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Eric
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INTRODUCTION

As proteomics technologies are reaching a plateau in the number of proteins that can be resolved and detected, pre-fractionation steps have become essential to increase the depth of proteomic analysis. So far, many pre-fractionation steps have been based on chromatography methods where the proteins are separated according to their individual physicochemical properties. Subcellular fractionation methods proved to be very potent protein pre-fractionation steps: they allow the representation of low abundance proteins, and they can be combined with chromatography steps. Moreover, as the isolated subcellular components also represent functional units, subcellular fractionation allows the proteomic analysis of protein subsets that are functionally related in a biologically relevant manner. The first three sections of this volume deal with different levels of subcellular organization that also correspond to specific methodological approaches.

In his keynote chapter, Thierry Rabilloud superbly introduces the first section with a thorough definition of membrane proteomics where he pinpoints key theoretical and practical issues of this field, thereby setting the stage for the next contributions. Miguet et al. address the first key issue: the quality of the membrane preparation; they introduce and validate a microparticle strategy for plasma membrane purification. Zahedi et al. deal with the second major issue, the resolving of the hydrophobic proteins found in biological membrane samples, which they solve through two-dimensional BAC/SDS-PAGE gel electrophoresis. To close the first section, Foster and Chan review the proteomics of lipid rafts, membrane structures that are involved in intracellular trafficking and signal transduction. They describe a clever validation scheme based on the sensitivity of lipid rafts to cholesterol disruption.

A central theme in the second section on organelle subproteomes is the variability of their composition and how it can be exploited and interpreted. Kavanagh et al. describe a state-of-the-art subtractive proteomics scheme that relies on an in silico purification step based on the comparison of organelle subproteomes. With this approach, they could demonstrate variations in subproteome content across tissues. In the next chapter on synatosome proteomics, Bai and Witzmann review the current efforts to correlate synaptic plasticity and variations in synaptic subproteome content...
Introduction

with a special emphasis on post-translational modifications. Finally, Olver and Vidal discuss how the proteomic analysis of exosomes would give clues to the molecular basis of their biogenesis and contribute to a better understanding of their function. Moreover, they propose that the variations observed in exosome protein content are useful for biomarker discovery.

The third section deals with protein complexes, which are considered as the molecular machinery that performs most cell functions. This area is certainly not a trivial one: there are several types of protein complexes and protein-protein interactions and it is not always clear which methodology is most suitable to use in either context. In their chapter, Boissy and Collura sort out for us the concepts and methods encountered in interactomics, guide us through data interpretation issues and share with us their insight on the very nature of interactions. They plead for a systematic integration of interaction maps with functional genomics and molecular genetics data: the potential of such an approach is strikingly demonstrated in the next two chapters. Collins and Grant examine the molecular architecture of membrane associated signalling complexes in the nervous system, they highlight the role scaffolding proteins within these complexes and point out to a few much needed construction rules. Aligning interaction and functional genomics data, they build a case for a modular organisation of large complexes into functional sub-networks. To complete the picture, based on a thorough review on the complexes of the photoreceptor cilia, Roepman and Wolfrum sketch out an approach to organize complexes in functional modules and investigate their interactions.

Assuming that the protein content of an organelle has been inventoried and its protein complexes characterized, the next step is to translate this knowledge into functionally relevant interpretations. This is the purpose of systems biology: if we consider organelles as systems that function and communicate with each other through their protein machinery, it makes sense to apply such an approach at the subcellular level. In their chapter, Caruso and Chevet prove that this concept can actually be applied to reconstruct the stress signalling network of the endoplasmic reticulum. They build an integrative signalling map that qualitatively accounts for the interactions of the stress network with other endoplasmic reticulum machineries and also with other organelles. On the quantitative side, Bruggeman et al. introduce a theoretical framework for subcellular systems biology and thoroughly review the relevant mathematical approaches. Drawing on their extensive experience of metabolic networks, they argue that a direct translation of subcellular units as modules within a mathematical model of the cell can be advantageous both for solving the problem and interpreting the results. Garcia Osuna and Murphy survey the current automated methods for high-throughput determination of protein subcellular location that are used to reconstruct subcellular anatomy at high resolution. These methods provide essential information on the dynamic aspect of subcellular events in individual cells: it would also be extremely interesting to combine them with the molecular switches described by Martini et al. in the next section.

This brings us to the fifth and last section of this volume, where the most recent technological developments in proteomics are reviewed. Martini et al. introduce
the emerging field of systems nanobiology that relies on ultrasensitive methods and instruments to investigate cellular processes at the single molecule level. An application, among others, is to monitor the dynamics of protein translocation from one subcellular compartment to another using two-photon laser scanning microscopy and a photoactivable GFP as a molecular switch. Faupel et al. review the current applications of biophotonic technologies to proteomics with a focus on mass spectrometry based molecular imaging. Tian et al. describe a fast-track approach for the characterization of antibody epitopes using Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS). The application of this method to the Amyloid Precursor Protein has important consequences for the study of intracellular processing pathways relevant to Alzheimer’s disease. Finally, Mueller et al. describe the interfacing of LC and MALDI-MS and – MS/MS, discuss its performance, and present selected applications in the proteomics field including the analyses of membrane proteins and protein interactions.