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Engineering and Mechanistic Insights into the Enhanced Activity of a Nitrile Hydratase - An Enzyme with a Non-standard Active Site

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Nitrile hydratase (NHase) is an enzyme that catalyzes the hydration of nitriles to amides. This biocatalyst contains post-translationally modified cysteines and a cobalt ion in its active site. It plays a crucial role in the industry, with over 2 million tons of amides produced annually using NHase. Despite its large-scale application, further improvements in activity and thermostability are needed to reduce production costs.

Here, I demonstrate how theoretical computational biophysics tools - such as ligand docking, molecular dynamics (MD), and protein structure prediction - were used to design more efficient NHase variants and elucidate the molecular mechanisms underlying their enhanced activity.

In the first study, we identified key gatekeeper residues and modified them to improve catalytic efficiency. The αTrp116 variant exhibited a 14-fold increase in the catalysis of 3-cyanopyridine. Docking and MD simulations provided molecular insights into this enhanced activity.

In a separate innovative approach, self-organizing protein scaffolds were fused with wild-type NHase, leading to a threefold increase in activity and a 28-fold improvement in thermostability. MD simulations of models generated using AlphaFold3 and LzerD explained the molecular basis of these enhancements.