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Introduction to the Minireview Series on Modern Technologies for In-cell Biochemistry*

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The last decade has seen enormous progress in the exploration and understanding of the behavior of molecules in their natural cellular environments at increasingly high spatial and temporal resolution. Advances in microscopy and the development of new fluorescent reagents as well as genetic editing techniques have enabled quantitative analysis of protein interactions, intracellular trafficking, metabolic changes, and signaling. Modern biochemistry now faces new and exciting challenges. Can traditionally "*in vitro*" experiments, *e.g.* analysis of protein folding and conformational transitions, be done in cells? Can the structure and behavior of endogenous and/or non-tagged recombinant proteins be analyzed and altered within the cell or in cellular compartments? How can molecules and their actions be studied mechanistically in tissues and organs? Is personalized cellular biochemistry a reality? This thematic series summarizes recent studies that illustrate some first steps toward successfully answering these modern biochemical questions. The first minireview focuses on utilization of three-dimensional primary enteroids and organoids for mechanistic studies of intestinal biology with molecular resolution. The second minireview describes application of single chain antibodies (nanobodies) for monitoring and regulating protein dynamics *in vitro* and in cells. The third minireview highlights advances in using NMR spectroscopy for analysis of protein folding and assembly in cells.

The ultimate goal of biochemistry is to understand how molecules work in a complex cellular environment at atomic level. Although we are still far away from this goal, great progress has been made toward characterization of metabolic identities of various cells and the behavior of many essential molecules in diverse intracellular contexts. Chemically induced dimerization of recombinant proteins has enabled dissection of signaling events in distinct intracellular locations with fine temporal resolution (1). Advances with cell sorting, single cell RNA sequencing, and computational methods revealed a rich diversity of cell identities and the existence of previously unknown cell subtypes (2, 3). Single molecule tracking, correlation spectroscopy, and time-resolved fluorescence energy transfer protein dynamics and interactions in cells in unprecedented detail and allow high-throughput screening for new protein modulators (4, 5). This thematic series aims to add to the excitement by highlighting recent methodological advances in three areas that differ markedly with respect to their scale and specific experimental goals but that, together, bring us closer to the ultimate goal of learning about cell function at the atomic level.

In the first minireview of this thematic series (6), Zachos *et al.* (6) focus on the advantages of primary human enteroids and organoids for studies of intestinal physiology and pathobiology. In recent years, it has become increasingly clear that studies directed toward the understanding and treatment of human disorders require experimental systems that not only accurately recapitulate metabolic states of tissues but also account for a significant genetic and metabolic variability between human patients. Stable organ-like cultures offer tremendous opportunity to explore the mechanisms underlying various pathogenic events at the molecular level and to use this information to develop personalized treatment regiments. The authors describe two major ways of generating three-dimensional primary organ-like cultures; they also compare the properties of colonoids (enteroids) and organoids, including differentiation, recapitulation of key morphological features of a normal tissue, and suitability for functional studies. The minireview provides examples of utilization of enteroids for quantitative analysis of ion transport and for studies of cellular and molecular events during host-pathogen interactions, as well as discussion of future ways to further optimize these exciting experimental systems.

Molecular movement is at the core of molecular function. Significant advances have been made in studies monitoring protein trafficking between cellular compartments, whereas analysis of protein conformational transitions in the cellular environment remains challenging. In the second minireview of this thematic series (7), Dmitriev *et al.* highlight recent studies of functional dynamics of membrane proteins using single-chain antibodies (also known as nanobodies or variable fragments of heavy chain-only antibodies). The utility of these reagents has been proven in the areas of structural biology, cancer research, and molecular diagnostics. It is based on high selectivity of nanobodies, as well as their relative ease of production and derivatization (8–10). The minireview describes new applications of these versatile reagents for fundamental mechanistic research, especially for studies of conformational landscapes of proteins in their native environment. The authors discuss the generation of single chain antibodies and illustrate how the unique properties of these antibodies enable their utilization for analysis of fast protein dynamics by solution NMR spectroscopy and the identification of difficult to capture transient protein interactions. Single chain antibodies enhance traditional fluorescence energy transfer methodology, because they can be used not only for detecting molecules and their interactions, but also for manipulating/altering proteins of interest *in vitro* and in cells. The minireview describes exciting examples of recent studies of cell signaling and receptor function, which are

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based on the nanobody-mediated capture of functionally relevant protein conformers within cells.

Biochemists today are acutely aware that many conclusions made in *in vitro* experiments require verification in cells because of the much greater complexity of cellular environments when compared with simple and well controlled *in vitro* experimental systems. The third minireview by Luchinat and Banci (11) provides an overview of the cutting-edge field of in-cell NMR spectroscopy. This rapidly developing research area has already yielded convincing results that illustrate the possibility of using the high resolution power of NMR spectroscopy in a wide range of host cells from various species. One exciting promise of this technology, originating from the analysis of the effects of molecular crowding on protein folding and structure, is to uncover functionally relevant weak protein-protein interactions. Another promise is to identify the role of the protein environment/locale in protein folding and assembly. The minireview also describes the experimental limitations of in-cell NMR as well as recent approaches that improve spectra quality and sample lifetime. In addition, the minireview discusses the first steps in developing a solid-state in-cell NMR spectroscopy, as well as applications of NMR spectroscopy for mechanistic studies of human pathologies in cells at the structural level.

The authors and I hope that you will enjoy reading about these innovative experimental approaches and the mechanistic insights that they have generated thus far. It is important to emphasize that the methods and reagents described in these minireviews, although currently fairly costly, are conceptually within the reach of many biochemical laboratories. We hope that this thematic series will stimulate further interest and will help to expand these technologies and their applications in mechanistic studies of molecules in cells.

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