TOWARDS THE *DE NOVO* DESIGN OF METALLOHYDROLASES

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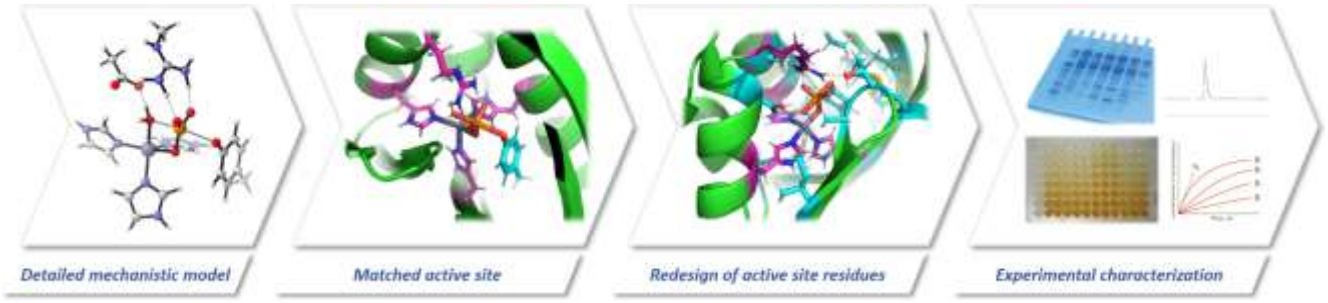
De novo design of proteins has enabled the exploration of vast regions of sequence space previously inaccessible by nature.[1] These proteins are computationally designed based on physical principles of protein structure and folding, and are tailored according to a specific function or property. Great advances have been made in the design of novel highly stable protein folds capable of hosting enzyme active sites.[2] That has in turn allowed us to undertake larger scale efforts of installing various enzymatic activity into these scaffolds. As a proof of principle we have undertaken efforts towards designing hydrolase (esterase and phosphatase) activity into the NTF2[2b] and the TIM-barrel[3] folds through the introduction of metal binding sites.

The computational enzyme design process has been performed at multiple scales, starting with high level quantum chemical computations that were used to obtained a detailed mechanistic model of the reaction in the form of an idealized enzyme active site (theozyme).[4] These insights have then been transferred to the enzyme design process where the algorithms in the Rosetta software have been employed to match and design the corresponding active sites into thousands of *de novo* scaffolds. Combined with smart filtering methods this approach has enabled us to narrow in on the most promising computational designs that were then carried forward to experimental testing. These proteins have been expressed in *E. coli* using the corresponding custom genes, isolated, their biophysical properties spectroscopically determined, and lastly their enzymatic activities measured using appropriate screening assays. The obtained information then feeds back into further rounds of computational design aimed at improving the activity and stability, as well as understanding what mutations are additionally beneficial for the desired activity.

Through the use of computational de novo enzyme design we are aiming to open up new avenues for mechanism-based development of novel enzymatic reactions for which currently no evolutionary trajectories exist.



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