Bornberglab

Molecular Evolution and Bioinformatics

Student project

Adaptive Laboratory Evolution

Mapping Epistatic Mutations Within Ancestral Sulfatases

Background: Understanding how enzymes adapt toward new functions can help to further develop laboratory evolution strategies to engineer enzymes for improved commercial performance. The latter procedures typically involve repeated cycles of introducing genetic diversity followed by screening/selection for improved function. This process is also known as directed evolution. One particular bottleneck in understanding adaptive evolution is the presence of epistatic effects, i.e. the same mutation can have widely different effects, depending on its genetic background. Directed evolution campaign typically consider a single protein and a single evolutionary path. In this project we will test the effect of mutations known to improve the catalytic performance of ancestral and extant arylsulfatases in 'ancestral enzymes' bridging the path between *Sp*AS1 and the common ancestor of aryl sulfatases and phosphonate monoester hydrolases (Figure 1).

Objectives: We have found several extant and ancestral sulfatases with improved activity towards 4-nitrophenyl sulfate (Figure 1). The aim of this project is to assess the impact of the mutations found in these sulfatases when introduced in the ancestral AS variants that we have reconstructed and explore what determines epistatic interactions between these evolutionary related enzymes.

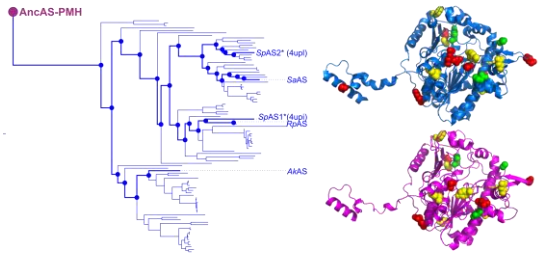

Figure 1: Phylogenetic tree of the AS part of the AS-PMH clade going back to the last common ancestor. Blue dots denote reconstructed ancestral sequences. Beneficial mutation sites on *Sp*AS1 (above) and the corresponding residues on the closest ancestor(below). Mutation sites are indicated in yellow (conserved in *SpAS1* and ancestor), red (not conserved between *Sp*AS1 and ancestor) and green (*Sp*AS1 mutated back to the residue present in the ancestor.

Requirements:

- Interest in evolution at the level of individual proteins
- Interest in molecular biology techniques
- Interest to work with isolated proteins

Methods:

- Creating mutants using site-directed and site-saturation mutagenesis.
- Biochemical and biophysical characterization of enzyme variants.

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Selected Literature: van Loo *et al* (2019) Balancing Specificity and Promiscuity in Enzyme Evolution: Multidimensional Activity Transitions in the Alkaline Phosphatase Superfamily. *J. Am. Chem. Soc.* **141**, 3701–387.

Bayer *et al* (2017) Specificity Effects of Amino Acid Substitutions in Promiscuous Hydrolases: Context-Dependence of Catalytic Residue Contributions to Local Fitness Landscapes in Nearby Sequence Space. *ChemBioChem* **18**, 1001–1015. van Loo *et al* (2019) High-Throughput, Lysis-free Screening for Sulfatase Activity Using *Escherichia coli* Autodisplay in Microdroplets. *bioRxiv* 479162, doi: https://doi.org/10.1101/479162.

Starr & Thornton (2016) Epistasis in protein evolution. *Prot. Sci.* **25**, 1204-1218.

Baier *et al* (2019) Cryptic genetic variation shapes the adaptive evolutionary potential of enzymes. *eLife* 8:e407891