

**Part I****Perspectives in Proteomics Sample Preparation**



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## Introduction

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A lot can happen to a protein in the time between its removal from an intact biological system and its introduction into an analytical instrument. Given the increasing sophistication of methods for characterizing many classes of post-translational modification, an increasing variety of protein-modifying processes need to be kept under control if we are to understand what is biology, and what is noise. Hence, the growing importance of sample preparation in proteomics. One might justifiably say that the generation of good samples is half the battle in this field.

Fortunately, proteomics provides us with good methods for studying sample preparation issues. Two-dimensional electrophoresis of plasma, for example, provides a visual protein fingerprint that allows the immediate recognition of sample handling issues such as clotting, platelet breakage, and extended storage at  $-20^{\circ}\text{C}$  (instead of  $-80^{\circ}\text{C}$ ). A deeper exploration of plasma using mass spectrometry-based methods provides a more comprehensive picture, though perhaps more difficult to understand.

Unfortunately, despite the power of these methods, we do not know as much about sample quality and sample processing as we need to. The general attitude to these issues in proteomics has been to focus on the standardization of a few obvious variables and hope that the power of the analytical methods allows the sought-for differences between sample groups to shine through. This short-cut approach is likely to be problematic. Not only do the unrecognized effects of sample preparation differences add noise to the background against which the biological signal must be detected, but the sample preparation effects themselves are occasionally confused with biology. Well-informed skeptics correctly suspect that variables as basic as how blood is drawn or stored can generate spurious biomarker signals if the case and control samples are not acquired in exactly the same way. At this point we do not have adequate definitions of what “in exactly the same way” actually means for any given analytical platform.

These problems point to a need to take sample preparation (including initial acquisition through all the steps leading up to analysis) as a mission-critical issue, worthy of time and effort with our best analytical systems. Published data on

differences between serum and plasma protein composition, the effect of blood clotting, is interesting but very far from definitive – and in fact specialists in blood coagulation can offer a host of reasons why this process is not easily controllable (and hence not especially reproducible) in a clinical environment. Even a process as widely relied on as tryptic digestion is not really understood in terms of the time course of peptide release or the frequency with which “non-tryptic” peptides are generated – aspects which are critical for quantitative analysis. These and a host of similar issues can be attacked systematically using the tools of proteomics, with the aim of understanding how best to control and standardize sample preparation. In doing so, we will learn much about the tools themselves, and perhaps resolve the paradox surrounding the peptide profiling (originally SELDI) approach: that is, why it seems to be so successful in finding sample differences, but so unsuccessful in finding differences that are reproducible. Perhaps peptide profiling is the most sensitive method for detecting sample preparation artifacts: if it is, then it may be the best tool to support removal of these artifacts and ultimately the best way to classify and select samples for analysis by more robust methods.

Obviously, it is time to take a close look at sample preparation in proteomics. The reader is encouraged to weigh what is known against what is unknown in the following pages, and contemplate what might be done to improve our control over the complex processes entailed in generating the samples that we use.