Cellular Biophysics

Rick Horwitz[1,*]
[1]Allen Institute for Cell Science, Seattle, Washington

Cellular biophysics is the branch of biophysics that studies cells from the perspective of a physicist or physical chemist by applying physical methods to interrogate cell structure and function, and developing models of cells using physics and physical-chemical principles. Early on, biophysics was usually practiced by physicists or other researchers with physics-based training who had changed fields, but by the 1960s many PhD programs in biophysics had been developed for undergraduate physics and physical-chemistry majors wanting to study biology.

After World War II, biophysics in general got a lift from the field of radiation physics, which was trying to understand the effects of radiation on life and genetic mutations. This came in the wake of H.J. Muller's Nobel Prize studies showing that x rays induced mutations in *Drosophila*. Another major area of biophysics research focused on emerging structural methods such as x-ray diffraction, and spectroscopic methods such as fluorescence and magnetic resonance. This was the advent of the field of molecular biophysics, and these methods were used to determine the structures and functions of individual molecules and contributed to the molecular biology revolution.

On the cellular side, however, there was great interest in the physiology of nerve and muscle cells, and understanding how molecular components drive cell function. Forces and electrical activity are topics of great interest to physicists, and biophysicists have played a major role in understanding them in biological systems. Nerve cells propagate spikes in electrical potential, called action potentials, across an individual cell, and these signals transmit information from one nerve cell to another nerve or muscle cell. These spikes can be initiated by electrical or chemical stimuli and are measured using electrodes. This research culminated in a Nobel Prize to Alan Hodgkin, Andrew Huxley, and John Eccles in 1963 (1).

Muscles generate force through a mechanism involving contraction of individual muscle cells. Our understanding of this process has been greatly enhanced by detailed structural studies of the organization of muscle cells using electron and light microscopy. Muscle cells form highly organized repetitive filamentous structures, and changes in the spacing of these repetitive structures during contraction form the basis of the sliding-filament hypothesis, which holds that the molecular components found periodically along the muscle fiber slide to affect contraction (2).

Microscopy: a major theme in cellular biophysics

The organization and activities of cells are major themes in cellular biophysics, and studies have focused on observing complex structures inside cells, detecting cellular activities, and extending methods developed to study purified biological molecules to microscope-based cellular measurements. Microscopy, which functions across multiple scales of time and spatial resolution, is at the center of these studies. The highly localized and often transient nature of cellular activities is an overarching theme that has emerged from live-cell microscopy and drives contemporary cellular biophysics. For example, some cellular receptors come together to form small bimolecular complexes when they become functionally active. Analogously, many signals that regulate cellular processes are generated from large molecular complexes that form transiently on scaffolds residing in specific locations. These molecular interactions produce new structures that change conformations, produce new functions, or create more efficient organizations resulting in enhanced activity (3). On a larger spatial scale, cellular components are often organized into discrete, readily visible structures, often referred to as organelles or molecular machines. These large, identifiable molecular machines make proteins (ribosomes) generate energy (mitochondria), protect and regulate genetic material (nucleus), and cause cells to contract (actomyosin filaments) (4). They also appear to occupy specific regions and act transiently.

One goal of contemporary cellular biophysics is to understand the molecular details of how cellular components organize to generate and regulate specific activities. Another goal is to determine how all of these diverse cellular activities and structures work together to produce characteristic and specialized cellular behaviors. Biophysicists are also developing mathematical and computational models that describe these cellular functions.

At present, microscopy is at center stage in the world of cellular biophysics, driven by the development of new microscopic methods and fluorescence reagents specialized for cellular imaging. Recent advances in light microscopy now allow us to view structures at previously unattainable spatial and temporal resolutions, image live cells in tissues and animals, and visualize many colors (and thus different molecules) in the same measurement. Amazingly, new

Submitted November 4, 2015, and accepted for publication January 15, 2016.

*Correspondence: rickh@alleninstitute.org

0006-3495/16/03/0993/4
http://dx.doi.org/10.1016/j.bpj.2016.02.002

highly sensitive cameras even allow for the visualization of individual molecules (5–9). Similar to advances in light microscopy, improvements in electron microscope tomography now allow us to see the molecular architecture of organelles in cells at a higher level of detail (10). Finally, measurements can be made in living cells of molecular attributes, including concentration, binding affinities, and diffusion and flow, which were previously studied by using purified components in test tubes (11). All of these measurements provide data that can be used to develop and test theories and mathematical models for complex cellular phenomena.

New fluorescence reagents complement advances in microscopy. They include genetically encoded tags that can be attached to biological molecules, as well as dyes and other fluorescence reagents that localize to specific cellular structures or sense biological activities, telling not only where a molecule or organelle is but also what it is doing at that time. These reagents allow us to localize and measure the positions and dynamics of molecules and the complexes in which they reside, as well as when and where cellular activities occur.

Biosensors are another useful tool that was developed to measure alterations driven by cellular processes. Fluorescence changes are induced in biosensors when molecules come into close proximity or undergo a conformational change (12,13). Similarly, optogenetic reagents allow perturbations of cellular function with great spatial and temporal resolution (14). In contrast, other microscopic methods probe the interaction of the cell with its exterior, sensing the forces that cells exert though their contacts with other cells or connective tissue components (15).

Looking ahead

These are exciting times in cellular biophysics, and this era of breathtaking progress and newly developed technologies points to a bright future for the field. We now have tools to address questions that have lingered for decades, and recent findings are raising new questions that are moving science down important and unexpected paths. Imaging in particular has benefited from significant advances, and our understanding of cellular organization and activities is becoming ever more refined and providing new insights into cellular processes such as cell differentiation. The development of biosensors that report the activities of various cellular machines and processes is still young, but these devices have already revealed how the machinery of the cell is integrated, coordinated, and regulated (16). New genome-editing technologies are being used to introduce genetic tags to specific proteins using the cell's own genome and regulatory apparatus, enabling researchers to obtain highly quantitative measurements regarding the numbers of proteins and organelles (17) present in a cell. Our ability to detect single molecules has already provided highly detailed insights into the mechanisms of specific cellular processes, such as cargo transport along microtubules (18). One goal is to develop mathematical or computational models for individual cellular processes. These models could be based on detailed physical-chemical principles, as has been done for some highly complex and integrated processes, such as membrane protrusion and cytokinesis in vitro (19). They could also be integrated whole-cell models, such as that described for the life cycle of a bacterium (20). The promise is that new methods in quantitative microscopy will provide better data, leading to models that are increasingly realistic and predictive.

The complexity of cells in terms of the numbers of different molecular machines and regulatory complexes, and the numbers of molecules that comprise them, has forced us to look at cells from the point of view of a single or small group of molecules at a time. This approach has been enormously productive, as each protein and complex of proteins becomes a source of fascinating new information as we learn more. However, each molecular machine or complex is comprised of many molecules, each cell has many different organelles and complexes, and there are many different kinds of cells, each exhibiting specialized behavior. In addition, tissues are comprised of many different cell types working together. This integrative behavior of cells is highly challenging and therefore largely uncharted territory.

The ever-growing complexity of understanding how cells work at a molecular level is driving researchers to work more collaboratively and form multidisciplinary teams. Many areas of specialization are needed to understand cellular functions and how they are altered by genetic and environmental factors. New multidisciplinary groups and institutes are being formed, and arguably the largest effort along this line is the Allen Institute for Cell Science, cofounded and supported by Paul Allen, the cofounder of Microsoft.

The Allen Institute aims to develop predictive computational models of cell behaviors and how they respond to environmental and genetic alterations. In its initial project, the Institute is focusing on live-cell imaging and using genome editing of induced pluripotent stem cells (iPSCs) to measure the locations and relative organization of cellular machinery, regulatory complexes, and activities, as well as the concentrations and dynamics of key molecules. iPSCs proliferate and can be induced to differentiate into different kinds of cells, including muscle, nerve, gut, and skin (Fig. 1). Using genome editing, investigators can inactivate a gene or change it by inserting either a mutation that mimics a disease or a fluorescence protein tag, which allows quantitative estimates of molecular number. Once developed and characterized, these cells will become a launch pad for the study of many different cell types by members of the Institute and the greater scientific community, to whom they will be distributed freely. The goal is to measure the changes that occur when cells execute their various activities, as well as when

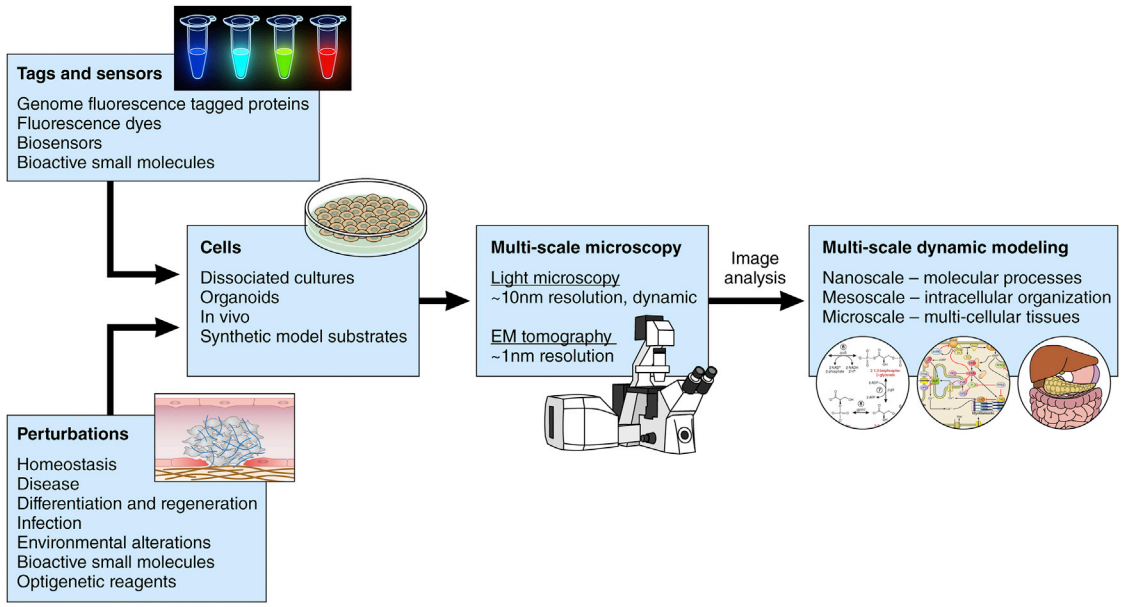

FIGURE 1 A systems approach to meso- and nanoscale imaging and modeling. To see this figure in color, go online.

they differentiate into specialized cells and respond to genetic and environmental alterations, including drug intervention. Investigators could then combine these measurements with other cellular data to develop computational models that predict cellular states and behaviors during homeostasis, regeneration, and disease.

To execute this program, the Institute will foster multidisciplinary research, with a strong focus on physical methods and approaches. This research will have a major biological component that includes the processing, genome editing, and differentiation of iPSCs. These cells will be used to understand different cell states and how these states change as the cells execute their characteristic behaviors and respond to different environments. The Institute will incorporate engineering aspects by bringing its activities to large-scale, automating, and integrating methodologies, and undertaking systems-level approaches. Physical science approaches will take center stage in the state-of-the-art microscopic methods and biosensors that will be employed. Computational and mathematical modeling will benefit from theoretical physics, computer science, and applied math and engineering approaches, as both systems- and physicochemical-level models will be employed. A novel product of the project, an animated cell, will be a visual output designed to integrate image data and existing structural data, and will show the dynamic inner organization and workings of a cell in unprecedented detail. It is also designed to integrate quantitative data on subcellular structures that can be visualized together, allowing the viewer to see, both en groupe and selectively, the relative positions of cellular structures and activities.

The Institute's model for research is defined by attributes that can be applied to similar ventures. These characteristics include the use of large-scale and integrative approaches, such as looking at effects on several cellular components rather than a specialized one. The Institute will share data, reagents, models, and tools openly with the community, and will focus on interdisciplinary team science with clear objectives and milestones.

Cellular biophysicists are working toward understanding cells as individuals and collectives, and how this drives tissue, organ, and organism functions. Such knowledge would help satisfy our innate human curiosity about how life works, and would also contribute significantly to regenerative medicine and disease therapies by elucidating tissue formation and identifying new therapeutic targets.

Cellular biophysics also drives innovation and economic growth. Efforts to understand the biology of the cell have driven the development of new technologies, including two-photon, confocal, light-sheet, and superresolution microscopies. These technologies will greatly impact the pharmaceutical industry by advancing drug discovery and improving diagnostic methodologies. Finally, the ability to model the cell and its regulatory pathways, connecting genomic, epigenetic, environmental, and other data with quantitative cellular data of the kind discussed here, holds enormous predictive promise, leading to a computational "cell clinic" where one can query what the effects of different alterations will be on cell and tissue function, building on the premise that most disease originates from alterations in cell function.

ACKNOWLEDGMENTS

The author thanks Paul Allen for his vision and support.

REFERENCES

1. Hodgkin, A. L., and A. F. Huxley. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* 117:500–544.
2. Huxley, H. E. 1953. Electron microscope studies of the organisation of the filaments in striated muscle. *Biochim. Biophys. Acta.* 12:387–394.
3. Scott, J. D., and T. Pawson. 2009. Cell signaling in space and time: where proteins come together and when they're apart. *Science.* 326:1220–1224.
4. Alberts, B., A. Johnson, …, P. Walter. 2002. Molecular Biology of the Cell, 4th ed. Garland Science, New York.
5. Helmchen, F., and W. Denk. 2005. Deep tissue two-photon microscopy. *Nat. Methods.* 2:932–940.
6. Keller, P. J., F. Pampaloni, and E. H. Stelzer. 2006. Life sciences require the third dimension. *Curr. Opin. Cell Biol.* 18:117–124.
7. Liu, Z., L. D. Lavis, and E. Betzig. 2015. Imaging live-cell dynamics and structure at the single-molecule level. *Mol. Cell.* 58:644–659.
8. Chen, B. C., W. R. Legant, …, E. Betzig. 2014. Lattice light-sheet microscopy: imaging molecules to embryos at high spatiotemporal resolution. *Science.* 346:1257998.
9. Hell, S. W., and J. Wichmann. 1994. Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy. *Opt. Lett.* 19:780–782.
10. Asano, S., B. D. Engel, and W. Baumeister. 2015. In situ cryo-electron tomography: a post-reductionist approach to structural biology. *J. Mol. Biol.* 428:332–343.
11. Wiseman, P. W. 2015. Image correlation spectroscopy: principles and applications. *Cold Spring Harb. Protoc.* 2015:336–348.
12. Weitzman, M., and K. M. Hahn. 2014. Optogenetic approaches to cell migration and beyond. *Curr. Opin. Cell Biol.* 30:112–120.
13. Giepmans, B. N., S. R. Adams, …, R. Y. Tsien. 2006. The fluorescent toolbox for assessing protein location and function. *Science.* 312:217–224.
14. Gautier, A., C. Gauron, …, S. Vriz. 2014. How to control proteins with light in living systems. *Nat. Chem. Biol.* 10:533–541.
15. Dembo, M., and Y. L. Wang. 1999. Stresses at the cell-to-substrate interface during locomotion of fibroblasts. *Biophys. J.* 76:2307–2316.
16. Welch, C. M., H. Elliott, …, K. M. Hahn. 2011. Imaging the coordination of multiple signalling activities in living cells. *Nat. Rev. Mol. Cell Biol.* 12:749–756.
17. Doudna, J. A., and E. Charpentier. 2014. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. *Science.* 346:1258096.
18. Park, H., E. Toprak, and P. R. Selvin. 2007. Single-molecule fluorescence to study molecular motors. *Q. Rev. Biophys.* 40:87–111.
19. Pollard, T. D., and J. Berro. 2009. Mathematical models and simulations of cellular processes based on actin filaments. *J. Biol. Chem.* 284:5433–5437.
20. Carrera, J., and M. W. Covert. 2015. Why build whole-cell models? *Trends Cell Biol.* 25:719–722.