Amine Transaminase Engineering based on Constraint Network Analysis

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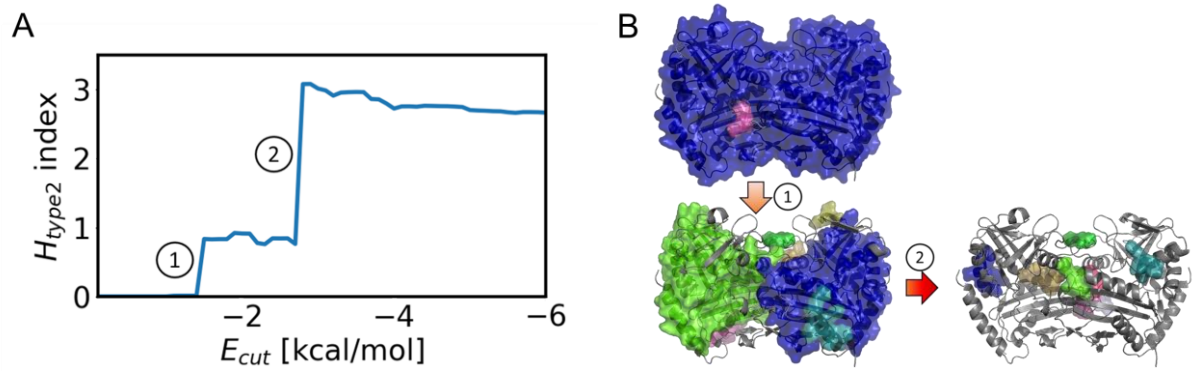
Amine transaminases (ATAs) are important enzymes for the production of chiral amines in the pharmaceutical and fine chemical industries. [1] However, the application of ATAs on novel substrates is often accompanied by several challenges. These include product and co-substrate inhibition effects as well as limited resistance to organic solvents needed for substrate solubilization. [2]

With our in-house software Constraint Network Analysis (CNA), we can study protein rigidity at the atomistic level and gain insights into structural changes during thermal unfolding. [3] This is achieved by monitoring changes in a cluster configuration entropy (H_{type2}) index while removing constraints between atoms according to a stepwise increasing cut-off energy (E_{cut}), to mimic the weakening of non-covalent interactions upon heating of the enzyme (Figure A). Based on this analysis, we aim to identify structural weak spots, i.e., residues that can be mutated to improve protein rigidity, in ATAs of fold type I and IV.

With the goal of stabilizing ATAs in reaction conditions with increased temperatures, organic solvents, and high co-substrate concentrations, we apply CNA on ATA structure ensembles generated via molecular dynamics simulations in both explicit water and mixed organic solvents. By applying this approach to ATA variants with known (de-)stabilizing mutations, we study changes in the unfolding behavior to ultimately propose novel stabilizing mutations.

Initial rigidity analysis of a dimeric fold type IV ATA representative (PBD-ID: 4CE5) shows a two-phase thermal unfolding process (Figure A), with the dimer interface destabilizing first, followed by decomposition of the rigid clusters in both monomer subunits (Figure B). This early insight can already be used to guide the engineering of this ATA to prioritize dimer interface stabilization, with the analysis of further ATA variants in progress.



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[3] C. Pfleger, et al., *J Chem Inf Model.*, **2013**, *53*, 1007-1015.