Graduate training in cellular biophysics

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INTRODUCTION

In this article I will outline some of the knowledge and skills that investigators in cellular biophysics find valuable in their work. Everyone has their own definition of cellular biophysics. For me it is hard to separate cellular biophysics from molecular biophysics, so let us accept the inevitable overlap and differentiate the areas on the basis of emphasis. Both include quantitative studies of the chemical and physical basis of cellular function. Both involve characterization of proteins, nucleic acids, and lipids and their interactions. But a molecular biophysicist is more likely to be interested in electrons, while a cellular biophysicist is more interested in macromolecular events that can be studied with a light microscope. Cellular biophysics emphasizes the molecular aspects that directly illuminate functions at the cellular level, while molecular biophysics emphasizes the properties of the molecules themselves. They really represent a continuum with a common goal.

I write from the perspective of almost 25 years of research on the molecular basis of cellular motility, an endeavor that started out as a purely biological fascination and has taken on a strongly biophysical tone as our understanding has broadened. I have a very practical view of training in cellular biophysics, since as an MD I had essentially no formal training in the subject. Instead, my students, postdocs, and I have learned on the job from the literature, books, and many generous colleagues. I have also had the opportunity to learn what challenges students while teaching a laboratory course during the summer at Woods Hole. Consequently, I will propose what one needs to know to do creative research rather than what constitutes a formal course of study. Although knowledge of this material will not replace a good idea, it does free one's thoughts from the constraints of ignorance and can lower one's natural inhibitions to try new things. Good ideas can come from many places, so I put a very high priority on developing broad interests in biology and a lifetime of reading widely about the natural sciences. Ostrich-like individuals with their heads stuck in the sand of their own subspecialty risk becoming fossilized.

WHAT IS A GOOD BIOLOGICAL PROBLEM?

Formal curricula almost always neglect the first and most important prerequisite for successful research, a good biological problem. Lest we not forget it here, I will start by considering some of the features of a good biological problem from my perspective as a cell biologist.

Good problems focus on important biological processes, offer growth potential, and are tractable experimentally. Some newcomers imagine that previous generations have already grabbed all of the good problems, but it is not true. The landscape is so vast in biological research that new knowledge creates new opportunities. As the frontiers broaden, so will the opportunities, at least for the foreseeable future. Not only are there essentially limitless details awaiting discovery; even more important, many of the general principles still need to be identified.

"Important biological processes" are those that cells depend on for their basic function. Some of the best problems deal with universal processes used by all cells such as the assembly of organelles, motility, signal transduction, and regulation of the cell cycle, but the parsimony of mechanisms at the molecular level has the consequence that investigation of specialized processes usually reveals some basic principles with wide applicability. For example, the wealth of information on muscle contraction has greatly enriched our understanding of all sorts of cellular motility.

There are so many genuinely fundamental questions in biology that no one should waste their time repeating established work in new organisms or with new methods unless they have an attractive hypothesis about why such repetitive work is likely to reveal new principles of general interest. The scientific community responds more enthusiastically to something novel. Discovery not only provides the most gratification in research, but discovery is the only way to gain the confidence of the community that is required to obtain the resources to continue our research. One must not waste good training and resources on boring problems.

There is another side to this argument espoused by my friend Evan Eisenberg of the NIH. When we were postdocs together, he claimed that the best way to become famous is to identify the most important experiment in one's field and to repeat it. Of course, he did not mean that one should literally repeat earlier work; rather one should try a creative variation of the important experiment in the hopes of expanding knowledge at the heart of a field. This approach does not have the novelty that I have advocated above, but it does focus one's attention on the important issues in a field. Since experiments are rarely replicated exactly, Evan is right that one has a good chance of finding something new that will command attention simply because of the focus on an issue of acknowledged importance.

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By "growth potential" I mean that one's hard work should be rewarded by expanding opportunities, not dead ends. Most research requires a substantial investment of thought, time, effort and financial resources. Ideally, each of our investments in research should open up new opportunities. Common examples include the discovery of a new process or molecule, the determination of a new structure, the identification and cloning of a new gene, the purification of a new cellular constituent, or the development of a new method. These contributions can elevate a field to a higher level that allows work to expand in more sophisticated ways. In many cases students or postdoctoral fellows have based their independent careers on the hard work they did to elevate their field. Obviously, one should not leave loose ends behind, but the scientific community places such a high value on novelty, that we all should strive to find something new.

By "tractable" I mean experimentally approachable. Many important problems with growth potential are not easy or someone would have taken them on already. What is usually missing is a good idea about what to do or how to get started, rather than the absence of the technology to do the work. Many fields are idea limited rather than technology limited. How many times have you said, "I wish that I had thought of that"? Since considerable physical labor is usually required to do novel research with growth potential, be sure that you have done some good thinking ahead of time. Make sure that your strategy is sound and that the outcome will meet your goals of contributing something novel and giving you the desired foothold for your future work.

Who should pick the experimental problem and the research strategy? It must be the person with the best idea. Since no one has a monopoly on good ideas, these decisions turn out to be team efforts. Ideally everyone in a lab is continuously hunting for good ideas. The group leader may have the best perspective, but everyone should contribute. In our laboratory the technical staff, students, and postdocs have all contributed to the overall direction of the research in addition to providing solutions to the everyday experimental problems. The only way for this synergism to develop is to have everyone informed about the details of their colleague's experiments through give and take at frequent laboratory meetings.

FORMAL COURSES

I will assume that all students entering cellular biophysics will have a general background in science and mathematics. For undergraduates, this will include courses in general chemistry, organic chemistry, and physics, each with a laboratory. This trio along with calculus is generally required for the advanced courses considered below. Upper level courses in physics and math may be helpful for some types of research in molecular biophysics but are not essential for most work in cellular biophysics. Some expertise with computers is essential, but I hesitate to recommend a formal course, since most individuals learn their computing skills informally on the job.

Biology

The aspiring cellular biophysicist needs to have a broad knowledge of biology to help identify a good research problem and the most advantageous experimental system to get the answers. Courses in general biology are necessary for exposure to the diversity of organisms and for orientation, but are not adequate for appreciating the merits of a research project. One needs to understand in some depth how cells work at the molecular level. The best way is probably a semester or year long course in cell biology. Either in this course or others, one must learn about classical genetics and molecular biology. Many biophysicists deal with topics like membrane channels that are covered in physiology courses, another important option to consider. Each student will need to be selective in choosing biology courses since the topics covered in cell biology, for example, differ widely from school to school.

What about an aspiring cellular biophysicist who comes from the physical sciences with little or no opportunity for formal training in biology? I would strongly recommend that they read and think about the material in one of the big, comprehensive cell biology books such as the "Molecular Biology of the Cell" by Alberts et al., 2nd ed. (1989, Garland Press, New York) or "Molecular Cell Biology" by Darnell, Lodish, and Baltimore, 2nd ed. (1990, Freeman Publications, San Francisco). Both of these books cover cellular biology at the molecular level in enough depth to provide an excellent basis for thinking about cellular biophysics. One shortcoming is that neither book consistently highlights the limits of our knowledge, so a naive reader is likely to get the impression that there are more answers than we actually have in hand.

Biochemistry

Every cellular biophysicist needs a good working knowledge of biochemistry, since virtually all experimental biologists are biochemists, at least in part. An upper division or graduate level biochemistry course is adequate to equip the cellular biophysicist with a working vocabulary and the general concepts. This will suffice for formal training, providing that students retain an active interest in the field and do their best to keep up with trends and new discoveries. Few will master biochemistry without a formal course, although thorough study of one of the major biochemistry books is a possible alternative. The books by Stryer (3d., 1988, W. H. Freeman and Co., New York) and Matthews and vanHolde (1st ed., 1991, Benjamin/Cummings Publishing Co., Redwood City, CA) are examples of the excellent biochemistry books that could be studied independently.

Biophysical chemistry

Every cellular biophysicist also needs solid grounding in physical chemistry, but it is not obvious where they should get this formal training. The problem is that many physical chemistry courses for chemists emphasize topics and approaches that are not particularly helpful in cellular biophysics. Coverage of physical chemistry with a life sciences thrust is recommended providing that this does not mean watered down physical chemistry. Alternatively, some biophysical chemistry classes have the right orientation, but they do not necessarily cover the essential topics. The most important topics are thermodynamics, chemical kinetics, spectroscopy (including scattering), optics, and transport processes (hydrodynamics). Quantum and statistical mechanics are less useful in cellular biophysics than the other topics, but will be valuable for selected students whose research involves these tools. The optics should include diffraction, lenses, image formation, and image processing as they apply to visible light, x-rays, and electrons. The student needs to understand general principles like the relation of lenses to Fourier transforms and microscopy to diffraction methods rather than the intricacies of any of these methods. For those who need to study the material on their own or who need a refresher, I like "Physical Chemistry with Applications in the Life Sciences" by D. Eisenberg and D. Crothers (1979, Benjamin-Cummings Publishing Co., Redwood City, CA). Another good book is Barrow's "Physical Chemistry for the Life Sciences" (1975, McGraw-Hill Inc., New York).

THEORY AND PRACTICAL KNOWLEDGE

Microscopy

Microscopy of all sorts is probably the most universally applicable technique in cellular biophysics, so it is regrettable that many users do not fully understand how microscopes work. I suppose that the problem is that none of the standard courses regularly takes responsibility for microscopy. Basic optics and the properties of light are covered in elementary physics, but image formation, methods of enhancing contrast, and quantitative fluorescence methods are not. The student usually has to shop around to find some instruction in microscopy. Fortunately, much of the basic theory is covered in books including the following: "Fundamentals of Light Microscopy" (M. Spencer, 1982, Cambridge University Press, New York); "Video Microscopy" (S. Inoue, 1986, Plenum Press); "Fluorescence Microscopy of Living Cells in Culture" (Y. L. Wang and D. L. Taylor, editors, 1989, volumes 29 and 30 of Methods in Cell Biology, Academic Press, New York); "Biological Confocal Microscopy" (J. Pauley, editor, 1989, Plenum Press, New York). A good source of information on fluorescent probe molecules is the catalogue "Molecular Probes" (R. Haugland, 1992, Molecular Probes Company, Eugene, OR).

One does not have to learn to operate each type of microscope, but all cellular biophysicists should know the capabilities of the range of modern microscopes, because this knowledge will help with the selection of the appropriate microscope for each application. It is useful to understand the principles of bright field, dark field, polarization, phase contrast, fluorescence, and differential interference microscopes, of basic image formation, video cameras, and video image processing, of confocal laser scanning microscopes, and of transmission and scanning electron microscopes. Scanning probe microscopy is already a valuable tool in materials science and will have an impact in biology once specimen preparation methods are mastered (see A. Engel, 1991, *Annu. Rev. Biophys. Biophys. Chem.* 20:79–108). Each type of microscopy involves specialized methods of specimen preparation, especially electron microscopy, and it is helpful to know in general what they involve.

Many research institutions have microscopy facilities that provide practical training in microscopy and specimen preparation. Good facilities will also offer theory to go with the practice. For advanced training intense courses in microscopic theory and practice are available at the Marine Biological Laboratory, Woods Hole, MA.

Purification and manipulation of DNA, proteins, lipids

To do molecular studies on cells, one must know the theory and practice of handling the major biological molecules: at least proteins and nucleic acids, but some knowledge of lipids can also be useful. One needs to know how to purify, manipulate, and analyze these molecules. In the case of proteins, this requires centrifugation, chromatography, electrophoresis, amino acid analysis, and protein sequencing. For DNA this requires library construction, cloning, sequencing, in vitro mutagenesis, DNA and RNA blots, and expression in bacteria and eukaryotic cells. For lipids this requires extraction, column chromatography, thin layer chromatography, gas chromatography, and the formation of vesicles and micelles. Most of this material is covered in graduate biochemistry courses, but the level is usually much too superficial to be of practical use. Individuals in most institutions and many laboratories are familiar with these methods, but I advise newcomers to be cautious about their sources of advice, since many successful practitioners do not fully understand the methods that they use.

Fortunately, many excellent books are available on biochemical methods, for example, "Protein Purification" by R. K. Scopes (1987, Springer-Verlag, New York) for proteins, "Molecular Cloning" by Sambrook et al. (2nd ed., 1990, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) for nucleic acid manipulations, and "Techniques of Lipidology", M. Kates (1986, Elsevier Scientific Publishers, Amsterdam) for lipids. The series "Methods in Enzymology" (Academic Press, New York) has covered all of these methods in detail over the years. I strongly recommend that you consult these or more advanced books the first time that you use a new method to give yourself the benefit of fully understanding the principles and the practical matters involved. If you do this reading consistently, you will soon become the expert in your lab on methods, since most people only have hit or miss, word of mouth training in many of the techniques upon which their work depends.

Although it is no longer usually included in the list of skills in handling macromolecules, I think that it is essential for everyone working with pure macromolecules to know the methods available for crystallization. The individual who does the purification is most likely to know the tricks required to keep a particular molecule happy during a crystallization trial, so they, rather than a professional crystallographer, should take the first crack at growing crystals suitable for structure determination by x-ray analysis. I claim that the actual manipulations are much easier than running a gel and the hardware required to set up some hanging drops for vapor diffusion crystallization trials is less expensive than lunch at a fast food restaurant. A detailed book by A. MacPherson on "Protein Crystallization" (1982, John Wiley and Sons, Inc., New York) is available for practical advice. An intense practical course in x-ray crystallography is available annually at the Cold Spring Harbor Laboratory.

Handling cells

Good sterile technique is required for both tissue culture and bacteriology and thus for everyone who clones DNA, expresses cloned proteins and prepares live cells for microscopy. More specialized techniques, including cellular microinjection, intracellular recordings, and patch clamping, are the mainstays of some types of cellular biophysics. A general knowledge of these valuable methods will suffice for the undifferentiated cellular biophysicist. Practical courses are available at most universities, the Marine Biological Laboratory and the Cold Spring Harbor Laboratory.

Kinetics

Every cellular biophysicist should understand how to use reaction rates to determine mechanisms. The standard steady-state enzyme kinetics covered in biochemistry classes is one way to look at the subject, but is not nearly as useful as a general approach as transient state kinetics. For example, transient kinetics can be used to evaluate not only enzyme mechanisms in more detail than possible with steady-state kinetics, but the same general approach can also be applied to the host of binding reactions and conformational changes that cells use to assemble their macromolecular components, to regulate genes and membrane permeability, and to transduce signals. You need to understand the meaning of rate constants, the relation of rate constants to equilibrium constants, and the experimental strategies used to evaluate rate constants. These include stopped flow and quenched flow rapid kinetics as well as single turnover and pulse-chase designs that are applicable to virtually every biological system. A laboratory section on kinetics is available as part of the Physiology Course at the Marine Biological Laboratory.

Spectroscopy

The cellular biophysicist must be knowledgeable about the theory and practice of UV-visible spectroscopy, fluorescence spectroscopy, and simple light scattering. Some of this is purely mechanical: how to take an absorption spectrum, how to choose the optimal wavelengths for a fluorescence measurement, what sample concentrations give reliable measurements, how to increase the signal-to-noise ratio. For fluorescence work, this usually requires an understanding of the chemical methods and basic fluorescent reagents available to label specific sites on biological molecules with fluorescent probes. Photoactivated fluorescence probes have recently emerged as particularly valuable probes.

It is also extremely useful to understand enough about the theory and practice of specialized spectroscopic methods so that one knows when and how to consult an expert colleague for a particular experimental application. The most important advanced methods are x-ray diffraction and multidimensional NMR methods for atomic structure determination. At some time in our careers, each of us is likely to find a valuable application for other specialized types of spectroscopy including ESR, NMR, IR, CD, mass spectroscopy, quasielastic light scattering, phosphorescence, fluorescence energy transfer, fluorescence life time, and fluorescence polarization, so it is useful to know what they have to offer.

KEEPING UP

Keeping up with advances in research is probably the most challenging task for most practicing biological scientists. Having become acquainted with biology in general, having mastered a particular field and (hopefully) having contributed something original, how should the cellular biophysicist keep up with the literature? Obviously, one must read carefully the emerging literature in one's own specialty, that goes without saying. Each of us knows well the three or four journals where we and colleagues in a field are most likely to publish new results. These new papers need to be read, not just xeroxed. (Faced with spiralling costs at the copy center at the Cambridge MRC Laboratory of Molecular Biology, Sidney Brenner once sent out an appeal to replace xeroxing with "memeroxing"!)

Since few individuals are disciplined enough to read the original literature on their own, I strongly recommend specialized journal clubs. A common format is to assign important papers on a rotating basis to the members of the group and have them lead an in depth discussion of one or two papers per session. I have had better results with a literature scanning format. Each of the 10 members of our journal club selects a different paper each week and has 10 minutes on the clock to present all of the figures in the paper to the whole group. The papers available each week are those that seem most interesting to me from a computerized literature search that casts a rather wide net in our field. This format allows each participant to see the data from more than 300 original papers each year. Although we cannot consider each in depth, the participants rapidly learn the skills to provide criticism and praise for their papers each week. In some ways, this journal club is even more valuable and certainly more cost effective than attendance at scientific meetings.

Keeping abreast with the action outside one's own field is more challenging, but no less important. It is advantageous to know in general where the action is in the whole field of cellular biophysics, because many of the most valuable insights come at the interfaces of different fields of study. Further, one needs to know how investigators are thinking in related fields to keep one's own ideas fresh. Participation in large national scientific meetings, especially careful attention at the symposium talks outside one's speciality, is an excellent way to keep up. Broad departmental journal clubs and seminar series are also valuable. Since one can read only a tiny fraction of the original literature, I recommend regular reading of one or more of the excellent review journals such as *Current Opinion in Cell Biology* or *Current Opinion in Structural Biology* (each of which systematically covers their whole field each year) or *Trends in Biochemical Sciences* or *Trends in Cell Biology* (which highlight exciting new work). Similar publications are available in genetics and neuroscience. A second essential reading assignment is to peruse *Nature* and *Science* each week. Not much of major importance escapes publication there either as original research or as news features. Finally, teaching (that oft maligned obligation of professors) is probably the best way to stay informed in the general field of cellular biophysics. Courses with biological content will provide much more educational benefit to the professor than the all too frequent biophysics courses that stress methods.

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