# *Functional Measurement of Biological Parts*

# Functional Measurement of Biological Parts

Matthew J. Lang and David C. Appleyard
*Department of Biological Engineering and the Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, mjlang@mit.edu*

**Abstract:** We present a parts perspective using biological components to form complex systems and discuss: connectivity, network formation, and motility. Challenges associated with characterization and assembly are discussed relative to assay development and instrumentation advances.

The core strategy of engineering is to integrate fundamental components, or parts, to form higher order systems. In one example, basic parts consisting of lenses, fibers, mirrors, filters, crystals, and gratings are combined using optical engineering principals to form a complex optical system like a laser or spectrometer. In electrical engineering resistors, capacitors and inductors are integrated into more sophisticated devices such as transistors, logic gates, microcontrollers and computers. Mechanical, Chemical, and Civil all have analogous combinations of parts that can be combined to form larger systems. Interchangability and interfacing between parts is supported through standardization and characterization. Foundational engineering principles enable mathmatical and physical descriptions of the individual parts and define the behavior of assembled systems; a ray may be traced through optical components, electrons through circuits or forces through structures.

Biology surely does not lack for parts nor for impressive higher order systems. Standards are ever present in biology: the length scale of a peptide bond or DNA basepair, the energy available from a molecule of ATP or glucose, the spacing of microtuble latice sites or the pitch of actin filaments, or the basic physical structure of the $\alpha$-helix or $\beta$-sheet. Systems approaches to biology have allowed the construction of rudimentary functions using genetically based parts providing some guiding principals for engineering. These strides in engineering have been enabled through great advances in molecular biology methods and tools such as sequencers and synthesizers. Yet many biologically based parts are still not at a stage where they are fully "hackable", having not been extensively characterized and standardized. However, the wealth of complex systems present from natural evolutionary means provide an abundance of parts and engineering principals to be mined. By rigorously examining these sytems we hope to ultimately have the ability to directly build from the ground up with biological parts.

Perhaps it is time for a "Parts Project" where we lay the foundation for building with biological components and seek full knowldege of the working relationships between functional modular domains. To do this the development of rigorous foundational principles, models, and the advancement of measurement tools for probing and manipulating biological component structures will be required. Molecular and cellular biophysics has provided advanced tools for probing the interworkings of biological motors and cell machinery, yet characterization of many of these parts is currently beyond our reach. However, where we have succeeded in probing a part, we have benefited greatly.

Three examples where characterization of an individual part has contributed to the understanding of the system as a whole or to the development of engineered biological structures are kinesin, actin, and DNA. Kinesin, a biological motor, travels along microtubules in the body transporting a variety of cargos. The force generation mechanism of an individual part, the kinesin motor head, has been elucidated and can be tied to understanding of the motility and stepping behavior of the protein and system as a whole [1,2]. This success can be used as a starting point to exaimine more complex motility systems like the mitotic spindle.

Actin, a cytoskeletal component forms filamentous structures which are critical to cell function. These actin networks can be built and studied *in vitro* by combining filaments with actin binding proteins to form a wide range of structures. We have studied the impact of the actin binding proteins filamin and $\alpha$-actinin which organize actin into networks of mesh like gels and bundles of filaments, respectively [3]. An optical trap based single molecule assay consisting of a surface bound actin filamment, an actin binding protein and a second bead-linked actin filament was used to test the strength of these individual linkages. When the filamin is pulled, both unbinding and unfolding of the protein occur and these events are manifested in higher order network responses of these systems.

Great strides have been made in measurment of a few classes of parts. Perhaps the best understood

978-1-55752-869-8/09/$25.00 ©2009 IEEE

are DNA and nucleotide based parts where ease in assay development has facilitated measurement. Most see DNA as a genetic template, the biological engineer also sees a useful element for connectivity and structure in an assay. Due to the ease of functionalization of DNA, attachment to small molecules or even surfaces can be achieved through antibody-antigen interactions, protein binding, or aptamers. DNA not only connects, but forms a linker of well defined length and mechanical properties. Like DNA, other biological parts can abstracted from their primary roles to form similar connective structures: amyloid fibers, actin, or the M13 bacteriophage.

To further the parts perspective of engineering biology we must address the degree of difficulty in setting up the wet-assays required to study these components and systems. A successful kinesin motility assay contains multitudes of items: motor proteins, bead based cargo, fuel, microtubules and buffers. Although this is a wonderfull assay it has taken many years and great effort to reach this point. We seek to minimize the time involved in developing these assays by focusing on common challenges. Often these assays are comprised of chemical linkers and handles which allow manipulation and examination of the biological parts using instrumentation like the atomic force microscope or optical trap. Our lab has strived to advance new strategies for connecting biological components and ease the hurdles in assay development for biophysics. Our approach is to use connectivity achieved through the M13 bacteriophage which forms a tough, stiff tether, about 1$\mu$m long, that can be genetically programmed for specific adhesion and simply produced in bacteria to achieve linkages to beads and proteins of interest [4].

As assays are improved so must the instrumentation and technology that is used to probe these parts of molecular and cellular machinery. We have focused on advancing single molecule biophysics instrumentation by carving out methods for combined optical trapping and single molecule fluorescence. The merging of these mainstay biophysics techniques is applicable for a new class of experiments for simultaneously probing a molecule's structure with force and reporting on the structure with single molecule fluorescence. This combination has been difficult to achieve due to accelerated destruction of fluorophores in the presence of the high photon flux of the trap and high background levels in fluorescence detection. We have developed an out of phase temporal innovation, a simple and general solution to trap-accelerated photobleaching [5]. We demonstrated the first combined coincident trapping and FRET measurement using a test system of a DNA hairpin and a donor and acceptor FRET pair positioned on opposite ends of the hairpin base [6]. These hairpins also form a fluroescence based force sensor "part" with binary on-off reporting of a force magnitude centered at $\sim$15 pN.

The parts perspective allows a framework for applying engineering principals to biology. This can lead to the advancement in the complexity of the biological systems we can design and to the better understanding of the systems present in nature. Progress on part based engineering is not limited to the characterization of individual parts, but also impacted by advancements in the assays and instrumentation used to measure and manipulate these systems.

The authors would like to acknowledge funding from the National Science Foundation Career Award 0643745 (M.J.L.), the National Institutes of Health grant 5R21NS058604-02 (M.J.L.) and Lincoln Labs ACC423 (D.C.A).

[1] A. S. Khalil, D. C. Appleyard, A. K. Labno, A. Georges, M. Karplus, A. M. Belcher, W. Hwang and M. J. Lang, "Kinesin's cover-neck bundle folds forward to generate force," PNAS **105,** 19246-19251 (2008).

[2] W. Hwang, M. J. Lang, and M. Karplus, "Force generation in kinesin hinges on cover-neck bundle formation," Structure **16,** 62-71 (2008).

[3] J. M. Ferrer, H. Lee, J. Chen, B. Pelz, F. Nakamura, R. D. Kamm and M. J. Lang, "Measuring molecular rupture forces between single actin filaments and actin-binding proteins," PNAS **105,** 9221-9226 (2008).

[4] A. S. Khalil, J. M. Ferrer, R. R. Brau, S. T. Kottmann, C. J. Noren, M. J. Lang and A. M. Belcher, "Single M13 bacteriophage tethering and stretching," PNAS **104,** 4892-4897 (2007).

[5] P. B. Tarsa, R. R. Brau, M. Barch, J. M. Ferrer, Y. Freyzon, P. Matsudaira and M. J. Lang, "Detecting force-induced molecular transitions with florescent resonant energy transfer," Angew. Chem. Int. Ed. **46**(12), 1999-2001 (2007).

[6] R. R. Brau, P. B. Tarsa, J. M. Ferrer, P. Lee and M. J. Lang, "Interlaced optical force-fluorescence measurements for single molecule biophysics," Biophys. J. **91**(3), 1069-1077 (2006).