Structural analysis of metabolic binding partners of SuhB

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Crystallographic fragment screening is a method to obtain insight into ligand binding sites in a protein. These binding events provide a starting point for drug discovery [1]. Ligand binding sites are not only important for drug development, but can also give insight of the function of a protein in the cell. As there are still many proteins with unknown cellular function, ligand or metabolic binding partners can provide a first starting point to deteminate the protein's function.

In this study, a metabolite screen was designed and assembled based on the 26 most abundant metabolites in *Escherichia coli* [2]. Therefore, the metabolites were concentrated to a 10x higher concentration than their intracellular concentration, and dried onto a 96-well plate for soaking expermients. The protein SuhB was chosen to validate the metabolite screen. Validation of the screen revealed four possible metabolic binding parteners for SuhB: 6-phosphogluconic acid, L(-)-malic acid, 3-phosphoglyceric acid and L-glyceric acid. 6-phosphogluconic acid binds to the active site of SuhB and is therefore an interesting target for further enzymatic activity assays. In an isothermal titration calorimetry measurement, the binding affinity of the metabolite to the protein could not be determined, probably due to too low affinity between the ligand and the protein. L-glyceric acid binds in the active site as well were usually a Mg^{2+} ion binds. As 3-phosphoglyceric acid and L(-)-malic acid bind between two symmetry mates in the crystal, it makes these hits rather uninteresting for further analysis.

The validation of the metabolite screen was successful, and the screen might be used in the future for the discovery of metabolic binding partners. 6-phosphogluconic acid might be a new binding partner of SuhB as it binds in the active site. Further analysis and especially validation of this binding event is needed to confirm the results of the screen.

1. Wollenhaupt, J., et al., *F2X-Universal and F2X-Entry: Structurally Diverse Compound Libraries for Crystallographic Fragment Screening.* Structure, 2020. **28**(6): p. 694-706 e5.

2. Bennett, B.D., et al., *Absolute metabolite concentrations and implied enzyme active site occupancy in Escherichia coli.* Nat Chem Biol, 2009. **5**(8): p. 593-9.