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Molecular imaging: *sine labore nihil*

Editorial overview

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Introduction

The field of molecular imaging, even when restricted to illuminating questions at the intersection of chemistry and biology, is large, diverse, and multidisciplinary. A common thread running through the field, however, is the development of biomolecular labels and strategies for identifying the location, concentration, activity, association partners, and lifetime of all biomolecules — proteins, nucleic acids, oligosaccharides, lipids, metabolites, etc. — in a living cell as a function of the cell's life cycle. Another unifying theme is the harnessing of the unique physics of light to illuminate the structures of macromolecular assemblies and the mechanics of complex molecular machines. Indeed, it is becoming increasingly clear that our understanding of biomolecular structure and function will be only as sophisticated as the tools with which these features are probed. This issue of *Current Opinion in Chemical Biology* highlights creative approaches that first, expand the chemical encyclopedia of small (and medium) molecule fluorophores that can be used for molecular imaging; second, broaden the manner in which these molecules can be exploited to produce a more complete picture of dynamic biomolecular behavior; third, provide new optical methods to further increase the scope and precision of our observations; and finally fourth, apply these multiple tools to delve deeper into the inner workings of the macromolecular assemblies and molecular machines that were barely characterized only a decade ago.

New molecules

Small-molecule fluorophores have a rich and fertile history and the advantages they possess as biomolecular labels are hard to ignore. Their small size relative to fluorescent proteins imparts a smaller 'price for peeking', that is, it minimizes the potential that the label will perturb the structure and/or function of the biomolecule of interest. In addition, small-molecule dyes are likely to permeate cells *via* passive diffusion or, alternatively, can be designed for cell impermeance, providing control over both time and place of visualization. Moreover, and perhaps most importantly, small-molecule fluorophores provide exceptional brightness, a broad range of excitation and emission wavelengths, and tunable photostability. As a result, the diversity of their optical properties exceeds those of most fluorescent proteins, whether found in Nature or produced by directed evolution. Three reviews in this issue highlight complementary approaches towards new small-molecule fluorophores that possess advantages for illuminating complex cellular or biomolecular function; a fourth describes recent advances in the development of quantum dots, highlighting their unique utility in time-resolved biophysical studies of mechanism. Wysocki and Lavis describe new, synthetically accessible fluorescent scaffolds that possess any of a number of interesting properties: unique emission wavelength, improved

photostability, pH independent emission, masked reactivity, and the potential for photoactivation. Lee, Vendrell and Chang highlight the power of combining diversity-oriented synthetic methods with clever screening approaches to discover, in an unbiased way, the molecular architectures required to achieve a particular imaging goal. With an eye towards developing new tools for subdiffraction-limit imaging (i.e. superresolution imaging) using photoconvertible fluorescent proteins, Lukinavičius and Johnsson describe a strategy in which self-labeling proteins are exploited to generate fluorophores that photoconvert upon interaction with another molecule or, alternatively, when irradiated with light. Finally, with the recognition that certain applications benefit from the unique photophysical properties of quantum dots, Bruchez highlights recent advances in their development and utility for tracking the dynamics of single molecules in reconstituted biophysical systems as well as on the surfaces of living cells.

New strategies

The growing interest in cataloging biomolecular associations and activities in a physiologically relevant context — in cells, tissues, and organisms — has led to the continued development of novel and creative strategies to detect and monitor these sophisticated events at increasing levels of spatial and temporal resolution. While experiments performed *in vitro* provide a wealth of information regarding *which* biomolecular associations and functions might exist within the cell, they often capture an oversimplified picture that lacks the subtleties of *where* (i.e. subcellular location) and/or *when* (i.e. cellular state) such biomolecular associations and functions occur. Providing this multidimensional information requires novel approaches that can accurately report the status of biomolecular interactions and functions in living cells with precise spatiotemporal resolution. This issue highlights four complementary approaches that advance the field closer to this goal. Lowder *et al.* review recent advances in visualizing protein associations and networks under increasingly native conditions using both fluorescent proteins and small-molecule fluorophores. Key, focused breakthroughs have effectively expanded the repertoire of tools available for generating protein interaction maps that are resolved in both space and time. Shekhawat and Ghosh focus on one strategy for studying protein interactions, in which a protein association event guides the assembly of an enzyme or fluorescent protein from its split polypeptide fragments, thus reconstituting an easily measurable enzymatic activity or fluorescent signal, respectively. The authors demonstrate how this concept can be applied to monitor higher-order complexes involving additional macromolecules and to identify potential inhibitors of protein-protein interactions. Building on this theme, Edgington, Verdoes, and Bogyo describe recent advances in the design and application of substrate-based and activity-based probes that illuminate protease func-

tion. In a final review for this section, Armitage focuses on what perhaps remains the steepest uphill battle, identifying tools to monitor the locations and activities of RNA and RNA-containing macromolecular complexes.

New optical methods

Not surprisingly, breakthroughs in our understanding of cellular and molecular biology correlate with major technical advances in microscopy. Starting with light microscopy, the development of electron microscopy, scanning probe microscopy, and, most recently, fluorescence microscopy has enabled researchers to visualize biology with continually increasing clarity and depth. The recent development of superresolution fluorescence microscopy is allowing researchers to resolve objects that are closer together than the diffraction limit of light, a feat that was considered impossible only a few years ago. Simultaneously, advances in optical methods that push beyond fluorescence microscopy have begun to emerge. Three reviews in this issue communicate the state-of-the-art in the development of fluorescence microscopy and new optical methods. Manley reviews the rapidly growing family of superresolution fluorescence imaging modalities that are currently available and provides a practical guide for choosing the fluorescent protein or small-molecule fluorophore that is most appropriate for use with the intended platform. This guide also serves as a primer for choosing a software package for image reconstruction. In their review article, Xu, Melia and Toomre discuss recent variations of superresolution imaging techniques that considerably extend the reach of the technology into three-dimensional imaging of live cells with high, ~500 ms per frame, time resolution. Combined with pulse-chase strategies, these new variants of superresolution imaging are allowing membrane trafficking to be imaged with high spatiotemporal resolution. In the final review for this section, Min describes two newly developed molecular imaging techniques, stimulated Raman scattering microscopy and stimulated emission microscopy, that enable high-sensitivity, label-free imaging of non-fluorescent species in living cells by harnessing the optical amplification aspect of stimulated radiation.

New applications

The combination of increasingly clever biomolecular fluorescence labeling schemes and stunning breakthroughs in the sensitivity and resolution of fluorescence microscopy has ushered in a new era in the molecular imaging of biological processes. Using reconstituted biochemical systems in which one or a few biomolecular components are specifically labeled with fluorescent molecules, complex processes like replication, transcription, splicing, translation, and protein degradation are increasingly investigated using *in vitro* single-molecule fluorescence approaches. These emerging and rapidly evolving studies are providing mechanistic insights into intricate biological processes that have remained difficult

or impossible to understand using traditional ensemble approaches. Beyond these *in vitro* applications, super-resolution imaging of fixed cells allows for the visualization of complex cellular structures, which would ordinarily be disrupted upon cell lysis, to be determined within their native environments at ever-increasing spatial resolution. Perhaps most exciting, however, are ongoing efforts to image biological processes in living cells with single-molecule superresolution in real time. The reviews in this issue provide cutting-edge examples of how these approaches are impacting our understanding of complex biological systems. Flors and Earnshaw focus on superresolution imaging of chromatin and discuss how these methods are providing high-resolution structural information on the higher-order organization of chromatin in cells. These studies are setting the stage for detailed investigations of replication, transcription, DNA-repair, among others using the native template on which these processes occur. The final three reviews focus on some of the most complex ribonucleoprotein molecular machines in the cell. In their review, Hengesbach, Akiyama and Stone describe recent efforts to elucidate the molecular mechanisms that underlie folding of the telomerase RNA, assembly of the telomerase ribonucleoprotein complex, and telomerase-catalyzed synthesis of protective telomere DNA repeats onto the ends of chromosomes using single-molecule imaging approaches. Perez and Gonzalez next provide an analysis of the mechanistic insights which have emerged from the rapidly expanding body of *in vitro* and *in vivo* single-molecule imaging studies of ribosomes and ribosome-catalyzed protein synthesis. The most

recent breakthrough in this area is an *in vivo* study in which ribosomes were imaged in living *Escherichia coli* cells with 40 nm spatial resolution and 4–50 ms time resolution! Finally, Hoskins, Gelles and Moore close this issue of *Current Opinion in Chemical Biology* by describing how the combination of chemical biology approaches for labeling proteins in cellular extracts and fluorescence imaging with single-molecule resolution is enabling detailed studies of what is perhaps one of the most complex and dynamic biochemical systems in the cell: mRNA splicing by the spliceosome.

Future perspectives

Increasingly, the interface of chemical biology with molecular imaging is providing exhilarating views of biological processes, both inside and outside living cells. Through the development of fluorescent proteins, small-molecule fluorophores, and innovative strategies for genetically encoding, deploying, and activating these fluorescent reporters inside living cells, chemical biology continues to push molecular imaging further and further into the world of biology. Likewise, the spectacular progress that is currently unfolding in the spatial and temporal resolution of molecular imaging is driving the field of chemical biology to develop ever more robust molecular reporters and labeling strategies of increasing sophistication and scope. If the reviews in this issue of *Current Opinion in Chemical Biology* are any indication, the fusion of chemical biology and molecular imaging promises to continue to reveal the breathtaking complexity of the biological world at molecular resolution.