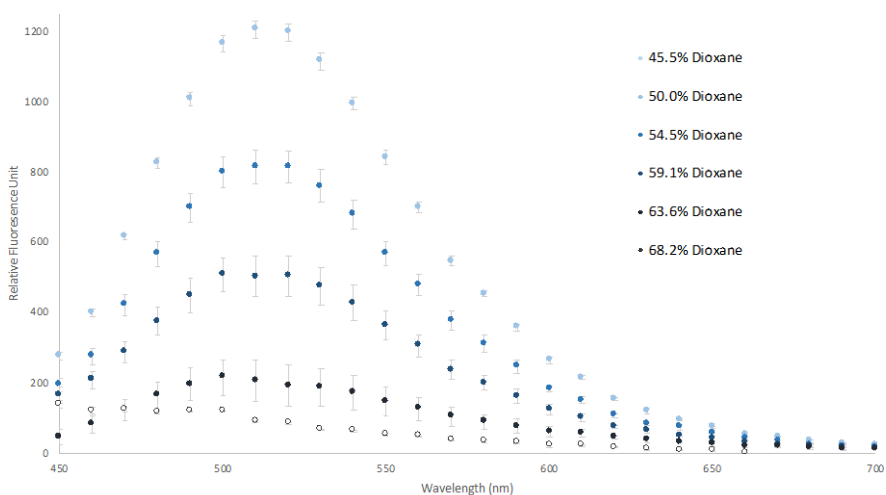
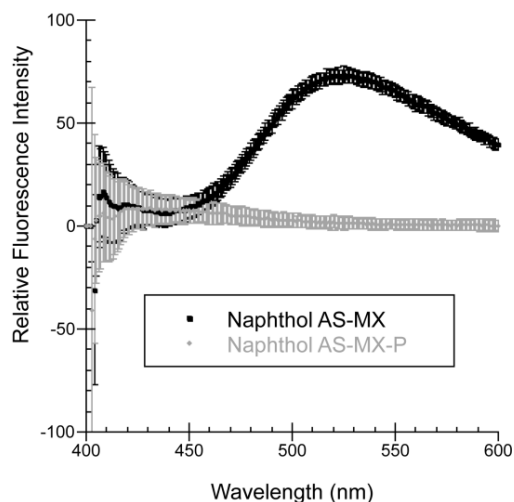


PRF#: 57384-UNI4

Project Title: Enzymatic Dephosphorylation in Organic Media

PI Name, Affiliation: Veronica R. Moorman, Kettering University

So far, progress has been made on both aims of the grant “Enzymatic Dephosphorylation in Organic Media”: 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. A detection system using the fluorescence of dephosphorylated naphthol AS-MX phosphate when excited at 388 nm was quickly identified as feasible because of its ability to be dissolved in organic and aqueous solvents. Protocols were developed for semi high-throughput fluorescence analysis. Differential fluorescence was seen in a range of dioxane percentages using Tris buffer as the aqueous component. An example, using a 80:20 dioxane:pH 8.0 Tris buffer mix, is shown aside. Subsequently, three plant phosphatases were tested and shown to be active up to ~60% dioxane when using Tris buffer (pH 7) as the aqueous component. The fluorescence spectra after naphthol AS-MX phosphatase in dioxane:Tris mixes were exposed to sweet potato purple acid phosphatase for 60 minutes are shown below. Work is now ongoing to quantitatively characterize this activity and to understand the structural stability required for activity in dioxane mixes. Additionally, another solvent will be tested in the near future to determine if the trends seen here in dioxane are universal.



This grant is allowing me to start a research program in new field of research that I would have not have been able to do otherwise. Because my background in macromolecular NMR was difficult to be translated into a research program at a small PUI, I needed to establish myself as an independent researcher in a field more accessible to undergraduates with the equipment that I have access to, which is difficult without the funds to collect preliminary data. Over the past year, I was able to purchase materials to work with organic media in the lab as well as some chemicals and enzymes themselves for the project. This helped me to establish a robust research program at Kettering which students could easily relate to the goals of allowing me to take on undergraduate students of two types: those who were paid by the grant and those who were not.

So far, five undergraduates have worked on the project. The first two students both got course credit for research while working part time on the project and thus did not get paid from the grant. The majority of their materials, however, were funded through the grant. They were able to get the lab set up for this kind of work, develop initial protocols, and were able to identify and start testing the fluorescence of naphthol AS-MX and naphthol AS-MX phosphate in dioxane mixtures. One has since graduated and is working full time as an assistant scientist at MI Bioresearch while he applies for graduate school in the upcoming year. Working on this project helped him solidify his interest in graduate school and the basic sciences. Two students worked full time on the project for 3 months each. One was funded entirely through this grant (but was only able to do one 3 month work term during the past funding period) while the other's salary and some materials were paid through an internal funding source for freshman students who are interested in research. They were able to collect systematic data on the utility of naphthol AS-MX fluorescence detection in dioxane mixes. One manuscript has been written that showcases the work of these four students and is being sent off for peer review presently. One of them will be working on this project again for another 3 months starting in October and will be writing his undergraduate thesis on this work. Either he or another interested student will continue on with the project after that point. Both of these students had originally wanted to go into medicine, but since their experience with basic research are now on a research track. The final student is an undergraduate of Kalamazoo College. She worked full time for two months as a part of an NSF REU site at Kettering. Her salary and much of her materials were paid for through the NSF, but supplementary materials were paid for from this PRF. Remarkably, this student was able to see activity of three different plant phosphatases in dioxane percentages of up to ~60% and has started the process of computationally modeling components of system. She has limited opportunities for research at her home institution and thus was extremely grateful for the opportunities that working on this project presented her. While her stipend was not funded through the project, she would not have had the opportunity to do this work had the project not previously been established nor would she have been able to test three different enzymes without supplemental funding.