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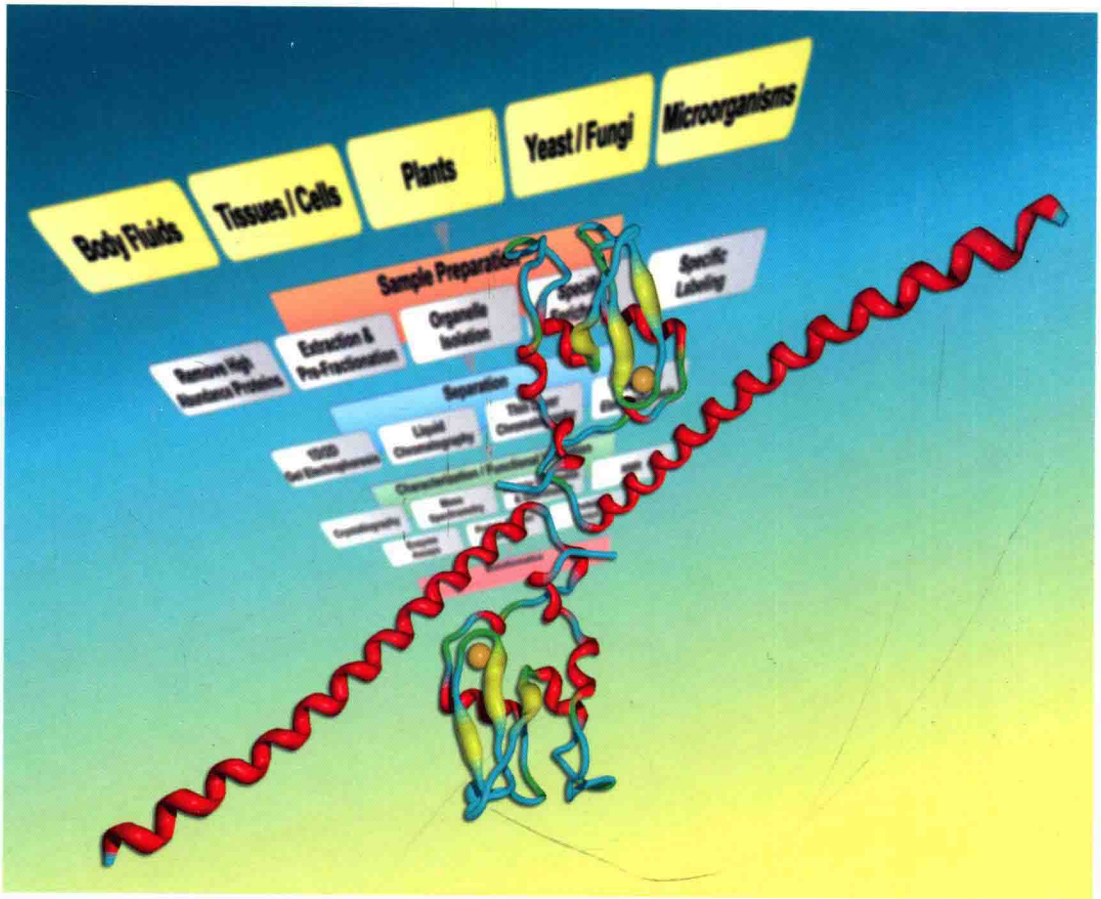
Proteomics Sample Preparation



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To my family
Vanessa, Annika, and Roswitha

Preface

Why is there a need to consider *Sample preparation* in proteomics? Following the successes of the genome era, researchers have switched their efforts to analyzing complex protein mixtures, hopefully to obtain deeper insights into the molecular development of diseases by comparing whole proteomes from healthy versus disease tissues, body fluid samples, or other sources. Proteomics was born on the waves of these advances and, as a consequence, enormous investments were made in many attempts to unravel the proteome for biomarker identification. The first wave of proteomics resulted in a re-arming of the laboratories which, by this time, no longer required vastly expensive equipment such as mass spectrometers. Inevitably, this surge of interest led to a vast number of reports in which biomarkers had, supposedly, been identified. The second wave of proteomics has been characterized more by the establishment of diverse methods and their combination, as so-called "standard proteomic workflows". Today, this subset of methodologies, databases and workflows appears largely to have been optimized, and the numbers of applications for the funding of studies and grants which include the catchword "proteomics" are rapidly increasing as the research teams continue their quests for meaningful data. Yet, the best way to obtain high-quality data and ensure consistency is not only to perform analyses in replicate but also – and more importantly – to standardize the methods of sample preparation.

What is meant by the term "proteomics"? Whilst this is to some extent a philosophical question, the answer depends heavily on an individual's point of view. Some researchers describe proteomics as a unique scientific area for the analysis of whole proteomes, as notably do clinical proteome scientists. Others define proteomics as a subset of methodologies that are valuable in the analysis of proteins, as proteins represent the most common drug targets today and are the molecules closest to the point of invention in living cells. Despite these differences of opinion, common sense among the scientific community decrees that sample preparation procedures must be kept as simple as possible. In this way, such procedures will go hand in hand with high accuracy and standardization. Clearly, proteomics – in contrast to genomics, which embraces sensitivity, abundance and a combination of different methods – depends on the state of the biological sample

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itself. The main question, therefore, is how to create an optimal workflow for each particular experimental set-up.

This book will provide those scientists on the third wave of proteomics – whether researchers or simply users of protein biochemical methodologies – with a comprehensive overview of the different requirements for sample preparation when using today's technologies. Hopefully, it will also provide any "beginners" in proteomics with some very brief "recipes" designed by well-known experts in each particular field.

I believe that this book will "sensitize" the need for sample preparation in proteomics, and will illustrate – with many useful practical applications – the problems which stem from the complexity of whole proteome samples. In this way it will provide solutions for those scientists who are new to this intriguing field of proteomics.

Jörg von Hagen

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List of Abbreviations

2-DE	two-dimensional electrophoresis
BGE	background electrolyte
CE	capillary electrophoresis
CGE	capillary gel electrophoresis
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate
cIEF	capillary isoelectric focusing
cITP	capillary isotachophoresis
CLOD	concentration Limit of Detection
CZE	capillary zone electrophoresis
DC	direct current
DMSO	dimethyl sulfoxide
EDTA	ethylenediaminetetraacetic acid
EOF	electroosmotic flow
FIA	field-amplified jnjection
HPLC	high-performance liquid chromatography
HUPO	Human Proteome Organisation
ITP	isotachophoresis
LE	leading electrolyte
LOD	limit of detection
MEKC	micellar electrokinetic chromatography
MIP	molecular imprinted polymer
MS	mass spectrometry
OPA/NAC	phthaldialdehyde/*N*-acety-L-cysteine
PC	personal computer
p*I*	isoelectric point
PVA	polyvinyl alcohol
RPLC	reversed-phase liquid chromatography
SDS	sodium dodecyl sulfate
SPE	solid-phase extraction
SPME	solid-phase micro-extraction

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