PRF#: 57384-UNI4
Project Title: Enzymatic Dephosphorylation in Organic Media
PI Name, Affiliation: Veronica R. Moorman, Kettering University

2019 Narrative Report

This grant entitled "Enzymatic Dephosphorylation in Organic Media" originally had two aims: 1) developing a protocol for keeping a phosphatase stable in organic media and 2) developing a protocol for keeping a phosphatase active in organic media. Progress was made on both aims during the first year of the grant (as reported in the 2018 narrative report) while the second year of the grant focused mostly on the second aim (described below) and disseminating research done during the first year. Notably, a portion of this work was published this year (and has been input into the ACS PRF Bibliographic Citations page): Kindl, Evan D.; Koschnitzke, Kevin E.; Blauwkamp, James A.; McCann, Sean M.; MOORMAN, VERONICA R. "Fluorescence of Naphthol AS-MX is Readily Detectable in Dioxane Mixtures" Science Matters, 2019, 5, (5), e201905000003. Finally, an extension was granted to spend down the remaining budget in the upcoming year while further working towards the original aims of the grant.

While a suitable substrate (naphthol AS-MX phosphate) was selected early, only one organic solvent (1,4-dioxane) was examined during the first year of the grant. Therefore, during this past year additional solvents were also investigated. Specifically, acetonitrile and DMSO were chosen as they are solvents often used with proteins and enzymes in the laboratory, while 1,2-dimethoxyethane and 2-methoxyethanol were used to compare with 1,4-dioxane. DMF was also used. Each of these five solvents were combined with multiple different v:v ratios with a single aqueous component (0.1 M Tris buffer, pH 7.0). Absorbance and fluorescence of the phosphatase product naphthol AS-MX was investigated as were the physical properties of the solvent mixtures themselves. Additionally, the activity of one enzyme, wheat germ acid phosphatase (WGAP), was tested in each solvent mixture to compare with the data collected the year prior. Fluorescence emission spectra of mixtures with DMSO and 2-methoxyethanol are shown in Figure 1 as examples. Currently, my undergraduate students are actively working to relate these relative fluorescence units to phosphatase activity and that to the containing solvent mixture properties. Additionally, because commercial WGAP is actually a mixture of partially purified proteins, this past year we have also worked towards identifying the active phosphatase isozyme(s) in the mixture to better be able to characterize both stability and activity.

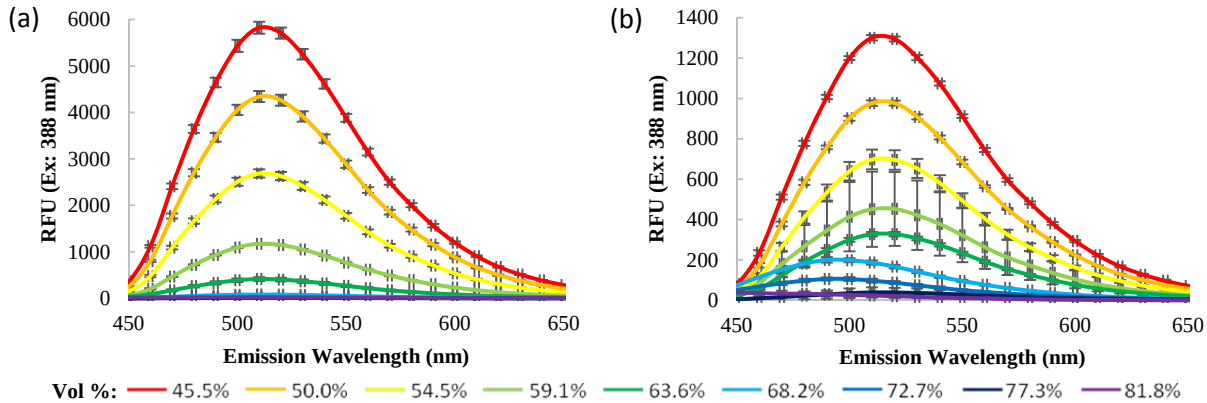

Figure 1. Wheat germ acid phosphatase activity as indicated through fluorescence (ex: 288 nm, em: ~510 mM) after three hours of incubation with 0.23 nM Naphthol AS-MX phosphate in different volume percentages of (1) DMSO or (b) 2-methoxyethanol.

As I am applying for tenure this year, this grant has been invaluable for driving my career forward. Not only can I point to a successful grant application and administration, but so far I have also been able to get one peer-reviewed publication from this work and have been able to fund numerous students working on the project. At a small, primarily undergraduate institution like mine, these are not trivial points. More broadly, because this grant allowed me to set up a robust research program, undergraduate students have starting inquiring to work with me and I have small questions that they can help answer right away, without me having to come up with a new project for each student, like I had done in the past. Additionally, because of the nature of this project chemistry and chemical engineering students are now interested in my projects, which brings additional researchers working with me and additional students in my biochemistry courses. This is particularly important at a school like mine where the majority of our students are engineering students.

During the past year, three undergraduate students have worked directly on this project. The first student worked full-time for three months at a time for two separate co-op terms. This student's salary was paid for by the grant as well as all of his supplies and materials. He previously had done three months of full-time work on the project during the previous year. Over these two years he was the primary researcher on all of the solvent testing. This funding has allowed him to do a research thesis, instead of having to do one with an industrial partner. As someone who is planning on applying for graduate school in the next year, this is invaluable to him. Additionally, being able to work on a project for so long has allowed him to develop a lot of skills that are not often worked on until graduate school, including independent and critical thinking. His authorship on the published paper will also help him as he goes forward into graduate school. The second student who worked on the project had just finished one year in college, but worked on the project for 8 weeks. Her stipend was provided through a different grant, but some of her materials were paid off of this grant. She worked with the first student on some of his projects (materials paid by this grant) and focused on identifying the exact proteins(s) in WGAP (materials paid by a different grant) for better quantification. This student grew tremendously over the course of her term and the grant funds really helped her to grow from a student mentality to a researcher mentality since we had the materials available to her to start following someone else's protocols to developing her own. Working in this system also helped her to see the importance of safety and disposal in a way that young students do not often understand. The third student worked on this project for class credit during two different 3 month terms. She is graduating after this current term, and thus these terms counted as her senior research. Since she was getting course credit for her work, she did not receive payment, but her materials were split between a small amount of money that the school provides for chemistry research students and from this PRF grant. This grant therefore allowed her to do the research she was able to do which allowed her help make decisions about how the project should go forward. She worked on characterizing naphthol AS-MX in solvent mixtures, including determining quantum yields. Additionally she was able to test some additional methods, including those related to IR and apparent pH. Without the PRF funding, her projects she would have only been able to work on quantum yields and would not have been able to take the project in the directions that she helped determine were the most useful. Additionally, as a dual chemistry/chemical engineering student, this project also helped to show her how her fields can intersect together and relate to the world at large. While she has a job lined up for when she graduates, working on this project has helped her to realize that she will likely want to go back to graduate school at some point. All of these undergraduates gave at least one departmental presentation on their findings, improving their scientific communication skills and exposing the department (which includes chemists, biologists, biochemists, and chemical engineers) to this research.