Project Leader: Erica L. Woodahl, Department of Biomedical and Pharmaceutical Sciences

Project Title: Structural and Functional Analysis of Genetic Polymorphisms in the P-glycoprotein (ABCB1) Drug Transporter

Project Abstract:
P-glycoprotein (P-gp), encoded by ABCB1, is an efflux drug transporter important in drug disposition throughout the body, particularly in the intestine, liver, kidney, and blood-brain-barrier. P-gp is thought to have multiple binding sites to accommodate the large diversity in its substrates. ABCB1 pharmacogenomic studies have shown that P-gp activity and disposition of P-gp substrates is altered by genetic variation. The goal of this proposal is to determine the structural and functional changes in the drug-binding and nucleotide-binding sites of P-gp due to ABCB1 genetic variation. The specific aims of are to 1) establish a ligand-based modeling approach to study genetic variation in P-gp and to 2) determine the structure of P-gp genetic variants with homology modeling. We will focus on nonsynonymous SNPs at nucleotides 1199 and 2677, which code for changes in the protein at amino acids 400 and 893, respectively, and have been shown to alter P-gp activity. We will use several experimental and modeling approaches to achieve our aims. We will generate pharmacophore models based on quantitative structure activity relationships (QSARs) using a dihydropyridine (DHP) scaffold. DHPs are known P-gp substrates. Kinetic parameters of DHP transport will be estimated using an in vitro recombinant ABCB1 cell system developed by Dr. Woodahl, which express either wild-type P-gp or a genetic variant of P-gp. We will further investigate ligand binding using spectroscopic and biophysical measurements. These experimental observables will be used to refine the pharmacophore models. Using the recent determined mouse P-gp X-ray structure, which shares 87% identity to human P-gp, we will construct homology models for human P-gp and its genetic variants. We will dock our pharmacophore models into the homology models to predict how genetic variation will change protein structure, substrate binding and transport. The iterative in vitro
screening and pharmacophore and homology model refinement will strengthen our predictions about the influence of genetic variation in P-gp transport. This project has the translational application to help identify substrates that will be most impacted by ABCB1 pharmacogenomics and minimize interindividual variability in P-gp substrates.