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# Editorial overview: Untangling proteome organization in space and time

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Current Opinion in Chemical Biology 2019, 48:

A1–A4

For a complete overview see the [Issue](#)<https://doi.org/10.1016/j.cbpa.2019.02.001>

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Ileana Cristea is a Professor at Princeton University. Her laboratory investigates mechanisms of cellular defense against viruses, as well as mechanisms evolutionarily acquired by viruses to modulate cellular processes. She has promoted the integration of the fields of virology and proteomics and has developed methods for uncovering virus-host protein interactions in space and time. This has allowed her group to bridge mass spectrometry developments to findings in virology, including in immune response and organelle remodeling.

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Kathryn Lilley is the Professor of Cellular Dynamic in the Department of Biochemistry, University of Cambridge, UK where she directs the Cambridge Center for Proteomics. Her research program creates and applies technologies to measure the dynamics of the transcriptome and proteome in high-throughput in space and time during critical cellular processes. Her group has also contributed many informatics tools to efficiently mine spatiotemporal proteomics data. In 2018, she was awarded the HUPO Distinguished Achievements in Proteomics Award.

It is well understood that the regulation of protein abundance is critical for numerous biological processes, but also linked to the progression of disease. Many proteomics methods have been developed for quantifying proteins in healthy and disease states. However, a critical aspect of protein regulation is when and where these changes occur. Furthermore, even if a change in protein levels is not observed, an alteration in function can still occur via the movement of that protein from one subcellular compartment to another. Protein translocation can be connected to changes in protein posttranslational processing, interactions with other biomolecules and structural changes, all of which result in alterations in protein functions. Apparent changes in localization may also arise from synthesis of a protein in another location or location-specific degradation. The understanding of the proteome organization in space and time has therefore become an important and not trivial task in biology in recent years.

Subcellular compartmentalization is among the most complex and interesting examples of molecular organization in life. However, acquiring knowledge about organization is challenging. A first fundamental step is discovering where proteins localize in different biological systems. This is followed by the more complex task to understand the dynamic regulation of protein localization during a biological process or following a perturbation. Recent years have demonstrated that proteomics is well situated to defining spatial and temporal protein function. In this special issue of *Current Opinion in Chemical Biology*, the significant contribution of proteomics to understanding proteome organization in space and time is highlighted. To illustrate the impact and breadth of proteomics in this field of research, this issue presents reviews of diverse methods developed to study protein localization, as well as their applications to fundamental biological and medical studies.

## Technologies and biological applications viewed through the prism of scale

The study of the relationship of proteins in time and space has been highly dependent on advances in technology. Emerging methodologies allow interrogation of data on a variety of scales, from tissue and cell type level resolution, through to whole cell atlases and the components of specific subcellular niches and protein complexes. Within this issue, these methodologies, their challenges and examples of their application to cell biology and disease studies, are reviewed at all scales.

## Organization at the tissue level

At one end of this scale, [Caprioli and co-workers](#) describe pioneering work using Matrix Assisted Laser Desorption Ionization (MALDI) as a tool to

image the spatial distribution of thousands of bio-molecules including proteins throughout tissue sections. This approach, together with other surface ionization methods form powerful imaging mass spectrometry methodologies. A common limitation of these methods is the ability to fragment whole proteins in the gas phase to enable their identification. In this review the authors focus on the recent advancements to overcome these limitations, and empower imaging mass spectrometry (IMS) technologies to inform on the tissue and cell type distribution of thousands of proteins per experiment.

An example of the biological relevance of understanding organization at the tissue level is provided by [Conlon et al.](#) The authors describe how congenital heart disease (CHD), one of the leading causes of structural birth defects, results from genetic defects that produce spatial and temporal abnormalities during the early stages of embryogenesis. They discuss the challenges in studying such defects due to their early presence in few cells, and how recent advancements in mass spectrometry technology now enable insights into the molecular and cellular basis of CHD.

### Cellular surface and extracellular regulation

At the cellular scale, protein organization occurs on multiple levels, playing essential roles in the establishment of cellular phenotypes and functions. Among these is the dynamic regulation of proteins at the cell surface, which is fundamental for biological processes such as progression through development, intercellular communication, propagation of cellular signaling, and host response to pathogens. [Wollscheid and co-workers](#) provide an informative overview of mechanisms that underlie the dynamic regulation of the surfaceome, that is, the cell surface protein content. The authors also discuss advanced microscopy and mass spectrometry-based technologies that can inform about alterations in protein abundance, posttranslational modifications, structure, and interaction networks at the cell surface, as well as methods dedicated to investigating the extracellular space.

### Subcellular organization within organelles

At the subcellular level, the partitioning of proteins and other chemical species within organelles is at the core of cellular biochemical reactions. Dynamic alterations in organelle composition are required during normal cell function, and in response to cellular stress. Conversely, dysregulation of organelles is linked to a large spectrum of genetic and non-genetic human diseases, as highlighted by [Yates and co-workers](#). Several manuscripts in this special issue focus on technological advancements or different biological aspects of subcellular organization.

[Lamond and colleagues](#) highlight several ways in which proteomics has begun to move into the 'Next Generation'

by assessing cells beyond their unidimensional, steady-state proteomes. Developing multidimensional methods that integrate cell biology, biochemistry, proteomics, and informatics have allowed for greater depth in characterizing both whole cell and subcellular proteomes, as well as in enhancing the quantification of protein isoforms. The authors review these signal enhanced proteomic approaches, as well as how the application of isotopic labeling has further enabled the determination of protein half-lives. Combining these techniques with additional experimental dimensions has facilitated the identification of localization-dependent protein turnover rates.

Further delving into the fundamentals of subcellular division, the [Wuhr group](#) elegantly reviewed the distribution of proteins between the nucleus and the cytoplasm, which is primarily regulated via passive diffusion or active transport through the nuclear pore complex. The authors propose that the propensity of proteins for nuclear transport can be effectively predicted by correlating information about physicochemical properties of proteins (e.g. shape, charge, hydrophobicity) with a quantitative mass spectrometry assessment of the abundance and interactions of importin and exportin proteins.

At even a more refined level of compartmentalization, [Lilley and colleagues](#) discuss recent developments in computational predictive analyses, proximity tagging, subtractive proteomics, and microscopy methodologies to define organelle-specific proteomes. Using the yeast *Saccharomyces cerevisiae* as a model organism, the authors demonstrate the robustness of hyperLOPIT, a mass spectrometry-based protein correlation profiling technique, for gaining a spatially-resolved cell wide map of the yeast proteome. The integration of data from orthogonal approaches, including fluorescence microscopy studies, complement the assignment of proteins that localize to singular subcellular compartments. Emphasizing the dynamic properties of proteins, as well as the associated technical challenges for investigating their functions, the authors identify that a majority of proteins in yeast have multiple localizations.

To further gain insight into the biological processes that are contained within specialized subcellular structures, it is of paramount importance to be able to characterize these structures in a manner that precludes contamination from other subcellular compartments with similar physico-chemical properties. In this issue, [Trost and colleagues](#) describe elegant workflows designed to robustly interrogate phagosomes using their uptake of latex beads in order to facilitate their enrichment. The authors also describe that, by using this approach, fine detail of the posttranslational modification status of the proteins residing in the phagosome suggests that it serves a significant role in cell signaling, in addition to being a degradative organelle.

With the growing number of spatial proteomics studies being performed across a broad range of scales, biological conditions, sample types, and quantification strategies, an unmet need has been the ability to systematically gauge and compare the levels of subcellular resolution and protein localization assignments across studies. The review by [Gatto and colleagues](#) discusses their developed computational tool, QSep, which both measures and compares the subcellular resolution of proteins across different experimental datasets. Such assessments can facilitate standardized guidelines for analyzing spatially-resolved proteomic datasets and better accommodate comparisons across experiments.

Parallel developments in chemical proteomics have provided a complementary platform to temporally study the subcellular distribution of proteins in cells. These approaches obfuscate the time intensive and labor intensive need for organelle fractionation, which can also suffer from specificity and coverage limitations. [Zhu and colleagues](#) poignantly review applications of reactive chemicals in live cells to covalently label and enrich for proteins in specific organelles, followed by their subsequent identification via mass spectrometry. This can be accomplished by selectively targeting reactive reagents and enzymes to organelles, as in activity-based protein profiling, proximity labeling via a peroxidase, and organelle-localizable reactive molecules. To further explore proteins localized within unique intracellular microenvironments, this review details the application of conditional proteomics to characterize zinc-rich subcellular compartments. The value of chemical proteomics approaches is also highlighted in this issue by [Weerapana and co-workers](#), with a focus on cysteine functionality. Cysteine residues have unique properties among the amino acids and adopt many diverse roles making them targets for drug action. Many of these roles are impacted by cellular localization. The authors emphasize not only chemoproteomics platforms to characterize cysteine functionality at an organelle level, but also how the unique physicochemical properties of different organelles impact cysteine function, paying particular attention to proteins that reside in the mitochondria and endoplasmic reticulum.

### Dynamic movement of proteins between organelles

Biological processes are mediated by the translocation of proteins between different subcellular compartments. There are significant technical challenges in capturing dynamic re-localization events. Determining confident re-localizations from within complex datasets rife with technical and biological variability can be a daunting prospect. Nevertheless, re-localization of proteins in response to extracellular information underlie many cellular mechanisms. [Toettcher and co-workers](#) discuss the precise temporal and spatial reorganization of proteins as

a defining property of signaling networks. They describe how challenges in studying these properties are now being overcome by recent innovations in the creation of optogenetic tools that can directly control individual signaling nodes and the resulting assembly of protein complexes both temporally and spatially.

Another important cellular process underpinned by spatio-temporal changes in the proteome is viral infection. In this issue, [Cristea and colleagues](#) describe the accumulating body of evidence that demonstrates the extent by which host protein re-localization is manipulated by the virus. By harnessing protein re-localization, the virus is able to drive changes in protein interactions and functions, thereby inhibiting host defense mechanisms and facilitating virus replication. The technical challenges of determining protein re-localization are discussed along with the value of integrating quantitative proteomics with advanced microscopy for understanding the biology of human virus infections.

### Protein complexes and localization-dependent protein interactions

Further down the subcellular scale, proteins within spatially restricted subcellular compartments form multiprotein complexes that serve as functional units. The dynamic association of proteins in such complexes underpins a significant number of molecular mechanisms, allowing one protein to carry out multiple functions. Capturing such dynamic events is confounded by the fact that many proteins function within distinct complexes and also operate with widely different levels of association, from stable interactions to transient associations over small time scales. [Ratray and colleagues](#) review the challenges of determining protein–protein interactions and focus on recent key technology developments that enable robust characterization of dynamic events within multiprotein complexes.

[Gingras and colleagues](#) provide an elegant review of the history and impact of proteomic methods for studying proximity-based protein interactions. Based on the biotinylation of proteins present in the proximity of a protein of interest, followed by their detection by mass spectrometry, these methods have revolutionized the ability to define direct and indirect protein interactions within a given subcellular space. Additionally, these methods offer alternative means to define subcellular composition at a suborganelle level, as shown for mitochondria. This review discusses experimental challenges and design considerations when using such methods, which include BioID and APEX.

Delving into defining precise direct protein–protein interactions within subcellular compartments, the [Bruce group](#) discusses advancements in approaches that couple chemical cross-linking with mass spectrometry.

Such methods allow the preservation of protein conformations and transient interactions, capturing structural information of systems that range from single proteins to complexes to proteome scale models. The authors comment on the important consideration that cross-linking results can represent a mixed population of conformations and protein complexes simultaneously present in the cell, and provide examples and a perspective for transitioning the use of this methodology from *in vitro* to in cell to *in vivo* contexts.

We thank all the special issue contributors for bringing to light the major impact that diverse proteomic methods have had on expanding the current understanding of proteome organization, and its dynamic regulation in health and disease conditions. We appreciate the constructive and candid assessment of the strengths and limitations of the current technologies and the future necessary improvements. We hope that this issue will promote future studies in this important field of science and stimulate new questions and scientific undertakings.