Computational design of proteins and enzymes with a physics-based approach

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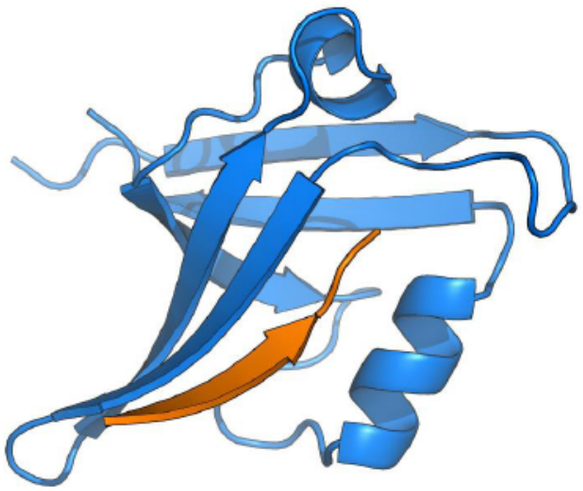
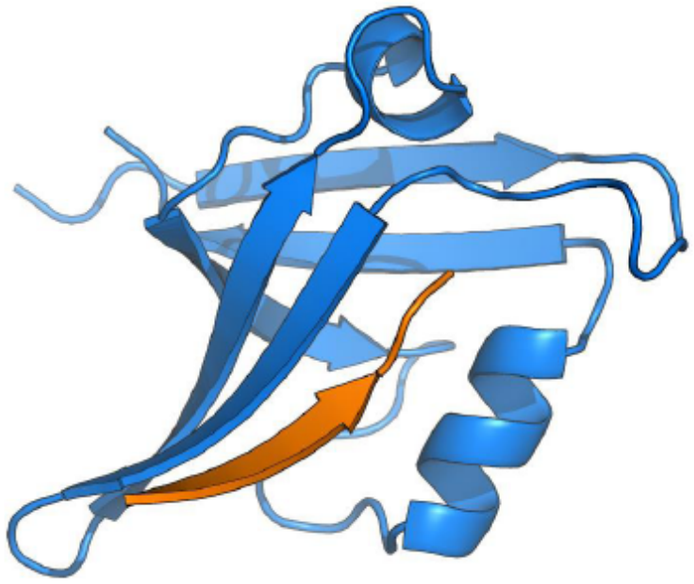
- Complete redesign of a PDZ domain
  YJ. Sun, T. Hou, E. Fuentes; University of Iowa



- Aminoacyl-tRNA synthetase design for the genetic code expansion
  G. Nigro, E. Schmitt, Y. Mechulam; Ecole Polytechnique

Complete redesign of a PDZ domain

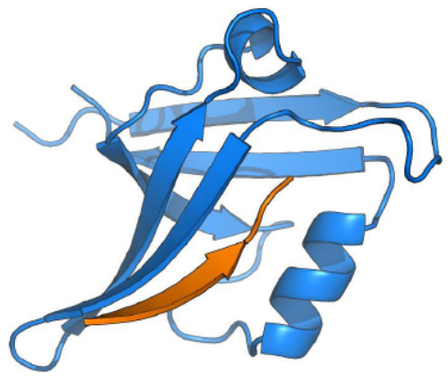
- Domain length: 83 aas
- Establish protein-protein interactions



- Mutate positions in a Monte Carlo exploration
- Proline, glycine, backbone, and 13 ligand binding positions not allowed to mutate
- All 61 others mutate freely: 10^{76} possible sequences

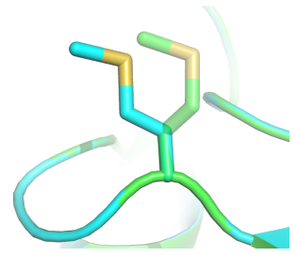
Computational framework

Experimental backbone structure

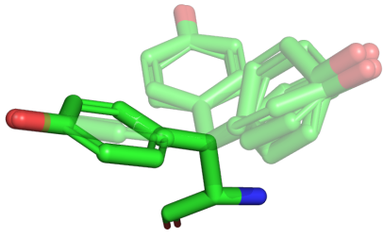


Monte Carlo moves

Change a rotamer or type



Rotamer library



Physics based energy function

$$E = E^{MM} + E^{GB} + E^{SA}$$

Replica Exchange MC sampling

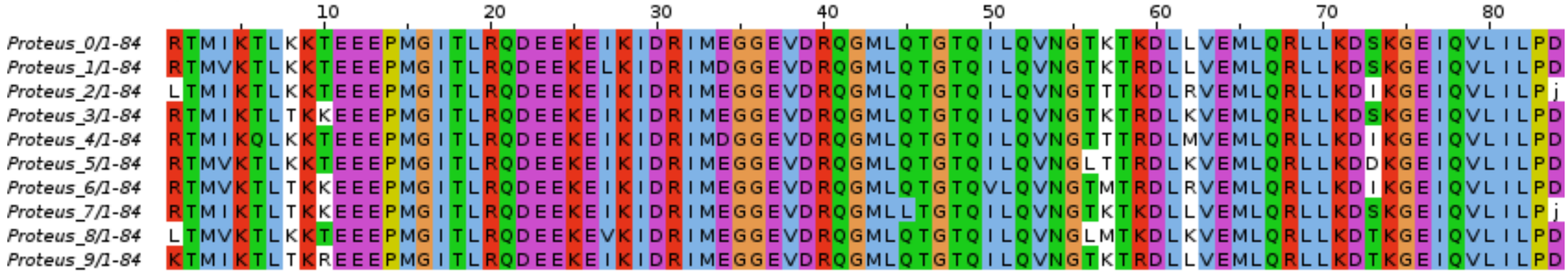
Empirical unfolded state

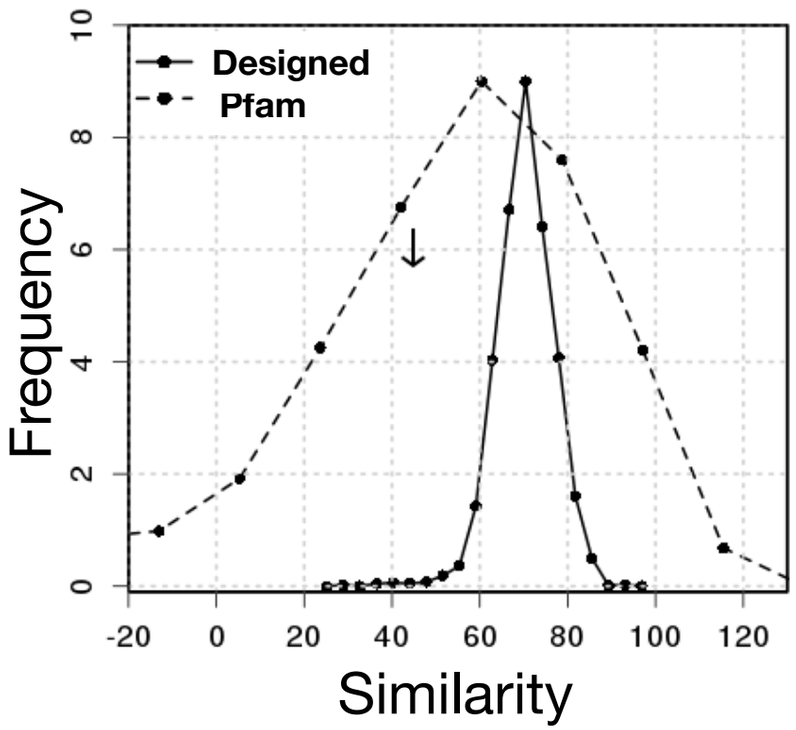
$$\sum_{aa} E^{uf}_{aa}(type_{aa})$$

Proteus software (https:proteus.polytechnique.fr)

Designed sequences ressemble natural PDZ sequences

10^4 sequences were obtained

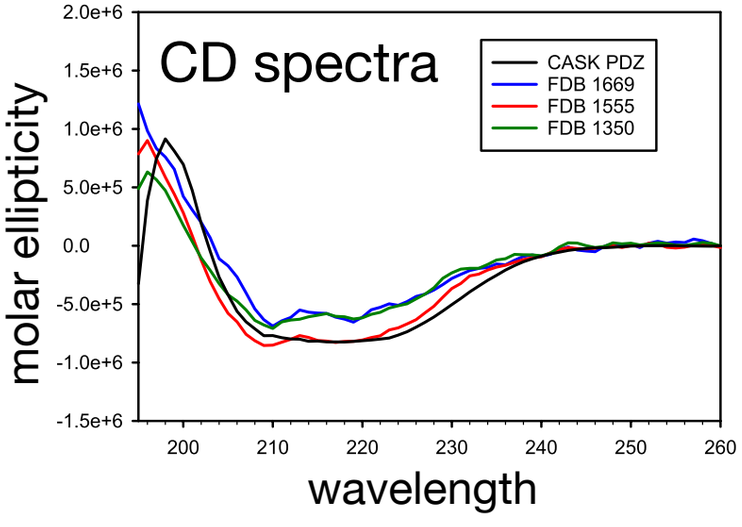


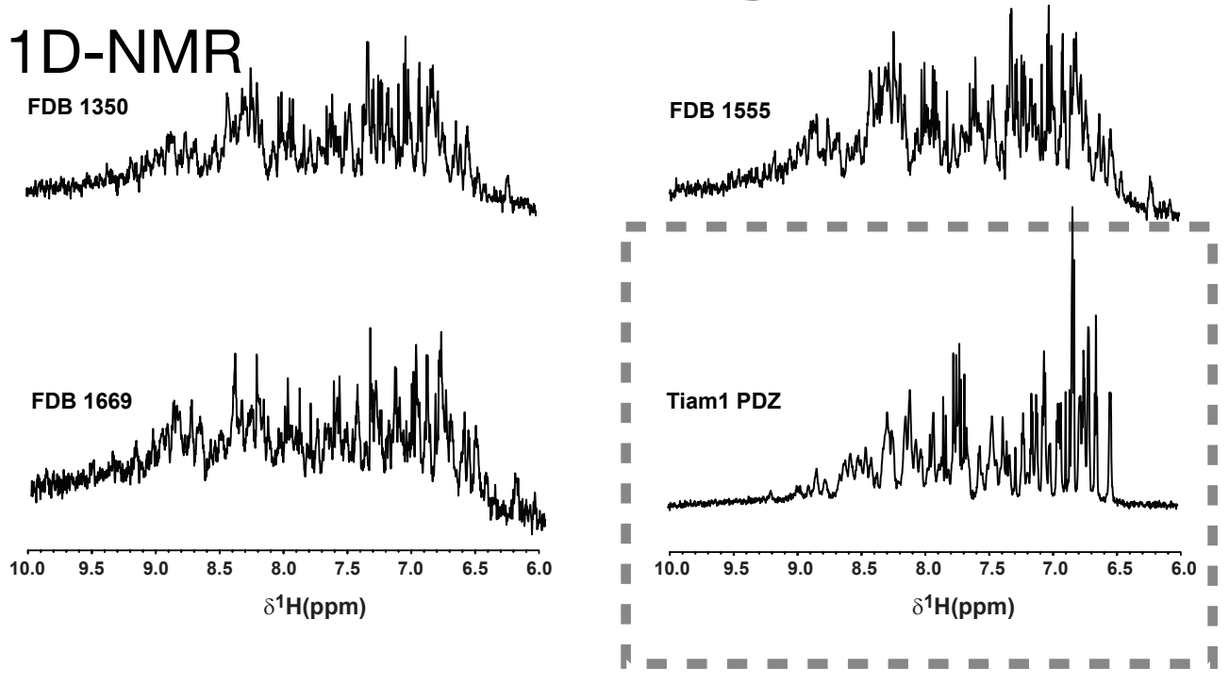


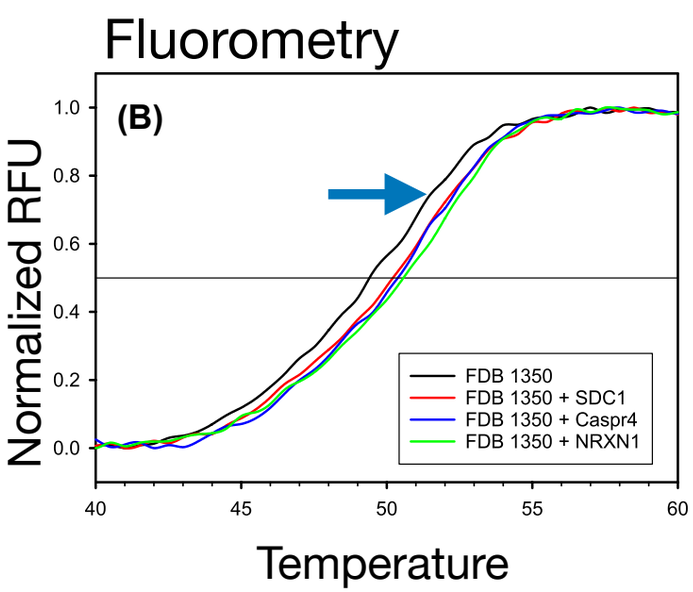
Core positions: Proteus vs Pfam

37% mean identity

3 sequences chosen for experimental testing
All 3 shown to fold correctly





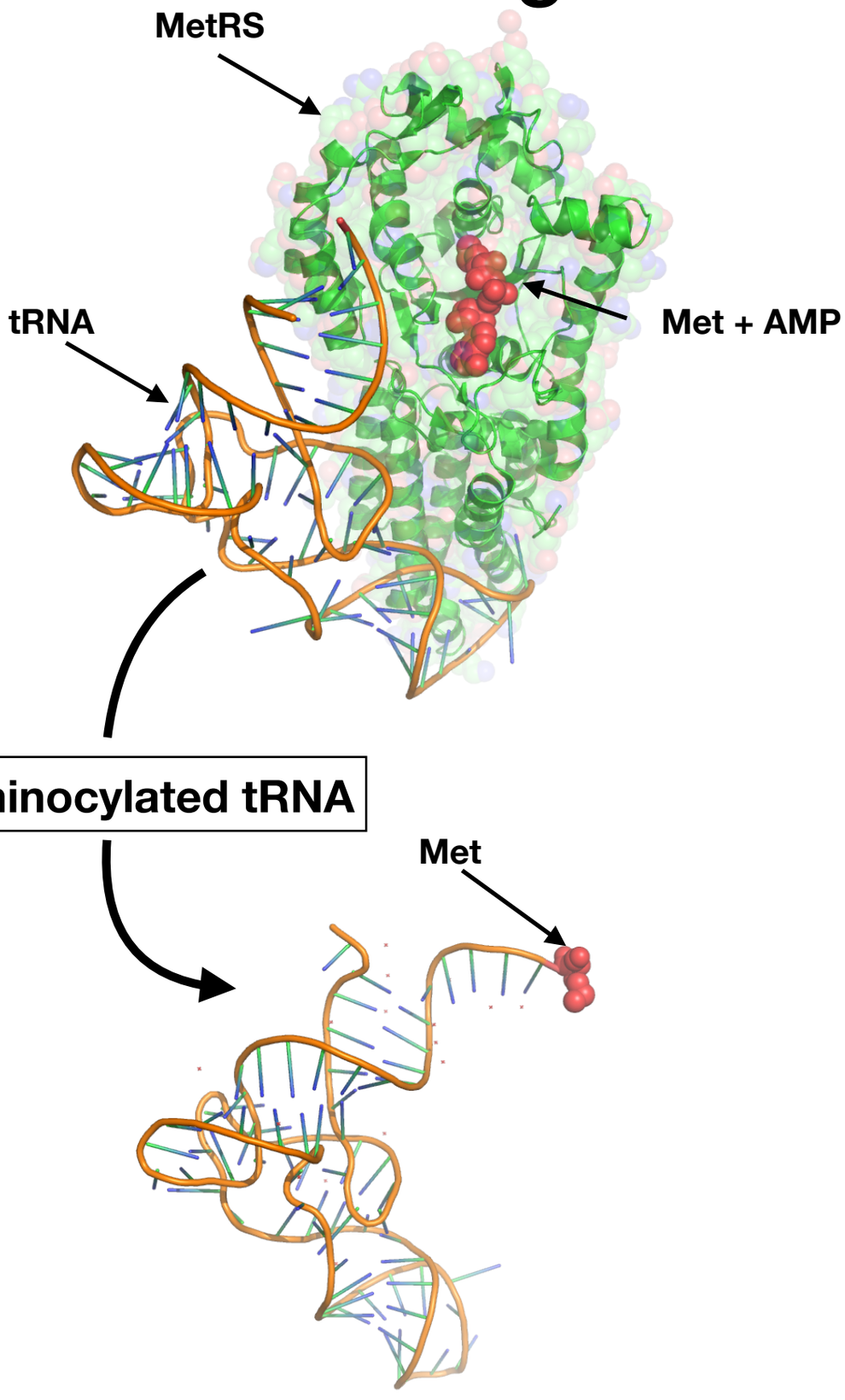


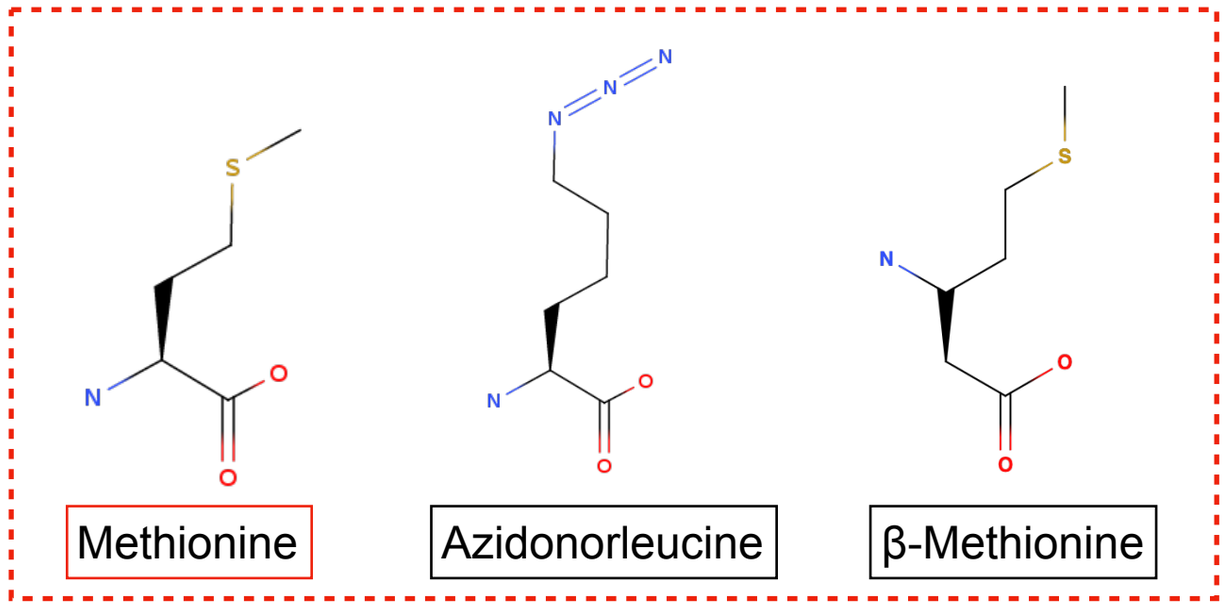
Thermal denaturation upshifted
by peptide binding

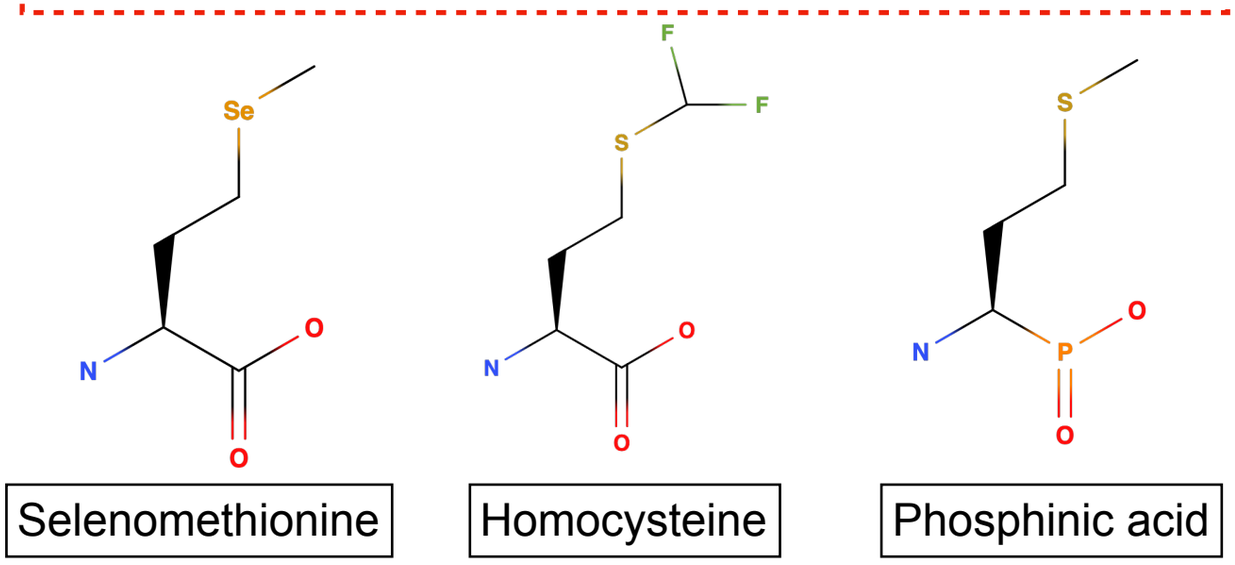
First protein redesign with a physics based energy function

Opuu, Sun, Hou, Panel, Fuentes, Simonson, under review, JACS

Methionyl-tRNA synthetase design for genetic code expansion







Design for affinity or catalysis is very difficult

- Large combinatorial space

- Simultaneous optimization directions: stability, affinity, catalysis

- Need to optimize bound/unbound difference:
  both positive and negative design

- Existing methods are heuristic or very expensive:
  optimize the bound state energy (Rosetta)
  exhaustive enumeration of states (Osprey)

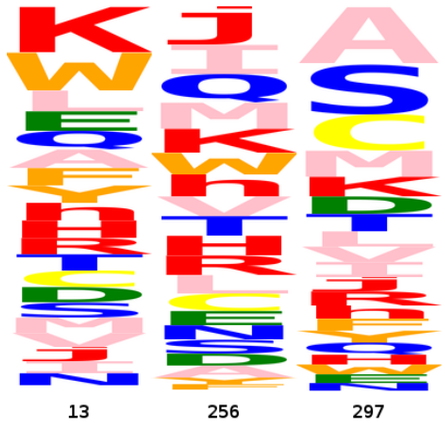
A rigorous method to sample by affinity: adaptive importance sampling

Villa, Panel, Chen, Simonson 2018; Bhattacherjee & Wallin 2013

1) Adaptatively flatten the free energy landscape of the apo state

Natural population

+bias



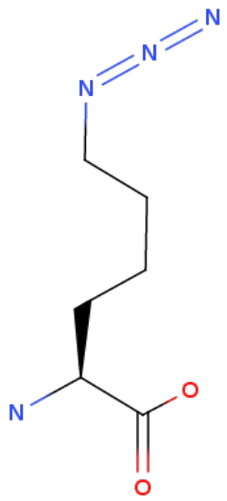
Flattened population

Flattened landscape

2) Simulate holo state with the same bias:
bias "subtracts out" the apo free energy

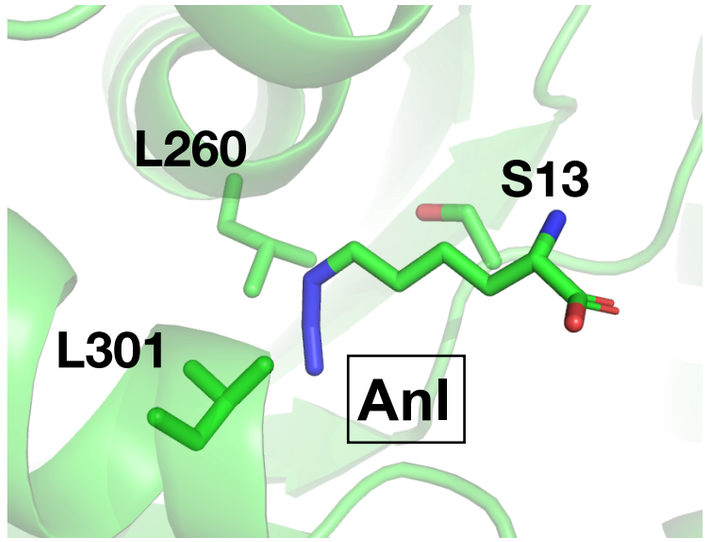
Sequences populated according to their binding free energy

As a test: redesign MetRS for Anl binding



Anl

Azidonorleucine
(Protein labeling)

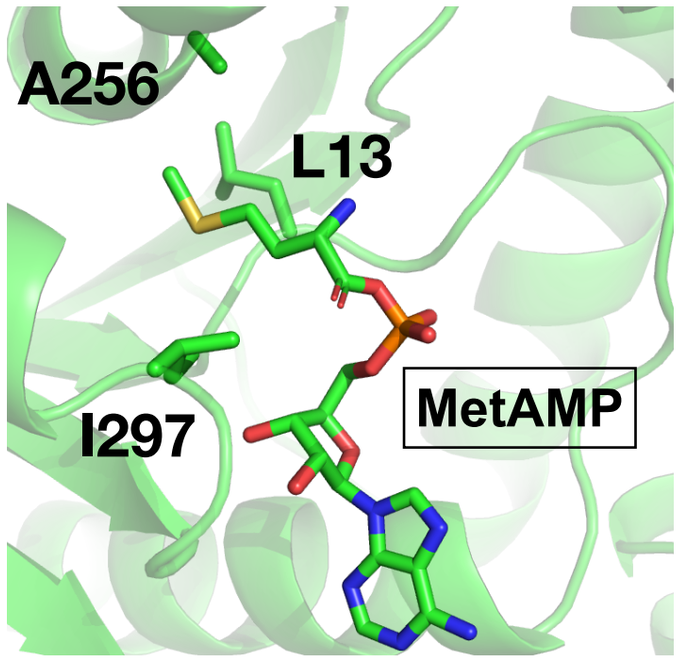


(Tanrikulu et al, 2009)

21 experimental variants
3 variable positions

5 of 6 most active variants are among the top 100 predictions

2nd test: redesign MetRS for Met binding

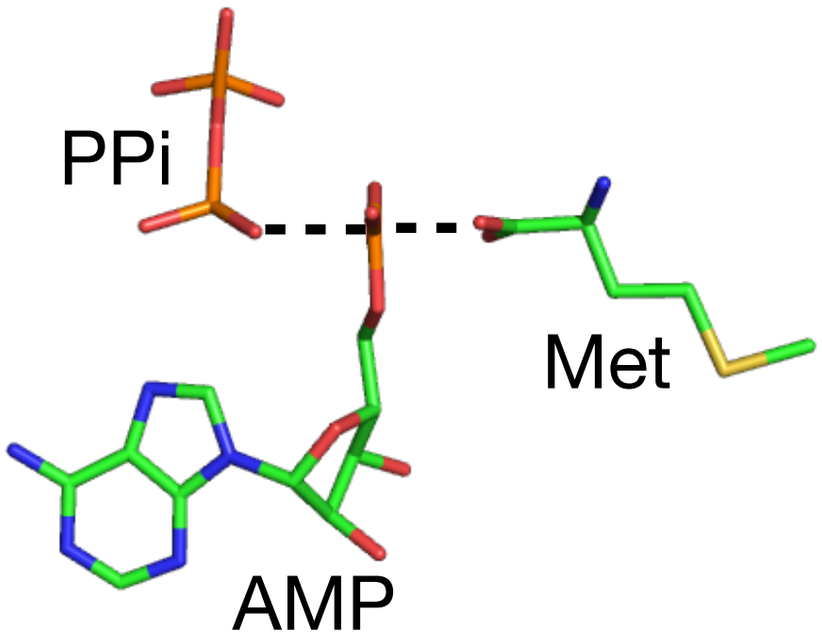


- 21 experimental variants
- 3 variable positions

- 5 active variants among top 40 predictions
- Computed affinities in good agreement with experiment:

0.9 kcal/mol mean error, 0.75 correlation

Adaptive importance sampling allows us to design for catalysis



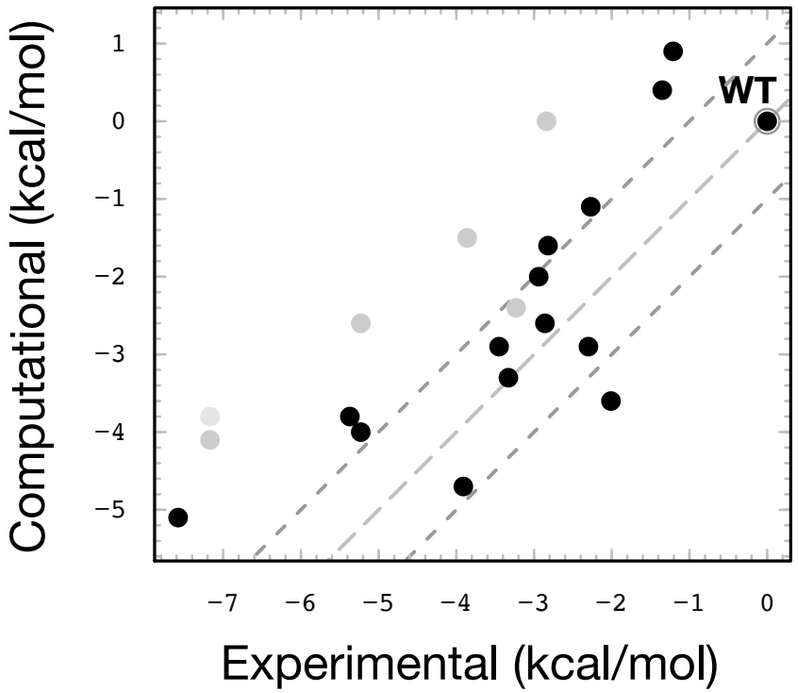
1) Flatten the landscape of the substrate complex

2) Sample the transition state complex, including the bias

Sequences populated according to their activation free energy

MetRS redesigned for catalytic power

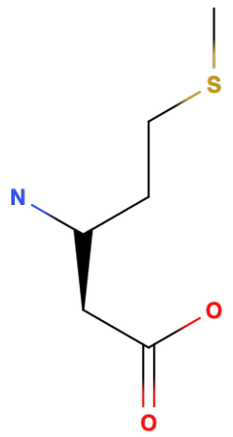
$$kT \times ln\left(\frac{k_{cat}}{K_M}\right)$$



- 21 experimental values
- 13 among top predictions
- Excellent agreement for k_{cat}/K_M
- 0.8 correlation, 1.1 kcal/mol mean error

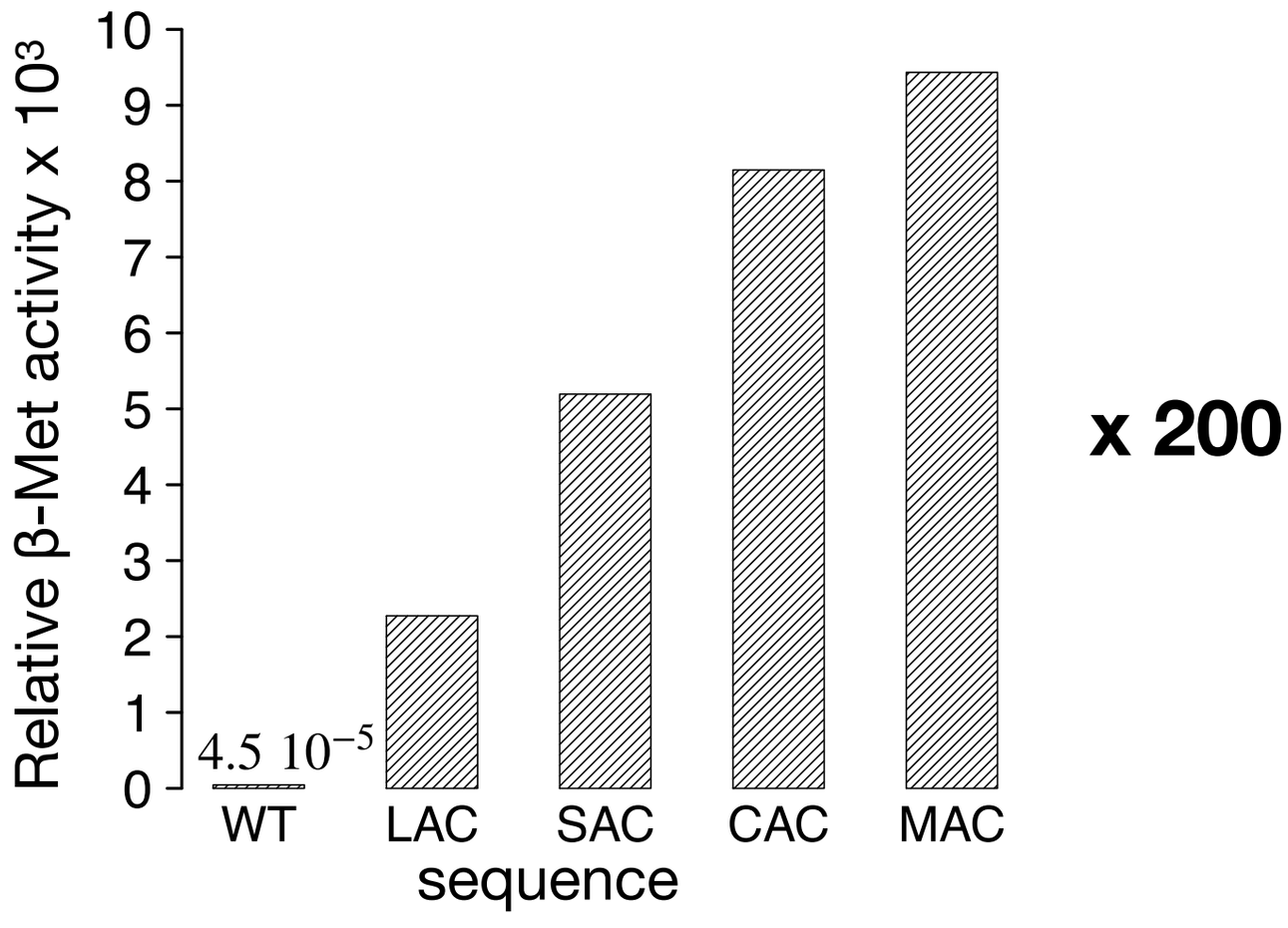
Opuu, Nigro, Villa, Gaillard, Schmitt, Mechulam, Simonson, accepted, Plos Comp. Bio.

Design MetRS for β-Met activity



β-Methionine

- 3 positions allowed to mutate (more underway)
- Good agreement for k_{cat}/K_M
- 4 variants discovered with improved selectivity



x 200

Summary & Acknowledgements

- First whole-protein redesign with a physics-based energy function

- First design of an enzyme for catalytic power
  - good agreement with experiment
  - predicted variants have improved β-Met selectivity

- **PDZ collaborators:**
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Titus Hou
Young Joo Sun

The University of Iowa

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