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Editorial overview: Macromolecular machines and assemblies: Rise and fall at the molecular level

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Katrina Forest, Ph.D. is a professor of Bacteriology at the University of Wisconsin-Madison. In the spring of 2015 she is also a Fellow at the Institut d'études avancées d'Aix-Marseille (IMéRA). Structural microbiology is the theme of the Forest Lab, with two main focus areas. The first is assembly and retraction of Type IV pili and, via collaboration, type II secretion systems. The second is exploitation of bacterial phytochrome photoreceptors for biotechnology applications and an attempt to understand the functional consequence of the unusual knotted topology of this microbial histidine kinase. The Forest lab also collaborates in diverse areas including characterization of recently described photoreceptors in fresh water lakes, quasiracemic crystallography for crystallization and structure determination, and chemical biology and drug discovery in the realm of infection. Dr. Forest is a member of the American Academy of Microbiology, and a co-director of UW-Madison's Biotechnology Training Program.

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Christopher Hill is a professor of Biochemistry at the University of Utah School of Medicine. His lab uses multiple approaches, especially X-ray crystallography, to study several biological systems including regulation of the proteasome, protein complexes that regulate nucleosome dynamics, and HIV biology. Recent advances include determining the structural mechanisms for how the UCH37 deubiquitylase is recruited to the very different proteasome and INO80 complexes where in one case it is held in an activated conformation and in the other case in a distinctly different repressed conformation. Another advance (currently unpublished) reveals the mechanism and biological consequence of interactions between histones H2A–H2B and the nucleosome reorganizer FACT. Studies on HIV biology include characterizing multiple structural proteins of the virion and characterizing proteins that function in the cellular ESCRT pathway, which functions in cytokinesis, endosomal sorting, and viral budding.

What is life but building up and tearing down of structures? History mirrors this struggle against entropy as cities rise and fall. Constructions provide protection and beautify, transport occurs, and defenses are mounted against onslaught, only to be tested and eventually fail. Cellular/molecular biology echoes these processes with multiple anabolic pathways, energy conversion mechanisms, inflammatory and immune responses, and reactions to diverse threats.

In this issue of Current Opinion in Structural Biology, leading experts review complex macromolecular assemblies that function on one hand in building and energizing cellular components, and on the other hand in mechanisms of attack (by viral and microbial pathogens) and defense. Techniques included are numerous, often used in combination, and include NMR, X-ray crystallography, negative stain and cryo electron microscopy, small angle X-ray scattering, molecular modeling, single molecule rotation assays, and 3D animation. Interestingly, as editors we did not need to make a concerted effort to seek out a broad range of *techniques* to feature. Rather, by focusing on exciting biological advances in which structural biology has profoundly contributed to our understanding of cellular processes, we naturally found ourselves with a ready mix of experimental approaches. This alignment is more than a happy coincidence, and underscores the fact that mechanistic understanding of biological processes requires information at many time and length scales, and that powerful experimental tools are increasingly available to provide complementary information that drives the development of testable, working models of biological systems in action.

The article by Iwasa gets right to the heart of this idea of working models by discussing 3D animation of macromolecular assemblies. It is widely appreciated that these animations can be stunningly beautiful and impactful ways to communicate complicated molecular insights. Less well appreciated, but perhaps of even more fundamental importance, Iwasa outlines how animations can help investigators refine hypotheses and design new experiments. In addition to outlining the process of creating 3D animations of complex macromolecular assemblies, this article provides specific suggestions for recently developed software that may make this enlightening practice accessible to molecular scientists who lack expert training in computer science.

Starting the theme of construction, Noinaj *et al.* review the current state of knowledge in the biogenesis of the outer membrane β barrel proteins of Gram-negative bacteria. Recent work has advanced understanding of the β barrel assembly machinery (BAM) from still-life images of individual components to

a growing appreciation of how this complex multiprotein pathway functions as a dynamic ensemble to target outer membrane proteins and catalyze their folding in the outer membrane. This review focuses on the role and potential mechanism of action of the essential protein BamA. Capitalizing on the availability of multiple different structures of BamA and homologues, the authors evaluate the hypothesis that dynamic motions of an external loop and the release of hydrogen bonding between the adjacent first and last β strands in the BamA barrel catalyze assembly of other outer membrane proteins.

Synthesis of a diverse array of secondary metabolites is carried out by polyketide synthase (PKS) complexes. These have striking sequence homology and enzymatic similarity to the fatty acid synthases, but rather than multiple rounds of addition of identical atoms from an Acetyl-CoA precursor, PKS enzymes sequentially synthesize and then add diverse extender units to the growing polyketide. Smith *et al.* review recent advances toward understanding the remarkable ultrastructure of these modular PKS systems and how their domain arrangements and motions define catalytic efficiencies. Pivotal work combining cryo electron microscopy and protein crystallography has moved the field from a collection of static pictures of bits and pieces of the PKS system to an exciting model for the impressively choreographed passage of the growing substrate along the synthetic pathway.

Rotary motors, including the well-known FoF1 ATPase, use the rotation of subunits to couple the energy flow of ions with conversion between ATP and ADP. In some cases these remarkable machines run in the direction of synthesizing ATP, such as in respiratory chemiosmosis, whereas others hydrolyze ATP in order to drive ions against a gradient. Iino *et al.* review how crystal structures of multiple states of *Enterococcus hirai* vacuolar ATPase (V1-ATPase) have been combined with elegant single molecule experiments on the same protein to explain how the conformational changes that accompany ATP binding, hydrolysis and product release lead to step-wise 120° rotations of the motor and consequently drive sodium ion pumping.

As we move from the build and energize side of this issue to the attack and defense viewpoint, the review by Lam and Jin on the Botulinum neurotoxin complex summarizes the current structural understanding of a the large Progenitor Toxin Complex (PTC), which was revealed by crystal structures to be a 14-subunit assembly that protects the Botulinum Toxin at its core from low pH and protease activity in the gut while simultaneously presenting a multivalent binding interface for the glycan surface of epithelial cells through hemagglutinin (HA) subunits. Remarkably, the structural similarity of one HA domain to cadherin suggested a devious mechanism by which Botulinum Toxin Complex interacts directly with E-cadherin to weaken epithelial cell lateral interactions and promote its own passage across the epithelial cell barrier for intoxication.

The innate immune system is an ancient protective pathway that leads to cytokine release and programmed cell death in response to external threats including many microbial-specific or viral-specific molecular patterns. The response to such molecular warning signals is the hierarchical construction of the inflammasome, a supermolecular complex whose activity must be tightly regulated to prevent inappropriate accumulation of cytokines, or premature launching of apoptosis. Hauenstein *et al.* describe structure-derived functional implications for this process. Recent progress includes explanations for the release of auto-inhibition of inflammasome components: when the DNA-receptor IFI16 binds DNA, its PYD domain is exposed and cooperative assembly of nucleoprotein filaments ensues; when the inflammasome adaptor and caspase activator NLRC4 is liberated from a coiled monomeric state, it recruits and initiates activation of caspase-1.

Continuing the topic of cellular responses to external threats Jia *et al.* review the structural understanding of several diverse and elegant antiviral host restriction factors, which act at multiple steps of the viral lifecycle, and the wily countermeasures evolved by HIV to circumvent these cellular strategies. A flood of important structures in recent years has broken open this field and taught us much about the molecular details of the host:HIV arms race. In just one example, the host protein TRIM5α recognizes internalized HIV capsids, which have a fullerene cone lattice structure that displays a continuously varying curvature. Structures of the human TRIM5α PRY/SPRY domain explain how adaptable capsid binding loops accommodate different curvatures. Moreover, the combination of TRIM5α domain crystal structures with EM observations of TRIM proteins in association with an HIV-1 capsid led to a striking model for the higher-order assembly of a TRIM lattice on the capsid surface.

In closing we invite you to be enchanted by the remarkable findings reviewed in this issue of Current Opinion in Structural Biology. Our ability to interrogate the macromolecular machines at the heart of living cells has advanced on every front over the past several years; higher spatial resolution, larger macromolecular complexes, more dynamic systems, less symmetry-constrained approaches, and computationally advanced integration of multiple types of data continue to push us to ask and answer more insightful questions about the inner workings of cells. The advent of user-friendly approaches to animation promises to help all biologists — not just card carrying graphic artists — to make the very most of these exciting results. Cellular machineries continue to construct, protect, and destruct at every moment in every cell. As our understanding of these systems deepens via structural biology we cannot help but be amazed.