

Nanoparticle Superlattice Processing: Monodispersed Building-Blocks & Single Crystal Films

by

Perapat Pete Gatenil

Submitted to the
Department of Materials Science and Engineering
In Partial Fulfillment of the Requirements for the Degree of

Bachelor of Science

at the

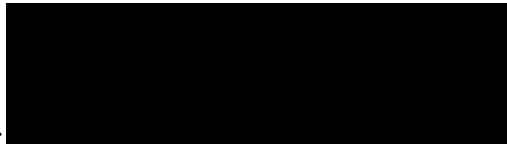
Massachusetts Institute of Technology

May 2023

©Perapat Pete Gatenil
All Rights Reserved

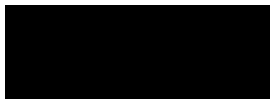
The author hereby grants to MIT permission to reproduce and to distribute publicly paper and electronic copies of the thesis document in whole or in part in any medium know or hereafter created.

Signature of Author.....



Department of Materials Science and Engineering
05/12/23

Certified by.....



Robert Macfarlane
Associate Professor of Materials Science and Engineering
Thesis Supervisor
05/19/23

Accepted by.....

James LeBeau
Class of '23' Associate Professor of Materials Science and Engineering
Chairman, Undergraduate Thesis Committee

Nanoparticle Superlattice Processing: Monodispersed Building-Blocks & Single Crystal Films

by

Perapat Pete Gatenil

Submitted to the Department of Materials Science and Engineering on May 12, 2023 in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science in Materials Science and Engineering

ABSTRACT

Nanocomposite tectons (NCTs) offer a versatile method for systematically modulating material properties at the nanoscale. These building blocks are created by grafting nanoparticles with end-terminated polymers comprising supramolecular binding groups, akin to DNA base pair hydrogen bonding. This approach enables the precise organization and assembly of NCT units into macroscopic materials. By adjusting nanoparticle core sizes, polymer brush lengths, and molecular recognition groups, NCTs can be tailored to produce superlattices with controlled crystalline structures, lattice spacing, and geometries. These superlattices have potential applications in photonics, plasmonics, and microfluidics. While NCTs can be produced in bulk scale, processing these materials into useful devices remains challenging. One major hurdle is the random aggregation of crystals during assembly, which makes it difficult to identify the properties of single NCT crystals. This study investigates methods to process NCTs and their assemblies into monodisperse crystallites and oriented crystal films, using density gradient centrifugation and scratch-directed assembly, respectively.

Density gradient centrifugation allows for the separation of NCT assemblies, comprising various-sized crystallites, into discrete monodisperse distributions. This technique was applied to NCTs composed of gold nanoparticles (AuNPs) and iron oxide nanoparticles (IONPs), grafted with a polystyrene (PS) brush end-terminated with either diaminopyridine (DAP) or thymine (Thy) – which serve as a complementary hydrogen bonding pair. Scanning electron microscopy (SEM) analysis of drop-casted assemblies demonstrated the effectiveness of density gradient separation in processing NCT crystallites into size-specific distributions while revealing a crucial relationship between crystallite size and shape.

Heterogenous nucleation provides a means for directing assembly in more controlled ways, for example via nano scratch-directed assembly. SEM analysis of scratched substrates revealed that NCTs in contact with scratches exhibit strong alignment and have the potential to form larger single-crystalline superlattices through oriented attachment.

Thesis Supervisor: Robert Macfarlane

Title: Associate Professor of Materials Science and Engineering

ACKNOWLEDGEMENTS

I would like to thank Professor Robert Macfarlane and his lab group for their support throughout my time doing undergraduate research. My gratitude goes to Theodore (Ted) Hueckel and Matthew Ye for their supervision and guidance for my project and training me on all the instruments and concepts for this project. I am also appreciative of Griffen Desroches for being my first graduate mentor in the Macfarlane lab. In addition to my lab group members, the lab instructors and professors I have had at MIT have all been vital to my education, skills, and future pursuits and cannot thank them enough. I would like to thank my peers and specifically Samuel Song, Amena Khatun, and Ningxin (Charlotte) Chen for persevering with me through our studies in materials science. Lastly, I would like to thank my parents and sister for their continuous support and love to get me through my four years at MIT.

TABLE OF CONTENTS	
ABSTRACT	2
ACKNOWLEDGEMENTS	3
TABLE OF CONTENTS	4
TABLE OF FIGURES	5
INTRODUCTIONS	6
<i>Supramolecular Interactions</i>	6
<i>Nanocomposite Tectons</i>	8
EXPERIMENTAL DESIGNS AND METHODS	12
<i>NCT Synthesis</i>	12
<i>UV-Vis Spectroscopy</i>	13
<i>DAP-Thy NCT Assembly Through Thermal Annealing</i>	13
<i>Solvent Exchange and Drying</i>	13
<i>Substrate Scratching</i>	14
<i>Substrate Growth</i>	14
<i>Density Gradient</i>	14
<i>SEM of NCT Assemblies</i>	15
RESULTS AND DISCUSSION	16
<i>NCT Assembly</i>	16
<i>Density Gradient Separation of NCT Crystallites</i>	17
<i>Scratch Directed Growth of NCT Winterbottoms</i>	28
CONCLUSION AND FUTURE WORKS	30
WORKS CITED	33

TABLE OF FIGURES

Figure 1. Crystal packing structures of various NCT systems	7
Figure 2. Assembly of DAP-Thy NCTs into Rhombic Dodecahedron Wulff Polyhedra	8
Figure 3. Effects of temperature cycles on assembly of NCTs	10
Figure 4. IONCTs assembling to form pyramidal shaped crystallites	11
Figure 5. Assembly of AuNCTS to form superlattices	17
Figure 6. Stabilization of NCT assemblies using Pluronic F-127	19
Figure 7. Assembled IO-NCTs running through NaCl density gradient at 1 r.c.f. over 180-Minutes	20
Figure 8. Optical microscopy images of NCT superlattices from top and bottom layers of – density gradient centrifugation	21
Figure 9. Size comparison of NCT assemblies before and after density gradient-Centrifugation	22
Figure 10. Crystallite structures formed from identical NCT building blocks	23
Figure 11. Assembled IO-NCTs of different sizes and shapes running through NaCl density-gradient at 1 r.c.f. over 150 minutes	24
Figure 12. Size comparison of crystal shapes that were separated using density gradient-Centrifugation	25
Figure 13. Assembled AuNCTs of different sizes and shapes running through NaCl density-gradient at 1 r.c.f. over 180 minutes	26
Figure 14. SEM of AuNCT assembly that became disordered after density gradient-Separation	27
Figure 15. Assembly of NCTs on silicon substrates	28
Figure 16. Distribution of crystal orientations with respect to scratch angles	29

INTRODUCTION

This project focuses on evaluating density gradient centrifugation and scratch-directed assembly as methods to process NCT assemblies into monodisperse building blocks and single-crystal films. As nanoparticle-based nanocomposites research is still in its early stages, much remains to be understood about the factors affecting and enabling controlled, large crystal growth with minimal variation between crystallites. Separating NCT assemblies into distinct shapes and sizes of superlattices enables applications that require size-specific building blocks. These monodisperse sets can be utilized to sinter crystallites together, forming sintered materials with uniform grain sizes. Grain size can alter the resulting material's mechanical properties and mass transport capabilities.¹ Developing a streamlined method for creating thin films could lead to nanocomposite coatings that exploit nanoscale phenomena, such as plasmonic or magnetic coupling.^{2,3}

Supramolecular Interactions

Supramolecular bonding groups are key components that facilitate the programmable ordering of NCTs. These non-covalent, reversible bonding groups can be manipulated independently of the polymer brush or the NCT core, making them ideal candidates for nanoparticle-based composites.⁴ Similar recognition groups can be observed in nature, such as DNA's Watson-Crick interactions, which have specific pairings that can dissociate and reform during replication or transcription.⁵ The dynamic nature of these bonds allows NCT assembly to be triggered or controlled by external stimuli, such as heat or pH, making nanocomposites versatile and responsive to their environment.²

The structure of an NCT assembly can be controlled by the type of supramolecular moiety used in a set of NCTs. Self-complementary groups, such as Thy-Thy bonds or self-complementary DNA sequences, tend to form close-packed face-centered cubic (FCC) lattice structures, as these maximize the packing of the NCTs and the complexes formed.^{2,6} In systems with complementary binding groups like Thy-DAP interactions, body-centered cubic (BCC) structures are favored, as this arrangement maximizes the number of complementary nearest neighbors. The choice of supramolecular moiety thus plays a crucial role in determining the final structure of the assembled NCTs.²

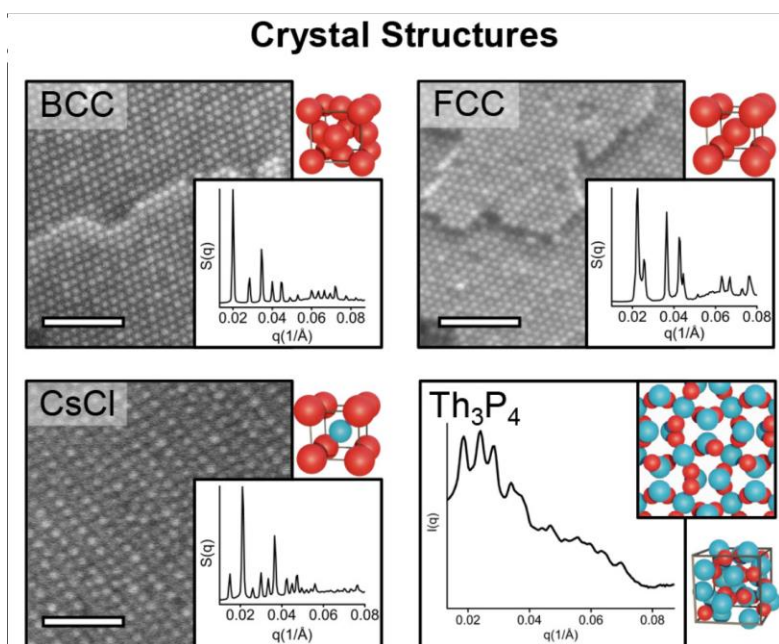


Figure 1. Crystal packing structures of various NCT systems. Depending on the supramolecular recognition group used to program assembly of the NCTs, different crystal packing structures can be achieved such as BCC, FCC, CsCl, and Th₃P₄. Courtesy of *Journal of the American Chemical Society*.⁴

Nanocomposite Tectons

NCTs are nanoparticle-based building blocks for nanocomposites, consisting of a nanoparticle core, a surrounding polymer brush, and a supramolecular moiety terminating each polymer. The core provides a sturdy foundation, determining the fundamental geometry and size of the NCT.⁷ The polymer brush functions as a deformable shell, controlling the minimum spacing between NCTs in an assembly. As mentioned earlier, the supramolecular groups surrounding the NCTs drive their assembly and packing to maximize favorable hydrogen bonding.² Each component of the NCT can be manipulated independently, making the resulting assembled nanocomposite highly tunable. In this study, NCTs are composed of AuNPs or IONPs and either a PS-Thy or PS-DAP brush, as seen in Figure 2a. In systems with binary bonding groups like Thy and DAP, NCTs assemble to form BCC symmetric unit cells, which ultimately form rhombic dodecahedron-shaped Wulff polyhedra (See Figure 2b).^{2,4,8}

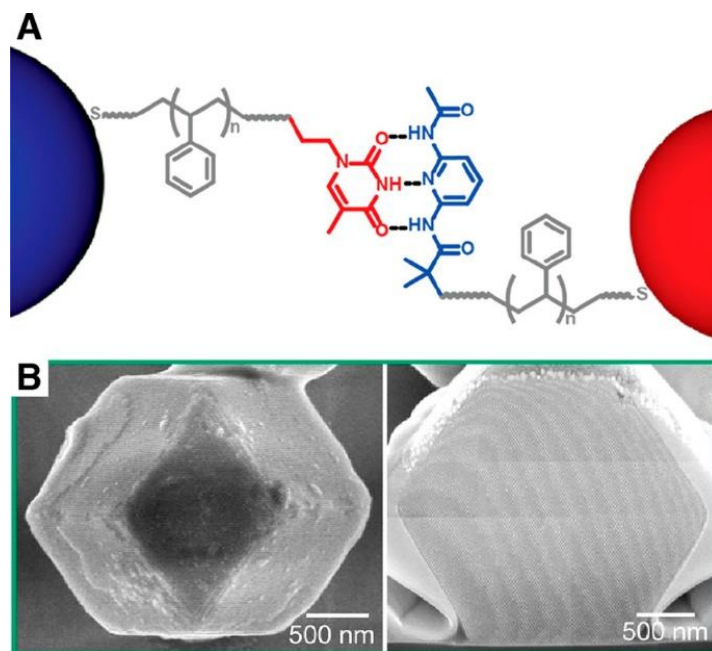


Figure 2. Assembly of DAP-Thy NCTs into Rhombic Dodecahedron Wulff Polyhedra. (A) NCTs are formed from a inorganic nanoparticle core with a PS brush that is end terminated with a supramolecular bonding group. A Thy functionalized NCT (Left) is complexing with DAP

functionalized NCT (right). (B) SEM of single crystalline, faceted crystallites. Courtesy of *Journal of the American Chemical Society*.⁴

The composition of individual NCTs makes them robust units for nanocomposites. In typical colloidal crystallization, low dispersity is crucial for ensuring long-range order in the resulting crystal; however, NCTs with high dispersity in both polymer brush length and nanoparticle core size can assemble into superlattices with long-range order.^{1,7} Although variations in polymer brush length and core size are expected to cause amorphous materials with short-range order due to insufficient packing, the inherent flexibility of the NCT polymer brush allows it to stretch and compress to maximize bonding pairs, further supporting crystallization.¹

The presence of hydrogen bonds and polymer flexibility aiding self-assembly of NCTs means that processing conditions of the assembly can influence the crystallites formed. By controlling the temperature of an NCT system, the hydrogen bonds between recognition groups can be forced to associate or dissociate (see Figure 3), ultimately controlling factors like crystallite growth rate or nucleation events.⁷ Previous work on the thermal hysteresis of NCTs and the resulting crystallites has shown that simply holding NCTs at a temperature just below the melting temperature (T_m) results in polycrystalline crystals with relatively small diameters of approximately 250 nm.¹ The polycrystallinity and small size are attributed to disordered crystallites maintaining a similar number of DAP-Thy bonds as perfectly ordered crystallites and a broad transition temperature.¹ A slow cooling rate through the transition range was used to decrease the number of nucleation events while allowing enough time for particles to assemble in an ordered fashion (see Figure 3d).¹

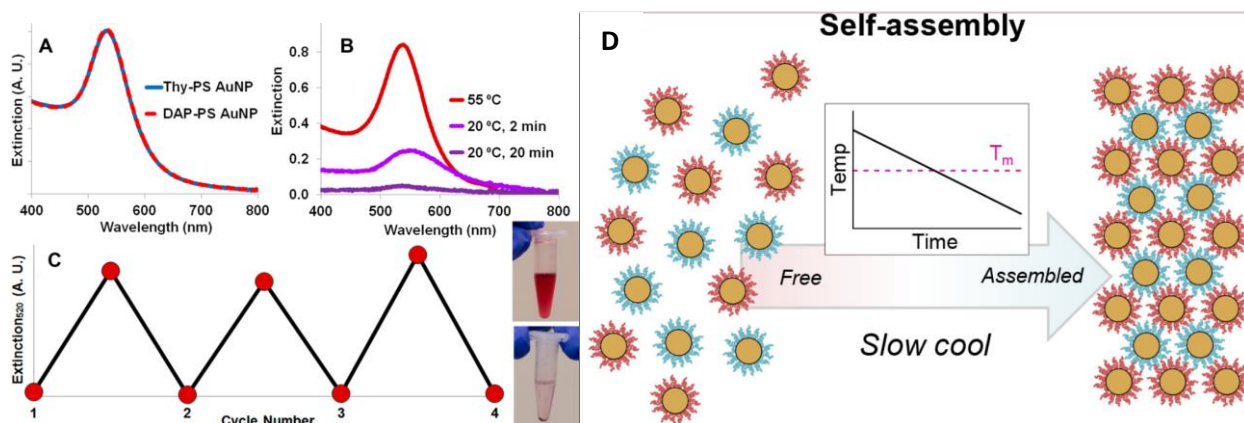


Figure 3. Effects of temperature cycles on assembly of NCTs. (A) UV-Vis spectrum of DAP and Thy AuNPs before assembly. (B) Cooling of a collection of DAP and Thy AuNPs at different temperatures and time points. Cooled NCT systems result in assembly and a drop in extinction coefficient of suspended NCTs. (C) Extinction coefficients can freely alternate between non-assembled suspension of NCTs and assembled NCTs through multiple thermal cycles. (D) Ramping down through the melting point of NCTs results in crystalline assembly of NCTs. Courtesy of *Journal of the American Chemical Society*.^{4,7}

The polymer brush also serves to control crystallites post-assembly. The superlattice spacing is dictated by the average brush length of the NCTs, which is entropically controlled by the number of available conformations the polymer has when its supramolecular end group is complexed to a complement and enthalpically controlled by the interactions with the polymer matrix. In previous work, drying out NCTs for characterization is a standard process where an antisolvent/unfavorable solvent is slowly introduced, causing the polymer brush to collapse and the crystallites to shrink. However, contracting the polymer too quickly results in a loss of crystallinity.⁹ Conversely, NCTs can be swelled with a favorable solvent, causing the polymer brush to swell up and the crystallite lattice spacing to grow.

Because NCTs assemble into ordered superlattices, it is possible to apply similar crystallographic concepts of heterogeneous nucleation and crystal growth to these systems to grow large, oriented crystals. Previous work has demonstrated the effectiveness of scratched

substrates in supporting nucleation, directing assembly, and aligning systems of liquid crystals and cells.^{10,11} Furthermore, substrates can be functionalized with recognition groups specific to NCTs to direct the formation of Winterbottom crystals, which account for both NCT-NCT and NCT-substrate interactions.² The surfaces from which Winterbottoms heterogeneously assemble can be functionalized with either one or both complementary groups to NCTs.² When both recognition groups are present on the substrate, the Winterbottom formed is truncated along the $\langle 110 \rangle$ plane of the Wulff polyhedron to form a flat-topped diamond structure. When only one group (either Thy or DAP) is present on the substrate, the Winterbottom formed is truncated along the $\langle 100 \rangle$ plane.

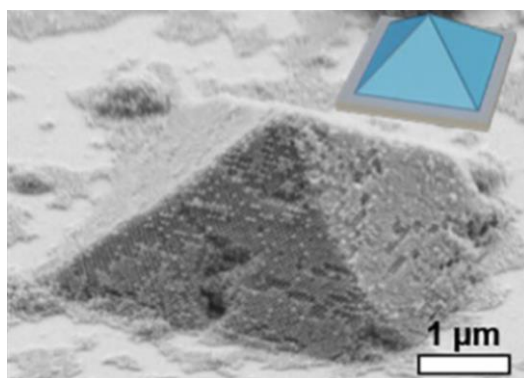


Figure 4. IONCTs assembling to form pyramidal shaped crystallites. Square Pyramidal shaped NCT Winterbottom formed from heterogeneously nucleating on a Thy-PS functionalized Silicon Substrate. Courtesy of *Journal of the American Chemical Society*.⁷

Understanding the kinetics of the assembly process is essential as well. By controlling growth conditions, such as temperature, solvent quality, or concentration, it is possible to tailor the crystal growth to achieve desired morphologies and sizes. This can lead to equilibrium Wulff polyhedra or more exotic stellated polyhedra, which arise due to rapid growth in the $\langle 100 \rangle$ vector. However, the growth observed in these systems cannot be simply explained as analogous to molecular systems, particularly because the expected growth trends are reversed. This makes

it critical to directly observe the properties of stellated crystals to determine their mechanism of formation.

NCTs offer a versatile approach for the creation of tunable nanocomposite materials with a high degree of control over their properties. By understanding the assembly mechanisms and optimizing processing techniques such as density gradient centrifugation and scratch-directed assembly, it is possible to produce monodisperse crystallites and single crystal films with desired properties. Further research in this area will help unlock the full potential of NCT-based nanocomposites in various applications.

EXPERIMENTAL DESIGNS AND METHODS

NCT Synthesis

In two 50 mL Falcon tubes, 7.2 and 7.4 mg of DAP-PS-SH and Thy-PS-SH were added to two 12 mL of acetone, respectively. To each tube, 12 mL of a nanoparticle suspension was added and immediately shaken on a vortexer for 30 minutes to avoid aggregation and ensure proper functionalization. The NCTs were purified by centrifuging first at 6000 r.c.f. for 2 min and resuspended in 4 mL of DMF. Each tube was then split into four 1.7 mL Eppendorf tubes totaling to four DAP-PS-AuNP and four Thy-PS-AuNP tubes. The NCTs were then cleaned again this time by spinning for 50 minutes at 9.5k rpm and recondensed into DMF twice. After the second DMF rinse, four DAP and four Thy particles were condensed down and resuspended into one 1mL tube of DAP and Toluene and one 1mL tube of Thy and Toluene. Each tube was spun down at 10.25k rpm for 55 minutes before resuspending in 800 uL of Toluene.

UV-Vis Spectroscopy

10 uL of DAP-PS-AuNP and Thy-PS-AuNP were each added to 190 uL of DMF to create a 40x dilution. UV-Vis spectroscopy was taken of each suspension with DMF as the baseline. Peaks were compared to obtain the optical dispersity and concentration of each suspension.

DAP-Thy NCT Assembly Through Thermal Annealing

After concentrations of NCT solutions were measured with UV-Vis spectroscopy, DAP and Thy-NCTs solutions were mixed together in 1:1 mole ratio to reach a final total volume of 500 uL. The sample was then heated to 65°C and cooled to 25°C at a cooling rate of .1°C/min in a thermal cycler.

Solvent Exchange and Drying

Directly drying NCTs in a favorable solvent results in loss of crystallinity from rapid polymer contraction. To prevent loss of crystallinity, slow collapse of the polymer is done by slowly adding in an antisolvent. The supernatant is replaced 3 times with toluene and a final 4th time in 100 uL of toluene. N-decane, a non-solvent, is gradually added to increase the content of n-decane by 10% (v/v) every 15 minutes. After reaching ~100% n-decane, the solvent is completely replaced with pure n-decane. Once pure n-decane is added, the solvent can be dried out without loss of crystallinity in the NCT assemblies.

Substrate Scratching

Diamond lapping paper was taped in place onto a flat top counter. A silicon wafer was placed above the lapping paper with a 5 kg weight resting on top. The wafer was slowly dragged across the lapping paper in one continuous motion without lifting the weight.

Substrate Coating and Functionalization

Substrates were coated in a thin layer of gold to create a surface with thiol groups. Substrates were then submerged into a Thy-PS solution for 24 hours to coat with supramolecular Thy groups.

Substrate Growth

Functionalized substrates were added to Thy-PS-AuNP and DAP-PS-AuNP solutions and thermally annealed with same cooling conditions for standard NCT assembly

Density Gradient

NCT separation into distributions of monodisperse crystallites was done using density gradient separation. 300 μL of 0.2% Pluronic F-127 solution were added to the assembled NCTs and subsequently sonicated for 30 minutes to break up any aggregates and stabilize the suspension of crystallites. Excess F-127 was removed by allowing crystallite suspension to settle down overnight and removing supernatant. After supernatant was removed, the pellet was vortexed and sonicated to ultimately resulting in a 100 μL suspension of NCT superlattices.

Five solutions for the density gradient were prepared using a 0.1% Pluronic F-127

solution and varying concentrations of NaCl leading to 6.0M, 5.4M, 4.8M, 4.2M, and 3.6M NaCl and 0.1% F-127 solutions. To create the density gradient, 2 mL of each solution was slowly pipetted onto into a 15 mL centrifuge tube where the NaCl concentration decreased from the bottom to the top of the centrifuge tube. After the layers of the gradient were pipetted into the centrifuge tube, 100 μ L of the Pluronic F-127 and NCT crystallites suspension was gently layered on top of the density gradient. The density gradient separation was then run using only gravity (1 g). After 150 minutes, layers of the density gradient were separated by slowly pipetting 1.5 mL from the top, followed by pipetting 0.5 mL layers, and lastly pipetting the bottom 1.5 mL layer into different 1.7 mL Eppendorf tubes.

Once separated, Eppendorf tubes were centrifuged for 15 minutes at 12 rcf and the supernatant was removed. DI water was then readded and this process was repeated three more times to remove excess salt. After washing away NaCl from the NCT assemblies, the crystallite suspensions were casted onto formvar-coated SEM grids for SEM characterization.

SEM of NCT Assemblies

Samples for SEM were prepared by drop costing collapsed NCTS onto a silicon wafer and allowing either the water or n-decane to evaporate depending on the solvent. Water was the solvent when NCTs were dropped through a density gradient while n-decane was the solvent just after assembly and collapse of NCT crystallites. SEM was conducted with a current of 1.0nA and accelerating voltage of 1.0kV.

RESULTS AND DISCUSSION

This project will be divided into three sections to discuss the independent results. The first section will cover the assembly of NCTs with different core compositions, demonstrating the formation of polydisperse crystallites. Characterization methods such as SEM, TEM, GPC, and UV-Vis will be used to study the individual components of NCTs, suspensions of NCTs, and assemblies of NCTs. The second section will evaluate the effectiveness of salt-based density gradient separation on refining the dispersity of assembled crystallites, using UV-Vis, optical microscopy, and SEM. In the third investigation of scratch-directed assembly, crystallite size and orientation will be compared under different scratching densities and characterized with SEM.

NCT assembly

NCTs were synthesized by functionalizing sets of AuNPs or IONPs with PS-Thy and PS-DAP. After functionalization, NCTs were resuspended in organic solvents such as Dimethylformamide (DMF) and Toluene, and excess polymer was washed away via centrifugation. The suspensions were characterized using UV-Vis to obtain their optical dispersities as measures of concentrations.

From the UV-Vis data, DAP and Thy coated NPs can be mixed in 1:1 stoichiometric ratios and allowed to assemble. When allowed to assemble, NCT superlattices would precipitate and sediment at the bottom, while the color of the solvent got clearer, indicating that the colloidal concentration was dropping (Figure 6b). The assembly was placed into PCR to thermally anneal and formed ordered crystallites, which were subsequently collapsed by slowly adding in a non-solvent to ensure crystallization was maintained. The superlattices were characterized with SEM to determine size distribution (Figure 6c).

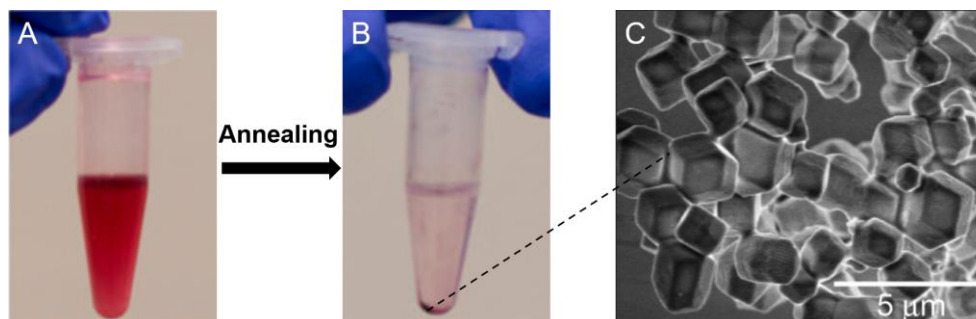


Figure 5. Assembly of AuNCTS to form superlattices. (A) Solution of un-assembled DAP and Thy NCTs. (B) Solution of assembled NCTs after annealing. (C) SEM of NCT assembly. Courtesy of *Journal of the American Chemical Society*.⁷

SEM of NCT assemblies shows that crystallites varied in size and aggregated together, forming grain boundaries. Although annealing is expected to yield large single crystalline superlattices and limit nucleation events, assembly of NCTs causes the particles to settle out of solution faster and sit at the bottom of the tube. Here, it is exposed to a lower concentration of free NCTs, and growth of the crystallites halts while new ones are formed above. The sedimentation of crystallites causes the NCTs to form clumps whose surfaces can still interact and bind to each other but without the energy necessary to reorient and maximize bonding. This ultimately drives aggregation and limited size control of crystallites.

Density Gradient Separation of NCT Crystallites

Previous work has demonstrated that centrifuging clusters of particles through a centrifugation gradient can separate clusters based on their relative size, mass, and surface area, which affect their sedimentation velocity.¹² An object's sedimentation velocity is directly correlated with its hydrodynamic radius and density as indicated by the following equation:

$$V_s = \frac{gd^2(\rho_{particle} - \rho_{medium})}{18\mu}$$

The density gradient can be tailored to match a set of NCTs, so that smaller crystallites with a slower sedimentation velocity remain in the upper region of the gradient, average-sized crystallites remain in the middle of the gradient, and larger crystallites sediment out faster at the bottom of the gradient. The more disparate the sizes, the more stark the separation between differently sized crystallites, and the finer the separation, even for crystallites with closely matched sedimentation velocities.

Before setting up a density gradient, aggregates of NCT superlattices must be broken up to stabilize a suspension of individual crystallites. This was done by adding Pluronic F-127, a triblock copolymer consisting of a central hydrophobic block of polypropylene linking two hydrophilic blocks of polyethylene glycol, to the suspension and sonicating in order to break apart clusters while coating crystallites in surfactant. The hydrophobic portion of the polymers lined the surface of the crystallites while the hydrophilic portions would extend out from the crystallites, which stabilize the polystyrene crystals in water and prevent them from aggregating or sticking to tube walls (see figure 1)

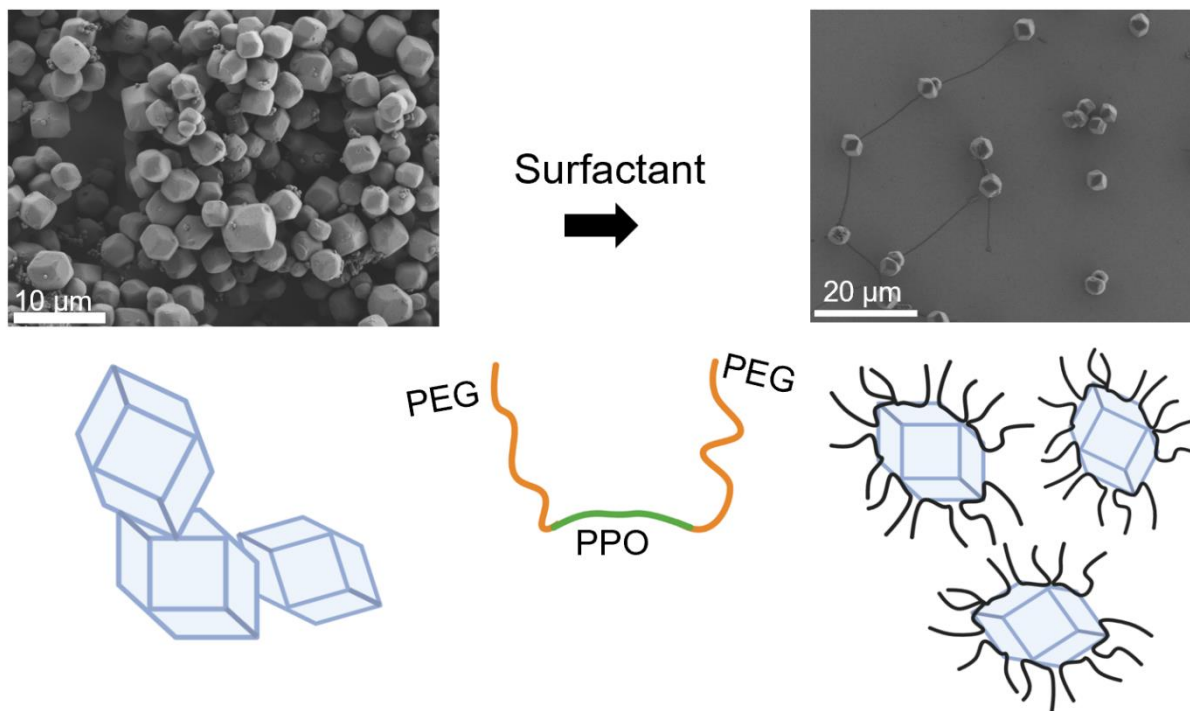


Figure 6. Stabilization of NCT assemblies using Pluronic F-127. NCTs aggregate after assembling and settling at the bottom (Left). Breaking apart aggregates and coating NCTs with surfactant results in a stable suspension of NCTs that are less prone to forming clusters and sticking to side walls (Right)

The density gradient separation for IONPs was performed using solutions of NaCl and F-127 in water. A portion of stabilized crystallites was added above the density gradient and allowed to sediment under gravity. The time evolution of the separation is shown in Figure 8:

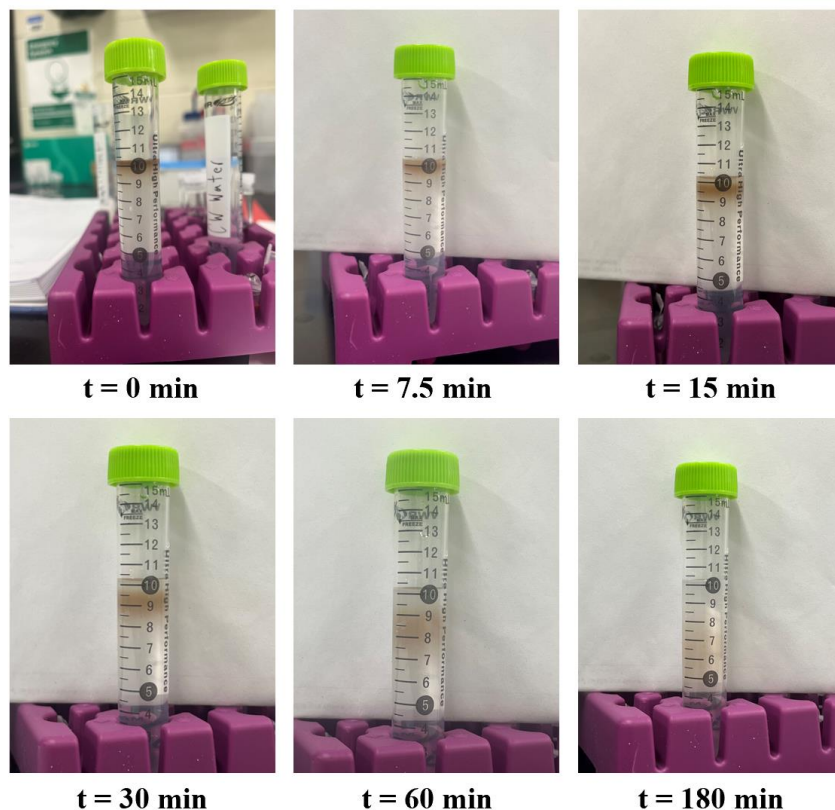


Figure 7. Assembled IO-NCTs running through NaCl density gradient at 1 r.c.f. over 180 minutes. Time stamps ($t = 0, 7.5, 15, 30, 60,$ and 180 min) are labeled for each image.

This separation resulted in a wide band instead of distinct bands for groups of monodisperse crystals. This indicates that the dispersity of crystallites ranged continuously from the largest to the smallest, rather than forming distinct distributions (unimodal, bimodal, etc.). The broadened band also confirms that NCT assemblies are consistent with Stokes' law. Although crystallites have the same density, differences in size led to variations in their hydrodynamic radii and Stokes' velocities.

The gradient was separated into 12 layers from top to bottom for characterization. Optical microscopy revealed a visible size difference between NCT superlattices from the top half of the gradient and those from the bottom half (Figure 9).

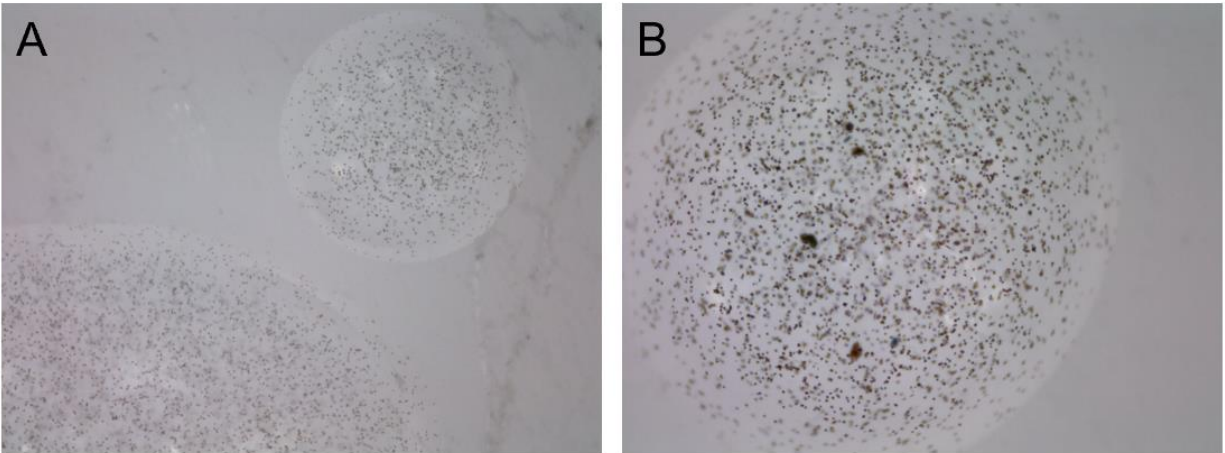
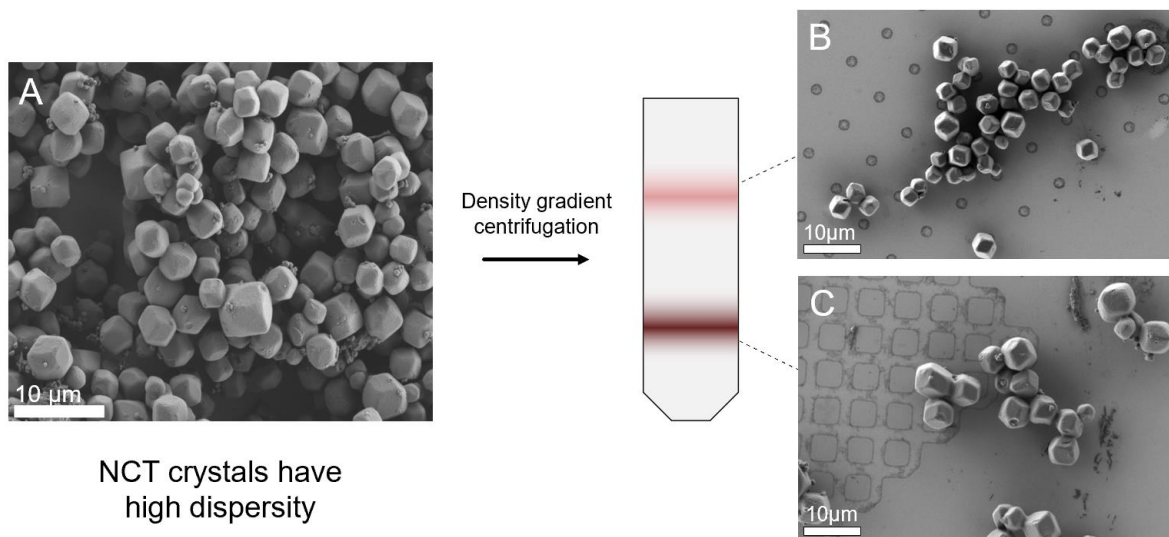


Figure 8. Optical microscopy images of NCT superlattices from top and bottom layers of density gradient centrifugation. (A) Top layer drop casted showing suspension of crystallites. (B) Bottom layer drop casted showing suspension of slightly larger crystallites.

To gain further insights into size-based separation using density gradients, SEM imaging was performed on different layers (Figure 10). The SEM images of crystallites that sedimented at the same height after 180 minutes were monodispersed. A comparison of crystallite sizes between layers revealed that crystallites from one layer were about XX times the size of those from another layer. This confirms the density gradient as an effective tool for refining assembled superlattices into monodisperse building blocks, which can then be used in size-specific applications and devices.



NCT crystals have high dispersity

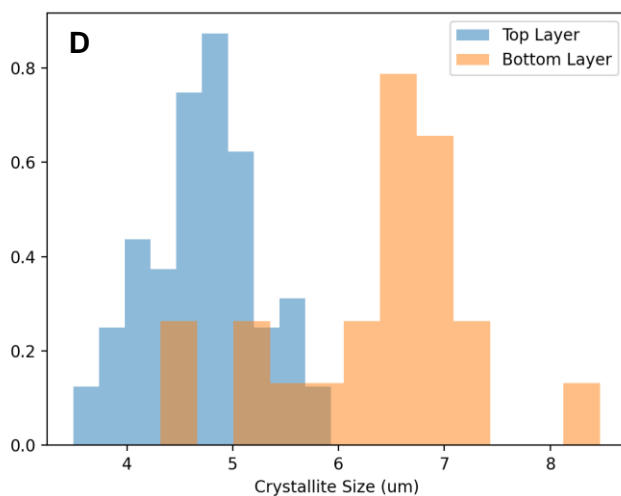


Figure 9. Size comparison of NCT assemblies before and after density gradient centrifugation. (A) Crystallites aggregated after assembly. (B) Top layer of crystallites after density gradient separation. (C) Bottom layer of crystallites after density gradient separation. (D) Histogram of crystallite sizes from top layer and bottom layer where bottom layer shows a distribution of larger crystallites than the top layer.

As previously discussed, NCTs can assemble into crystallites of various shapes and sizes, even when using the same compositions and annealing processes. Under certain conditions, such as higher NCT concentrations or lower cooling rates, non-Wulff structured crystallites are more likely to form. For example, IO-NCTs assembled under specific conditions formed hexapods, dendritic structures, and rhombic dodecahedra (Figure 11a). The polydisperse distribution of

crystallites with different shapes was stabilized using the same method as before, preparing them for density gradient separation (Figure 11b).

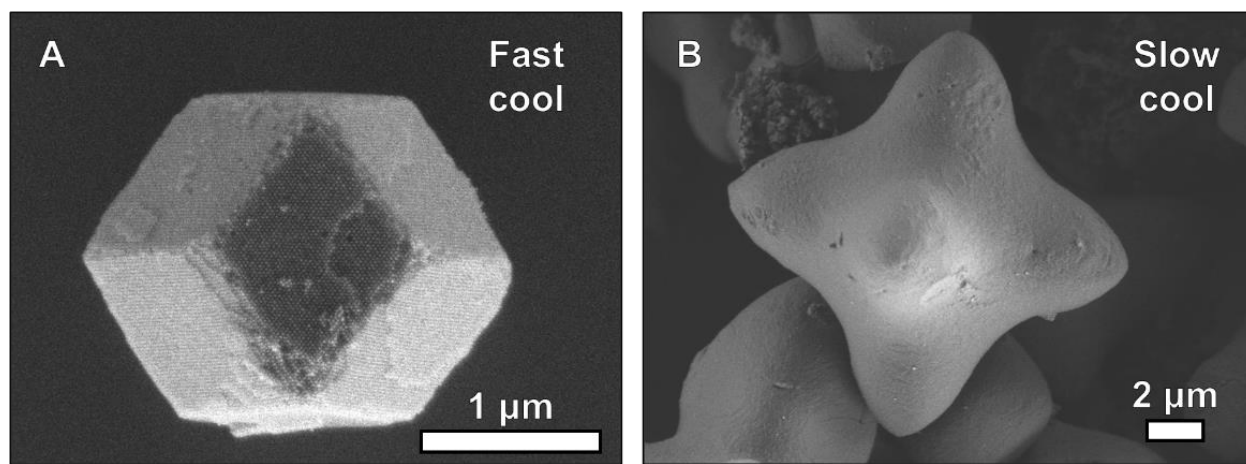


Figure 10. Crystallite structures formed from identical NCT building blocks. (A) Rhombic Dodecahedra shaped crystallite formed from fast cooling conditions. (B) Hexapod shaped crystallite formed from slow cooling conditions.

A similar solution of NaCl and F-127 in water was used for the distribution of hexapod and rhombic dodecahedra-shaped crystallites (Figure 12).

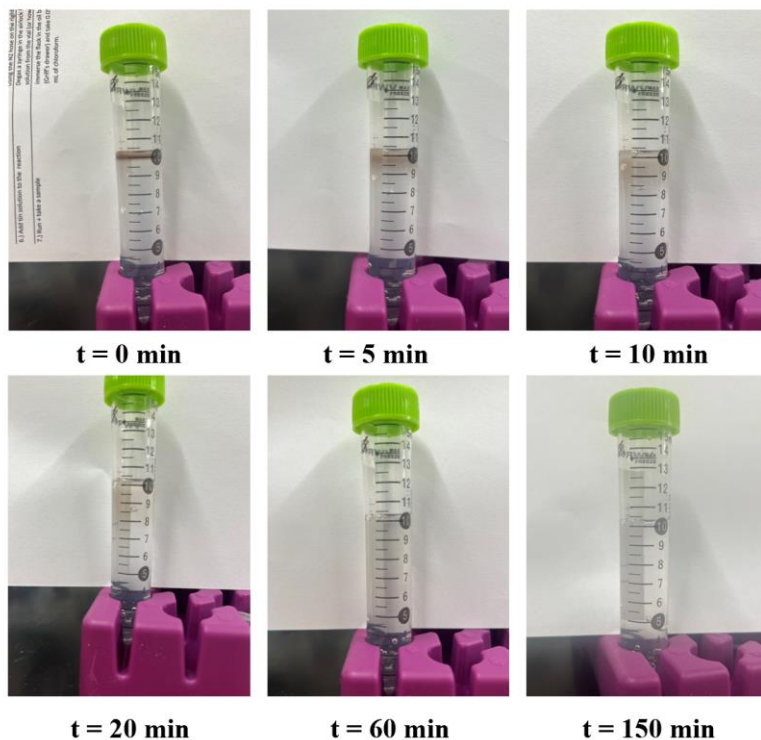


Figure 11. Assembled IO-NCTs of different sizes and shapes running through NaCl density gradient at 1 r.c.f. over 150 minutes. Time stamps ($t = 0, 5, 10, 20, 60,$ and 150 min) are labeled for each image.

The separation band for this set of NCTs widened and dropped more quickly, becoming indistinguishable from the gradient solution at times $t = 60$ and $t = 150$. This is associated with a larger overall size, wider size distribution, and various superlattice geometries in the NCT assembly. A larger average size leads to a larger hydrodynamic radius and faster sedimentation velocity. A broader distribution of sizes and shapes results in a more varied range of crystallite diameters and Stokes' velocities, causing a faster spreading of the NCT band. The gradient was separated into 19 layers from top to bottom for characterization.

SEM observation of the 5th and 19th layers from the top revealed that the 5th layer contained monodisperse, rhombic dodecahedron-shaped crystallites, while the 19th layer had monodispersed, hexapod-shaped crystallites. Moreover, superlattices from the 19th layer were

noticeably larger than those from the 5th layer. The correlation between size and shape indicates that assembly conditions leading to larger crystals also result in non-equilibrium shapes.

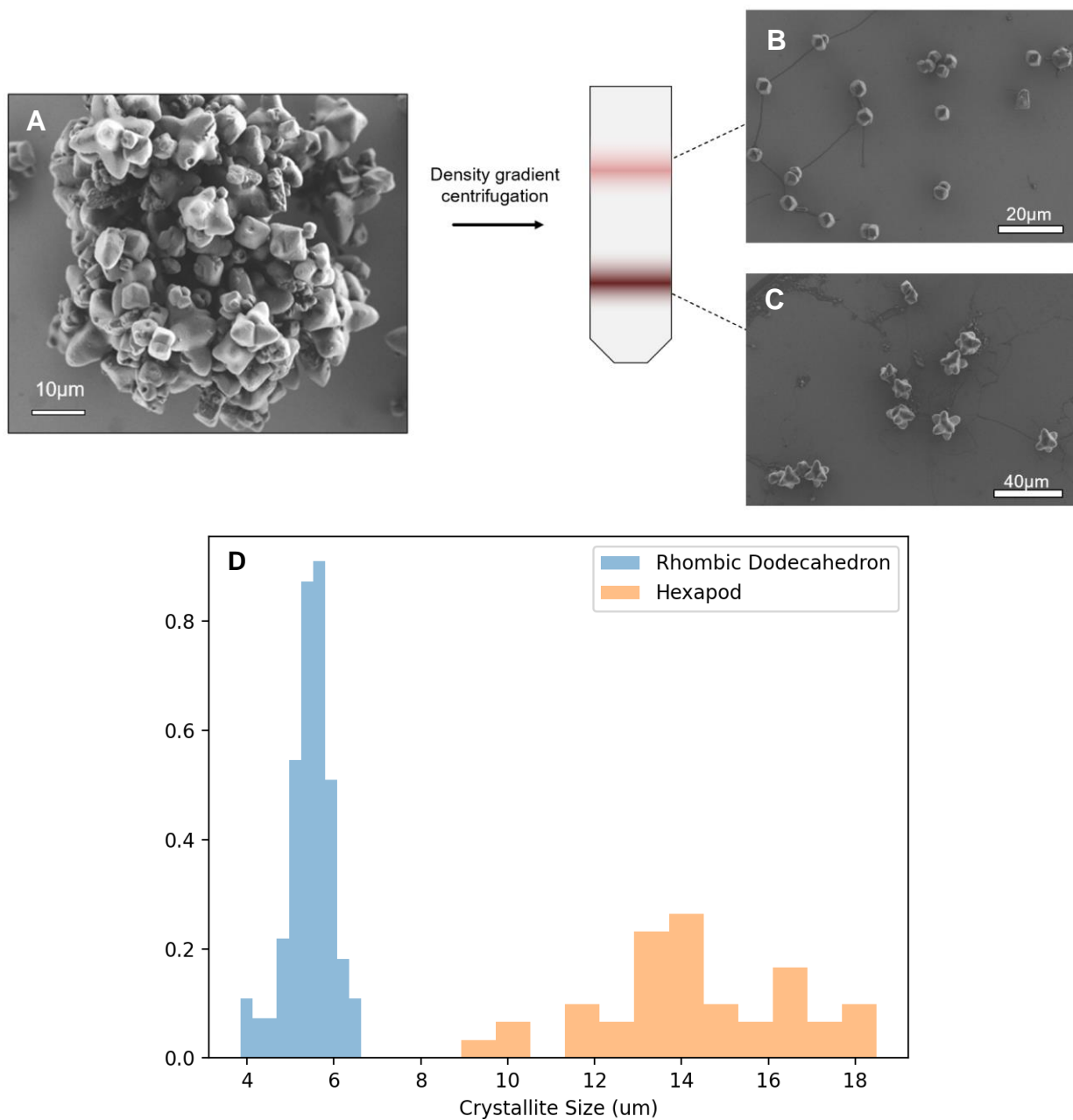


Figure 12. Size comparison of crystal shapes that were separated using density gradient centrifugation. (A) Rhombic dodecahedra and hexapods aggregated together after assembly. (B) Top layer of density gradient showing smaller rhombic dodecahedra shaped crystallites. (C) Bottom layer of density gradient showing larger hexapod shaped crystallites. (D) Histogram comparing sizes between hexapods and rhombic dodecahedra.

Dendritic growth is influenced by concentration or growth speed gradients. The size dependency suggests that larger crystals can penetrate concentration gradients, enabling rapid growth and overexpression of the $\langle 100 \rangle$ growth vector. This also indicates that slow cooling mainly leads to dendritic growth due to the production of larger crystals, as opposed to rapid cooling, which results in numerous smaller crystals that cannot form stellate structures. Moreover, these findings emphasize the effectiveness of density gradient separation as a reliable method for filtering building blocks based on both shape and size.

As previously discussed, NCTs can also be made using AuNP cores. Au-NCTs were stabilized using a similar solution of Pluronic F-127 to break apart aggregates, but solutions of CsCl and F-127 were used for the density gradient to better match the denser AuNP core (Figure 14).

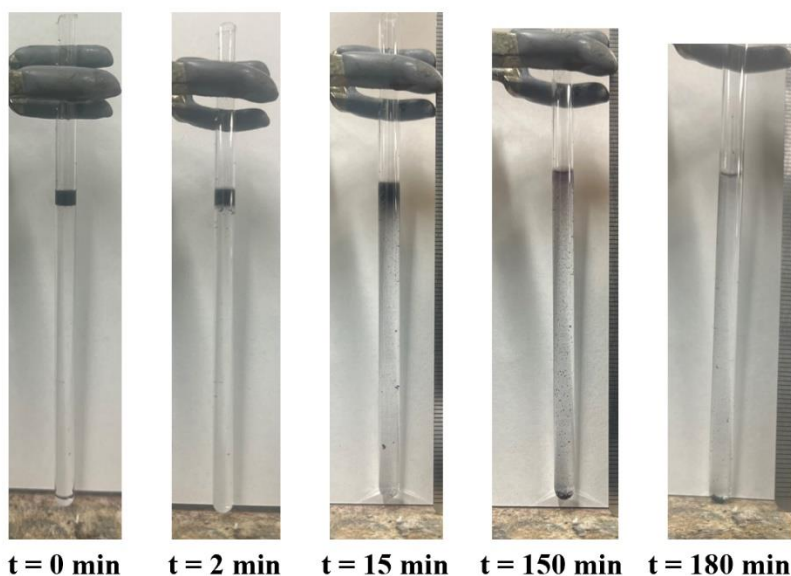


Figure 13. Assembled AuNCTs of different sizes and shapes running through NaCl density gradient at 1 r.c.f. over 180 minutes. Time stamps ($t = 0, 2, 15, 20, 150, \text{ and } 180 \text{ min}$) are labeled for each image.

The separation band for this NCTs remained at the top for most of the time the separation was conducted with visible particles falling through immediately and clinging to the side of the tube. The visibility of individual specks that clung to the side of the tube indicate aggregation of crystallites despite already treating them with surfactants. The aggregation is a sign that the NCT crystallites were no longer stably suspended in the CsCl and F-127 solution.

SEM of the crystallites that remained at the top and those that sedimented at the bottom confirm the instability caused by CsCl. The crystallites that sedimented no longer had the characteristic Rhombic Dodecahedron shape expected of a BCC lattice and resembled a collapsed NCT superlattice (Figure 15).

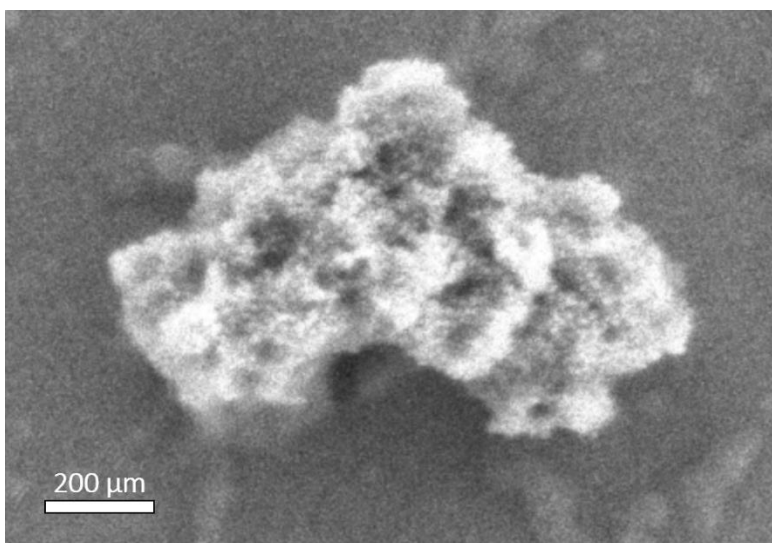


Figure 14. SEM of AuNCT assembly that became disordered after density gradient separation (Bottom Layer).

The density gradient relying on salts are a potential issue when trying to process NCTs. As observed in the AuNCT system, a CsCl density gradient disrupts stability of NCT crystallite suspensions so the superlattice distribution does not separate based off size. Furthermore, the CsCl solution may have interfered with the hydrogen bonding necessary to facilitate self-assembly of crystallites as indicated by the non-faceted superlattice post-density gradient

(figure). Although salt-based density gradients work as an effective way to separate shape and size of IO-NCT superlattices, it is not robust for systems that are more electrostatically sensitive.

Scratch Directed Growth of NCT Winterbottoms

Utilizing nanoscratches has proven to be a cost-effective and efficient method for directing the crystallization and orientation of various systems, such as liquid crystals, nanotubes, and even cells.^{10,11,13} Silicon wafer substrates were systematically scratched, and the scratch density and gold coating for each substrate are presented in the table below:

After scratching and gold coating, substrates were functionalized with PS-Thy to promote heterogeneous nucleation from the surfaces. Assembly was performed using AuNP-NCTs, and substrates were placed into PCR tubes before annealing to initiate directed assembly on the surfaces rather than free homogeneous assembly (see figure 16).

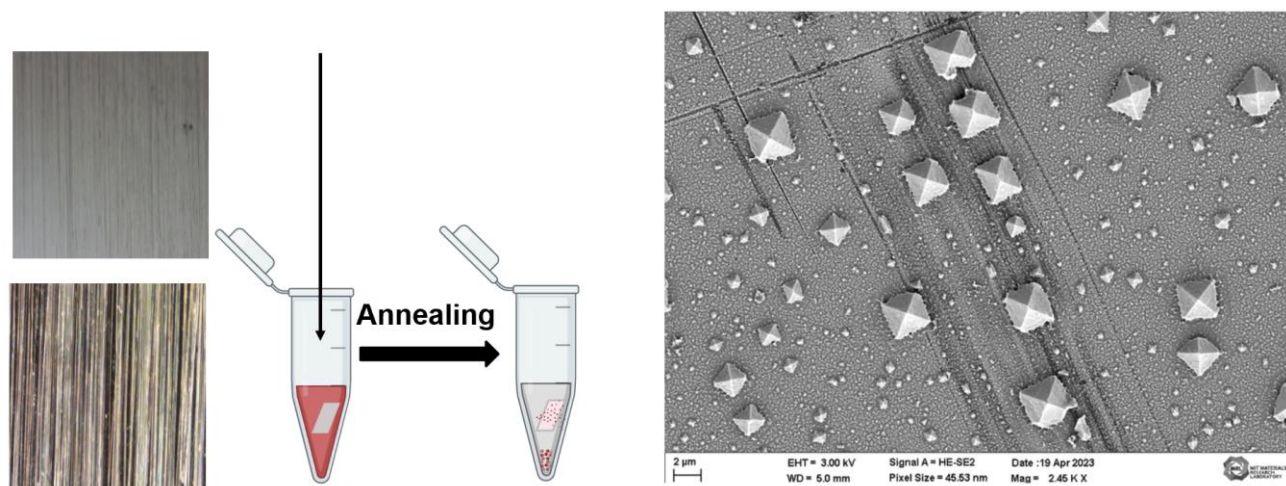


Figure 15. Assembly of NCTs on silicon substrates. Functionalized substrates were scratched and annealing in the presence of AuNCTs. Winterbottoms formed on the substrate on scratched and unscratched surfaces.

SEM analysis of the substrate-directed growth reveals the formation of Winterbottoms, which aligns with previous work on heterogeneous assembly of NCTs. However, Winterbottoms formed along a nanoscratch or a series of nanoscratches appear to have the $\langle 100 \rangle$ plane in line with the scratch. The relative proportions of Winterbottoms were compared. Winterbottoms that grew in contact with a scratch are likely to be angled 45 degrees with respect to the scratch orientation. Winterbottoms that nucleated purely from the substrate without scratch assistance had a moderately uniform distribution of angles. This supports the reliability of scratches to direct crystal nucleation and growth to be oriented.

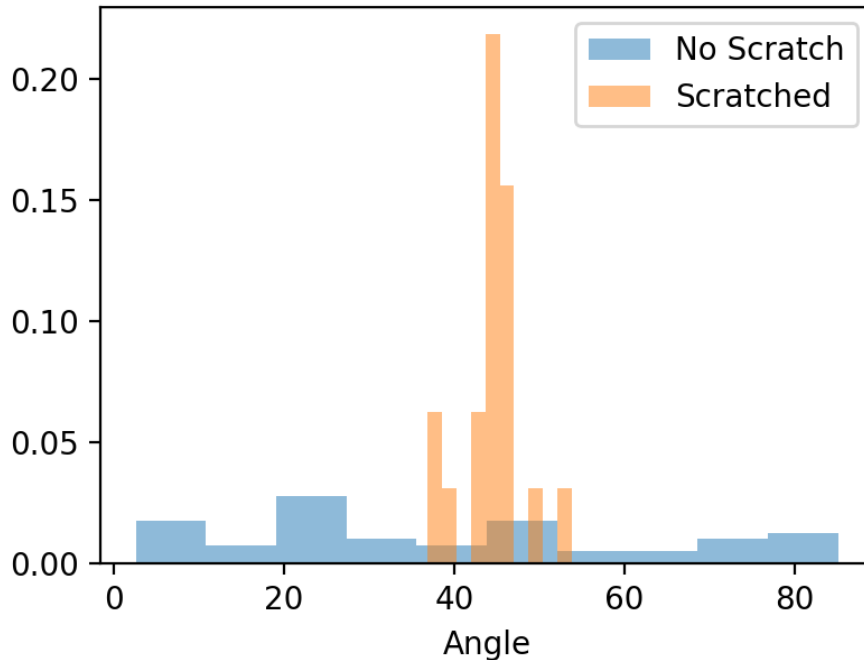


Figure 16. Distribution of crystal orientations with respect to scratch angles. Winterbottoms that grew in contact with a scratch were compared to Winterbottoms that grew without assistance from scratch orientation.

Having control of orientation is the first step in growing large thin-film crystals. Typically, crystals growing into each other without any directed growth will form grain boundaries since their lattices will not match. By orienting the crystals, crystals that grow into

each other will allow merge into larger, single-crystalline superlattices and continuous growth will form single-crystal films of NCTs. Just in this early validation of scratch directed growth, merging of crystals can be speculated from the final crystallites observed (figure). Normally, winterbottoms would form a pyramidal shape based off their plane of truncation and would grow symmetrically outward from the point. However, the structure seen in figure x shows an oblique triangular prism. This shows signs that two adjacent Winterbottoms grew into each other but their boundaries were aligned so that they merged.

CONCLUSION AND FURTHER WORKS

NCTS are promising building blocks for future nanocomposites and nanotechnology due to their tunability and self-assembly. Although much is yet to be uncovered about NCT-based materials, there is plethora of techniques to be tested on these systems to compare and understand more of nanocomposite behavior. This work investigated the application of density gradient centrifugation and scratch-directed assembly on the effects of processing NCT superlattices to have better shape and size control.

NCTs can be self-assemble to form superlattices and utilizing pair-wise recognition groups results in BCC structures. NCT assembly is affected by concentration and annealing conditions, with an increase in both resulting in larger crystallite growth. BCC crystal growth can be influenced to produce shapes such as rhombic dodecahedra, square pyramids, and hexapods. Current work is being set to further the work and understand more about shape control and large crystal growth.

Density gradient centrifugation is a common technique used to separate systems of multiple distributions. Applying this method to process NCT assemblies showed early success in

being able to exert control of crystallite shape and size. Salt-based density gradients sifting IONCTs processed separate into rhombic dodecahedra and hexapods of varying sizes. In comparison, salt-based density gradients need more fine tuning for AuNCTs as these systems. This problem arises from the AuNPs being denser and requiring a denser salt, and although the salt has the same ionic charges, this difference results in a dissociation of recognition groups between NCTs.

The current work is a valuable proof of concept for processing monodisperse building blocks by density gradient centrifugation. Current problems encountered were the continuous distribution of crystallites throughout the density gradient as opposed to a distinct bands of monodisperse sets. For the future, a denser gradient that was more close matched to the density of NCTs could be used to have larger variations in stokes' velocity and settling depth in order to produce more definitive separations. Another problem was the use of ionic component to form the density gradients. Because of the sensitivity of hydrogen bonds in supramolecular recognitions groups, temperature and pH can result in dissociation and disordered crystallites as observed previously. This can be studied in the future by using non-ionic or non-salt-based density gradients to achieve separation without disrupting the order of the assembly.

Scratch assisted growth has been shown to be vital in growing crystals that are oriented with each other. Not only was orientation achieved, but onset of crystals merging with each other was observed possibly signifying the chance to grow large, single crystal films. The current system was only tested on AuNCTs, although not much is expected to change between Au and IONCTs, it would be valuable to test the ability of nanoscratches to guide various NCT superlattices to be aligned with each other. More work could be done in testing different nanoparticle based nanocomposites to understand how robust grooves are in aligning crystal

growth. Additionally, future studies could attempt to grow larger crystals that grow into each other to observe limitations that scratches have in maintaining growth. This would further the capabilities of nanocomposite synthesis and control for scaling and implementation into devices.

WORKS CITED

- (1) Santos, P. J.; Cheung, T. C.; Macfarlane, R. J. Assembling Ordered Crystals with Disperse Building Blocks. *Nano Lett.* **2019**, *19* (8), 5774–5780. <https://doi.org/10.1021/acs.nanolett.9b02508>.
- (2) Li, R. L.; Thrasher, C. J.; Hueckel, T.; Macfarlane, R. J. Hierarchically Structured Nanocomposites via a “Systems Materials Science” Approach. *Acc. Mater. Res.* **2022**, *3* (12), 1248–1259. <https://doi.org/10.1021/accountsmr.2c00153>.
- (3) Zornberg, L. Z.; Lewis, D. J.; Mertiri, A.; Hueckel, T.; Carter, D. J. D.; Macfarlane, R. J. Self-Assembling Systems for Optical Out-of-Plane Coupling Devices. *ACS Nano* **2023**, *17* (4), 3394–3400. <https://doi.org/10.1021/acsnano.2c08344>.
- (4) Lee, M. S.; Yee, D. W.; Ye, M.; Macfarlane, R. J. Nanoparticle Assembly as a Materials Development Tool. *J. Am. Chem. Soc.* **2022**, *144* (8), 3330–3346. <https://doi.org/10.1021/jacs.1c12335>.
- (5) Bates, S. *Base Pair*. Genome.gov. <https://www.genome.gov/genetics-glossary/Base-Pair> (accessed 2023-05-03).
- (6) Santos, P. J.; Macfarlane, R. J. Reinforcing Supramolecular Bonding with Magnetic Dipole Interactions to Assemble Dynamic Nanoparticle Superlattices. *J. Am. Chem. Soc.* **2020**, *142* (3), 1170–1174. <https://doi.org/10.1021/jacs.9b11476>.
- (7) Zhang, J.; Santos, P. J.; Gabrys, P. A.; Lee, S.; Liu, C.; Macfarlane, R. J. Self-Assembling Nanocomposite Tectons. *J. Am. Chem. Soc.* **2016**, *138* (50), 16228–16231. <https://doi.org/10.1021/jacs.6b11052>.
- (8) Lewis, D. J.; Zornberg, L. Z.; Carter, D. J. D.; Macfarlane, R. J. Single-Crystal Winterbottom Constructions of Nanoparticle Superlattices. *Nat. Mater.* **2020**, *19* (7), 719–724. <https://doi.org/10.1038/s41563-020-0643-6>.
- (9) Santos, P. J.; Gabrys, P. A.; Zornberg, L. Z.; Lee, M. S.; Macfarlane, R. J. Macroscopic Materials Assembled from Nanoparticle Superlattices. *Nature* **2021**, *591* (7851), 586–591. <https://doi.org/10.1038/s41586-021-03355-z>.
- (10) Kim, D. H.; Suh, A.; Park, G.; Yoon, D. K.; Kim, S. Y. Nanoscratch-Directed Self-Assembly of Block Copolymer Thin Films. *ACS Appl. Mater. Interfaces* **2021**, *13* (4), 5772–5781. <https://doi.org/10.1021/acsami.0c19665>.
- (11) Suh, A.; Yoon, D. K. Nanoscratching Technique for Highly Oriented Liquid Crystal Materials. *Sci. Rep.* **2018**, *8* (1), 9460. <https://doi.org/10.1038/s41598-018-27887-z>.
- (12) Manoharan, V. N.; Elsesser, M. T.; Pine, D. J. Dense Packing and Symmetry in Small Clusters of Microspheres. *Science* **2003**, *301* (5632), 483–487. <https://doi.org/10.1126/science.1086189>.

(13) Shin, M. J.; Im, S. H.; Kim, B.; Choi, J.; Lucia, S. E.; Kim, W.; Park, J. G.; Kim, P.; Chung, H. J.; Yoon, D. K. Fabrication of Scratched Nanogrooves for Highly Oriented Cell Alignment and Application as a Wound Healing Dressing. *ACS Appl. Mater. Interfaces* **2023**, *15* (15), 18653–18662. <https://doi.org/10.1021/acsami.3c00530>.