Amine Transaminase Engineering based on Constraint Network Analysis

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Amine transaminases are versatile biocatalysts

As a special subgroup of ω-transaminases, amine transaminases (ATAs) reversibly transfer amino groups from a donor molecule to a carbonyl acceptor to produce either (*S*)- or (*R*)-enantiomeric products. Conveniently, the cofactor phosphatidyl-5-phosphate (PLP) is automatically recovered in this process[1,2] (Figure 1). This inherent sustainability makes ATAs popular targets for producing small molecule building blocks or late-stage functionalization of products in pharmaceutical and fine chemical industries[3, 4].

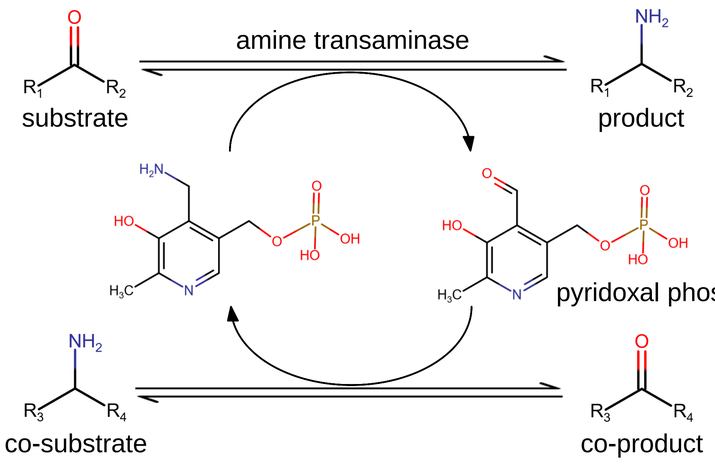

Figure 1: Amine transaminase catalysis

Studying ATA rigidity to predict structural weak spots

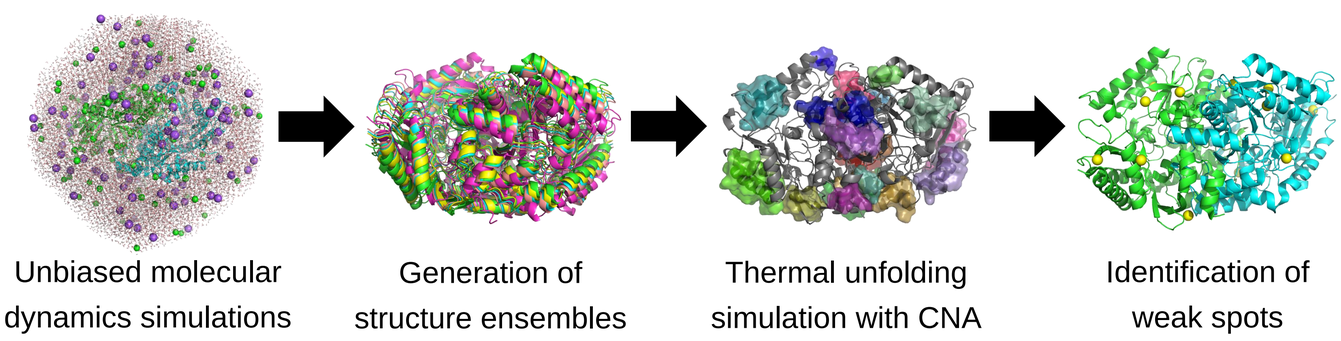

Figure 2: Structural weak spot identification

Since efficient enzyme stabilization of newly discovered ATAs remains challenging, we aim to establish our in-house software Constraint Network Analysis[5] (CNA) as a method to facilitate ATA engineering. By applying CNA on structure ensembles generated in unbiased molecular dynamics simulations, we study changes in enzyme rigidity during simulated heating. This in turn allows the identification of structural weak spots (Figure 2).

Guiding ATA engineering with CNA

Different ATAs show different rigid cluster decompositions in thermal unfolding simulations:

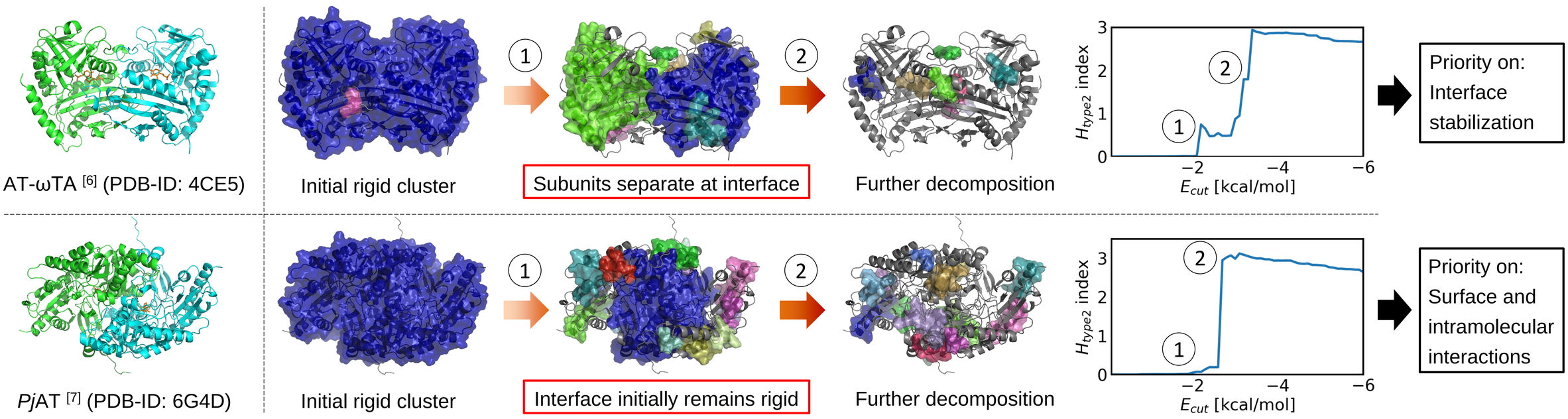

Figure 3: Thermal unfolding simulations with CNA for different ATAs

Current application of our approach on multiple ATA variants will give insights on:

- Identification of specific weak spot residues for enzyme stabilization
- Prediction of changes in T_m upon introduction of mutations
- Effects of organic solvents and co-substrates on ATA structural stability

<u>Thermal unfolding simulations (TUS) with CNA:</u>

Atoms and bonds are modelled as “body and bar networks”

- TUS = bond removal according to energy cut-off (E_{cut})
- Changes in cluster configuration entropy (H_{type2}) index reveal phase transition events
  - E_{cut} at phase transition relates to protein T_m

Summary

By studying the rigidity of different ATA variants using our CNA approach, we can provide unique insights into protein stability to guide ATA engineering projects. We aim to establish CNA as a tool to provide researchers with valuable insights on ATA thermostability and solvent resilience to ultimately allow more focused and efficient engineering of currently unexplored or newly discovered ATAs.

In an extension of this project, we will further explore opportunities to combine information gained from CNA with other computational protein descriptors and experimental data to develop innovative enzyme engineering strategies.

References

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Acknowledgements

The authors gratefully acknowledge the computing time granted by the John von Neumann Institute for Computing (NIC).