Biophysics: Past, Present, and Future

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Biophysics, the discipline at the intersection of biology and physics, is entering an era of rapid developments. This comes as several growing trends in biological researches converge. The first is that biological researches have been moving beyond phenomenological observations and into the underlying physical reasons. The second is that the researches are becoming less qualitative and descriptive and more quantitative and predictive. Lastly, not only the direct participants of a particular biological process but also the cellular contexts, including "bystander" macromolecules in the subcellular localization and entire signaling networks, are becoming the targets of research.

Tools and concepts from physics were instrumental in propelling the early development of biophysics.[1] The resulting fruits include the molecular structures of the many thousands of proteins and their complexes and the basic understanding of electron transfer, enzyme catalysis, protein folding, and a host of other fundamental biochemical processes.

The complexities of biological systems and processes present ever more opportunities for biophysicists to push the frontiers of research. Currently, exciting discoveries are being made about subcellular structures and dynamics by super-resolution imaging and about the physical basis of how protein liquid–liquid phase separation regulates cellular functions.

With the accumulation of knowledge in the past decades, it will be possible to build structural and physical models of cells and predict the fates of cellular processes with higher and higher accuracy.

Past

Among the many achievements of biophysics in the last several decades, especially worth celebrating are the tools that have been developed for characterizing the structures and dynamics of biomacromolecules. All these tools were rooted in physics and have been perfected to tackle the challenges presented by biomacromolecules.

Indeed, many of the powerful tools for investigating biomolecules were initiated by physicists. X-ray crystallography provides a telling example. X-rays were discovered by Wilhelm Röntgen (1901 Nobel Prize in Physics) and their diffraction by crystals was first demonstrated by Max von Laue (1914 Nobel Prize in Physics). The subsequent mathematical formulation of the diffraction pattern by the Braggs, father and son (1915 Nobel Prize in Physics), ushered in the new field of X-ray crystallography. This made possible the determination of the structure of penicillin by Dorothy Hodgkin (1964 Nobel Prize in Chemistry), the first protein structures by Max Perutz and John Kendrew (1962 Nobel Prize in Chemistry), the structural model of DNA by Francis Crick, James Watson, and Maurice Wilkins (1962 Nobel Prize in Physiology or Medicine), and the structures of the photosynthetic reaction center (1988 Nobel Prize in Chemistry), ion channels (2003 Nobel Prize in Chemistry), RNA polymerase II (2006 Nobel Prize in Chemistry), the ribosome (2009 Nobel Prize in Chemistry), and G-protein coupled receptors (2012 Nobel Prize in Chemistry). Similar paths can be traced for nuclear magnetic resonance spectroscopy (1902, 1943, 1944, and 1952 Nobel Prizes in Physics; 1991 and 2002 Nobel Prizes in Chemistry; and 2003 Nobel Prize in Physiology or Medicine), electron microscopy (1986 Nobel Prize in Physics and 1982 Nobel Prize in Chemistry), and single-molecule techniques such as atomic force microscopy (1986 Nobel Prize in Physics) and optical tweezer (1997 Nobel Prize in Physics).

On the theoretical side, the development of quantum mechanics and its application first to chemical systems and then to biological systems have undergone a similar migration (1918, 1922, 1929, 1932, and 1933 Nobel Prizes in Physics; 1998 and 2013 Nobel Prizes in Chemistry). Nowadays computation has become a *bona fide* partner of experiment in addressing mechanistic questions and in making quantitative predictions on biophysical properties.

Present

The areas in which biophysicists are working presently are so diverse, no single person can be familiar with all of them. Hence no attempt is made here to present an all-encompassing picture of the state of the art. Instead a few selected areas are briefly surveyed. The selection reflects the author's familiarity and perhaps bias. It is hoped that the surveys nevertheless convey an overall sense of the exciting current developments in biophysics.

Until recently, a gap in spatial resolution existed between X-ray and electron crystallography (sub-nanometer resolution) and optical microscopy (hundreds of nanometers resolution). The latter resolution is set by the diffraction limit, at which the images of two point-like light sources lose distinction due to blurring resulting from diffraction (such an image is referred to as the point spread function). Super-resolution imaging (2014 Nobel Prize in Chemistry) breaks the diffraction limit and thereby produces structural information in the 10-nm range or below. Samples must be tagged by fluorophores, which emit light of a red-shifted color upon illumination by a laser.

Breaking of the diffraction limit can be achieved in several ways. One, known as stimulated emission depletion (STED), uses two laser beams to effectively reduce the size of the point spread function.[2] A low-intensity beam brings the fluorophores in the

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focal region from the ground state to the excited state, which upon spontaneous returning to the ground state would produce fluorescence. A high-intensity, red-shifted beam brings the excited fluorophores back to the ground state without emitting fluorescence. The latter beam has a donut-shaped intensity profile, such that fluorescence emission is turned off everywhere except in a small part of the diffraction-limited focal region.

A second strategy relies on the fact that, while two point sources cannot be distinguished below the diffraction limit, the location of a single point source can be determined at a much higher precision. Several variants of this strategy have been implemented, including photoactivated localization microscopy (PALM),[3] stochastic optical reconstruction microscopy (STORM),[4] and fluorescence photoactivated localization microscopy (fPALM).[5] The basic idea is to use a low-intensity laser to excite a sparse population of fluorophores to allow for precise determination of their locations. A second laser then switches off these excited fluorophores. Subsequently the first laser is turned on again to excite a different sparse population of fluorophores and the process is repeated. By collating the locations of all the fluorophores, the structure of the target system can be reconstructed.

Super-resolution imaging is now widely used to visualize various subcellular structures, including the three-dimensional (3D) organizations of chromatin (a level of DNA condensation into a chromosome) in different epigenetic states (posttranslational modifications of chromosomal proteins dictating active, inactive, or repressed states of DNA transcription).[6] In addition to static structures, super-resolution imaging can also be used to track dynamic processes, such as the diffusion of fluorescently tagged particles in subcellular environments, down to a temporal resolution of sub-milliseconds.[7]

Cell biologists have identified many membraneless intracellular "bodies" implicated in regulating various cellular functions.[8,9] The physical nature of these protein droplets has come into focus in recent years.[10–13] It is now known that these droplets represent a high-density phase of protein solutions, and their formation is similar to the condensation of water vapor (corresponding to the ordinary dissolved phase of protein solutions) into the liquid phase. Much like the vapor–liquid phase transition of water, the liquid–liquid phase separation of protein solutions is reversible and this reversibility is well suited for regulating cellular functions. The link between phase separation and regulation has sparked great interest.[14,15] Some protein components are selected into droplets while others are excluded, though the mechanism is still murky. Moreover, cells apparently use a variety of means, including pH change and phosphorylation/dephosphorylation, to modify the liquid–liquid phase boundary and thereby control droplet formation. Computational methods are now emerging to address these critical questions and calculate liquid–liquid phase equilibria.[16]

Fifty-five years ago, Monod and Jacob,[17] with great foresight, proposed the concept of allostery, whereby effector binding at a distal site changes the functional activity (eg, substrate binding affinity or catalytic efficiency) of a protein at the active site. Further development by Monod, Wyman, and Changeux led to a seminal paper in *Journal of Molecular Biology*.[18] Allostery is now recognized as a universal regulatory mechanism, for biological systems ranging from single-domain proteins to multi-component complexes, and effectors can be small molecules as well as macromolecules.

Monod, Wyman, and Changeux hypothesized that allosteric effects were mediated by conformational transitions of the proteins. An alternative idea was that effectors induce changes in the broadness of the free energy basin of the protein conformational state, without shifting the basin minimum position.[19] Both emphasized the thermodynamic consequences of effector binding. However, when effector binding changes the free energy landscape of a protein in conformational space, the change not only affects thermodynamic properties but also dynamic properties, including the amplitudes of motions on different timescales and rates of conformational transitions.[20] Possible roles of conformational dynamics in mediating allosteric effects are being probed by NMR spectroscopy, molecular dynamics simulation, and other approaches. Among the mysteries are the directionality of allosteric communication and the relation between protein motions on different timescales.

The reversible folding of proteins demonstrated by Christian Anfinsen (1972 Nobel Prize in Chemistry) and the early success with protein structure determination by crystallography helped instill the idea that each protein is predestined to a unique 3D structure. However, around the turn of the century, it became clear that a substantial fraction of proteins are either wholly unstructured or contain such regions, which have since been referred to as intrinsically disordered proteins (IDPs) and intrinsically disordered regions (IDRs).[21–23] IDPs/IDRs are involved in numerous regulatory and signaling functions and are associated with many diseases.[24] The significant conformational freedom of IDPs and their malleability in response to environmental factors make the conformational characterization extremely challenging, and the help of computation becomes essential. Intrinsic disorder also complicates the mechanisms of association to and dissociation from cellular targets, which provide a fertile ground for experimental and computational studies.[25]

Future

Starting in the 1960s, biology became "molecular." Now and into the future, perhaps it can be stated that biology will be "physical." Questions of how intra- and intermolecular interactions mediate functional mechanisms at different spatial and temporal scales will come to the fore. Structural models of subcellular compartments and even whole cells are starting to be built and will surely become more and more realistic.[26] With input from and validation by experiments, it will be possible to predict the time evolution of cellular processes with higher and higher accuracy.

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