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RESEARCH ARTICLE

# Adaptive landscape flattening allows the design of both enzyme: Substrate binding and catalytic power

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## Abstract

Designed enzymes are of fundamental and technological interest. Experimental directed evolution still has significant limitations, and computational approaches are a complementary route. A designed enzyme should satisfy multiple criteria: stability, substrate binding, transition state binding. Such multi-objective design is computationally challenging. Two recent studies used adaptive importance sampling Monte Carlo to redesign proteins for ligand binding. By first flattening the energy landscape of the apo protein, they obtained positive design for the bound state and negative design for the unbound. We have now extended the method to design an enzyme for specific transition state binding, *i.e.*, for its catalytic power. We considered methionyl-tRNA synthetase (MetRS), which attaches methionine (Met) to its cognate tRNA, establishing codon identity. Previously, MetRS and other synthetases have been redesigned by experimental directed evolution to accept noncanonical amino acids as substrates, leading to genetic code expansion. Here, we have redesigned MetRS computationally to bind several ligands: the Met analog azidonorleucine, methionyl-adenylate (MetAMP), and the activated ligands that form the transition state for MetAMP production. Enzyme mutants known to have azidonorleucine activity were recovered by the design calculations, and 17 mutants predicted to bind MetAMP were characterized experimentally and all found to be active. Mutants predicted to have low activation free energies for MetAMP production were found to be active and the predicted reaction rates agreed well with the experimental values. We suggest the present method should become the paradigm for computational enzyme design.

## Author summary

Designed enzymes are of major interest. Experimental directed evolution still has significant limitations, and computational approaches are another route. Enzymes must be stable, bind substrates, and be powerful catalysts. It is challenging to design for all these properties. A method to design substrate binding was proposed recently. It used an adaptive Monte Carlo method to explore mutations of a few amino acids near the substrate. A