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Gel-Free Proteomics

Methods and Protocols

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Preface

Proteomics by means of mass spectrometry has rapidly changed the way that we analyze proteomes. Electrospray ionization mass spectrometry coupled with nanoscale peptide liquid chromatography in particular is currently by far the most used proteomics technology. Mass spectrometry-driven, and thus gel-free proteomics, techniques all start by digesting a proteome or an isolated sub-proteome into peptides, these being readily analyzable by mass spectrometers. Whereas the generated peptide mixtures are very information rich, given that almost all extracted proteins will finally be represented by one or more peptides, such mixtures are also very complex as they hold tens of thousands of peptides present in highly different concentrations. Contemporary mass spectrometers are still not able to fully cope with such highly complex mixtures of analytes, and therefore several intelligent solutions have been proposed, many of which are described here. Especially cumbersome turned out to be the analysis of modified peptides, since these are present at much lower levels as non-modified peptides and are thus outcompeted for ionization and detection.

Gel-Free Proteomics: Methods and Protocols addresses contemporary methods for gel-free proteome research with a special focus on differential analysis and protein modifications.

Chapter 1 starts with a perspective overview of gel-free proteome analytical approaches, explaining their raisons d'être, potentials, and pitfalls. Given that proteomics is typically used to discover differentially expressed proteins or protein modifications, a section of this book deals with isotope labeling approaches for gel-free proteomics. Metabolic labeling of organisms is described in **Chapter 2**. Of note is that not all proteome samples can be labeled metabolically and hence various ways of introducing mass tags post-metabolically are described in **Chapters 3**, **4**, **5**, and **6**. Finally, this section of the book ends with a description of the PSAQ method in which fully isotopically labeled proteins are expressed in cell-free systems and then added to proteomes to be analyzed as internal standards for subsequent absolute quantification of proteins (**Chapter 7**).

Reproducible and highly effective sample preparation is of key importance for proteome research. Our field especially needs reproducible protocols for isolating organelles and membrane proteins, and these are described in **Chapters 8** and **9**, respectively. Further, a protocol for comprehensive proteome analysis by the so-called GeLCMS method – i.e., separation of proteins by SDS-PAGE, followed by LC-MS/MS analysis of in-gel digested proteins – is described in **Chapter 10**. A promising new tool for gel-free proteomics is the metalloendopeptidase Lys-N. In **Chapter 11**, a protocol is given that exploits this Lys-N protease to enrich for amino-terminal peptides and phosphorylated peptides, as well as more basic peptides for detailed analysis of proteomes. Further, a protocol that details the use of diagonal chromatography for the identification of newly synthesized proteins is given in **Chapter 12**.

A large section of this book is dedicated to the analysis of protein modifications. Protein phosphorylation is without a doubt the most extensively studied protein modification, with high numbers of approaches reported. An overview of phosphoproteomics approaches is given in **Chapter 13**, and one of the most used approaches – enrichment

of phosphorylated peptides on titanium dioxide beads – is presented in **Chapter 14**. Protein processing by endoproteases and aminopeptidases creates novel protein amino termini, and the introduction of several technologies by which amino-terminal peptides are specifically enriched or recognized upon tandem mass spectrometric analysis was recently observed in the field of protease degradomics. The positional proteomics strategy that enriches for amino-terminal peptides by affinity removal of the biotinylated internal peptides is described in **Chapter 15**. A somewhat opposite strategy during which protein amino termini are biotinylated and affinity enriched is given in **Chapter 16**. This procedure exploits the fact that blocking a protein alpha-amino terminus by, for instance, acetylation is a typical co-translational modification, implying that the alpha-amino termini formed following protease action are not blocked and can here be biotinylated and further enriched for analysis. **Chapters 17** and **18** describe procedures from the Overall lab by which protease specificities are characterized with high detail using peptide libraries (PICS, **Chapter 17**) and by which internal peptides are removed from protein digests using high molecular weight and soluble polymers (TAILS, **Chapter 18**).

Protein glycosylation is a prominent protein modification that is reported to affect more than one-third of all proteins. In this book the use of lectins to affinity isolate glycosylated proteins and peptides is described (**Chapter 19**), next to two methods to specifically isolate glycopeptides carrying sialic acid (**Chapters 20** and **21**). Further, a novel approach for enriching for O-glycosylated peptides is given (**Chapter 22**). Finally, this section of the book ends with a protocol to characterize ubiquitination (**Chapter 23**).

A key feature of mass spectrometry-driven proteomics is the enormous amount of data that are generated per experiment. Not surprisingly these data pose high demands on data storage, analysis, and interpretation. A critical viewpoint on the various challenges that bioinformaticians face when confronted with large-scale proteomics data is presented in **Chapter 24**. Further, quantification of proteomics data aiming at identifying regulated proteins was recently automated by the introduction of several software tools. A case study on the use of several of these tools is presented in **Chapter 25**.

All procedures needed to perform gel-free proteomics are described in *Gel-Free Proteomics: Methods and Protocols*. These range from sample preparation, isotope labeling for differential proteomics, enrichment technologies for modified proteins and peptides, and bioinformatics. As such we hope that this timely and critical overview of the promises of gel-free proteomics will be a guide for researchers who are both new to the field and already working on some aspect of proteomics.

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