THE PROTEIN SOCIETY

# EDITORIAL

# Molecular machines one molecule at a time

*When it is the case that a wheel with teeth engages the screw furrow, then for every one turn the screw is turned, it will move one tooth of the wheel.*
***Mechanics***, Hero of Alexandria, 1st Century CE.

Machines have exerted a strange fascination for humans throughout history. Perhaps the realization of the "transformative" power of certain simple devices and tools (a wedge, an axe, an inclined plane, etc.) by means of which some action could be either amplified, converted into a different one, made to act in another direction, or somewhere else in space, is the origin of this enthrallment. The word *machine* derives from the Greek Ionian term *mechane* to designate an engine or contrivance; mechane, in turn, likely derives from the Hebrew *mekhonot*, a term used in the Torah to describe 4-wheel water carts built by Solomon for the Temple. The term in Latin is *machina*. In the third century BCE, Archimedes described and classified them as levers, pulleys, or screws, and discovered the *mechanical advantage* of the lever. Nearly 400 years later, in the first century CE, Heron of Alexandria (10–85 CE) described 5 mechanisms for "moving a given weight by a given force"; he included the lever, the windlass, the screw for power, the wedge, and the tackle block (pulley). The Greeks' only provided a "static" description of machines as the concept of mechanical work and its equivalence to energy was still not understood. It was in the 16th century that this idea permitted the description of machines in dynamical terms. In 1600, Galileo in "*Le Mechaniche*" ("*The Mechaninal Devices," "The Machines"*) provided the first complete dynamic theory of machines.

Already in 1647, in "*La description du Corps Humain*," René Descartes proposes that the body works like a machine, through a number of automated functions. But it was the invention of the microscope, in the late 16th century, that permitted scientists to use this instrument to observe and describe for the first time the microscopic organization of living matter. This newly acquired capability brought about a profound revolution in the biological sciences. In 1666, in his treatise "*De Viscerum Structura*," Marcello Malpighi—the "father" of microscopic anatomy—wrote *"The operative industry of Nature is so prolific, that machines will be eventually found not only unknown to us but also unimaginable by our mind."* This revolution in Biology was paralleled by the one ongoing in Physics, where Galileo first, and then Newton, refined the concepts of inertia, force, torque, mass, acceleration, and work that were to become the appropriate terms to describe the operation of machines.

The mechanical paradigm is recurrent in biology. During the industrial revolution, the need to understand the efficiency of vapor and combustion engines would eventually lead Carnot (1796–1832) to establish the foundations of thermodynamics. Meanwhile, during the 1780s Laviosier and Laplace, through carefully designed experiments on the utilization of oxygen and production of carbon dioxide and heat by the human body, concluded "Respiration is therefore a combustion, admittedly very slow, but otherwise exactly similar to that of charcoal."

In the last two decades, as a result of the great advances in structural biology and biophysics, a new understanding has emerged about the mechanical nature of the cell. We know now that this basic living unit has a modular architecture in which many of its central functions (replication, transcription, translation, splicing, protein degradation, energy generation, motility, etc.) are performed by interconnected and highly coordinated protein machines. These are assemblies of 5 or more polypeptide chains, contain various parts with specialized functions, and provide a localized environment where chemical species can interact and react in highly specific fashion. A protein machine is then a molecular "device" that, like its macroscopic counterpart, performs highly specialized functions requiring the conversion of chemical energy into mechanical work. This process—almost invariably—involves parts that

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move in some precise manner to produce forces, torques and displacements. Large research efforts are being directed today to characterize how these parts move, what forces and torques are generated in the process, and how is energy transformed and channeled from its chemical source (binding energy, bond hydrolysis, and chemical gradients) to its mechanical output.

Single-molecule methods of mechanical manipulation and fluorescence detection are ideally suited to address these questions. Because signals are not ensemble averaged, these methods permit investigators to follow in real time not just the *dynamics of the mean of a population of molecules* (what we know as "kinetics") but the actual *molecular trajectories* of individual machines and their time-course dynamics. Using single-molecule fluorescence spectroscopy experiments, scientists can encode the dynamics of protein machines and their moving parts in the optical signal (quantum yield, fluorescence resonance energy transfer efficiency, etc.). Similarly, mechanical experiments allow them to follow movements and displacements of machine parts in real time while—simultaneously—determine the energies involved in those displacements. Very recently, several groups have begun to combine these two approaches in a co-temporal manner, using instruments that can simultaneously monitor mechanical (force, torque, and displacements) and optical (fluorescence) signals.

The articles included in this issue of *Protein Science* by several leaders of the field represent a limited set of the many applications of single-molecule methods to the study of molecular machines. Nonetheless, the list spans a broad range of systems and approaches, constituting a comprehensive exposition of the subject in 7 original and 9 review articles.

Several contributions describe the use of single-molecule manipulation, detection, and/or visualization methods to follow the dynamics of molecular motors in gene expression. Naufer *et al*. use optical tweezers to study the activity of *Escherichia coli*'s DNA polymerase III [DOI: 10.1002/pro.3152]. The authors investigate how forces above 30 pN switch the activity of the enzyme from its polymerizing $\alpha$ subunit to its proofreading exonuclease $\epsilon$ subunit. The results are consistent with a model in which recognition of the 3′ end by either the $\alpha$ or $\epsilon$ subunits depends on the primer stability, which is modulated by force or mismatches. Hodeib *et al*. review the use of magnetic traps to uncover the molecular mechanisms underlying the operation of helicases [DOI: 10.1002/pro.3187]. These protein machines constitute a diverse family of essential enzymes that convert the energy of nucleotide hydrolysis to mechanically separate nucleic acid strands in DNA, RNA or DNA/RNA hybrids. The authors also discuss the coordination of these motors with other proteins in support of diverse cellular processes. Lin *et al*. use a dual platform of single-molecule fluorescence and optical tweezers to investigate the translocation on single-stranded RNA and DNA of the hepatitis C virus helicase NS3, an essential enzyme of the viral reproductive cycle [DOI: 10.1002/pro.3136]. This combined approach reveals the existence of a new mode of translocation by NS3 that involves nucleic acid looping and that is sensitive to the applied force. The enzyme is seen to alternate between translocation and looping modes but the function of this translocation modality is not known.

Rudnizky *et al*. review their use of optical tweezers to "unzip" nucleosomes at the promoter of two model genes *Cga* and *Lhb* [DOI: 10.1002/pro.3159]. The authors describe the role of DNA sequence and histone modification H2A.Z on the spontaneous, thermally induced mobility of nucleosomes, and discuss their potential effect on the dynamics of transcription initiation and elongation.

Two of the articles review in a complementary manner the wealth of information that has resulted from the use of single-molecule methods to study transcription initiation in prokaryotes. Alhadid *et al*. emphasize the use of single-molecule FRET methods to monitor the changes in conformation of the template and the RNA polymerase, and the general spatial relationships between the enzyme and the promoter, during the early phase of transcription [DOI: 10.1002/pro.3160]. Besides describing the main results obtained from the use of FRET to characterize the dynamics of RNA polymerase during transcription initiation, Marchetti *et al*. review the use of force-based methods, such as scanning force microscopy and magnetic tweezers to quantify the energy driving the RNA polymerase during this process [DOI: 10.1002/pro.3183]. These authors also show how single particle tracking *in vivo* provides direct quantification of the populations of the enzyme among the various phases of transcription and their localization in the cell.

Vörös *et al*. utilize scanning force microscopy and magnetic tweezers to determine the effect of two lac repressors (Lac I) separated by 400 bp on a transcribing RNA polymerase [DOI: 10.1002/pro.3156]. The authors find that a single Lac I bound to an operator site can act as an effective roadblock to transcription, its strength depending on the affinity of the repressor for that site. Surprisingly, the effect is greatly potentiated if the repressor is bound to both operator sites in a DNA loop configuration, but its efficiency as a roadblock no longer depends on the Lac I's affinity for the operator sites. The results suggest that DNA looping can play an important mechanical role in the control of gene expression.

Methods of intracellular fluorescence labeling of coding and noncoding RNA molecules are becoming

an essential tool to map the abundance and distribution of these molecules among molecular machines in the cell. Custer and Walter compare two such methods: (a) use of T7 RNA polymerase to body label an RNA with a cyanine dye and (b) use of yeast poly A polymerase to place 2′-azido modifications in either the body or poly A tail of an RNA [DOI: 10.1002/pro.3108]. Using single-molecule fluorescence microscopy, these authors show that, unlike the case for body labeling with T7 RNA polymerase, both yeast polymerase strategies result in fully functional RNAs.

What molecular events control the fidelity of protein synthesis by the ribosome, the largest and most complex molecular machine of the cell? What are the tradeoffs among accuracy, efficiency, and energy expenditure during translation? Prabhakar *et al*. review the vast information gathered during the last two decades on the dynamics of elongation and termination in prokaryotes through the use of biochemical, single-molecule, and structural methods [DOI: 10.1002/pro.3190]. Despite the intricate network of movements and coordinated interactions uncovered, much remains to be understood about these portentous machines, and new and more complex operations are envisaged for their eukaryotic counterparts.

Protein folding has long been and continues to be an exciting area of research in biophysics. Although most of these studies have emphasized how fully synthesized proteins attain their most stable, equilibrium configuration, more recently attention has turned to understanding what factors control the folding of polypeptides during their biogenesis in the ribosome. The discovery of synonymous mutations, for example, highlights the importance of time-dependent, nonequilibrium processes in the attainment of the native state of both single-domain and multidomain proteins. As pointed out by Liu *et al*., co-translational folding can reduce the complexity of folding by promoting sequential, domain-wise folding [DOI: 10.1002/pro.3189]. The nascent chains remain close to the ribosome's surface and are not released until their synthesis is complete. Using optical tweezers, these authors investigate the folding in solution and on the ribosome of the *E. coli*'s five-domain protein elongation factor G (EF-G) in *E. coli*. While interactions among unfolded domains interfere with productive folding in the full-length protein, the ribosome is seen to modulate the rate of folding and the stability of the N-terminal G-domain, preventing its misfolding. Mártonfalvi *et al*. investigate the folding of skeletal muscle titin, known to play an important elastic and structural role in the sarcomere [DOI: 10.1002/pro.3117]. These authors use high-resolution optical tweezers to monitor the mechanical unfolding and refolding of the protein. They show that the protein can generate force in search of the native state and characterize force fluctuations that they identify as resulting from transitions between unfolded and molten-globule states. They speculate that the molten-globule state may reduce the amount of energy dissipation associated with changes in the extension of the molecule under force during muscle contraction and relaxation.

Do chaperones guide and promote folding or simply suppress aggregation? Do they interact with partially folded chains along their folding pathway? Do they operate by modifying the chains entropy, or by stabilizing key transition states of the polypeptide along the folding pathway? Avellaneda *et al*. address these questions emphasizing the contribution of single-molecule methods to understand ATP-independent chaperones such as trigger factor and SecB, and the ATP-dependent chaperone Hsp70, Hsp90, and GroEL [DOI: 10.1002/pro.3161]. Ramirez *et al*. discuss the use of optical tweezers to investigate how BiP—a chaperone of the Hsp70 family—alters the folding of protein MJ0366 [DOI: 10.1002/pro.3137]. They show that BiP binds to the unfolded state of MJ0366 in a reversible manner preventing the formation of tertiary contacts within the substrate. Their single-molecule data is consistent with the idea that ATP hydrolysis regulates the binding to—and release from—the chaperone of the client protein.

Two reviews describe important advances in understanding protein machinery associated with the cell membrane. Hu *et al*. discuss the sensing, transmission, and biochemical response to mechanical signals from the extracellular matrix to the interior of the cell, and between cells through focal adhesions [DOI: 10.1002/pro.3188]. Cells sense and respond to mechanical signals through mechanosensing proteins, resulting in enzymatic activation, as in titin kinases, or in the exposure of binding domains to associate with other protein partners, as it happens with tallin and vinculin in focal adhesions. Single-molecule mechanical experiments *in vitro* and *in vivo* in conjunction with super-resolution imaging are rapidly changing our understanding of these processes. Protein folding-directed membrane fusion by the SNARE complex plays a central role in as diverse processes as intracellular transport and trafficking, neuronal vesicle exocytosis, and viral membrane fusion to cells. Yongli Zhang reviews the use of high-resolution optical tweezers to follow the folding trajectories of the (vesicle) v-SNARE and the (target) t-SNARE complexes *in vitro* [DOI: 10.1002/pro.3116]. These experiments first demonstrated that the zippering of the SNARE coiled-coils machinery is able to generate enough force—and do so sufficiently rapidly—to promote synaptic vesicle membrane fusion.

In a succinct and elegant contribution, Howard Berg reviews what has been learned from the use of single-molecule methods about the operation of *E. coli*'s flagellar motor and its adaptability to external chemical and mechanical environments [DOI: 10. 1002/pro.3055]. The motor, as it turns out, can change the stoichiometry of some of its key components to match the fraction of the time that it rotates clockwise (its operating point) to the concentration of chemical signals. Similarly, it can change the number of its force-generating units to tune its torque generation to the viscous load of the medium. Much work remains to understand the molecular processes underlying the adaptability of this machine.

I wish to thank all authors for their excellent contributions. Their effort has resulted in this exciting perspective of the state-of-the-art application of single-molecule approaches to the study of molecular machines. I also wish to thank Brian Mathews, Editor-in-chief at Protein Science, and Anar Murphy, Managing Editor at Wiley, whose amazing efforts and diligence have made this volume a reality.

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