

Chapter 2

Specificity of Detection Is the Key Attribute of Selected Reaction Monitoring

Abstract Selected reaction monitoring uses tandem mass spectrometry to create instrumental conditions where only specific peptides can be detected. The two stages of the mass spectrometer are synchronized so that a signal is seen only when a molecular ion with a specific m/z is formed *and* that molecular ion fragments to a product ion with a specific m/z . The specificity of the analysis is increased by the unique amino acid sequence that defines a protein's identity and the effects of that unique amino acid sequence on the other parts of the overall assay, including the protein digestion and liquid chromatography. The result is the ability to selectively measure, with confidence and accuracy, any protein in a complex mixture.

2.1 Defining Selected Reaction Monitoring

The goal of this book is to describe a relatively new tool for measuring the amount of a protein in a biological system. This tool is selected reaction monitoring. The *reaction* in selected reaction monitoring is the collision induced dissociation of an ion formed in the electrospray ion source of a mass spectrometer. The tandem mass spectrometry experiment selects that ion, known as the precursor ion, in the first stage of m/z analysis, transmits those ions into a collision cell where the collision induced dissociation reaction occurs. Product ions formed by the fragmentation reaction are selected in the second stage of m/z analysis. Therefore, to produce a signal at the detector, a peptide must generate a molecular ion of the m/z selected by the first stage of mass analysis *and* fragment efficiently under the collision conditions being used to produce a fragment ion with the m/z selected in the second stage of mass analysis. This process is shown schematically in Fig. 2.1.

These experiments can be carried out in any type of tandem mass spectrometer, including instruments using either tandem in space or tandem in time arrangements, but the experiment is most often associated with triple quadrupole mass spectrometry

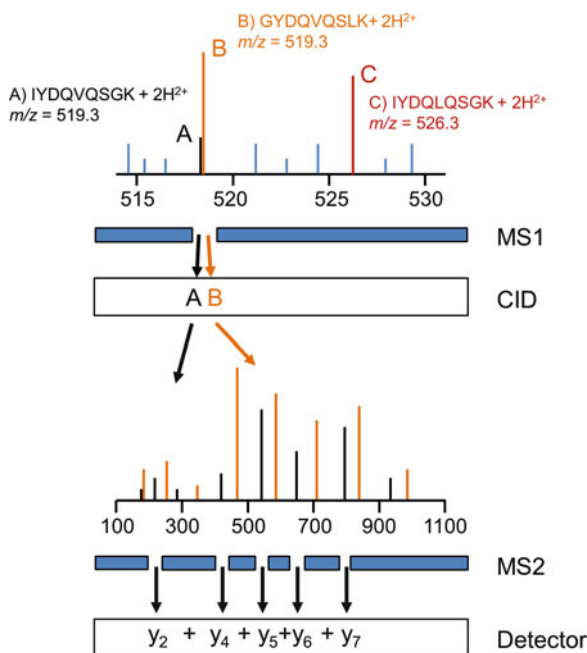


Fig. 2.1 A schematic illustration of the specificity of tandem mass spectrometry. A series of doubly charged peptide molecular ions are formed by electrospray ionization. The peptide ions are designated A, B, and C. Several ions of unknown origin are also illustrated. These ions may be other peptides, fragment ions of other peptides formed in the ion source, noise, plus many other possibilities. The first stage of mass analysis, MS1, transmits a single m/z of 519.3 which allows peptides A and B to enter the collision cell. All noise and peptide C are eliminated by MS1. In the collision cell, both molecular ions fragment into a series of product ions. The respective y-ion series are shown for each peptide. The second stage of mass analysis, MS2, steps between a predetermined set of m/z , allowing those fragment ions to reach the detector. In this illustration, an optimum set of y-ions from peptide A are selected and give a series of signals that are totaled to produce the overall signal for the peptide. Peptide B is eliminated in the second stage of mass analysis, so the entire signal recorded at this time is due to peptide A. This process can be completed in 25 ms or so, allowing up to approximately 40 peptides to be monitored in a 1 s cycle

systems. In most cases, selected reaction monitoring is used as a method to record chromatographic data in either liquid chromatography (LC) or gas chromatography (GC) experiments.

Some investigators use the term multiple reaction monitoring to reflect the nearly universal tendency to monitor more than one reaction in an experiment. The term selected reaction monitoring is the preferred term that will be used here. Based on the International Union of Pure and Applied Chemistry the term multiple reaction monitoring is deprecated [1]. Abbreviations for this experiment include SRM and MRM, but we will not use these abbreviations here believing that fewer abbreviations will enhance the clarity of our presentation. A short list of the abbreviations that we do use is given in the front material.

2.2 Specificity of Analysis Is the Key Attribute

In the early 1980s, as the field of tandem mass spectrometry was beginning to flourish, McLafferty described the ‘four Ss’ of good trace analyses—sensitivity, selectivity, speed, and \$ [2]. A continual point made through a series of papers published by McLafferty and the other pioneers in the field was that tandem mass spectrometry offered the potential for a remarkable increase in selectivity of detection that could have a dramatic influence on the effectiveness of the analytical method—increasing the accuracy, lowering the limit of detection, increasing the speed, and lowering the cost. Therefore, understanding the concept of selectivity, and the role it plays in an analytical method, is crucial to understanding the strengths of measuring protein abundance by selected reaction monitoring.

By definition, quantitative analyses are designed to determine the amount of a certain chemical entity (the analyte) in a sample. To accomplish this goal, quantitative methods measure some parameter and relate that measurement to the amount of the analyte. Thus, a key assumption in all quantitative methods is that the parameter being measured is due to the analyte and only the analyte. Other compounds that also contribute to the measurement are defined as interferences. The number and magnitude of these interferences can vary greatly. Severe interferences can render an assay unusably inaccurate. In other cases, more moderate levels of interference will require some element of compensation to maintain accuracy. Finally, some interferences can be so minor that the assay remains accurate and useful.

There are many approaches to increasing the selectivity of an assay. For example, steps can be added to the sample processing that remove the interferences from the final sample that is analyzed. A related strategy, if the analysis uses chromatography as a component, would be to increase the resolving power of the chromatography with tools like longer columns, specialized columns, gradient elution, ultrahigh pressure, or a combination. However, the most fundamental tool to minimize the potential for interferences in an assay is to increase the selectivity of the parameter being measured. In other words, measure a parameter that is as unique as possible for the analyte of interest, thereby discriminating against all other components of the sample.

Selected reaction monitoring is an example of measuring a parameter that is as unique as possible. The uniqueness of the parameter measured in selected reaction monitoring is derived from the two linked stages of m/z analysis, also called mass analysis. To generate a signal, the chemical entity *must both* (a) produce an ion with the m/z selected by the first mass analyzer and (b) fragment to produce a product ion with the m/z selected by second mass analyzer. Yost described this process as having ‘high information content’ [3]. This high information content reflects the fact that each stage of mass analysis is highly discriminating and linking the two stages defines an additional chemical characteristic.

2.3 The Specificity of Selected Reaction Monitoring Is Ideal for Targeted Quantitative Proteomics

The effectiveness of selected reaction monitoring in the analysis of proteins has three major elements (1) the uniqueness of a protein's amino acid sequence, (2) the conversion of the protein to peptides that preserve the amino acid sequence information, and (3) the ability of an LC-tandem mass spectrometry experiment to use the unique properties derived from a peptide's amino acid sequence.

The amino acid sequence of a protein is a fundamental attribute that distinguishes that protein from all other proteins. Although many proteins have similar isoforms and transcript variants, all differ in some way at the amino acid sequence level. One should also appreciate that while the proteome of any species is large, it is finite and well-defined. Analysis of the human genome indicates approximately 22,000 human genes and the human RefSeq database contains approximately 32,000 known protein coding transcripts [4]. Digestion of these proteins with a protease such as trypsin, an inherent part of the mass spectrometry experiment, translates these unique amino acids sequences into a large set of unique peptide sequences that can be traced back to the parent protein. This link between a peptide sequence and the parent protein is the foundation of the protein identification process, whether the peptide amino acid sequence is determined by tandem mass spectrometry or its predecessor Edman degradation [5]. Essentially all proteins, when digested with trypsin, will produce at least one and most likely many peptides with unique amino acid sequences. Selectively detecting these peptides serves as the critical marker for the parent protein both qualitatively as the protein of interest and quantitatively showing how much of the protein is in the sample.

The amino acid sequence gives each peptide a distinctive set of chemical characteristics that determine its behavior in the LC-tandem mass spectrometry experiment. These chemical characteristics are the hydrophobicity of the peptide, number of basic amine groups, molecular weight, and fragmentation pattern (in no particular order). Differences in hydrophobicity are reflected in the chromatographic retention and the response in electrospray ionization, which affect the detectability of a peptide. The number of basic amine groups determines the favored charge state. Finally, the most important factors, molecular weight and fragmentation pattern, are utilized by the tandem mass spectrometer and constitute the reaction monitored in the selected reaction monitoring experiment.

2.4 Turning the Specificity of Selected Reaction Monitoring into a Functioning Assay

The remainder of this book describes how our lab uses these elements to build and execute selected reaction monitoring experiments with sufficient specificity to accurately and precisely measure the amounts of multiple proteins in complex samples. The assays that are developed take advantage of all contributors to specificity.

The process begins with careful selection of the peptides to be measured and the tandem mass spectrometry conditions needed to carry out those measurements, including which peptides to use, which fragmentation reactions to monitor, and the collision energy needed to maximize the efficiency of those reactions. The design process is described in Chap. 3. As stated in that description, the method is presented as a methodical multistep process that appears tedious and time consuming—it is neither. In fact, a possibly unappreciated part of the design process is that most proteins have a good number of suitable peptides to utilize. The sample preparation method described in Chap. 4 also contributes. We use a short-run SDS-PAGE method that provides an effective clean-up of the sample through the protein-specific migration into the gel and subsequent washing of any remaining small molecule contaminants. Finally, as described in Chap. 5, the chromatographic separation of the peptides is used to create a modest time window in which each peptide is monitored. This scheduling based on each peptide's specific retention time improves the recognition of the correct signal by ignoring other signals that might otherwise generate confusion. Scheduling also allows the assembly of multiple protein-specific methods into a multiplexed assay. Taken together, sample preparation, chromatographic separation, plus the high specificity of selected reaction monitoring creates the strong link between the parameter being measured and the analyte of interest. This strong link, and the resulted accuracy and precision, is the fundamental power of targeted quantitative proteomics.

References

1. IUPAC (nd) Standard definitions of terms relating to mass spectrometry. Physical and Biophysical Chemistry Division. International Union of Pure and Applied Chemistry. http://mass-spec.lsu.edu/msterms/index.php/Selected_reaction_monitoring
2. McLafferty FW (1981) Tandem mass spectrometry in trace toxicant analysis. *Biomed Mass Spectrom* 8:446–448
3. Yost RA, Fetterolf DD (1983) Tandem mass spectrometry (MS/MS) instrumentation. *Mass Spectrom Rev* 2:1–45
4. Pruitt KD, Tatusova T, Brown GR, Maglott DR (2012) NCBI reference sequences (RefSeq): current status, new features and genome annotation policy. *Nucleic Acids Res* 40:D130–135
5. Kinter M, Sherman NE (2000) Protein sequencing and identification using tandem mass spectrometry. Wiley, New York

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