we mentioned that in the chemotaxis process a cell typically detects and moves along a chemical gradient. Gravitaxis is a similar process by which a cell, organism, or colloid moves in response to gravitational forces. This was accomplished in our system by simply tilting our entire 3D magnetic coil apparatus by approximately 16° which induced a large enough gravitational force so that colloidal motion could be observed within our experimental time scales. A schematic can be seen in Fig. 7-13. The tilt was achieved by simple propping up one side of the optical breadboard and inserting the magnetic coil winding structure underneath the breadboard. The same procedure was followed, as described previously, to create the disordered obstacle substrate and actuate the magnetic field. The same concentration of active particles and density of posts was consistent with the previous studies conducted in the absence of gravitaxis.

![Diagram](image)

Figure 7-13: A) Scenario of a spinner under an externally applied gravitational force with no magnetically actuated magnetic field or posts. B) Scenario of a spinner under an externally applied gravitational force, a magnetically actuated magnetic field, but no posts. C) Scenario of a spinner under an externally applied gravitational force and magnetically actuated in the presence of posts.

In the absence of obstacles and no magnetic field, as seen in Fig. 7-13, the ferromagnetic particle simply rolls down the incline due to gravitational forces which are opposed by Stokes’ drag and frictional forces between the particle and the ITO coated glass substrate. As seen in Fig. 7-14A, the particle simple translates along the direction of the incline which is defined as the positive x-
direction in this system. Interestingly, in the absence of obstacles and when a rotational magnetic field is applied, the motion of the spinner is quite different in both magnitude and direction.

Figure 7-14: A) Experimental trajectories of scenario of a spinner under an externally applied gravitational force with no magnetically actuated magnetic field or posts. B) Experimental trajectories of scenario of a spinner under an externally applied gravitational force, a magnetically actuated magnetic field, but no posts. C) Experimental trajectories of scenario of a spinner under an externally applied gravitational force and magnetically actuated in the presence of posts.

When the external magnetic field is applied, the spinners no longer simply translate along the incline direction but instead, deviate from its principal translation path depending on the rotational direction of the applied magnetic field. This behavior can be attributed to the Magnus effect. The Magnus effect can be observed when a baseball pitcher throws a curveball. By inducing spin to the ball during the throwing motion, this spin causes the ball to deviate from its path and curve away or toward the batter depending on the applied spin. This is analogous to what is observed here in Fig. 7-14B. When the spinner is embedded in a disordered, obstacle filled environment on an inclined plane and the rotational magnetic field is actuated, we observe yet another distinct pattern of motion or transport. The spinners still exhibit a persistent overall motion directed in the positive X-direction (i.e. in the direction of the gravitational force, down the inclined plane). This is due to the externally applied gravitational force. One can see that this persistent, driven motion
in the positive X-direction is a distinct, unique transport behavior from calculating the correlation function along X and Y positions for the scenarios: i) tilt with no applied field and no obstacles, ii) tilt with an applied field and no obstacles, iii) tilt with an applied field and obstacles, and iv) no tilt with an applied field and obstacles as seen in Fig. 7-15.

![Figure 7-15: Correlation function along X (A) and Y (B) along the experimental spinner trajectories for: i) tilt with no applied field and no obstacles (blue), ii) tilt with an applied field and no obstacles (red), iii) tilt with an applied field and obstacles (green), and iv) no tilt with an applied field and obstacles (yellow).](image)

There is a clear shift in the correlation decay for the case of no externally applied tilt and the case where gravitaxis is present. The correlation in X decays much more rapidly when there is no tilt present than when tilt is applied in the case of an applied field in an obstacle filled environment. This should be the case, as in the flat scenario, the transport is dictated by the disorder and arrangement of the obstacles or posts. If one is able to average over many trajectories as we have done here (18 independent trajectories), the correlation should be less than when an external driving force is applied. Thus, forcing the spinner to translate in a principal, positive X direction.

We also characterized the motion of the spinners under the externally applied gravitational force by examining the mean square displacement of the spinners as can be seen in Fig. 7-16. Here, we can see several key features to the motion of the spinners in three scenarios of i) tilt with no obstacles and no applied field, ii) tilt with no obstacles and an applied field, and iii) tilt with obstacles and an applied field. All of these scenarios approach a ballistic regime of motion because as mentioned in the previous Chapter, the slope of Log (MSD) vs Log (time) is $\alpha$ and when $\alpha = 2$ the motion is ballistic. What is interesting is the increase in the transport and translation of the spinners when an external field is applied. The spinners move more when rotating and rolling down an inclined plane when compared to simply rolling down the plane. Additionally, we see a similar, although smaller increase, in the motion when the disordered obstacle filled environment
is introduced. This should make sense intuitively because now in addition to the Magnus effect, we also get enhanced transport due to the hydrodynamic interactions of the spinners with the fixed obstacles. We eliminate, almost entirely, scenarios of trapping in a highly ordered environment because the externally applied force essentially functions to push the spinner to explore different post environments. So, even if the spinner becomes stuck in a highly ordered local environment, the gravitational force will eventually push the spinner out of the highly ordered environment which would have previously induced localization.

![Graph showing MSD of spinners](image)

Figure 7-16: MSD of spinners where there is: i) tilt with no obstacles and no applied field (blue), ii) tilt with no obstacles and an applied field (red), and iii) tilt with obstacles and an applied field (green).

As discussed in previous Chapters, we have explored the role that the actuation protocol (specifically changing the rotational direction and duration of the actuation time) can play in changing the emergent behavior of spinners. Hence, we sought to test if changing the rotational direction clockwise and counter-clockwise for different actuation periods, \( \Delta t \), could affect the transport of spinners in the disordered environment under an additional gravitational force. This was of interest because the spinners move in a flat, disordered environment due to hydrodynamic interactions with the obstacles. However, it takes time to build up fluid flow and subsequent interactions with the obstacles or posts. This is very similar to the spinners embedded in a dense passive medium where the minimum time scale to induce yielding and dislocation motion was on the order of 10-30s. Thus, we investigated the effect that changing the rotational direction of the applied field every period
Δt for Δt’s of 10, 30, and 60s and examined the motion of the spinners (still under an externally applied force). The diffusive behavior of: i) Tilt, field, posts, Δt = 360, ii) Tilt, field, posts, Δt = 60, iii) Tilt, field, posts, Δt = 30, iv) Tilt, field, obstacles, Δt = 10, and v) Tilt, no field, no posts can be seen in Fig. 7-17. What is immediately apparent is there is a transition from super-diffusive behavior (although reduced translation) observed for spinners when the Δt = 10 and 30s, to a regime where translation is enhanced and approaches the ballistic regime at long times for spinners where the Δt = 60 or 360s. This indicates that there is a critical continuous actuation minimum time scale of around 30-60s required to couple to the disordered obstacle filled environment in order to interact with the posts hydrodynamically and break the rotational symmetry of the system. It is also interesting to examine the long-time behavior of Δt = 10 and 30s and compare that to the baseline case where there is tilt and no applied field. The slope of the Δt =10 and 30s case does not approach the ballistic regime. Whereas, when there is tilt and no applied field at long times, it approaches ballistic motion which is to be expected. This means that at longer times one will expect a cross-over in the MSD curves. This is significant because this indicates that at certain times, for spinners where Δt = 10 and 30s, the spinners can actually do work against the system (i.e. they can move up against the incline). Here, the spinners are able to do so because the rotational direction is changed so rapidly that as they are close to or in contact with a post. It is feasible that as they rotate around the post, the rotational direction is switched and the spinner moves back and retraces that portion of the spinner path.

The effect of changing the actuation protocol on the spinner transport properties can also be seen when the MSD is compared for the flat and the tilted scenario as seen in Fig. 7-18. When Δt = 10 and there is no tilt, the motion of the spinner is essentially diffusive as α ≈ 1. However, once tilt is introduced the spinner exhibits a more super-diffusive type of behavior. However, the spinners still do not approach the ballistic regime as observed when Δt = 360s and there is an
additional applied gravitational force. It should also be noted that there is enhanced diffusion for \( \Delta t = 360 \)s compared to \( \Delta t = 10 \)s in the flat scenario.

### 7.5 Conclusions

In summary, we have demonstrated that active spinning matter exhibits a disorder-induced delocalization transition as well as anomalous super diffusive transport in such disordered substrate, even approaching ballistic motion. This super diffusive behavior does not scale with the frequency of rotation of the applied magnetic field but does strongly depend on the local order of the environment relative to the spinner. We confirm this super-diffusive behavior in a simplified model Brownian dynamic simulation and also confirm the disorder induced delocalization using more detailed Lattice-Boltzmann simulations. We also investigated how the transport properties of the spinner can be tuned via the addition of an externally applied gravitational force and the actuation protocol of the rotating magnetic field. The transport of the spinners in a disordered obstacle filled environment approaches the ballistic motion regime at long times. We see enhanced diffusive motion of spinners under an applied gravitational force (even in the absence of obstacles) due to the Magnus effect. However, we see a transition in the diffusive properties from super diffusive behavior when the actuation period, \( \Delta t \), is 10 or 30s to a more ballistic type of motion observed at longer times when \( \Delta t \) is 60s or longer. This mechanism of disorder induced transport is extremely robust and might be important in regulating transport in different systems that display vorticity.

What is even more interesting are some preliminary simulation systems that we have investigated which hint that ballistic transport is achievable in spinner systems for certain obstacle filled environments with special symmetries. Now that we understand the fundamental role that the local order of the obstacle filled environment plays, we can design (at least currently in the simulation...
system and in the future, the experimental system as well) environments with special symmetries that serve to couple successive hydrodynamic interactions with the spinner and the environment to constantly induce spinner transport. Not only would the spinner never become trapped, but one could engineer the environment to provide optimal spinner transport across the media. Of course in the long term, thermal fluctuations or noise in the system may eventually cause the spinners to deviate from this ballistic type of motion, but ballistic motion is possible for some time scales.
CHAPTER 8
SUMMARY AND OPEN QUESTIONS

8.1 Summary of thesis

8.1.1 Active Tribological Probes Summary

In this thesis, I developed several novel synthetic experimental model active matter systems that were able to effectively mimic the rich emergent non-equilibrium behavior exhibited by many vital biological systems and processes. In Part I, I was inspired by the biological process of chemotaxis and the emergent directed collective motion exhibited by cells which sense and then move along a chemical gradient present in the environment. To investigate the underlying fundamental physical phenomenon that may play a role in guiding or determining such non-equilibrium behavior, I developed a novel experimental synthetic system capable of synthetically reproducing chemotactic behavior. This model active matter system was composed of a single active unit. The active unit was a doublet of superparamagnetic particles functionalized with streptavidin ligands and a PEG brush on the surface of the particles. The particles were placed on a substrate with a gradient in the density of biotin binding sites. A rotating magnetic field was applied to drive the superparamagnetic doublet to walk, hence walkers. The walkers aligned their magnetic moments with the applied magnetic field and proceeded to rotate. Translation occurred due to the effective friction between the walkers and the substrate which converts some of the rotational motion into translational motion. The amount of rotational motion that is converted into translational motion scales with the effective friction between the object and the substrate. This scenario is analogous to a tire rolling much further on dry asphalt than on an icy road due to the difference in effective friction between the tire and the road. In this microscopic scenario, the effective friction is determined simply by the strength and the density of reversible bonds between an object and a substrate. In this case, it is the interaction of the streptavidin ligands on the surface of the walkers and the biotin binding sites on the glass substrate. When the walker encounters a biotin binding site, the effective friction is increased and the walker translates more when compared to the case where there is no biotin binding site and the effective friction is reduced. I demonstrated that the walkers utilized this fundamental principle of effective friction to sense gradients in the coefficient of friction and the chemical gradient of biotin ligands. The walkers are not only able to detect a gradient in the coefficient of friction but are also able to move along these gradients as well. By performing a stochastic 1D random walk, the walkers are able to move along gradients in the coefficient of friction and drift towards regions with a high density of biotin binding sites and larger coefficient of friction. The walkers are able to exhibit such emergent directed collective motion behavior because the symmetry of the system is broken due to the gradients in the density of biotin binding sites. I illustrate that on average, the walkers tend to translate a larger distance when moving towards a region with a high coefficient of friction or a high
density of biotin binding sites when compared to moving away from regions with a high density of biotin binding sites. This difference in translation distance is due to the difference in the density of biotin binding sites and thus, the effective friction. I also determined that the walkers can become trapped in these regions with a high coefficient of friction, a behavior reminiscent of cells becoming trapped in their own chemoattractant which contributes to the formation of biofilms. The strength of this trap can be tuned by changing actuation period of the walkers and the chemotactic drift behavior can be tuned by the frequency of rotation of the applied magnetic field. This is the first reported synthetic system capable of effectively performing chemotaxis with biological ligands in a non-equilibrium fashion. Additionally, it clearly illustrates the fundamental role friction can play in biological processes like chemotaxis.

I then investigated whether this ability to detect differences in effective friction, due to different interaction affinities, was ubiquitous and not unique to the biotin-streptavidin system. To investigate the full spectrum of interaction strengths, $K_d = 10^{-5} - 10^{-15}$, I had to develop another artificial model active matter system. The chemotactic or tribotactic system utilizing doublets of superparamagnetic walkers was unable to probe unpassivated biotin-streptavidin interactions as the torque applied is not enough to break this bond (approximately 100pN is required to break the bond). To overcome this limitation, I changed the active unit from superparamagnetic doublets to single ferromagnetic particles that roll across a substrate, hence rollers. I developed a 3D magnetic coil apparatus to enable 3D motion, reduce coil overheating due to high duty cycle experiments, and apply a larger magnetic field strength. I also developed a dimensionless parameter, the rolling parameter, to characterize the effective friction induced by the interaction between the ligands on the surface of the particle and the corresponding ligand or receptor on the substrate. The rolling parameter is essentially the ratio of the observed displacement divided by the maximum theoretical displacement and it varies from 0-1. A value of 0 corresponds to a scenario of perfect slipping and no translational motion, or essentially a substrate with no effective friction. This scenario is never experimentally realized as there always exists some finite contribution of hydrodynamic friction. A value of 1 corresponds to perfect rolling and an extremely large effective friction between the object and the substrate (i.e. a very high binding affinity). Between these two scenarios, the rollers exhibit some stick-slip rolling behavior. I was able to show that these rollers are able to distinguish between different binding interaction strengths by measuring the effective friction for electrostatic, hydrophobic, antibody-antigen, metal ion coordination, and biotin-streptavidin interactions. The effective friction, scaled with the binding affinity of the interaction and the rollers, could also distinguish between binding affinities within these general classifications. For example, the rollers could distinguish the interaction affinity between Protein A and IgG, IgY, and IgM. For the metal ion coordination interactions, different affinities could be distinguished depending on the metal ion used. I investigated how the rolling parameter can be modulated by the frequency of rotation of the applied magnetic field, magnetic field strength, density of ligands on the substrate and on the surface of the rollers, and the addition of competitive binders. These free parameters open up the possibility of obtaining quantitative information on the binding kinetics and affinity of these...
interactions.

I also investigated protein-lipid interactions on soft substrates, supported lipid bilayers (SLB). The lipids of interest were signaling lipid (known as phosphatidylinositol phosphates (PIPs)) interacting with multiple proteins, specifically Auxilin 1 (PTEN) and Auxilin 2 (GAK). Both are critical proteins involved in the clathrin mediated endocytosis pathway. To establish a baseline for this new system, the well characterized proteins DrrAWT, DrrAK568A, 2XFYVE, and PH-δ were found to interact preferentially with PI4P, PI4P, PI3P, and PI45P2 which corresponds to the literature data. The measured rolling parameter, scales qualitatively with the measured binding affinities, Kd. This confirms that even in this new system, on a soft substrate of SLBs, the rollers can still measure differences in effective friction due to different binding affinities. I then measured the effective friction of Auxilin 1 and Auxilin 2 on the lipids bilayers decorated with 10% of PI, PI3P, PI4P, PI5P, PI35P2, PI34P2, PI45P2, and PI345P3. Auxilin 1 shows preferential binding to PI3P and Auxilin 2 binds preferentially to PI4P and PI34P2 which is consistent with biological observations for the preferred affinity of these proteins and the corresponding signaling lipids. It should be noted that due to the weak nature of the binding of Auxilin 1 and Auxilin 2 to these signaling lipids, it has previously been very difficult to measure the interactions in-vitro in a reproducible manner. However, we have been able to do so here.

8.1.2 Emergent Non-Equilibrium Phenomenon in Complex Passive Media Summary

In Part II, I was inspired by the emergent dynamical and non-equilibrium steady state behavior of a number of different biological systems and processes like the volvox algae, cells moving collectively as sheets during wound healing, and the emergent vortices of spermatozoa. These are complex biological systems composed of many active units. Therefore, to better understand the underlying fundamental physical phenomenon determining these emergent behaviors, I developed a novel synthetic experimental active matter system that can effectively mimic some of the emergent collective dynamical and phase behavior exhibited by many biological active matter systems. Here, instead of the model active matter system being composed of a single active unit (microwalker or roller), the system is composed of two active ferromagnetic particles embedded in a 2D monolayer of passive particles of the same size and composition. The ferromagnetic particles are made active by a rotating magnetic field that rotates around the axis normal to the monolayer plane causing the particles to spin, hence spinners. This mode of motility was inspired by the motion of the dancing volvox algae. I also wanted to investigate emergent behavior in active systems with both active and inactive components because in biological systems, the active components (e.g. the cell) are often surrounded by inactive, passive, and even abiotic components all of which are non-motile. Thus, studying the emergent behavior in such a model system can potentially help distinguish which behavior is due to purely physical phenomenon and what behavior requires presumably biological or biochemical stimuli.

In the absence of a dense passive monolayer when the spinners are made active, they simply
rotate and spin in place and do not interact unless initially positioned less than 4D apart. If the spinners are positioned less than 4D apart, they will almost instantaneously snap together to form a dimer due to magnetic dipole-dipole interaction. The magnetic dipole-dipole interaction does not come into play at larger initial distances as the force decays as $\frac{1}{r^3}$. When the spinners are embedded in a dense passive media and made active, an attractive interaction between spinners emerges. This interaction is extremely long ranged and can be felt up to 20D apart, a range which is virtually unheard of in soft matter systems. This attractive interaction emerges due to the mechanical properties of the passive media and the activity of the spinner. I illustrated this by showing that as the density of the passive monolayer decreases below a critical density, this long range attractive interaction vanishes. This critical density corresponds to when the passive monolayer no longer behaves like a solid but behaves like a fluid. The ultra-long range attractive interaction only emerges when the passive monolayer behaves mechanically as a solid. If the passive monolayer does not behave mechanically like a solid, the spinner will be unable to transmit stress to the passive monolayer which is required to yield and erode the region of passive particles between the two spinners (which we refer to as the bridge). This emergent long-range interaction also appears only when the frequency of rotation of the applied magnetic field is at least 1Hz. Below 1Hz, the passive monolayer is able to dissipate energy faster than the rate of energy input. However, at frequencies of 1Hz and larger, the passive monolayer can no longer dissipate energy fast enough so this energy must be alleviated by the collective cooperative movement of passive particles in the monolayer. This is usually manifested in the form of yielding of the passive monolayer, specifically the bridge. It should also be noted that the mobility of the passive particles in the monolayer follows activated dynamics so there should be a minimum time scale associated that is required to induce yielding events in the passive monolayer. This time scale was found to be on the order of 10-30s of continuous stress input on the passive monolayer to induce dislocation motion or yielding.

The range of this emergent interaction can also be tuned by changing the composition of the passive monolayer and changing the mode of motility of the active units. By doping the passive monolayer with 10 and 25% of dopant particles of smaller and larger sizes, the range of spinner attraction decreased by 5D. The addition of these dopant particles effectively alloys the passive monolayer. Smaller dopant particles function as interstitial defects and induce a tensile stress on the surrounding colloids (or atoms in this analogy). The larger dopant colloids produce a compressive stress on the surrounding colloids. This increase in the density of defects in the passive monolayer increases the resistance to dislocation motion and thus, reduces the probability of yielding of the bridge as the spinners are actuated. Thus, the range of interaction decreases as the passive monolayer is increasingly doped regardless of the size of the dopant. The formation of spinner dimers at distances less than 4D apart can also be eliminated by changing the mode of motility of the active units. By changing the mode of motility of the passive particles from spinners to tops, dimer formation can be prevented while still retaining the emergent long range attractive nature previously described for spinners. The extra component of the magnetic field, parallel to the normal of the dense passive monolayer, effectively serves to frustrate the alignment of the magnetic
moments of the ferromagnetic particles during dimer formation. Thus, the tops will effectively repel at distances below 4D. However, the tops will still stress and erode the passive bridge monolayer at distances above 4D and in fact, will attract at comparable distances compared to the spinners embedded in a dense passive monolayer.

This emergent interaction is not due to the magnetic nature of the active particles. I have confirmed this both experimentally and in Lattice-Boltzmann simulations as well where spinning non-magnetic active units are embedded in a dense passive monolayer. The interaction again depends on the mechanical properties of the passive medium and the activity of the spinners. Interestingly, a force reversal was observed in the simulation system depending on the rotational nature of the spinners. In the experimental system, due to the magnetic nature of the spinners, only co-rotating spinners can be investigated. However, in the simulation system, co-rotating and counter-rotating spinners can be investigated. Co-rotating spinners repel in the absence of a dense passive medium (due to secondary flows) and attract when embedded in a dense passive monolayer. Counter-rotating spinners exhibit a force reversal. Counter-rotating spinners attract in the absence of a dense passive monolayer and repel when embedded in a dense passive monolayer. The dynamics of spinner aggregation in systems with many active spinner units embedded in a dense passive monolayer was also investigated. The aggregation behavior of spinners resembles classical 2D coarsening. Interestingly, in the simulation system spinner, aggregation behavior did not appear to depend on the spinner concentrations investigated here. However, in the experimental system, it was found that there was a characteristic cluster size at which the dynamics of aggregation was maximized. Spinner clusters with a radius of approximately 45μm, minimize Stokes drag while maximizing the stress applied on the passive medium.

Finally, I investigated the transport of spinners in a crowded disordered post filled environment. I was inspired by several theoretical and artificial experimental studies that examined the emergent behavior of active matter systems under some type of confinement, either via interactions with ratchets or fixed posts or obstacles, and how these fixed obstacles or posts could affect or even change the emergent behavior exhibited by active matter systems. I was also inspired by a study of C.elegans swimming in a dense array of PDMS pillars. The efficiency and speed of C.elegans swimming was modulated by the density, geometry, and symmetry of the PDMS pillar lattice. I observed an emergent disorder induced transport of spinners embedded in a dense array of fixed posts or obstacles. In the absence of obstacles, the spinners in a Newtonian fluid are simply diffusive. However, once the spinners are embedded in a dense disordered array of fixed obstacles, the rotational symmetry of the spinners is broken and the spinners begin to move and translate across the post filled environment. In fact, the spinners exhibit super-diffusive transport regardless of the rotational frequency of the applied magnetic field. If, however, the spinner is initially embedded in an ordered array of posts, then the rotational symmetry of the system is not broken. The spinner becomes highly localized and effectively trapped, exhibiting sub-diffusive transport, behavior characteristic of a trapped particle. In this system, the spinners effectively undergo a delocalization transition. The spinners are highly localized and trapped when surrounded by an
ordered array of posts and become delocalized when the posts become disordered. Moreover, I observe this behavior in a model simulation system where the order and position of the posts can be highly controlled. When the post lattice is perfect (rectangular or hexagonal), the spinners become highly localized. Yet, once noise is inserted in the lattice, the spinners become delocalized. The transport properties of the spinners in this disordered post-filled environment can also be tuned via the addition of another external driving force, gravity, and the actuation time scale protocol utilized to actuate the spinners. Under continuous actuation and the additional driving force of gravity, the spinners approach ballistic transport. However, if the actuation protocol of the applied field is modulated, specifically by changing the actuation period of rotating the spinners clockwise and counter-clockwise, the transport properties of the spinners under the external driving gravitational force can be tuned. If the actuation period is small, less than 60s, the spinners can actually do work against the system and the transport is reduced when compared to baseline scenario of a spinner being acted on by an external gravitational force. However, if the actuation period is larger, 60s or greater, then the near ballistic behavior is recovered.

8.2 Open questions and future work

8.2.1 Design guidelines for active tribological probes and artificial experimental active matter systems

The primary motivation and goal of this thesis was the development of novel artificial experimental model systems that could effectively mimic the rich emergent non-equilibrium behavior exhibited by many vital biological systems in order to better understand the biological system of interest. In Part I, I was particularly interested in developing active tribological probes that were capable of exhibiting directed motion characteristic of biological organisms undergoing chemotaxis. I accomplished this by exploiting the concept of effective friction due to binding interactions between an object, the magnetic active tribological probes, and the substrate. The concept of friction proved to play a vital role in performing chemotaxis synthetically and from this general understanding, I have identified several features that can serve as guidelines to designing active tribological probes which are summarized in Fig. 8-1.

- **Roller size or footprint:** The effective friction between the active tribological probe and substrate is a function of the strength and density of reversible bonds between the probe and the substrate. This area of interaction is denoted as the footprint of the probe. The footprint of the probe scales with the size of the probe. Thus, the larger the footprint, the larger the density of binding site and larger effective friction felt.

- **Ligand type:** The effective friction scales with the binding affinity of the interaction of interest, so changing the interaction between the probe and surface can change the effective friction and thus, the translation of the probes on the surface. It should be noted however,
that the binding kinetics of the interaction of interest also affect the effective friction and more work needs to be done to elucidate the precise nature of this relationship.

- **Ligand density**: The effective friction scales not only with the binding affinity of the interaction but also with the density of ligands either on the probe or on the substrate. The effective friction as a function of ligand density typically takes on a sigmoidal shape with a precipitous drop over a very small range in ligand concentration.

- **Linker length/competitive binding**: An extremely long linker length will typically reduce the effective friction as will a very short linker. If the linker is too short, depending on the interaction of interest, the ligand will not exhibit enough flexibility to find the receptor of interest. If the linker is too long, it will easily find the receptor of interest. Yet, the linker may be able to find another receptor before the probe rolls and pulls on the bond, thus reducing the effective friction felt. The effective friction can also be reduced via the addition of a competitive binder, like imidazole for the metal ion-coordination binding interactions.

- **Hard or soft substrate**: The mechanical properties of the substrate can not only influence
the underlying effective hydrodynamic friction between the probe and the substrate, but can also limit the range of effective friction values that can be measured. One would anticipate a soft substrate, like a SLB, to exhibit a lower baseline value for hydrodynamic friction than a hard glass substrate. Although, in this circumstance the PC SLB baseline measurements were comparable between the soft and hard substrate. Additionally, for the soft substrate, one cannot reach extremely large rolling parameter values like those measured for the biotin-streptavidin interaction on a hard substrate because lipids can be removed from the SLB.

- **Magnetic controls**: Changing the strength of the magnetic field (B), magnetic moment of the probe (m), or the rotational frequency of the rotating magnetic field (ω), we can modulate the effective friction of the interaction of interest. Increasing the frequency of the rotating magnetic field above the time-scales associated with the $k_{on}$ rate will virtually eliminate the effective friction due to the interaction. If the magnetic field strength or the magnetic moment is too small, the torque may be too small to break the interaction as was the case for biotin-streptavidin in the tribotaxis system.

- **External controls**: The effective friction can be modulated by changing external factors like the temperature or the pH of the environment. The $k_{on}$ rate will increase as the temperature increases and this will directly change the Kd and thus, the effective friction. Changing the pH can change the amount or type of electrostatic charges in the system and this can lead to effective changes in the rolling parameter due to these electrostatic interactions.

In part II, I was particularly interested in developing hybrid active-inactive matter systems that were capable of mimicking some of the rich emergent non-equilibrium collective dynamical and phase behavior exhibited by many active matter systems. I found that a combination of activity, mechanical properties of the environment, order of the environment, composition of the environment, and mode of actuation all play a crucial role in determining the type of emergent behavior exhibited by these active matter systems. From this general understanding, I have identified several features that can serve as guidelines to designing hybrid active-inactive soft matter systems as summarized in Fig. 8-2.

- **Active unit size and shape**: The size and shape of the active agent can determine the emergent behavior observed in the active system, particularly if the shape is anisotropic. In particular, the shape can lead to flocking or clustering depending on the shape of the active unit.

- **Active unit concentration**: Typically, exotic emergent non-equilibrium behavior emerges when the concentration of active units increases beyond a critical threshold value. In the systems investigated in this thesis, the emergent behavior changes depending on the concentration of active spinners from the formation of dimers to clusters of spinners aggregating and attracting in a coarsening-like fashion.
Figure 8-2: A variety of different parameters can be modulated or multiplexed to design future soft synthetic active matter systems.

- **Motility**: The mode of motility can change the emergent behavior of active matter systems. Spinners will form dimers and clusters when embedded in a dense passive environment. However, if the motility is changed from spinning to tops, the magnetic dipole-dipole interaction at small distances is removed and the tops will attract to form very loose active associations but will no longer form tight clusters of purely active components.

- **Mechanical properties of environment**: With active spinning units, emergent behavior is only observed when the mechanical properties of the passive medium transitions from behaving like a Newtonian fluid to behaving mechanically more like a solid. The emergent behavior depends on the ability of the spinners to erode the passive medium. Hence, this interaction can also be modulated by inserting dopant passive particles of different sizes to impede dislocation motion and thus, reduce the interaction range of the spinners by making it more difficult to erode the passive monolayer.

- **Actuation protocol**: To erode the passive monolayer, the spinners must be able to stress the passive monolayer continuously in order to induce dislocation motion. By changing the actuation protocol, specifically the period of continuous actuation in one direction, the emergent attraction can be eliminated. Similarly, changing the actuation protocol can change the transport properties of spinners surrounded by a disordered array of fixed posts or obstacles.

- **Magnetic controls**: Many emergent behaviors depend on the level of activity of the active units. Here, this is most easily changed by changing the frequency of rotation of the applied
magnetic field or possibly in the future, by the strength of the magnetic field.

- **Environment**: The environment plays a vital role in determining the emergent behavior of the active system. In a dense passive environment, we observed attraction. In a disordered environment filled with fixed obstacles, disorder induced transport was observed.

These are just several ways that these soft experimental active matter systems can be tuned in order to observe different emergent non-equilibrium behavior and better understand the fundamental physical phenomenon driving this behavior in the more complicated biological system of interest. However, beyond these design guidelines, there are several outstanding questions that must be solved and several open questions that must be addressed in order to better understand if the findings presented in this thesis can be extended directly to biological systems.

### 8.2.2 High throughput binding assay and moving to 3D in-vivo

Perhaps the most important remaining open question, as it pertains to the work done in Part I, is further developing the active tribological probe system to function as a high throughput binding assay. More specifically, to develop this technique so that a relationship between the rolling parameter and quantitative $K_d$ values, as well as $k_{on}$ and $k_{off}$ rates, can be measured directly using this technique. The preliminary work done with theory and simulation on this system suggests that the rolling parameter scales proportional to the logarithm of the $k_{off}$ and linear on the $k_{0}$.

However, these results are preliminary and more work must be done to further develop the model to obtain quantitative numbers for the binding affinity and binding kinetics, or at the very least to guide us in determining what sweep of experiments must be conducted to investigate these parameters. This is not a trivial problem to solve. Imagine that we were able to obtain three different ligand-receptor interactions with three different affinities ranging from micro-molar to pico-molar, all of which were extremely well characterized, the binding affinities and kinetics known and reproducible using a variety of different measurements (this is almost never the case), and these interaction do not exhibit any mutations that could change the binding affinity. One might deduce that the binding kinetics can be measured by doing a series of frequency sweeps and observing the change in the rolling parameter, specifically when the effective friction returns to the baseline value. Yet, thus far this does not seem to be the case. In fact, it appears that the binding affinity information may lie in how the rolling parameter decays but extracting numerical values from this decay is unclear. One might also deduce the binding affinity by rolling a fully coated probe on a fully coated substrate. However, this measurement can be misleading as well. It is very difficult to do an apples to apples comparison because the size of the ligand-receptor pairs are almost always different. As such, the density of sites on the substrate and the probe will fluctuate and therefore, the concentration will not always be consistent. The binding affinity and thus the rolling parameter is always dependent and inextricably linked to the concentration of ligand-receptor pairs. Even measuring $k_{off}$, which should be easily accomplished by doing a sweep of magnetic field strength, again depends on this concentration and getting numerical values is not
immediately intuitive. Thus, it is vital to further develop this binding model in order to find the correct series of experiments that can be run in conjunction to obtain numerical values for binding affinity and binding kinetics.

Another open question and a natural progression or extension of the tribotactic walkers system is investigating the ability to perform tribotaxis in a functionalized 3D collagen-like matrix. Now that I have developed a 3D magnetic coil apparatus, the tribological probes can be made to move in 3D. This should be done first in a simple, transparent gelatin matrix with different mechanical properties to ensure the probes can move in such a system. In fact, one can easily test if the probes can perform durotaxis (a process by which directed cellular motion is determined by gradients in the mechanical properties of the substrate) by creating a gel that exhibits an anisotropic Young's moduli [289, 290, 291, 292, 293, 294]. By randomly rolling the probes in the three principal directions, the probes should exhibit directed motion or drift along the direction with the largest stiffness. In doing so, they can perform durotaxis in a similar manner to cells. Regardless, once motion is established in the model gelatin network, the complexity of the system can be increased by moving to a collagen network (to mimic the ECM) which can then be functionalized with many different types of receptors, ligands, or cytokines as seen in Fig. 8-3 [295, 296].

Figure 8-3: A) Schematic of lymphocytes in ECM. B) Investigating tribotactic drift in 3D ECM due to gradients in IL2 receptors.
Gradients in the density of these receptors can be created using simple microfluidic devices and by performing a 3D random walk. The probe should exhibit directed or net motion in the direction where the density of binding sites is largest. These experiments can be conducted on a number of different ligands, receptors, and cytokines that are crucial to cells when performing chemotaxis and we can confirm if friction does indeed still play a role in this more complex system. Finally, we can even move in vivo by using a well characterized and understood model organism that is also transparent, C.elegans. The probes can be inserted in the C.elegans intestine and functionalized with lectins, or a corresponding receptor, that binds to the lining of the intestine to explore if the probes can again either perform chemotaxis or translate within the intestine and between the tissues. This can actually be done quite simply as C.elegans have been shown to be able to digest the type of magnetic beads that we utilize as probes and still survive [297, 298, 299, 300, 301]. Such an experiment would serve as an excellent in vivo test and it would be interesting to see if we could induce behavior in the biological organism that was not previously accessible in the absence of the synthetic soft active matter probes.

8.2.3 Incorporating biological components in hybrid active matter systems

In terms of open questions in the realm of active matter systems, imagination and the ability to design and develop novel active units is the only limitation. The 3D magnetic coil apparatus can enable virtually any mode of motion desirable. However, resources must be allocated toward the development of active units in house. The emergent behavior of active systems is related to the size and shape of the active agent. Being able to produce non-interacting, magnetically driven units of different shapes and sizes is crucial. It can be accomplished by adapting the techniques utilized to create patchy colloids. However, presently we have yet to fully develop and integrate such techniques in order to create our own active units. In doing so, we can confirm if some of the exotic emergent states predicted by purely theoretical simulation systems are experimentally achievable.

Personally, the more interesting open question to address in active matter is taking the fundamental insights gained from investigating the simplified model synthetic system and applying those concepts to more complex systems with biological components. Or to be more specific, to integrate some biological components, either active or inactive, into the synthetic systems and observe whether the same emergent non-equilibrium behavior is observed. For example, if the dancing volvox algae was embedded in a dense passive environment does an emergent attractive interaction emerge between the algae? The algae spin in place and exhibit the same mode of motility as the magnetically actuated spinners. So, if emergent attractive interaction depends on activity and the mechanical properties of the medium, we should observe some degree of emergent attraction between algae. Similarly, it would be extremely interesting to replace the spinners as the active unit in a disordered post filled environment with C.elegans to observe if the same order induced localization and disorder induced delocalization is observed with C.elegans, as was observed with the spinners. The same essential components are there, the hydrodynamic interaction with the posts
and breaking of the symmetry, but there are several other factors with C. elegans to consider as well. We could also insert synthetic components in the biological system of interest and observe whether new emergent behavior can be observed. Can we induce clustering of volvox algae, spinners, and subsequent non-equilibrium phase segregation between active and passive components? Can percolating cell swarm networks be formed by inserting fixed inactive posts randomly throughout the environment? There are any number of hybrid active matter systems that can be developed which combine both synthetic and biological components. These systems could prove crucial in helping to determine what emergent behavior is due to purely physical phenomenon and what behavior requires biological/biochemical stimuli.
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