SOAR Research Proposal – Summer 2015

Characterization of Polyproline with Capillary Electrophoresis

Faculty: Alison Holliday, Assistant Professor of Chemistry
Student: John Barr
Project Start Date: June 1, 2015
Length of Project: 10 weeks

Description of the project:
In biological systems, form usually dictates function. As a result, the shape – or conformation – of a protein can determine how it interacts with other molecules. This interaction, in turn, can result in a change in biological activity. Amongst the building blocks of proteins, the amino acid proline is unique in that it easily adopts two different spatial arrangements in proteins: *cis* and *trans*. Other amino acids are overwhelmingly found in the *trans* form. The presence of proline is thus key to the conformation and interactions of many proteins, and the folding of proline-containing proteins to form these conformations needs to be understood.

In collaboration with investigators at Indiana University, I have investigated the solution-dependent folding of polyproline using ion mobility spectrometry.[1] For the first time, intermediates were observed in folding, and so a more complete picture of how and why a (small) protein folds was generated. Ion mobility spectrometry, however, is a gas-phase separation technique; the protein folds in the solution, but the actual analysis of its shape takes place after removing the solvent. Calculations and simulations help us to understand the connection between what is seen in the gas phase and what was present in the original solution.

We have just purchased a capillary electrophoresis (CE) instrument at Moravian College. Capillary electrophoresis has a similar mechanism for separation as ion mobility spectrometry: it separates chemicals on the basis of their size (shape) and charge. However, unlike ion mobility, CE separates chemicals in solution. The first purpose of this summer research is thus to investigate whether we can use CE to confirm the results obtained by ion mobility. This semester, John is working on optimizing CE for detection of polyproline. His next step will be to see if we can observe and resolve the intermediate conformations of polyproline as it folds from its form in one solvent system to another.

The second purpose of this research is to analyze the binding of polyproline in solution. In particular, we will be looking at the interaction of polyproline with a WW2 protein, which is known to bind with proline residues in proteins.[2] As the polyproline-WW2 complex will have a different size and charge than either WW2 or polyproline alone, separation should be possible. In

[1] L. Shi, A.E. Holliday, H. Shi, F. Zhu, M.A. Ewing, D.H. Russell, D.E. Clemmer, "Characterizing intermediates along the transition from polyproline I to polyproline II using ion mobility spectrometry-mass spectrometry," *Journal of the American Chemical Society*, **136**, 12702-12711 (2014).
[2] X. Ramirez-Espain, H. Oschkinat, M.J. Macias, L. Ruiz, P. Martin-Malpartida, "Structural Characterization of a New Binding Motif and Novel Binding Mode in Group 2 WW Domains" *Journal of Molecular Biology*, **373**, 1255-1268 (2007).

combination with the folding experiments described above, we will investigating the amount of complex formed by each intermediate in the folding process. This research does not depend on observing resolvable intermediates in the first part of the project, as WW2 protein can be introduced at various time points in solvent-induced folding, corresponding to intermediate formation time found in ion mobility experiments. By doing this experiment at different temperatures, we can determine thermodynamic properties of the binding, including equilibrium constants. In May, John and I will be going to Indiana University for a week to work on complementary ion mobility studies.

Roles and responsibilities:

- Alison Holliday will be available to train John on the use and troubleshooting of the CE instrument and the analysis of resulting data.
- To start each day, John will have a meeting (~30 minutes) with Alison and the other two members of the research group (who are working on a different project). Results will be reported and discussed and plans for the day will be proposed and discussed.
- John will participate in periodic phone meetings with our collaborators at Indiana University and may provide powerpoint slides to summarize his results for the group.
- John will maintain a laboratory notebook that will include regular and complete entries, such that another student could follow his experimental progress. This includes ideas behind experiments, details of experiments (including solution preparation), the location of any electronic data files containing results or analysis, and a summary of results from each experiment. The notebook will be submitted to Alison upon completion of the research project.
- John will prepare a brief (<5 page) report to summarize his summer progress on the project.
- John is likely continuing research during the 2015-2016 academic year, so a poster may not be required at the end of the summer. Instead, a poster would be presented at the Annual Student Scholarship and Creative Endeavors Day in Spring 2016.

Project timetable

Week 1-2 Analysis of polyproline-13 (Pro13) size distribution at various time points in the folding process. Continued analysis of data obtained in Indiana (May 17-23).

Week 3: Analysis of Pro13 and WW2 protein mixtures in aqueous solution.

Week 4-5: Analysis of Pro13 and WW2 protein mixtures at various time points after dilution from an organic solvent system into an aqueous solution.

Week 6-8: Variation of the solution and capillary temperature for the time-dependent analysis of Pro13 and WW2.

Week 9: Calculations of thermodynamic and kinetic data.

Week 10: Repeat analyses, as required. Write a <5 page report for submission.

The project timetable that I have proposed is ambitious given the nature of experimental science, but completion of a small part of it would be significant.

Student engagement in discipline-appropriate scholarly research
Analytical chemistry involves the development and testing of new methods or instrumentation to observe and quantify chemical, biological, and physical systems and processes. John will be engaged in analytical chemistry laboratory research that includes planning and performing experiments involving new instrumental methods, analyzing significant amounts of data, and reading the primary literature to contextualize his findings and guide his choice of experimental conditions.

Contributions to the Discipline and Opportunities to Share Work
An understanding of protein folding is essential to understanding how biological reactions take place. Our ion mobility research was the first to directly observe folding intermediates by any experimental technique, and their observation in a solution environment would be both important and unprecedented. If the first portion of this project works, it would be publishable in a very good journal.

The second part of the project is more easily attainable and is not dependent on success in the first part of the project. Although CE has been previously used to analyze protein binding,[3] it is a relatively new application. By combining CE and ion mobility data, a more complete picture of binding is possible. Time-dependent differences in binding affinity could provide indirect evidence of difference intermediates in the folding of polyproline.

John will be sharing his results both within the Moravian research group and with our collaborators at Indiana University. He may have the opportunity to present his work at a local or national conference, and will be required to present his results during the Annual Student Scholarship and Creative Endeavors Day in spring of 2016.

[3] E.g. V.A. Galievsky, A.S. Stasheuski, S.N. Krylov, "Capillary Electrophoresis for Quantitative Studies of Biomolecular Interactions," *Analytical Chemistry*, **87**, 157-171 (2015).

SOAR Statement of Purpose
Characterization of Polyproline with Capillary Electrophoresis

Name: John Barr
Majors: Chemistry and Mathematics
Graduation: Class of 2016
Requesting On-Campus Housing
Mentor: Dr. Alison Holliday

"What do you want to be when you grow up?" is a question commonly asked to children. It was also a question that I never had a good answer to while I grew up. That was, until I took my first class in chemistry. What I do know now, is that I will be studying Chemistry for the rest of my life. My passion for the sciences has lead me to focus on mainly chemistry, mathematics and physics courses while attending Moravian, and has made me aspire to go to graduate school as well as earn my doctorate in chemistry. From here I will begin my career as a scientist as I perform studies of my own and push the boundaries on what we know about the universe we live in.

This experience doing research will be invaluable to me. All of my current experiences come from following a previously made lab procedure with expected results. This kind of independent work would push me to a higher level than that, as I work on a project with results that have yet to be determined. It would allow me to work in a lab and develop methods to overcome the unexpected challenges and problem solve through barriers to get to the needed results. Working on this project will let me apply what I learned here at Moravian to gain experience in what my entire career will based off of. This project will give me a great background when I move up to higher levels of work, such as graduate programs, as it will have already given me experience at that level of work.

I am currently working with Dr. Holliday in developing an analytical technique to study a unique protein, polyproline. This SOAR project, based off of our current work, will be to use capillary electrophoresis to separate out different sized and shaped polymers of polyproline and detect each discriminable polymer through UV-VIS detection. The expected outcome would be to use this method to determine the behaviors or characteristics of the chemical as it reacts with different peptides by studying the change in our detection signal. The expected outcome of our research would be a great way to expand our knowledge in both analytical and biochemical fields of chemistry.