Effects of engineering nonnative ligand binding into *E. coli* phosphoenolpyruvate carboxykinase

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Enzyme engineering has long been promised to be the technique that revolutionizes chemistry, from catalyzing industrial processes economically to transforming byproducts into useful materials to synthesizing natural products and pharmaceuticals. However, making mutations in a protein can lead to both intended effects along with unintended ones. My project sought to explore enzyme engineering by altering the active site of an *E. coli* metabolic enzyme, phosphoenolpyruvate carboxykinase (EcPCK), to accommodate a ligand different than its native substrate CO_2. EcPCK is interesting because the enzyme catalyzes formation of a carbon-carbon bond between phosphoenolpyruvate and CO_2; by replacing CO_2 with a different one carbon substrate, different four carbon substrates can be formed by this enzyme scaffold.

Mutants were generated through both rational design and gene shuffling techniques, then characterized through the use of protein crystallography (PX) and small angle x-ray scattering (SAXS). Binding of two nonnative ligands across five different EcPCK variants was observed with PX and characterized the structures to 1.5 Å resolution or better. These variants exhibited a high degree of ligand selectivity: even though the two nonnative ligands were very similar (thiosulfate and methanesulfonate), the mutants only bound to one and not the other, providing an interesting case of molecular recognition. The SAXS experiments were able to follow the closing of EcPCK upon nucleotide and nonnative ligand binding in solution; varying degrees of compactness in solution upon ligand binding are observed by SAXS. Certain mutations also appear to inhibit this conformational change which is necessary for catalysis. From these results, the mechanistic trigger behind the conformational change of EcPCK is also being investigated. Combined, the PX and SAXS results shed light on not just fundamental enzymatic structure-function relationships, but also on how to exploit enzyme molecular recognition and conformational change for therapeutic uses, such as for inhibitor design in drug development.