SOAR Research Proposal – Summer 2016

Polyproline folding: effect of chain lengths and interactions

Faculty: Alison Holliday, Assistant Professor of Chemistry
Student: Amanda Miller
Project Start Date: May 31, 2016
Length of Project: 10 weeks

Description of the project:
Most proteins fold into complex three dimensional structures on an incredibly fast timescale; the fastest fold in less than a microsecond. Misfolding may lead to disease, but misfolding is very rare. Similar to a stream going down a hillside, it is thought that a protein folds by picking out the pathway with the lowest barriers to reach its (energetically) downhill destination. However, until recently, no one had experimentally observed the route itself.

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*. In contrast to other protein folding events, the *cis* to *trans* conversion of proline (which can be initiated by changing its solvent environment) is a slow process, as the energy barriers are quite high. The folding of a short protein entirely composed of prolines (a polyproline) takes place on a timescale of hours. As a result, I and my collaborators at Indiana University, recently reported the observation of the first intermediates in the folding of a protein, a polyproline chain, using a technique called ion mobility spectrometry.[1] 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.

Over the past year, John Barr'16 has developed a technique using capillary electrophoresis to observe polyproline folding. Capillary electrophoresis (CE) has a similar mechanism for separation as ion mobility spectrometry: it separates chemicals on the basis of their size (shape) and charge, but CE separates chemicals in solution. We were able to confirm the results from ion mobility spectrometry, and we are the first group to observe multiple intermediates in protein folding directly in solution. We will be submitting this work for publication very soon

Amanda will be working on further analysis of this system. The first question she will be answering is: does the number of prolines in the chain affect the folding mechanism? Ion mobility research on a polyproline with seven prolines[2] shows that it folds by a very different mechanism than one with thirteen prolines; no intermediates were observed for the seven proline polyproline. Using CE, we will explore the range of polyproline chain lengths between these extremes and see if there is a progressive change in the mechanism or an abrupt cut-off in the appearance of observed intermediates.

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[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] L. Shi, A.E. Holliday, N. Khanal, D.H. Russell, D.E. Clemmer, "Configurationally-Coupled Protonation of Polyproline-7," *Journal of the American Chemical Society*, **137**, 8680–8683 (2015).

The second purpose of this research is to analyze the binding of polyproline to other proteins in solution. Proline binding is involved in cell signaling pathways, and it is often accompanied by changes in the proline from *trans* to *cis*. We will be looking at the interaction of polyproline with a WW2 protein.[3] As the polyproline-WW2 complex will have a different size and charge than either WW2 or polyproline alone, separation should be possible. In combination with the folding experiments described above, we will investigating the amount of complex formed by each intermediate in the folding process as polyproline folds from an all-*cis* form to an all-*trans* form. WW2 protein can be introduced at various time points in solvent-induced folding, corresponding to the intermediate formation time found in our earlier experiments.

Roles and responsibilities:

- Alison Holliday will train Amanda on the use and troubleshooting of the CE instrument and the analysis of resulting data.
- To start each day, Amanda will have a meeting (~30 minutes) with Alison and the other member(s) of the research group (who will be working on a different project). Results will be reported and discussed and plans for the day will be proposed and discussed.
- Amanda will participate in periodic Skype meetings with our collaborators at Indiana University and may provide powerpoint slides to summarize her results for the group.
- Amanda will maintain a laboratory notebook that will include regular and complete entries, such that another student could follow her 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.
- Amanda will prepare a brief (<5 page) report to summarize her summer progress on the project.
- A poster would be presented at the Annual Student Scholarship and Creative Endeavors Day in Spring 2017.

Project timetable

Week 1-2 Analysis of the 13 proline polyproline (Pro13) size distributions at various time points in the folding process. Learning how to run the instrument, set-up the experiment, and collect and analyze the resulting data.

Week 3-6: Analysis of Pro7 – Pro12 size distributions at various time points in the folding process.

Week 7: Analysis of Pro13 and WW2 protein mixtures in aqueous solution.

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[3] 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).

Week 8-9: Analysis of Pro13 and WW2 protein mixtures at various time points after dilution from an organic solvent system into an aqueous solution.

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. Amanda 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 her findings and guide her choice of experimental conditions.

Contributions to the Discipline and Opportunities to Share Work

I anticipate that the analysis of polyproline folding as a function of chain length will be publishable in a good analytical chemistry journal. It may be presented in conjunction with the work of our ion mobility collaborators.

Although CE has been previously used to analyze protein binding,[4] it is a relatively new application. Time-dependent differences in binding affinity could provide evidence of the role of intermediate structures in the biological activity of proteins.

Amanda will be sharing her results both within the Moravian research group and with our collaborators at Indiana University. She may have the opportunity to present her work at a regional conference (the Eastern Analytical Symposium), and will be required to present her results during the Annual Student Scholarship and Creative Endeavors Day in spring of 2017.

[4] 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).

Title of Project:
Polyproline folding: effect of chain lengths and interactions
Name:
Amanda Miller
Major:
Biochemistry
Expected Date of Graduation:
May 2017
Mentor:
Dr. Alison Holliday
On-campus Housing:
Yes

The SOAR program peaked my interest when I found out I could get paid to conduct new, interesting research that I would thoroughly enjoy. I am a junior majoring in biochemistry and this decision of my major first stemmed from an interest in chemistry in high school and further turned into biochemistry because I became more intrigued of how chemistry takes place in organisms. I find Dr. Holliday's polyproline folding research so exciting because it has the possibility to be incorporated into learning about different mechanisms and reactions that occur in the human body. The research focuses on the analysis of proline13's chain lengths and interactions of *cis* and *trans* conformations and everything in between. If the research goes well I hope to also monitor proline13's binding to other molecules at its different conformations. This is a process no one has visualized before and if successful, the analysis can be groundbreaking. Some of the classes I have taken while at Moravian have given me a favorable background on basics of the topic. While taking Biochemistry I, I learned a great deal about proteins and their properties, further preparing me for this research. I hope to incorporate this experience into my future and it can hopefully provide me with a smart outlook on what career decision will best suit me. After I graduate from Moravian, I hope to engage in further research opportunities in either graduate school or a job. In doing SOAR I hope to gain a strong idea of which option would best fit. It will also aid in my decision of whether I would like to continue in this area of research or perhaps veer in a different direction. All things considered, I believe SOAR will be a great opportunity for my education now as well as my decision making in the future.