Benson Lab: Cys-Tyr Crosslink formation in BF4112 Adam Hilbrands, Jackson Ross, Elysa Wolf

One need only briefly browse through an intro-level organic chemistry textbook to realize that there is a tremendous amount of chemistry than happens between only hydrogen, carbon, oxygen, and nitrogen. Biochemistry takes those interactions to further levels, requiring hundreds of amino acids to determine the shape, function, and properties of a single protein. Of course, there are only twenty amino acids that make up proteins.

With such a massive scope and variability to proteins, more chemistry is required of our amino acids. Fortunately, this chemistry does exist. Our beloved amino acids can be modified in a variety of ways by adding various compounds and functional groups, changing the charge of the acid, and bonding with other amino acids.

The Benson lab focuses on the last option. Our protein comes from the bacteria *Bacteroides Fragilis*, which resides in our stomach and intestines. The area of interest is an active site at the bottom of the beta sheet. This active site has a metal ion coordinately bonded to a water, two histidines, and a glutamate. Above the metal ion is a tyrosine. It is this tyrosine we want to focus on.

Next to the tyrosine is a cysteine. The two are not crosslinked in *B.F.* (like how they are in other proteins like *Galactose Oxidase*), the formation of which is the objective of our lab. Forming this crosslink, and the subsequent identification and quantification of, takes up the grunt work of the lab.

For a brief overview of how the research goes: we will grow colonies of *E. Coli* and hijack them to produce our desired protein (with desired mutations) then purify the mix to get only our protein. A treatment of various chemicals strips out all the metal from the active site to make sure we control which metal binds at the active site. More chemicals reduce the metal ion (either from copper two plus to one plus or iron three plus to two plus) in order to bind it to the tyrosine. Exposure to oxygen oxidizes the tyrosine, forcing it to draw electron density from the sulfur on the nearby cysteine (in other words, to form a bond).

We identify this crosslink in a variety of ways. UV-vis absorbance reveals that the crosslink absorbs at three hundred and seventeen nanometers; SDS-page gels show a higher mobility band of faster protein (the crosslink changes the shape enough to allow the crosslinked-protein to move faster through the gel); excitation at three hundred and seventeen nanometers produces fluorescence at three hundred and sixty seven nanometers. It is this last method that lets us quantify how much crosslink was formed.

The rest of the research is performing this same assay and quantification on various mutations of the protein. These mutations function as a control and reference (e.g., some mutations cannot form crosslink at all, while others cannot form dimers through other active sites).

Our results do indeed show that we are forming the crosslink, and most of the mutations are doing what we want (we have had a little trouble with the one that should only form the crosslink at the active site). Further work will involve looking more closely at the cys-tyr crosslink, specifically what happens after the formation with the metal (does the metal ion oxidize or is it forced to stay at the lower oxidation state) and the tyrosine (how does its redox potential change now that it’s bonded to the cysteine).

Though I intend to pursue graduate school in physics and quantum materials, this lab has still been an incredible benefit to me. I have refined my lab techniques and learned more about the scientific method and process, and met other professors and researchers from different and interesting projects. I hope that the experience I learned this summer will continue to grow during the school year and for many summers to come.