# Communicating Research to the General Public

At the March 5, 2010 UW-Madison Chemistry Department Colloquium, the director of the Wisconsin Initiative for Science Literacy (WISL) encouraged all Ph.D. chemistry candidates to include a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance to a wider audience that includes family members, friends, civic groups, newspaper reporters, state legislators, and members of the U.S. Congress.

Ten Ph.D. degree recipients have successfully completed their theses and included such a chapter, less than a year after the program was first announced; each was awarded $500.

WISL will continue to encourage Ph.D. chemistry students to share the joy of their discoveries with non-specialists and also will assist in the public dissemination of these scholarly contributions. WISL is now seeking funding for additional awards.

Wisconsin Initiative for Science Literacy

The dual mission of the Wisconsin Initiative for Science Literacy is to promote literacy in science, mathematics and technology among the general public and to attract future generations to careers in research, teaching and public service.

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# HYDROPHOBIC MODIFICATION OF PEPTIDES TO ENHANCE ELECTROSPRAY IONIZATION MASS SPECTROMETRY ANALYSIS

By

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# CHAPTER 5

## Summary of "Hydrophobic Modification of Peptides to Enhance Electrospray Ionization Mass Spectrometry Analysis" for the Wisconsin Initiative for Science Literacy

In order to understand how biological systems function in both healthy and disease states, it is critically important to study the role that proteins play. A protein is a large biomolecule made up of a long chain of amino acids. There are 20 common amino acids and the sequence of amino acids that make up a given protein dictate its chemical properties and function. Protein functions in an organism range from catalyzing enzymatic reactions to transporting substances, regulating gene expression to providing structure and strength for cells and tissues.[1] The large scale study of the proteins in a cell or organism is known as proteomics. Proteomics research includes identifying the proteins present in a cell or organism, measuring how the amounts of various proteins change in time, and deciphering how the profile, or pattern, of proteins changes as the result of a perturbation such as disease.

One of the most popular techniques used for analyzing proteins is mass spectrometry because it offers speed, sensitivity to detect small amounts of proteins, and the ability to perform large scale studies. In the typical mass spectrometry-based proteomics experiment, proteins are extracted from cells or tissues and then broken up into smaller chains of amino acids known as peptides. The resulting mixture of peptides is then separated on a reversed-phase chromatography column prior to entering the mass

spectrometer.[2,3] The reversed-phase chromatography column is packed with microscopic beads that have large carbon chains bound to them. Peptides that are hydrophilic (water-loving) have low affinity for the long carbon chains on the beads and only minimally interact with them. These peptides have higher affinity for the aqueous (water-based) solution flowing through the column and as a result flow through the column first. Hydrophobic (water-fearing) peptides have higher affinity for the long carbon chains on the beads and have stronger interactions with them. Consequently, these peptides take longer to flow through the column and reach the mass spectrometer. Separation of the mixture of peptides prior to mass spectrometry ensures that only a few types of peptides enter the mass spectrometer at any given time, simplifying the analysis.[4,5]

There are three basic parts to a mass spectrometer. The first part is a vaporization and ionization source that vaporizes the molecules of interest into the gas phase and charges them, forming gas phase ions. The second part of the mass spectrometer is a mass analyzer that separates the ions based on their mass-to-charge ratios. Most mass analyzers work on the principle that charged molecules (ions) can be manipulated by electric and magnetic fields, which is why the molecules need to be charged in order to be analyzed. The last components of the mass spectrometer are a detector to detect and count the ions and a computer to process the results. In mass spectrometry, determining the mass-to-charge ratio of an intact ion is informative, but is typically not enough information to confidently identify the ion. To confidently identify an ion, the ions are fragmented into pieces and the mass-to-charge ratios of the fragments are measured as well. Since many types of ions, including peptides, fragment in a characteristic way, the

mass-to-charge ratios of the fragment ions combined with the mass-to-charge ratio of the original ion is enough information to identify the original ion.[4,5]

The vaporization and ionization step is particularly important because only gas phase ions can be analyzed by mass spectrometry. If a molecule cannot be vaporized or ionized then it cannot be detected by a mass spectrometer. Electrospray ionization (ESI) is one of the most popular vaporization and ionization techniques for biological molecules such as peptides. During ESI, a liquid solution containing the peptides of interest is pumped through a metal needle with a very fine tip. A large potential difference (voltage) is held between the tip of the metal needle and the inlet of the mass spectrometer. The large potential difference causes a fine mist of charged droplets to be produced as the solution containing the peptides is pumped through the metal needle. The charged droplets are attracted to the inlet of the mass spectrometer. As the charged droplets traverse the region between the end of the metal needle and the inlet of the mass spectrometer, they begin to evaporate and shrink in size. Eventually, the droplets become so small that the forces of surface tension on the droplets cannot sustain the forces of repulsion of the charges on the surface of the droplet. This results in a "Coloumbic explosion" as the droplets break apart into smaller, daughter droplets and release gaseous peptide ions. The newly formed gaseous peptide ions are drawn into the sample inlet of the mass spectrometer where they are then mass analyzed and detected.[4-7]

Unfortunately, in most cases, not all of the peptides in an ESI droplet get ionized. Since the excess charge on an ESI droplet resides on the surface, hydrophobic peptides that spend more time on the surface of the droplet are more likely to get charged (ionized) than hydrophilic peptides that spend more time in the interior of the droplet. Hydrophobic

peptides spend more time on the surface of the droplet in order to minimize the interactions they have with the water molecules in the droplet. For a solution containing equal amounts of hydrophobic and hydrophilic peptides, the detected signal from the hydrophobic peptides will be larger than the signal due to the hydrophilic peptides because more of the hydrophobic peptides will be ionized. This means that it takes a greater amount of a given type of hydrophilic peptide to produce the same signal as a given type of hydrophobic peptide.[8-15] Consequently, it is very difficult to detect low levels of hydrophilic peptides which may be important for understanding various diseases.

As a solution to this problem, we have developed a novel method of chemically modifying peptides to make them more hydrophobic. The chemical reactions we have developed allow us to modify almost all peptides in a mixture with long carbon chains. This causes the peptides to have higher affinity for the surface of the droplets produced by ESI. As a result, more molecules of a given type of peptide are ionized producing higher signal. Increasing the hydrophobicity of peptides also leads to higher affinity for the packing material inside the reversed-phase chromatography column. The increase in affinity for the packing material inside the chromatography column is important because if peptides are extremely hydrophilic, they have such little interaction with the column that they are said to be unretained. These peptides are usually flushed from the column during the loading and washing steps and are not detected as a result. Increasing the hydrophobicity of peptides and their affinity for the packing material inside the chromatography column ensures that the peptides will not be lost during the washing and loading steps.

The use of the chemistry we developed to hydrophobically modify peptides led to large enhancements in signal from the mass spectrometer. In one case, a type of peptide that we had hydrophobically modified showed signal that was 34 times greater than the signal from an equal amount of the unmodified form of the same type of peptide. When we used reversed-phase chromatography to separate a mixture of peptides that had been hydrophobically modified, the modified peptides took much longer to flow out of the column than when the same mixture of peptides was not hydrophobically modified. This indicates that hydrophobically modified peptides had increased affinity for the packing material inside the chromatography column. We discovered that some types of peptides are unretained in the chromatography column and/or ionize so poorly that they are not detected unless they are hydrophobically modified. On other hand, some types of peptides that are detected in the unmodified form are not detected after they are hydrophobically modified. This could be caused by the peptides sticking to the column and not coming off because they are now so hydrophobic that they have very high affinity for the packing material inside the chromatography column. If we analyze the same mixture of peptides in the unmodified form and then after hydrophobic modification, we detect more of the types of peptides present in the mixture than if we only analyze either the unmodified or modified form of the mixture.

The improvement in signal and chromatographic retention achieved through hydrophobic modification allows for the detection of smaller amounts of peptides and allows for the detection of the types of peptides that are lost unless hydrophobically modified. This will allow for the detection of peptides and proteins that are biologically important but to date have been difficult to study because of poor ionization or

chromatographic retention. We hope others in the field of mass spectrometry-based proteomics will use the method of hydrophobic modification that we have developed to improve their own analyses, leading to a wealth of new knowledge.

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