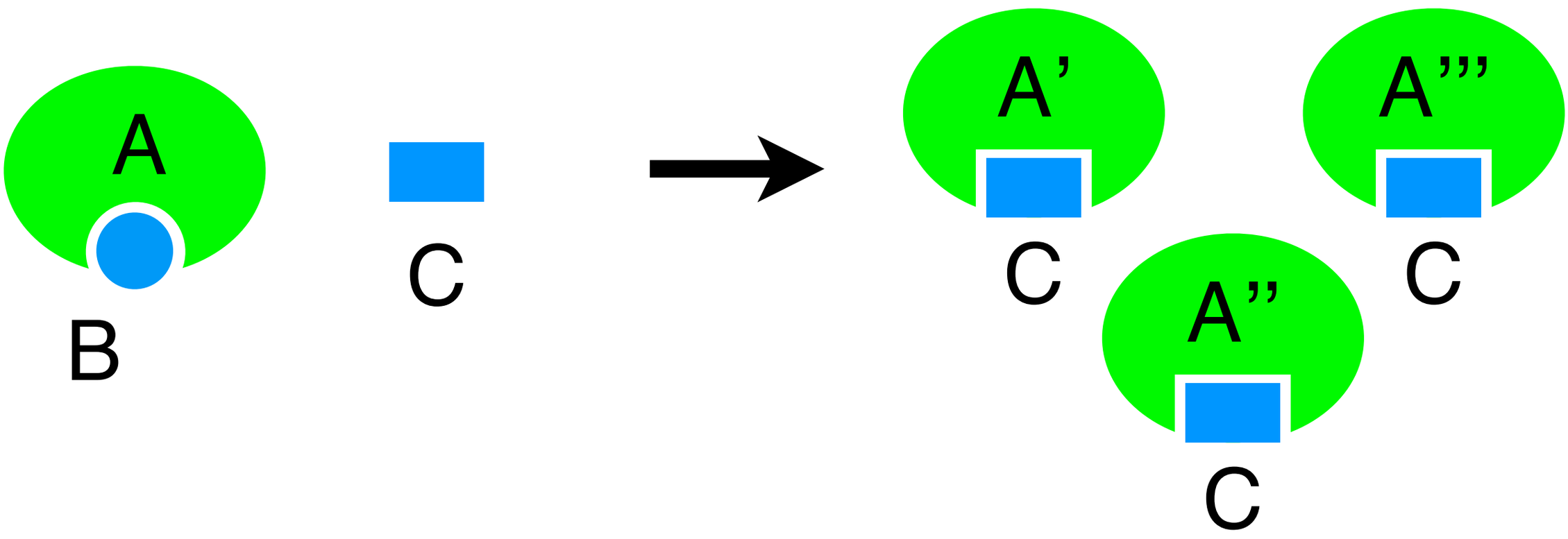
Computational redesign of enzyme substrate specificity using coupled side-chain backbone moves

Noah Ollikainen
RosettaCon 2013
Kortemme Lab, UCSF

DSM
BRIGHT SCIENCE. BRIGHTER LIVING.

UCSF
University of California
San Francisco

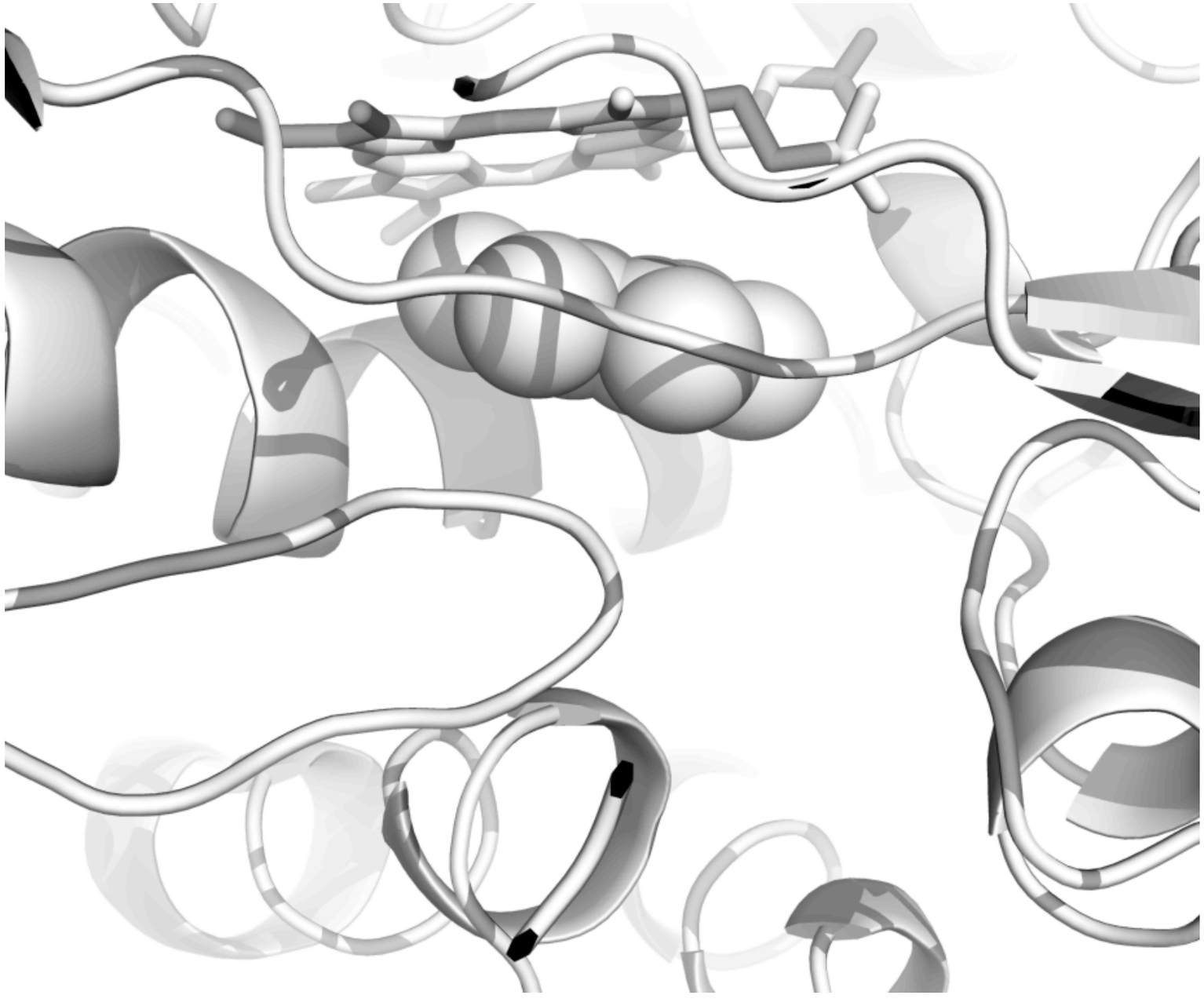
Can we use Rosetta to predict mutations that redesign enzyme substrate specificity?



Input: Wild-type enzyme complex (A–B) and desired substrate (C)

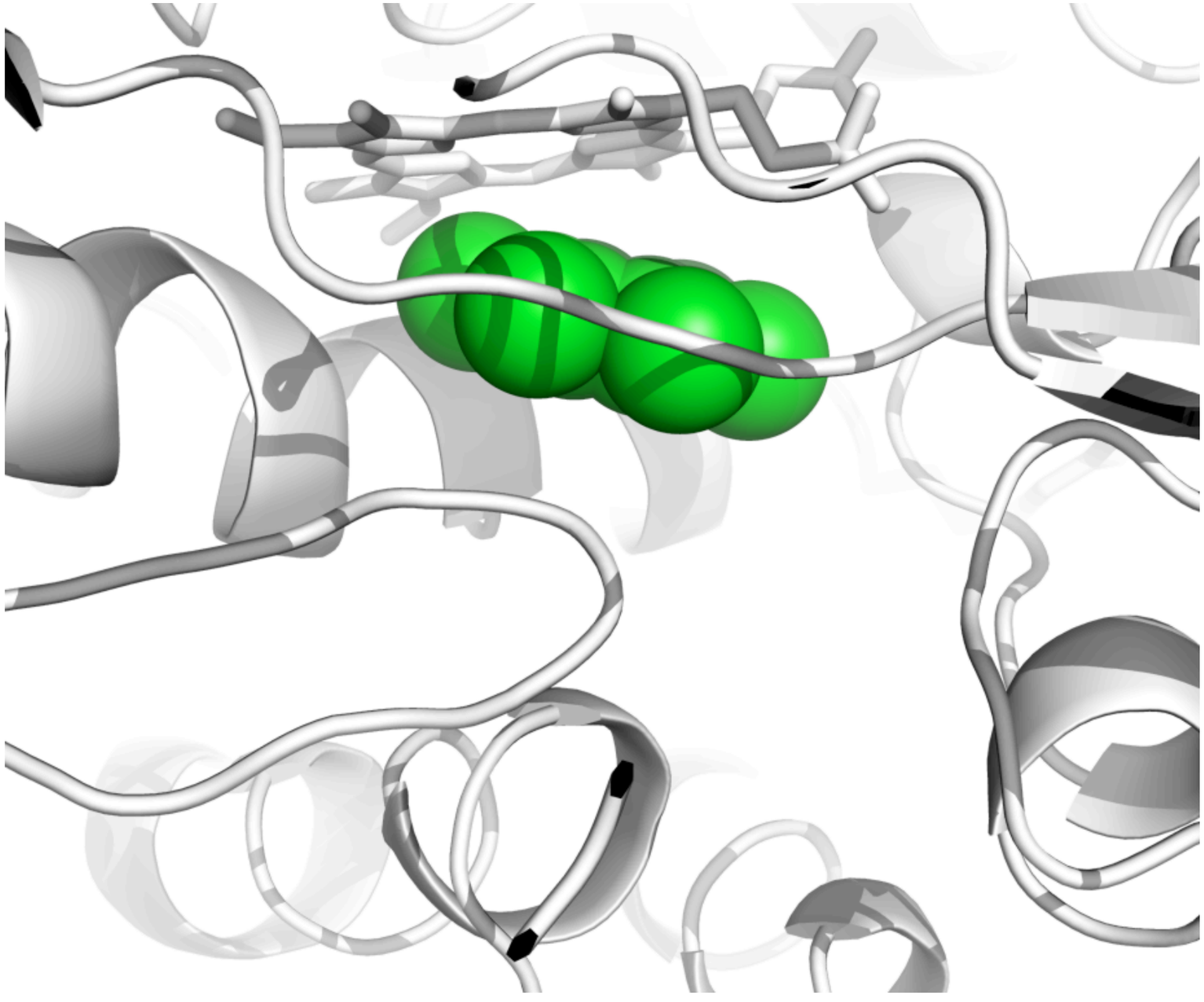
Output: Mutant enzymes with specificity redesigned toward desired substrate

Challenge: efficiently sampling protein sequence and substrate conformational space at a high resolution



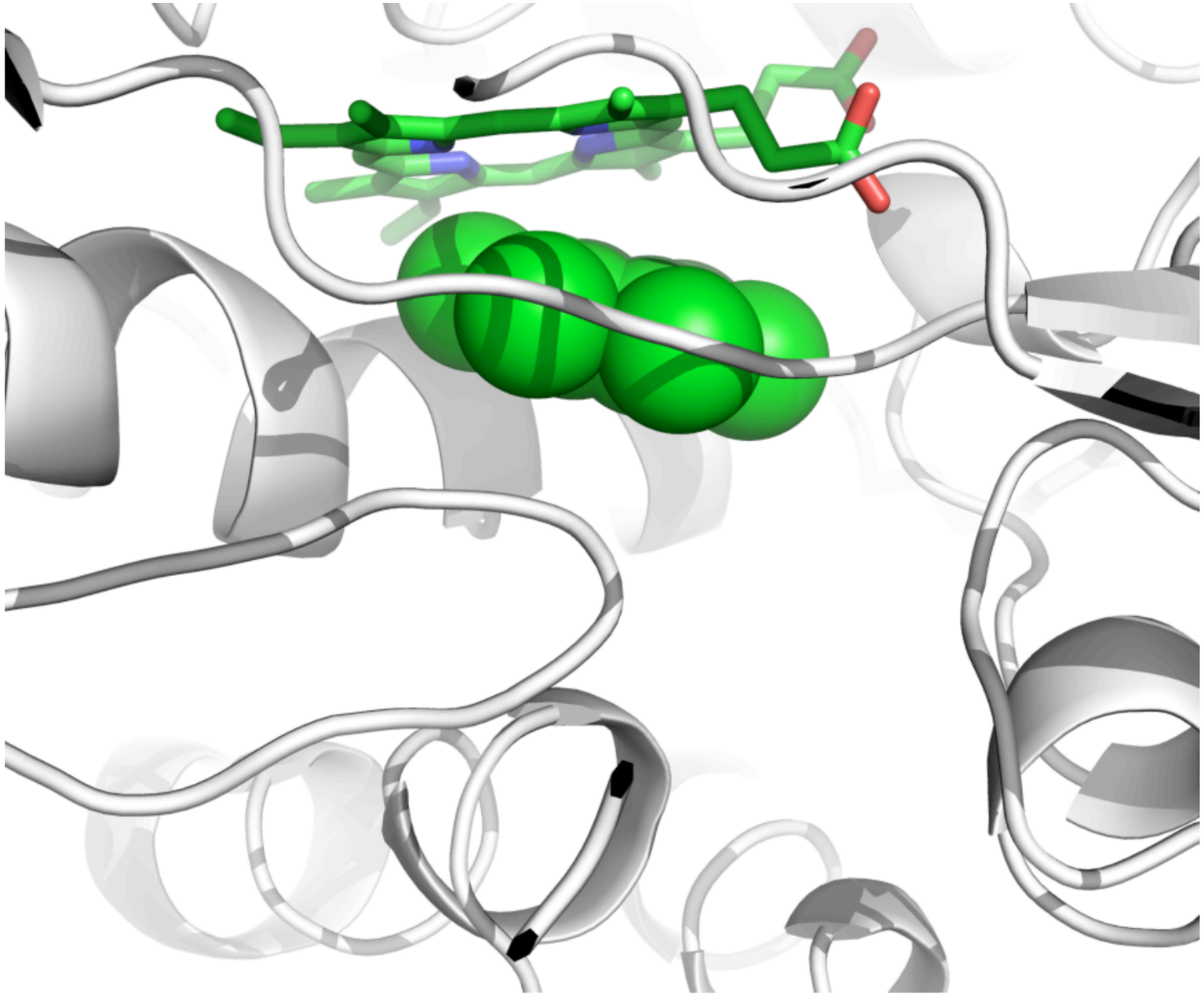
- Substrate rotation & translation
- Substrate flexibility
- Catalytic constraints
- Backbone flexibility
- Active site amino acids
- Second shell amino acids

Challenge: efficiently sampling protein sequence and substrate conformational space at a high resolution



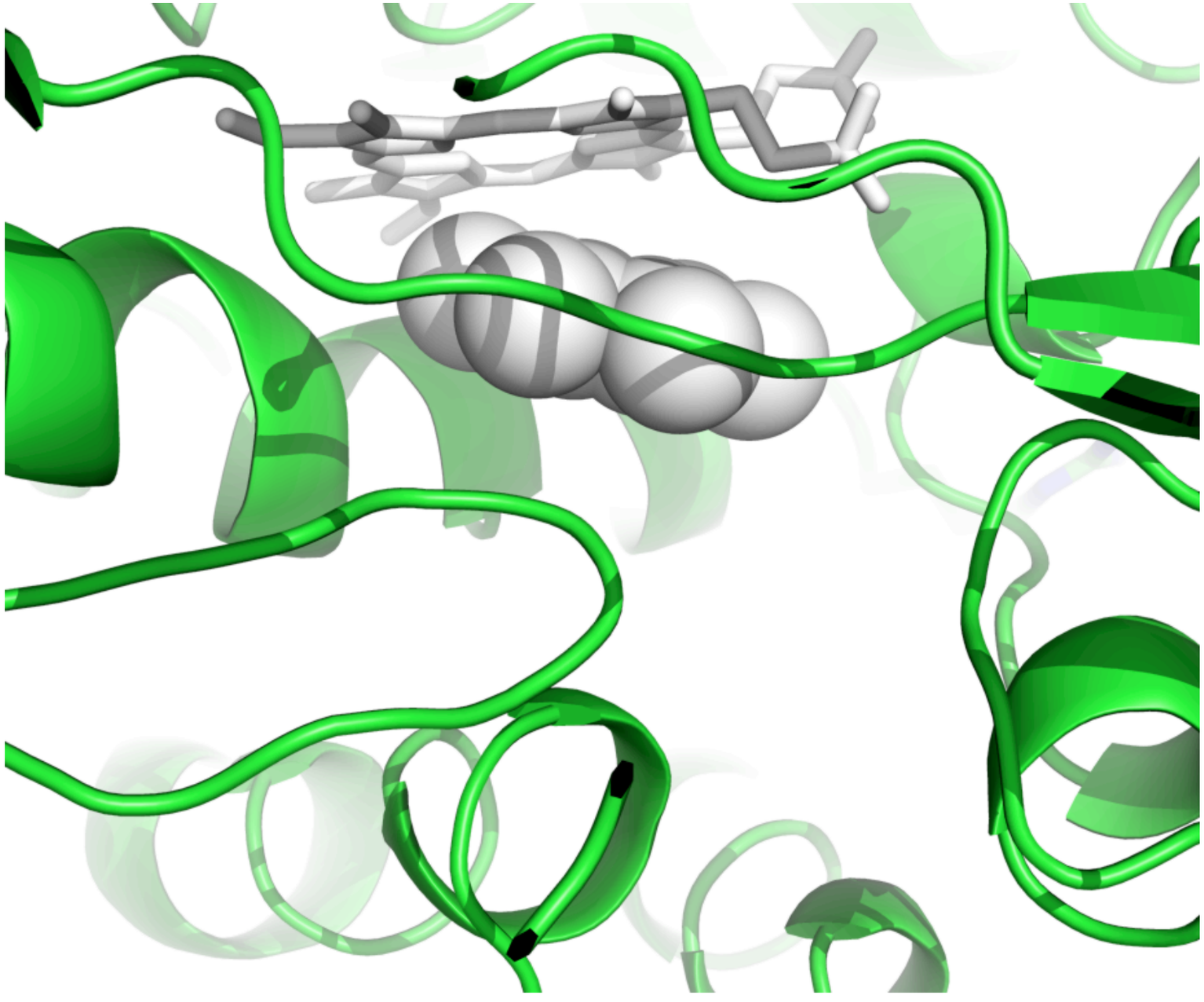
- **Substrate rotation & translation**
- **Substrate flexibility**
- Catalytic constraints
- Backbone flexibility
- Active site amino acids
- Second shell amino acids

Challenge: efficiently sampling protein sequence and substrate conformational space at a high resolution



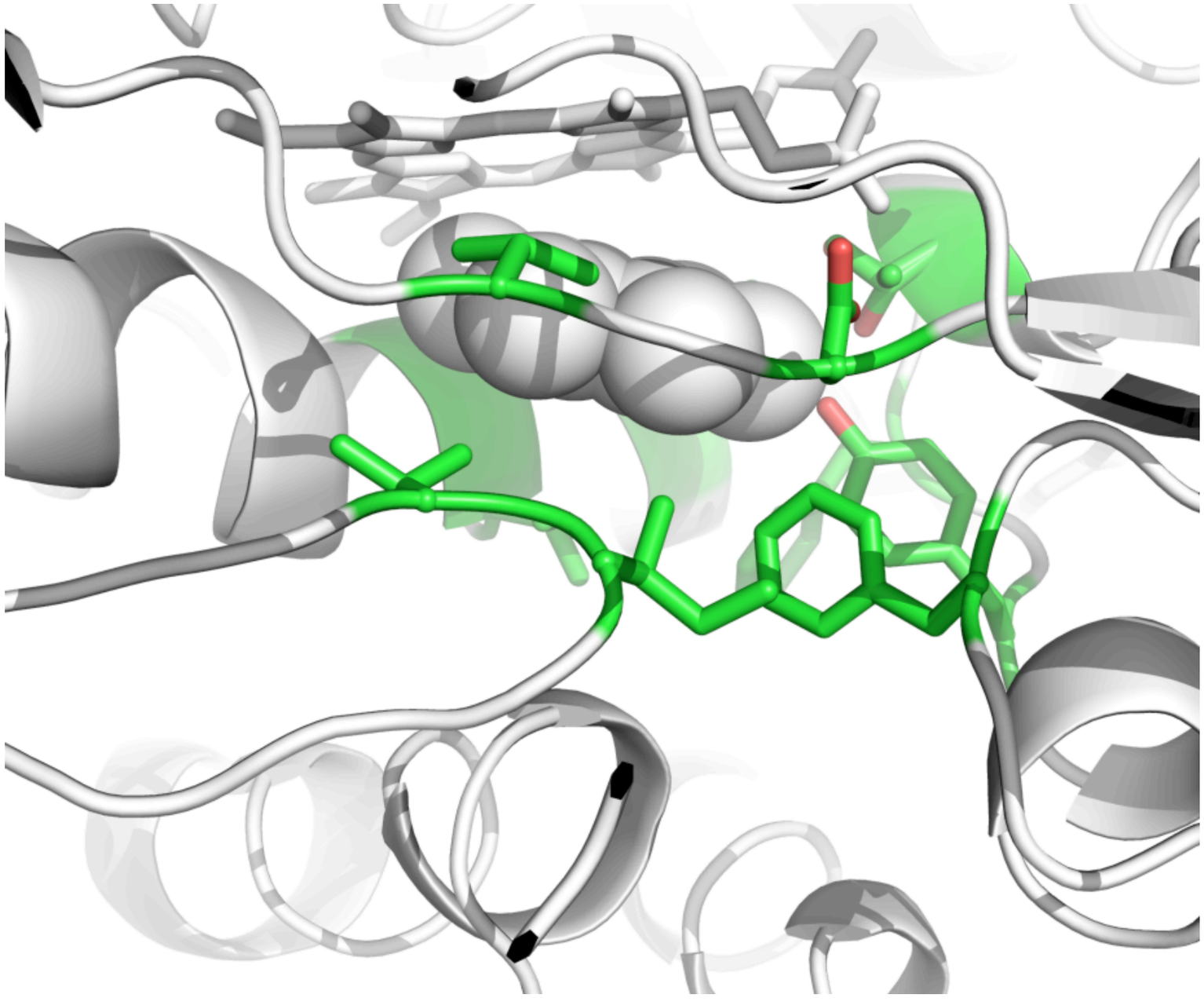
- Substrate rotation & translation
- Substrate flexibility
- **Catalytic constraints**
- Backbone flexibility
- Active site amino acids
- Second shell amino acids

Challenge: efficiently sampling protein sequence and substrate conformational space at a high resolution



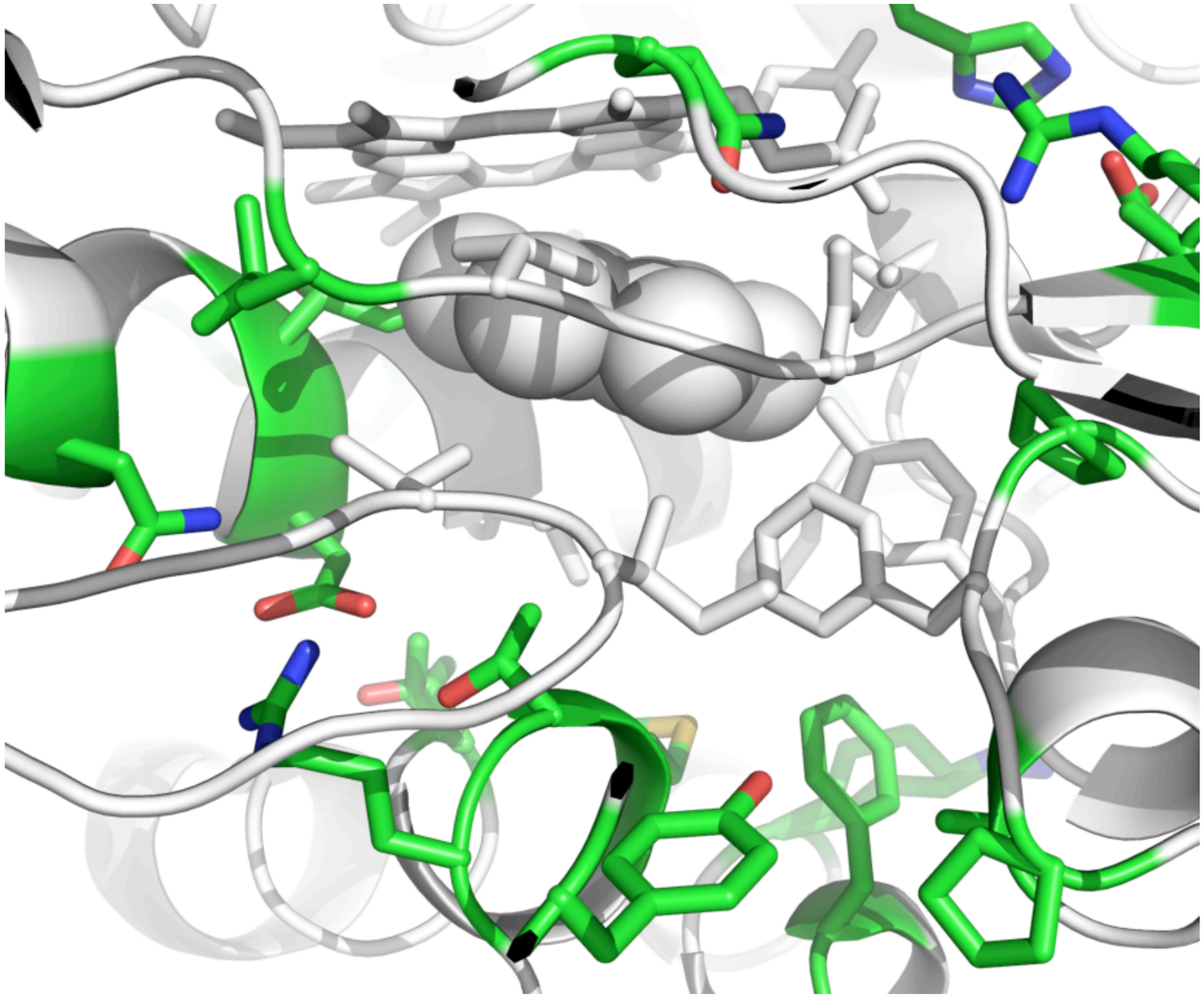
- Substrate rotation & translation
- Substrate flexibility
- Catalytic constraints
- **Backbone flexibility**
- Active site amino acids
- Second shell amino acids

Challenge: efficiently sampling protein sequence and substrate conformational space at a high resolution



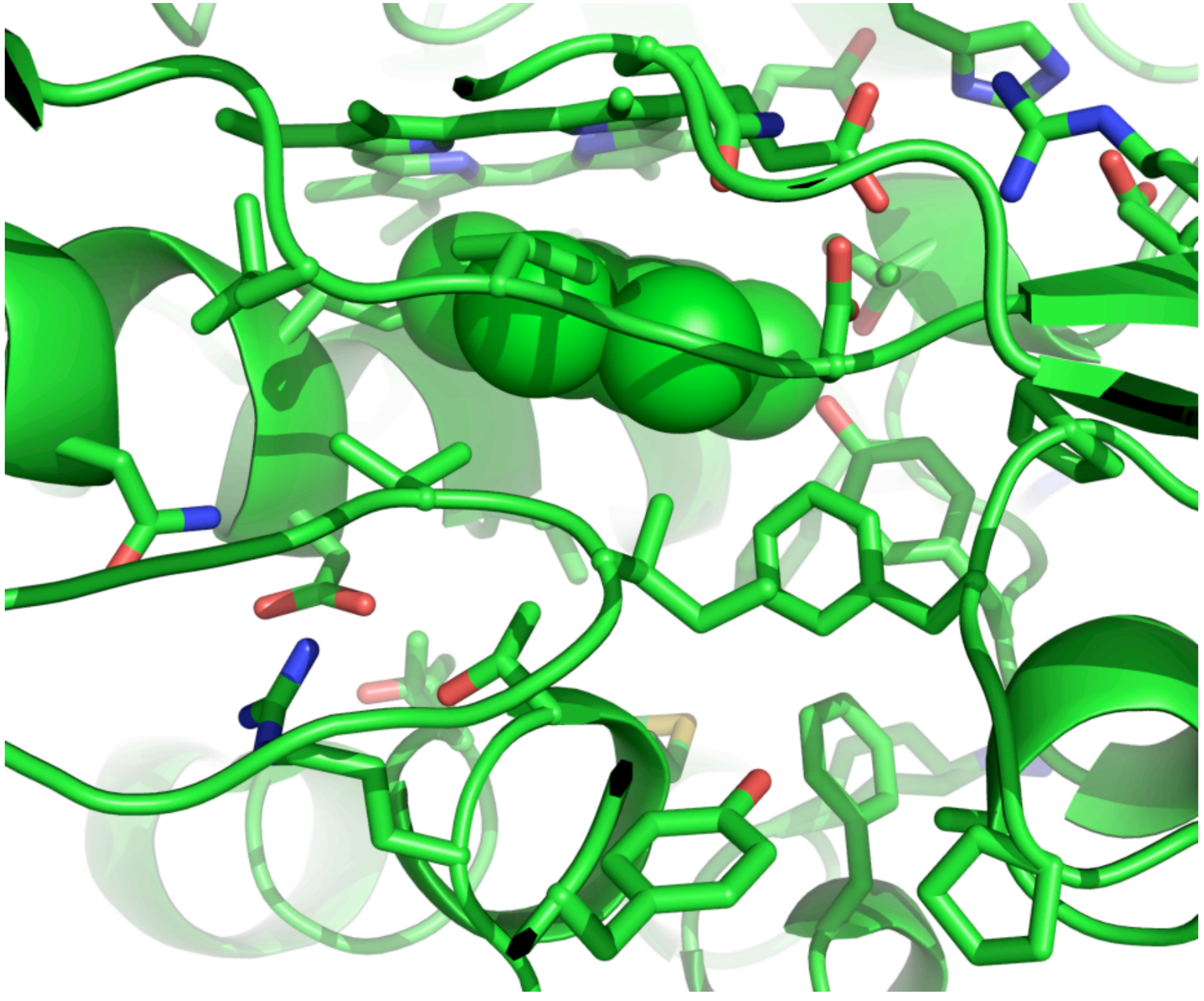
- Substrate rotation & translation
- Substrate flexibility
- Catalytic constraints
- Backbone flexibility
- **Active site amino acids**
- Second shell amino acids

Challenge: efficiently sampling protein sequence and substrate conformational space at a high resolution



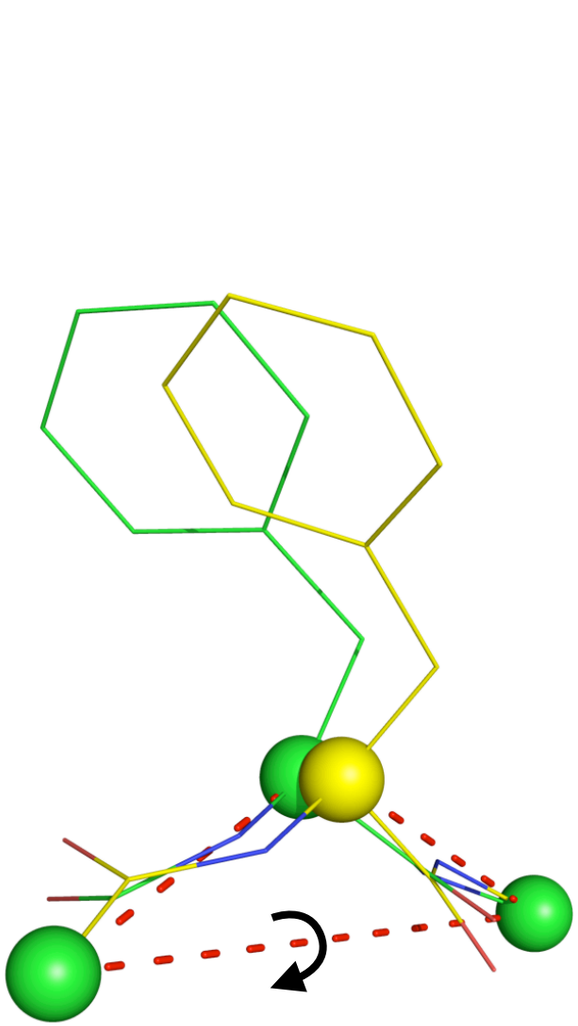
- Substrate rotation & translation
- Substrate flexibility
- Catalytic constraints
- Backbone flexibility
- Active site amino acids
- **Second shell amino acids**

Challenge: efficiently sampling protein sequence and substrate conformational space at a high resolution

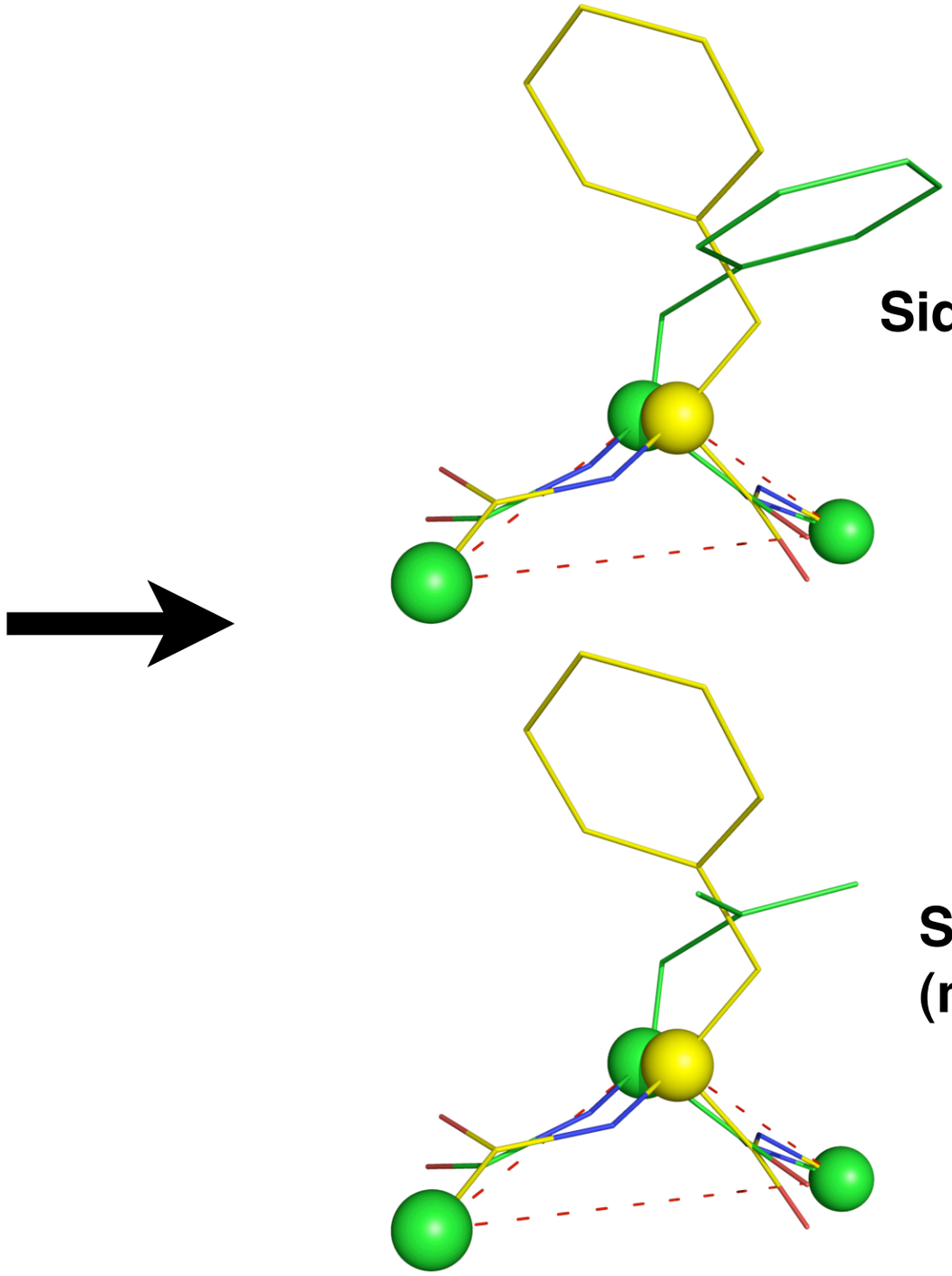


- **Substrate rotation & translation**
- **Substrate flexibility**
- **Catalytic constraints**
- **Backbone flexibility**
- **Active site amino acids**
- **Second shell amino acids**

Protein backbone flexibility can be coupled with sequence and side-chain conformational sampling.



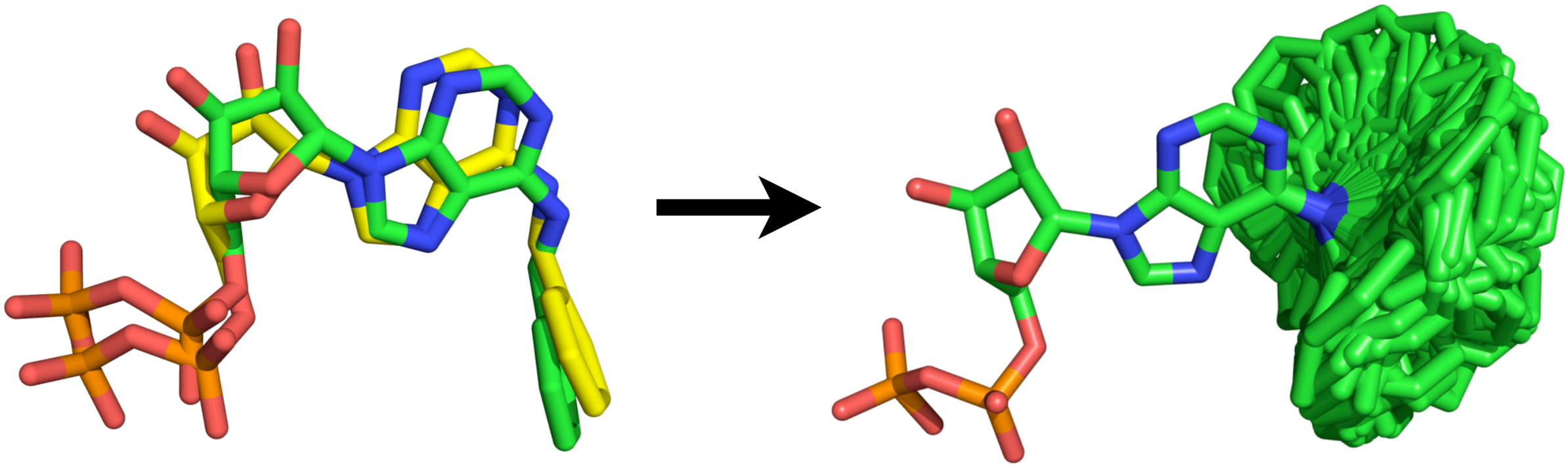
Backrub move



Side-chain repack

Side-chain design (mutation)

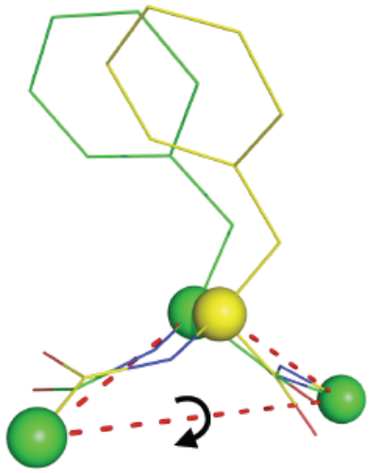
Substrate rigid-body rotation and translation can be coupled with substrate conformational sampling.

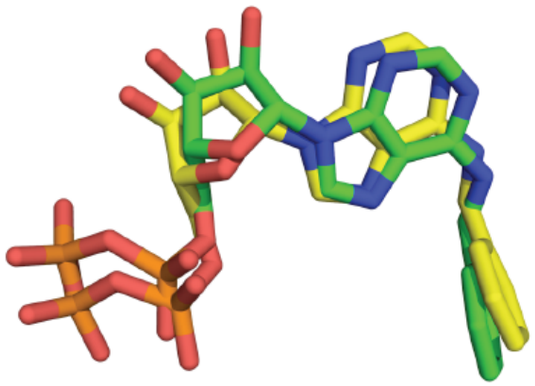


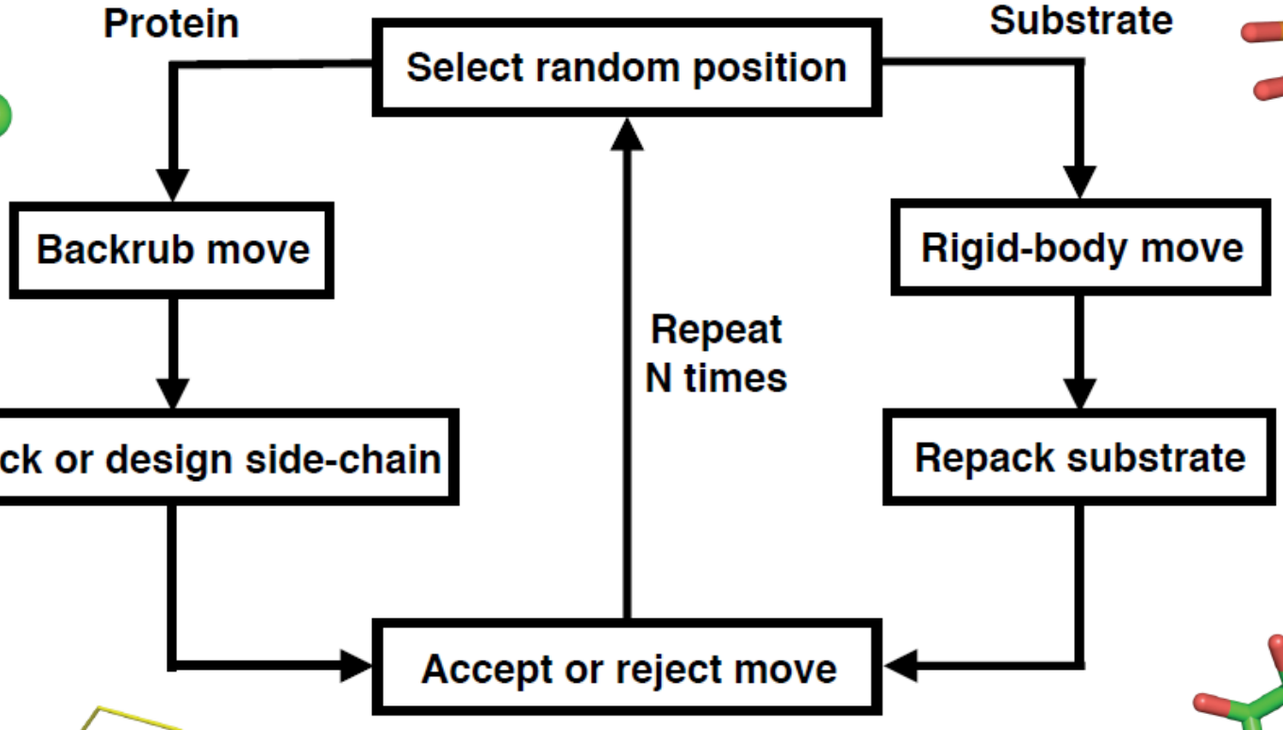
Rigid-body rotation/translation

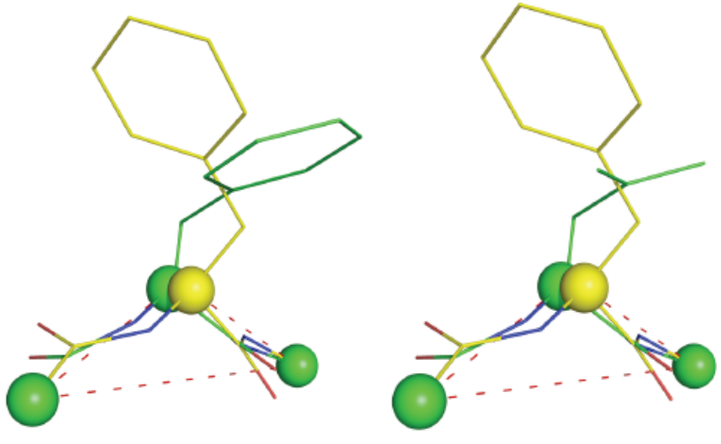
Repack substrate

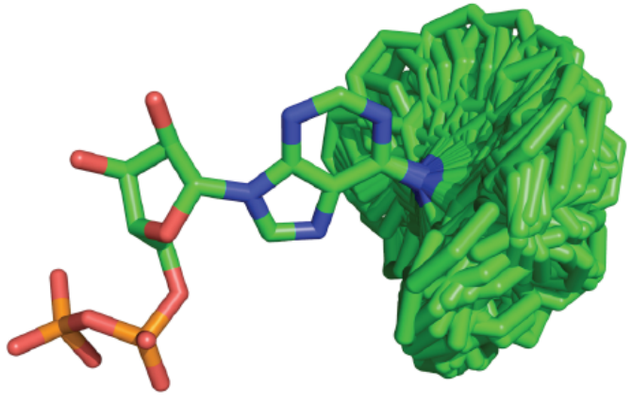
Monte Carlo sampling method can efficiently find low energy sequences and conformations.



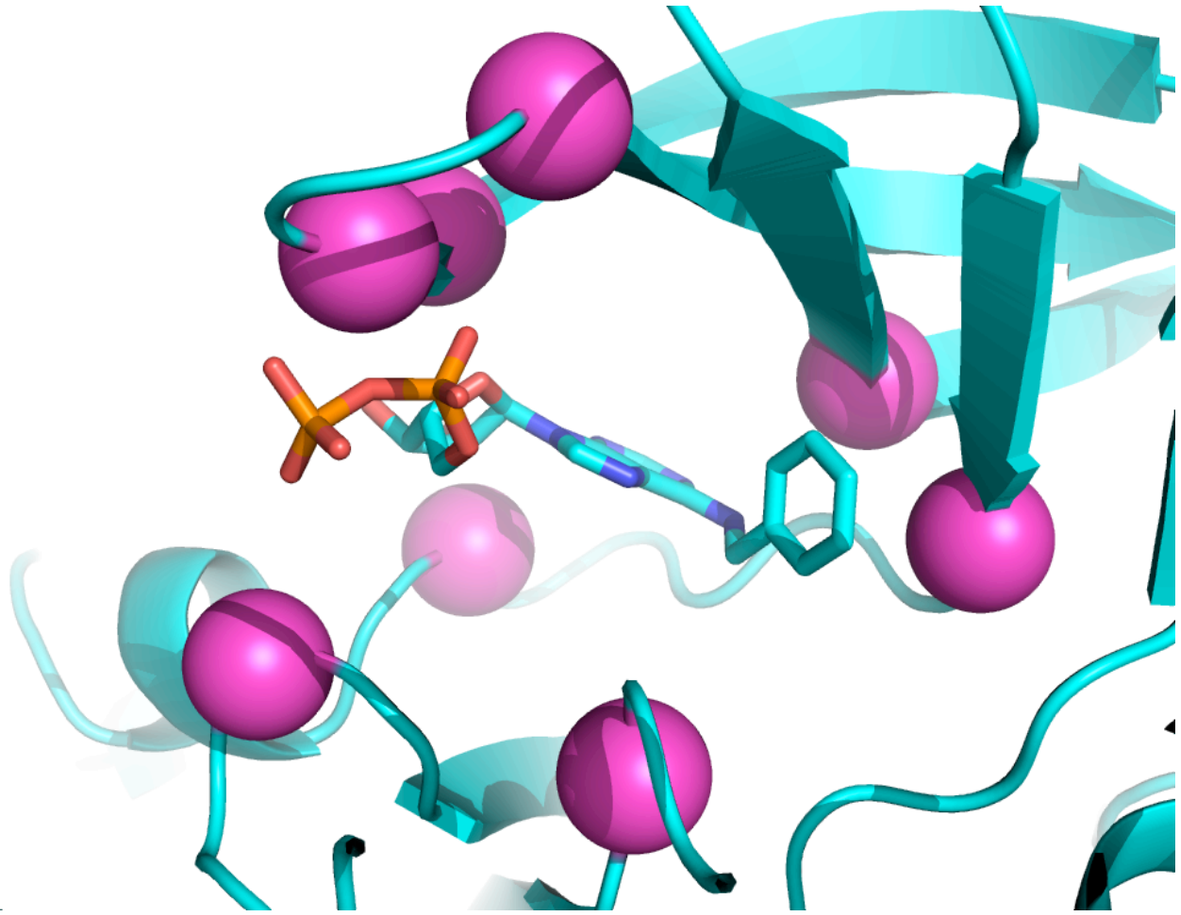




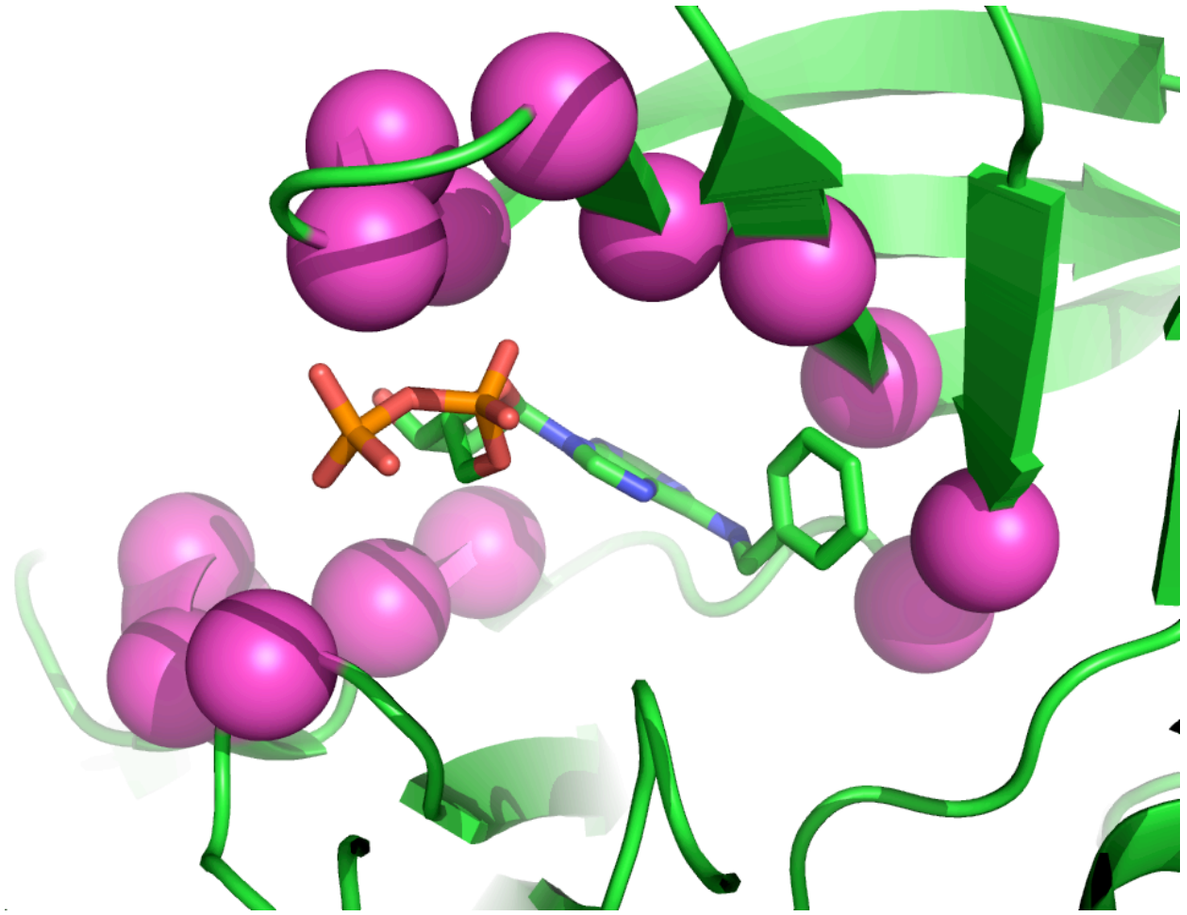




Unbiased sampling of mutations leads to accumulation of alanines and glycines.



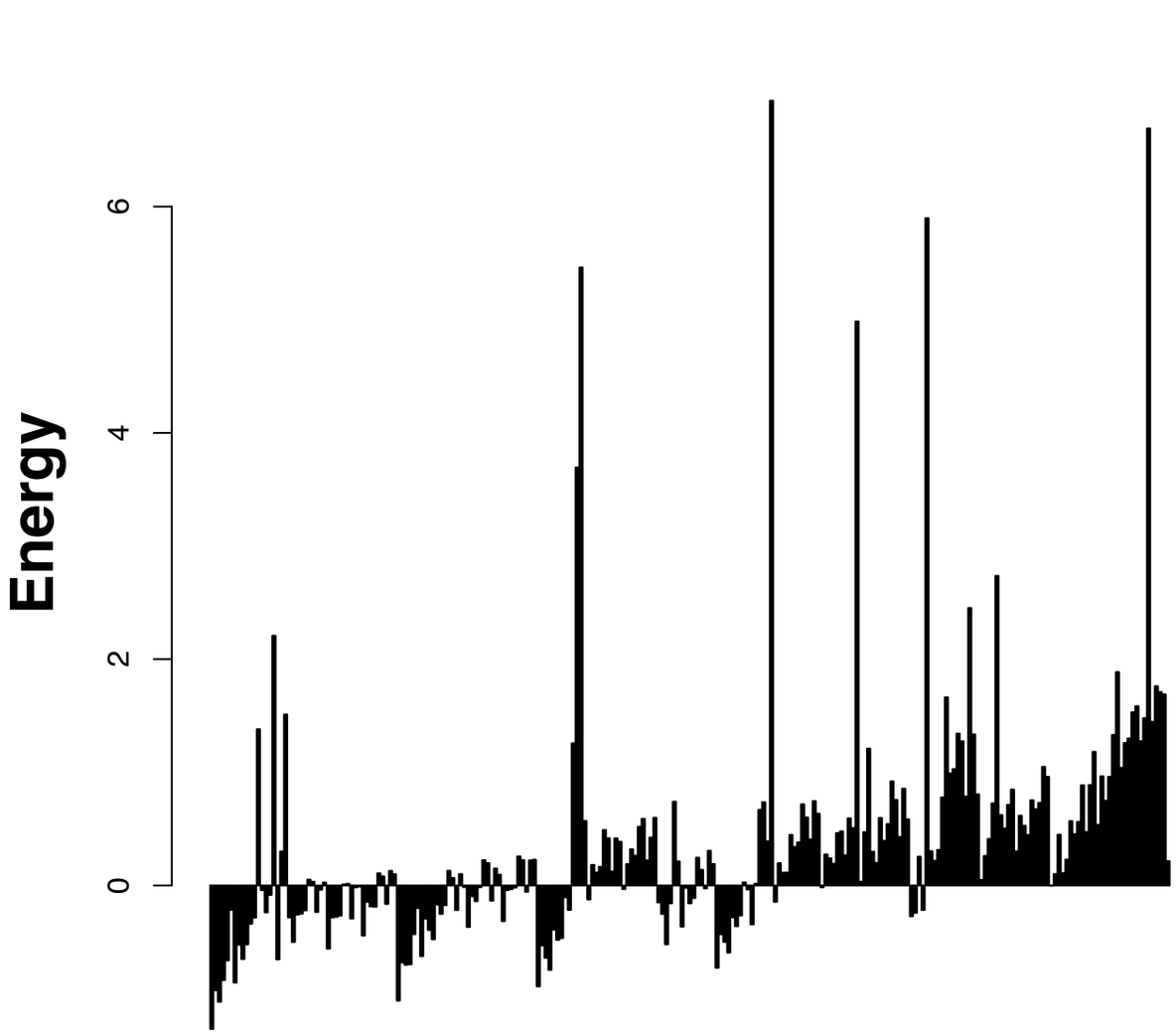
Wild-type: 8 ala+gly

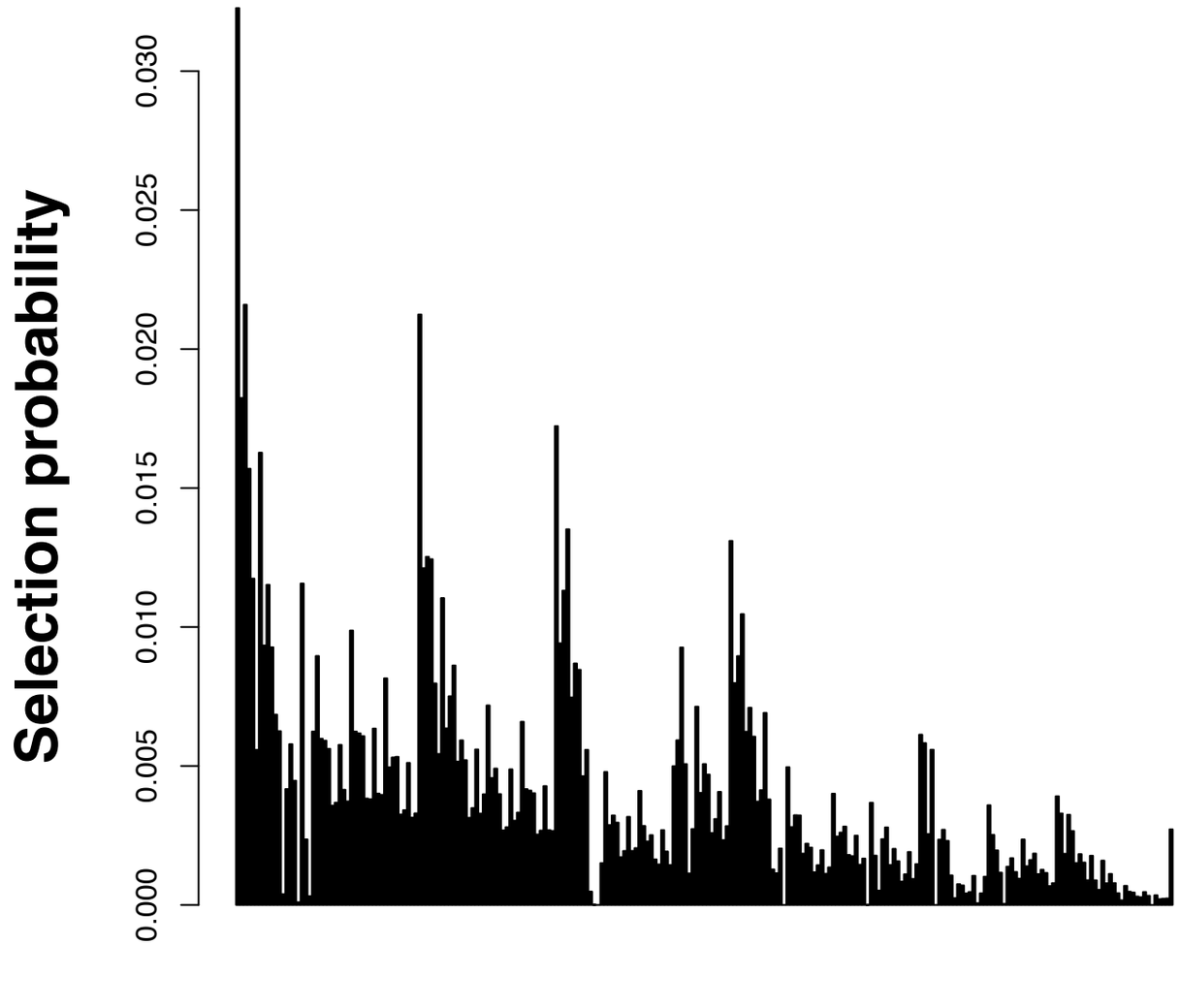


1000 MC steps: 15 ala+gly
Acceptance rate: 9%

A random rotamer of a large or flexible amino acid is more likely to result in a clash than an alanine or glycine.

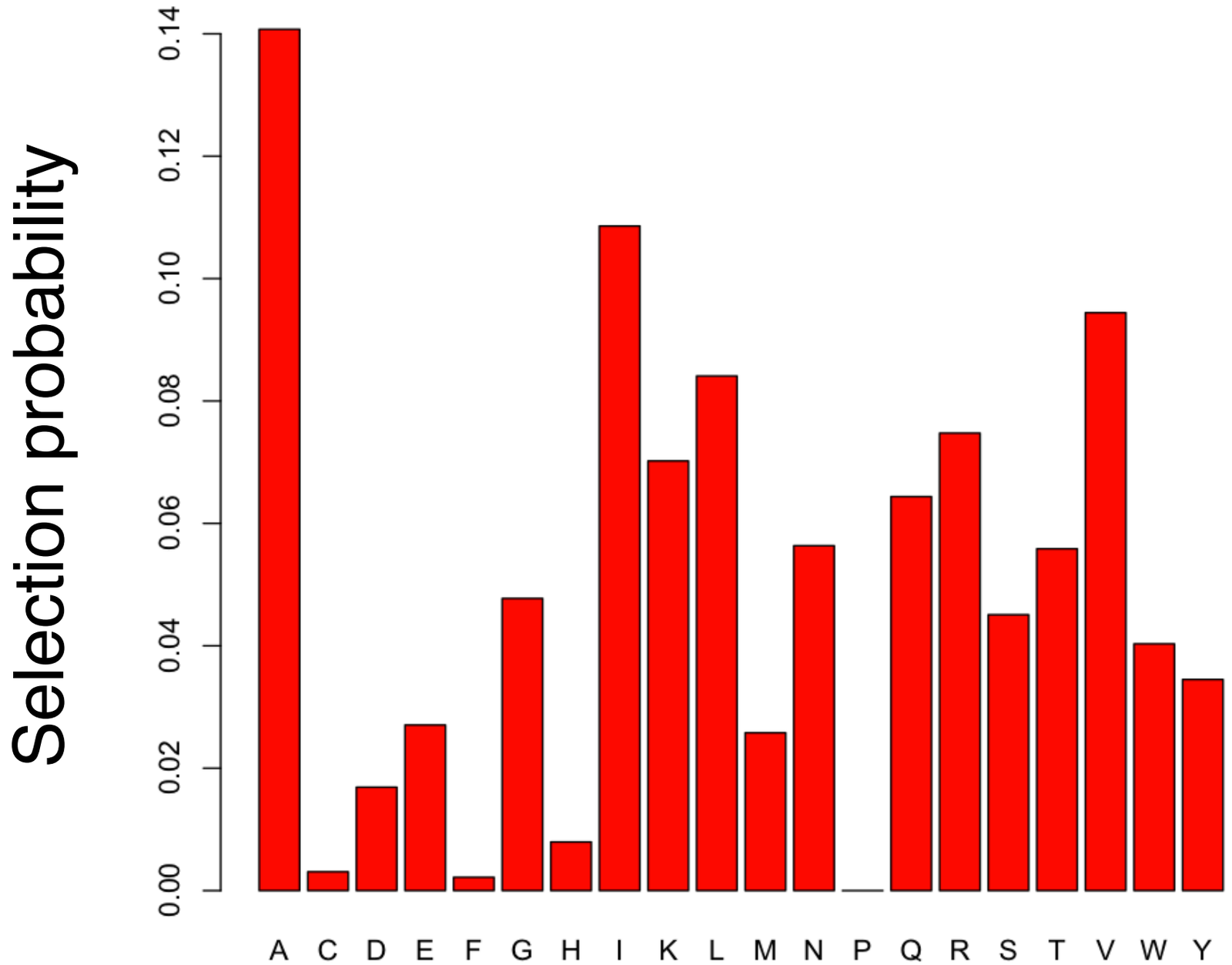
Boltzmann weighted rotamer trials biases rotamer selection based on energies.



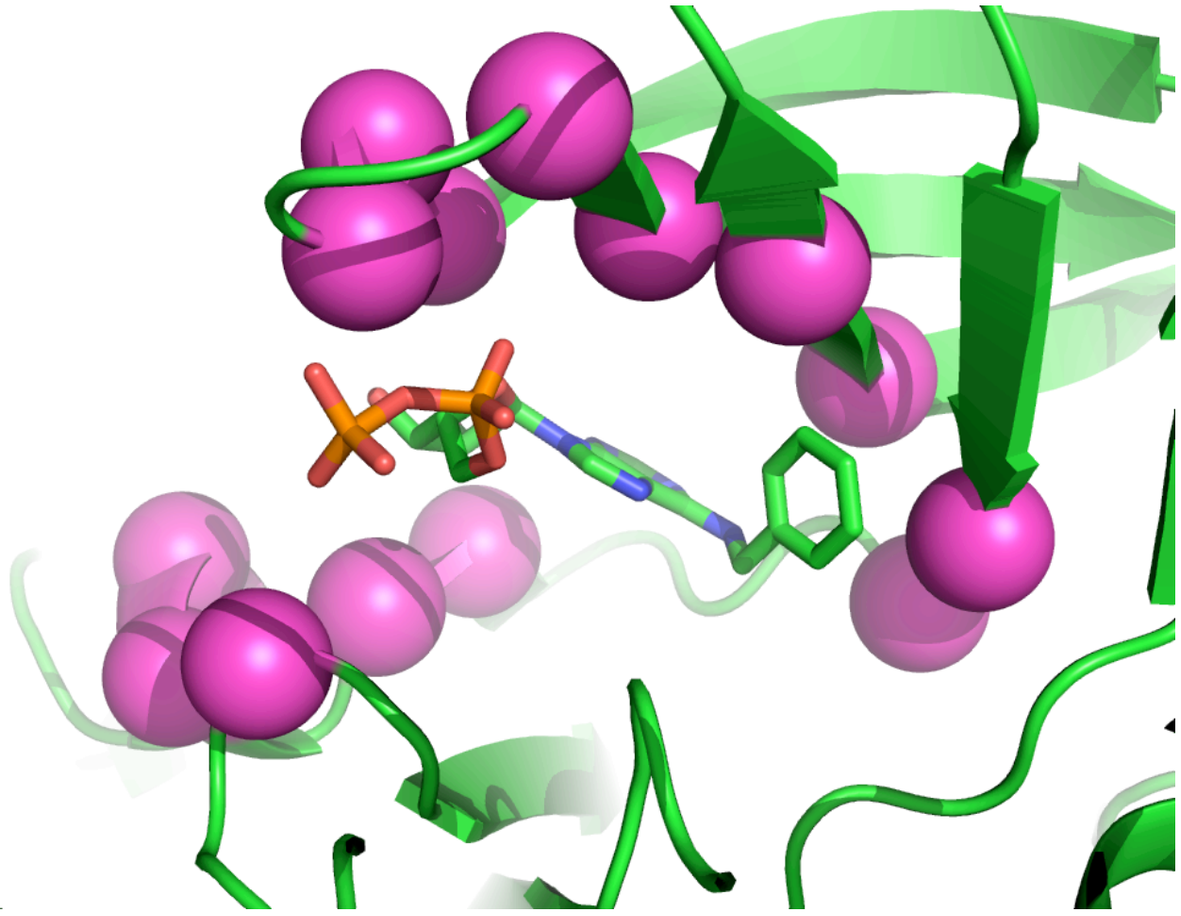


$$P(E_i) = \frac{1}{\sum_i e^{-\frac{E_i}{kT}}} e^{-\frac{E_i}{kT}}$$

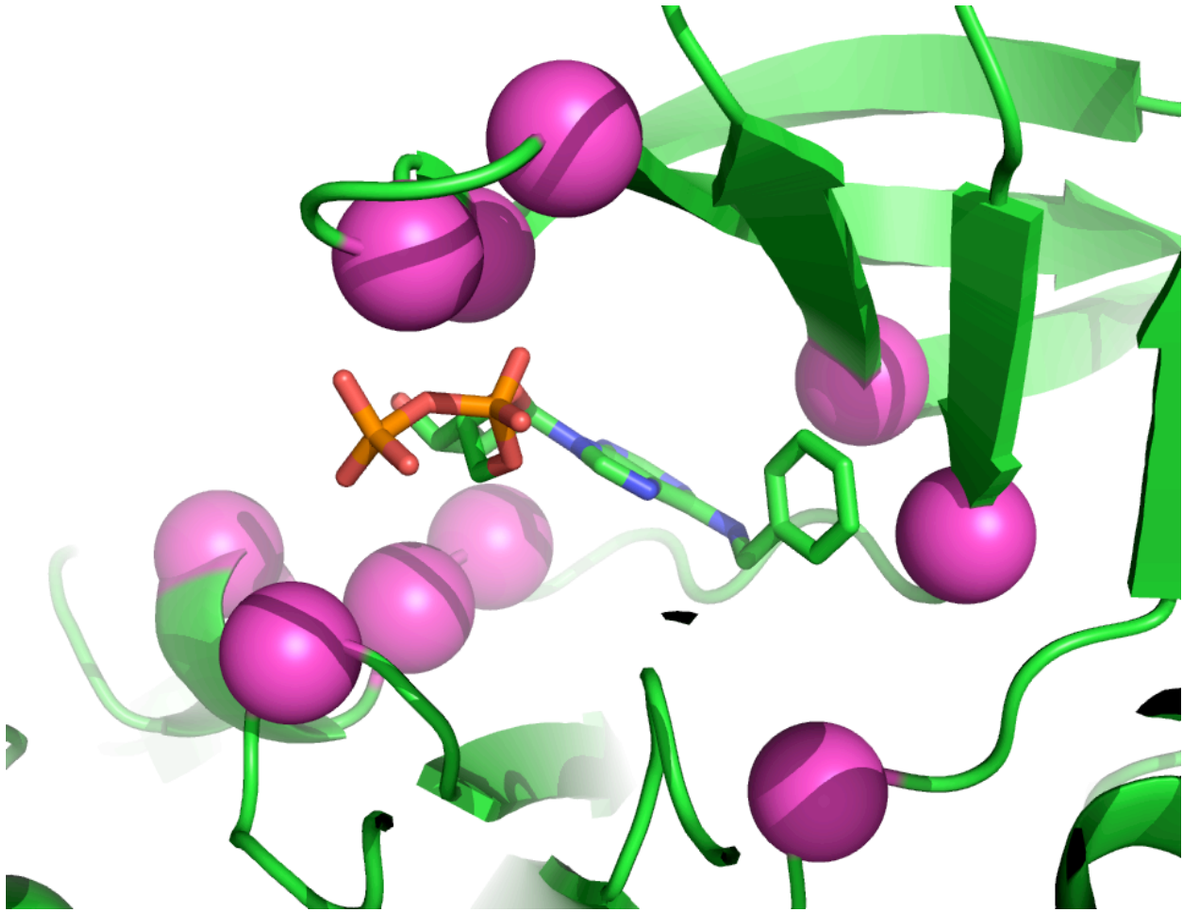
Potential amino acid mutations are compared using selected rotamers for each amino acid.



Boltzmann weighted rotamer trials increase acceptance rate and help with alanine/glycine problem.

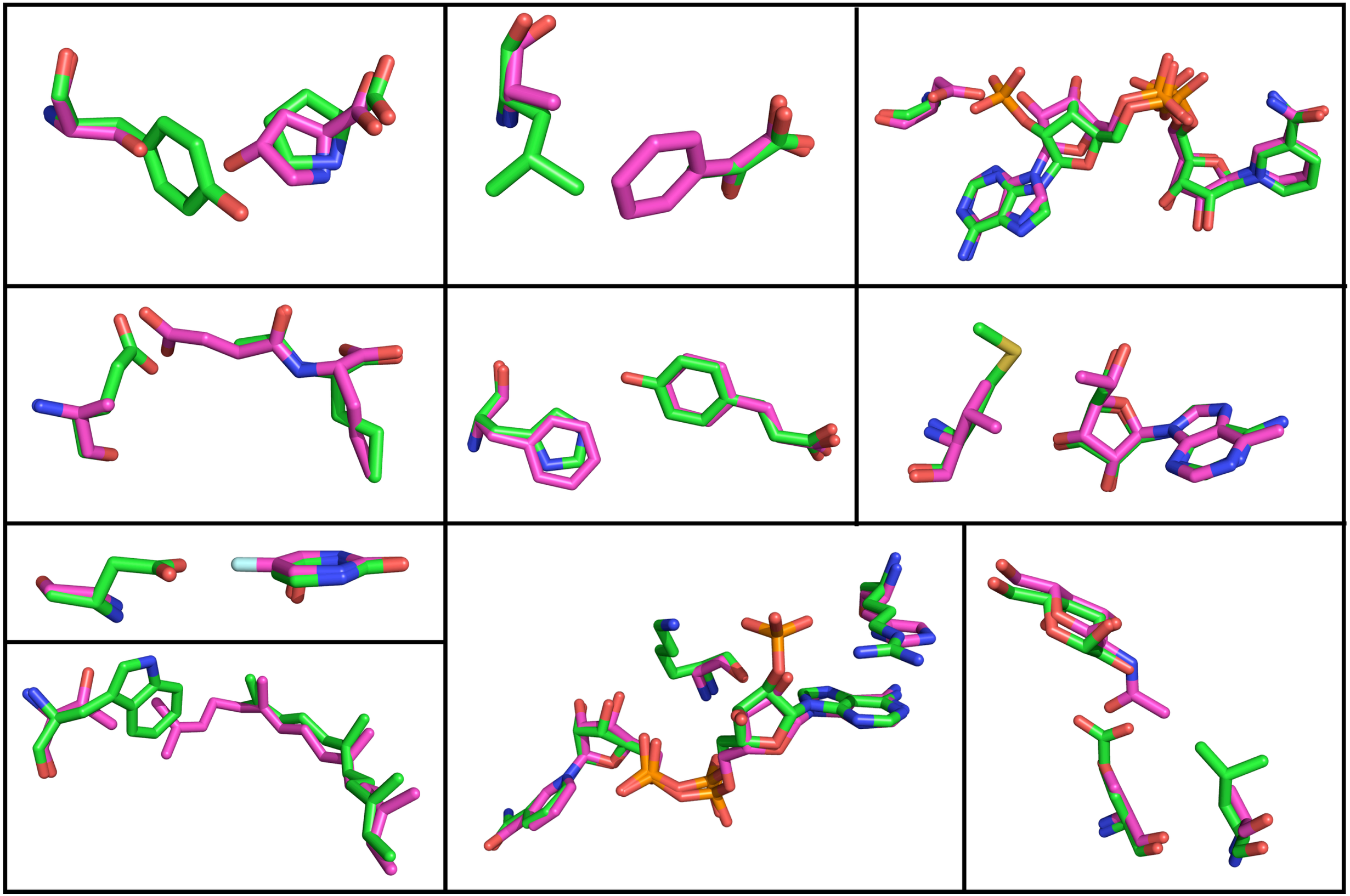


Unbiased sampling
1000 MC steps: 15 ala+gly
Acceptance rate: 9%



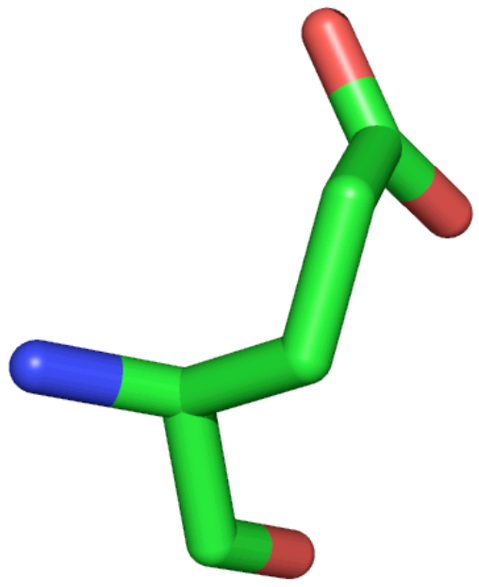
Boltzmann weighted rotamer trials
1000 MC steps: 10 ala+gly
Acceptance rate: 50%

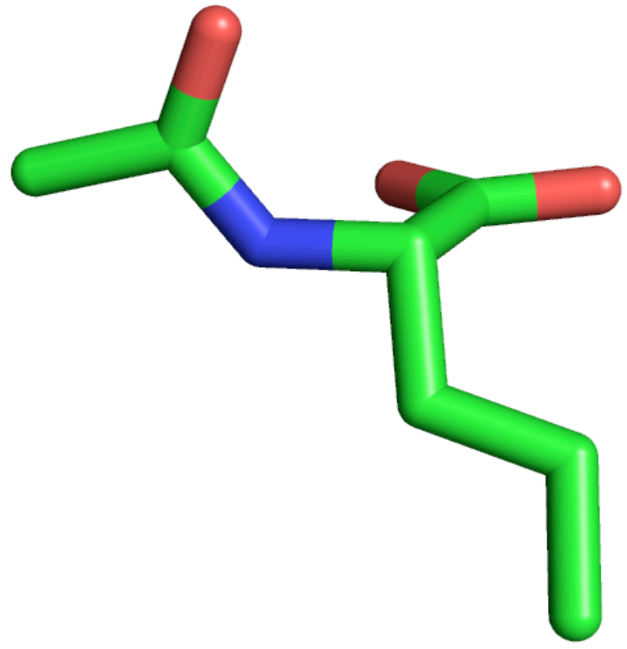
Benchmark: 10 enzymes with known specificity altering mutations



acetyl-ornithine transcarbamylase

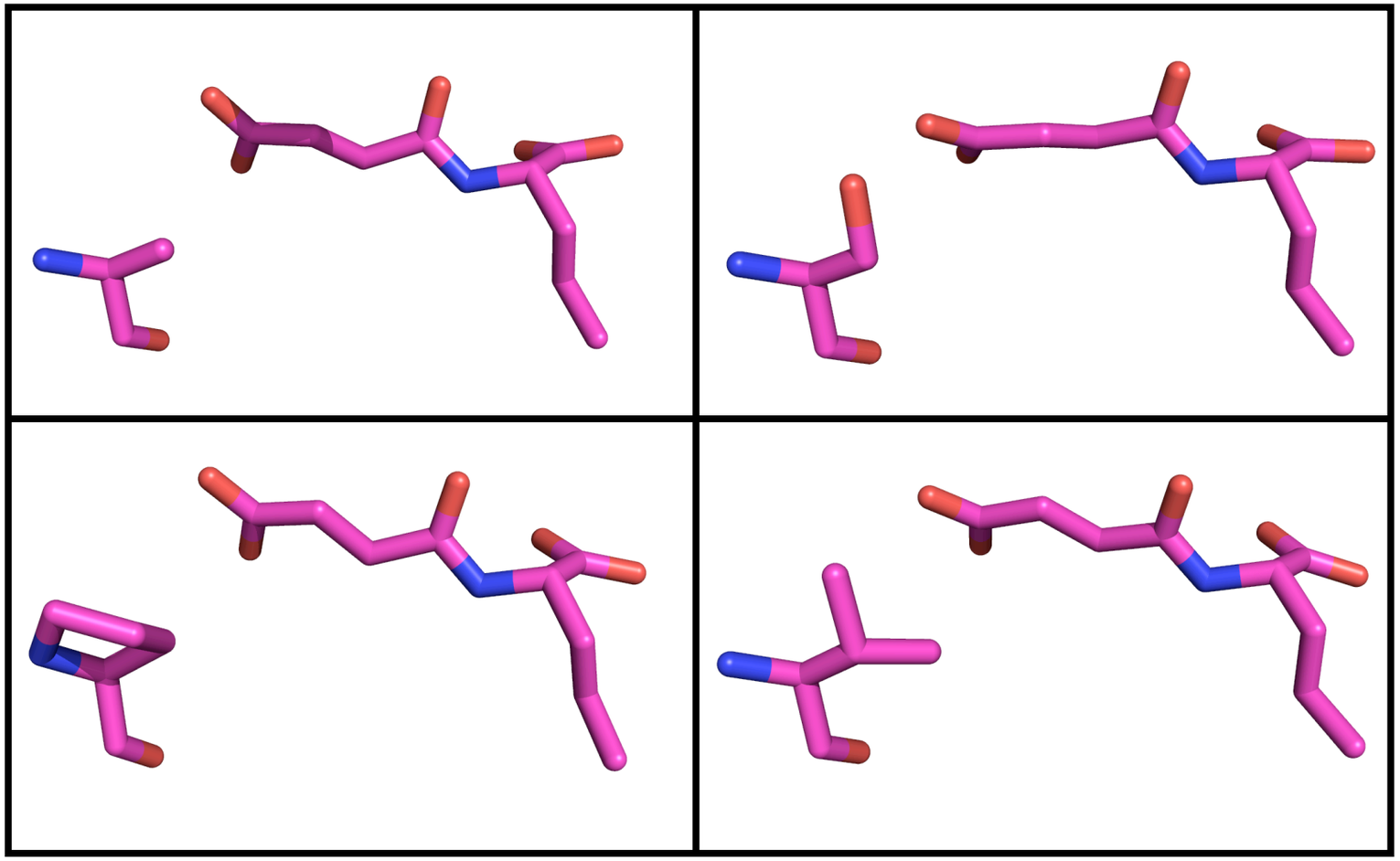
Wild-type

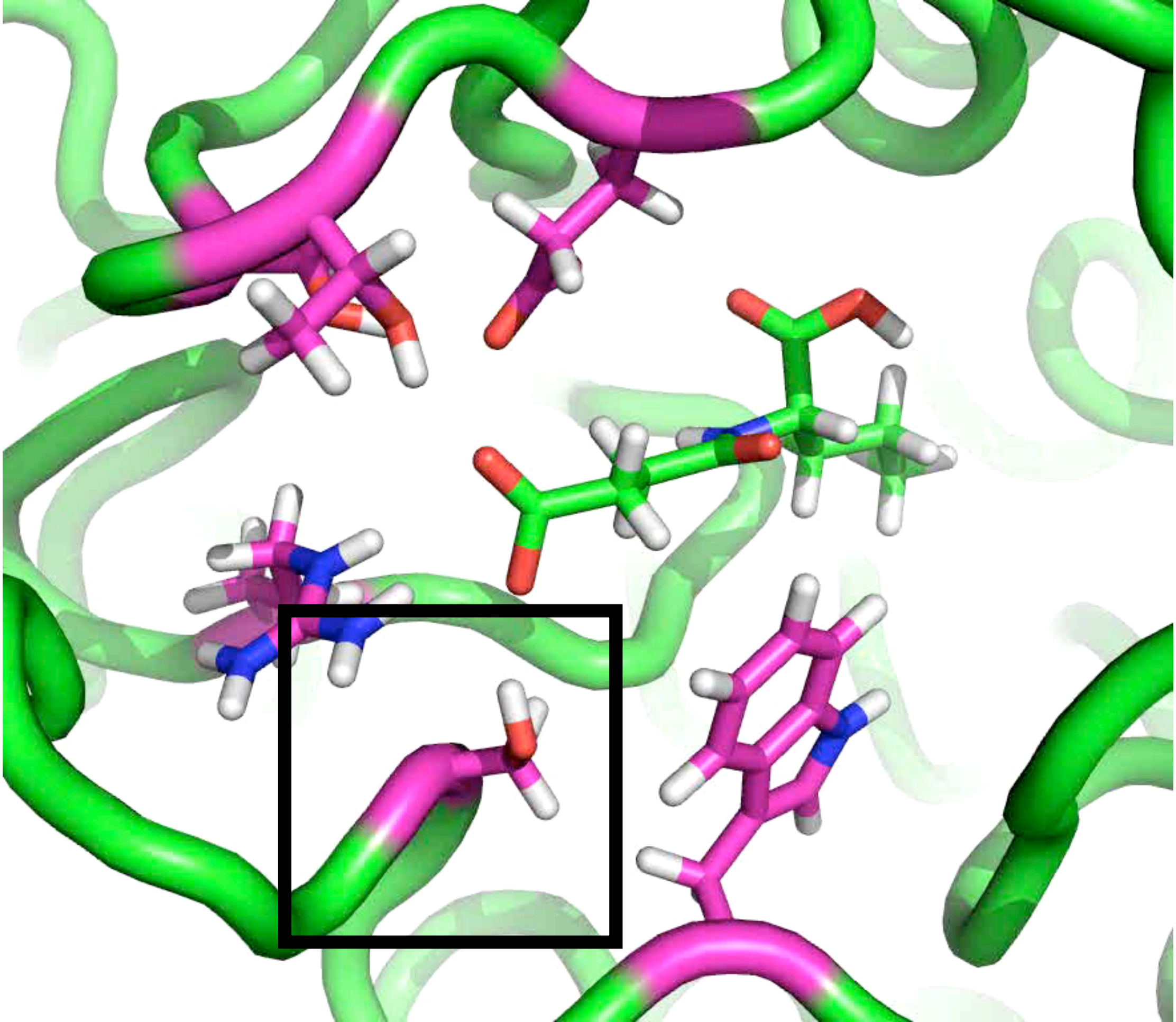




succinyl-ornithine transcarbamylase

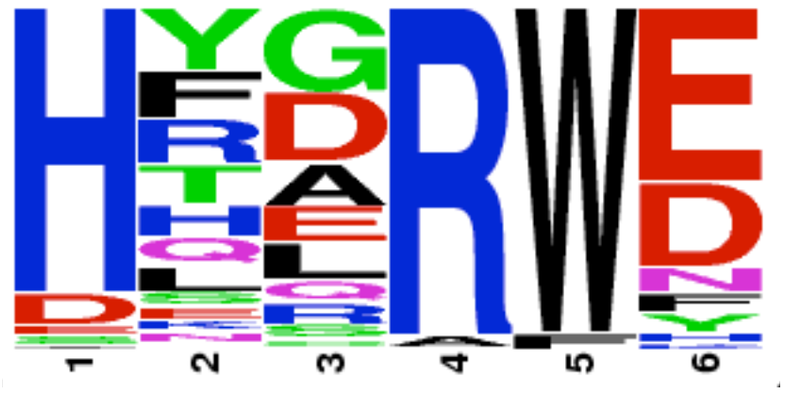
Mutant





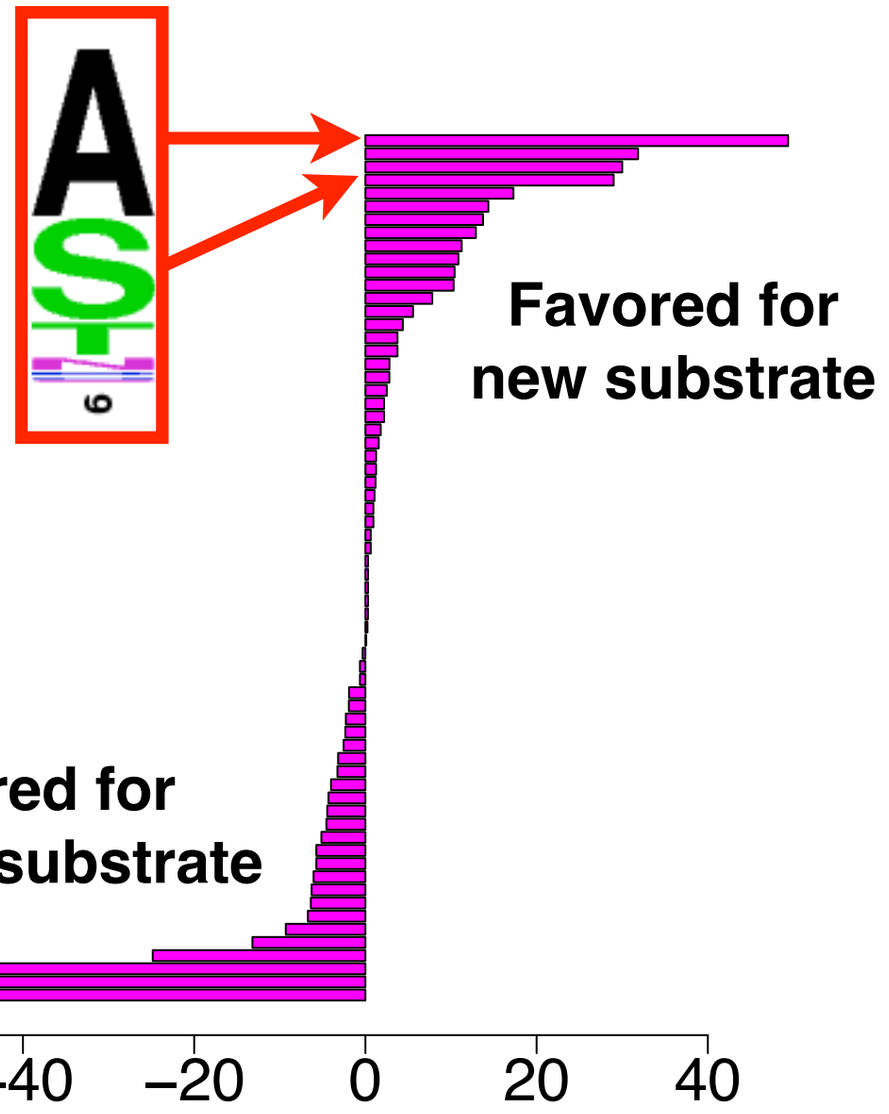
Wild-type

Original Substrate



Mutant

New Substrate

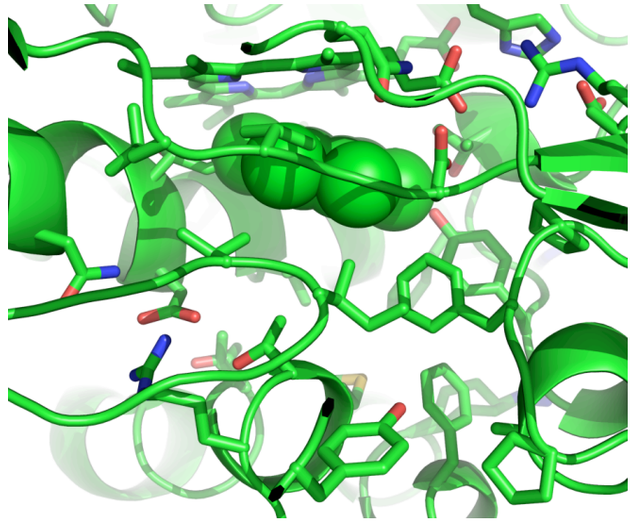


Mutation #	PDB ID	Mutation	Found Mutation?	Enrichment Percentile
1	2FZN	Y540S	YES	94%
2	1FCB	L230A	YES	72%
3	1ZK4	G37D	NO	0
4	3KZO	E92A	YES	100%
5		E92S	YES	97%
6		E92P	NO	0
7		E92V	NO	0
8	2O7B	H89F	YES	76%
9	1PK7	M64V	YES	79%
10	1K70	D314S	YES	92%
11		D314G	YES	8%
12		D314A	NO	0
13	2H6F	W602T	NO	0
14	1A80	K232G	YES	65%
15		R238H	YES	97%
16	3HG5	E203S	YES	99%
17		L206A	NO	0

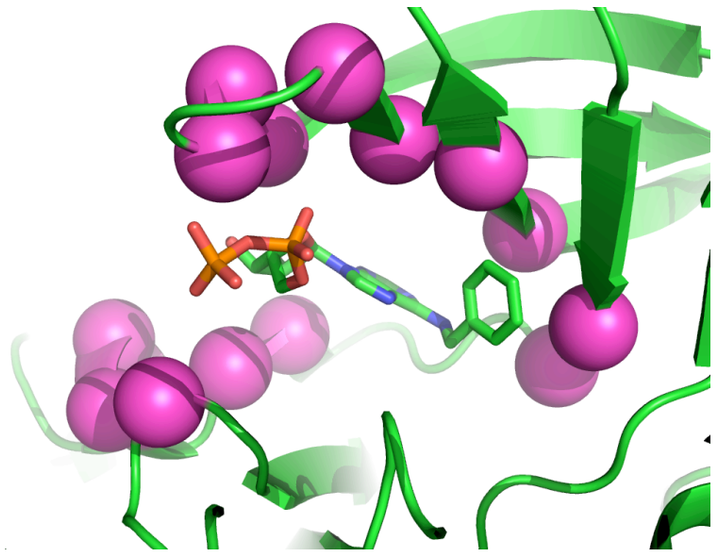
Mutation #	PDB ID	Mutation	Coupled Moves	Fixed Backbone
1	2FZN	Y540S	YES	NO
2	1FCB	L230A	YES	NO
3	1ZK4	G37D	NO	NO
4	3KZO	E92A	YES	NO
5		E92S	YES	YES
6		E92P	NO	NO
7		E92V	NO	NO
8	2O7B	H89F	YES	NO
9	1PK7	M64V	YES	NO
10	1K70	D314S	YES	NO
11		D314G	YES	NO
12		D314A	NO	NO
13	2H6F	W602T	NO	NO
14	1A80	K232G	YES	NO
15		R238H	YES	YES
16	3HG5	E203S	YES	NO
17		L206A	NO	NO

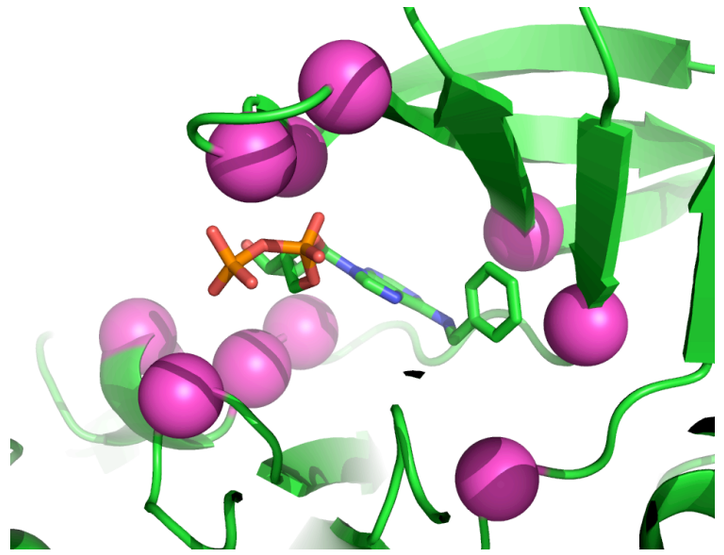
Summary

Challenge of efficiently sampling large conformational space at high resolution makes specificity redesign difficult.

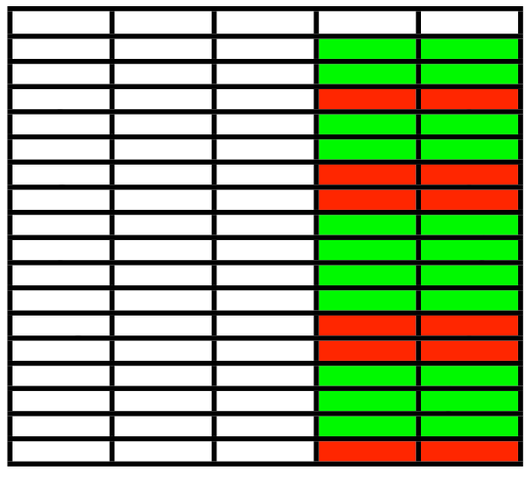


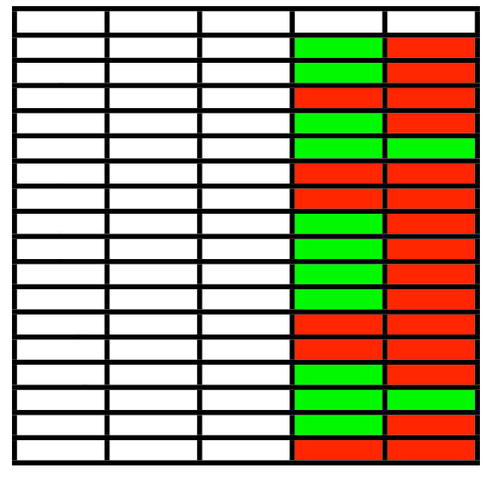
Boltzman weighted rotamer trials helps to avoid accumulation of alanines and glycines.





Coupled side-chain / backbone moves improves prediction of specificity altering mutations.



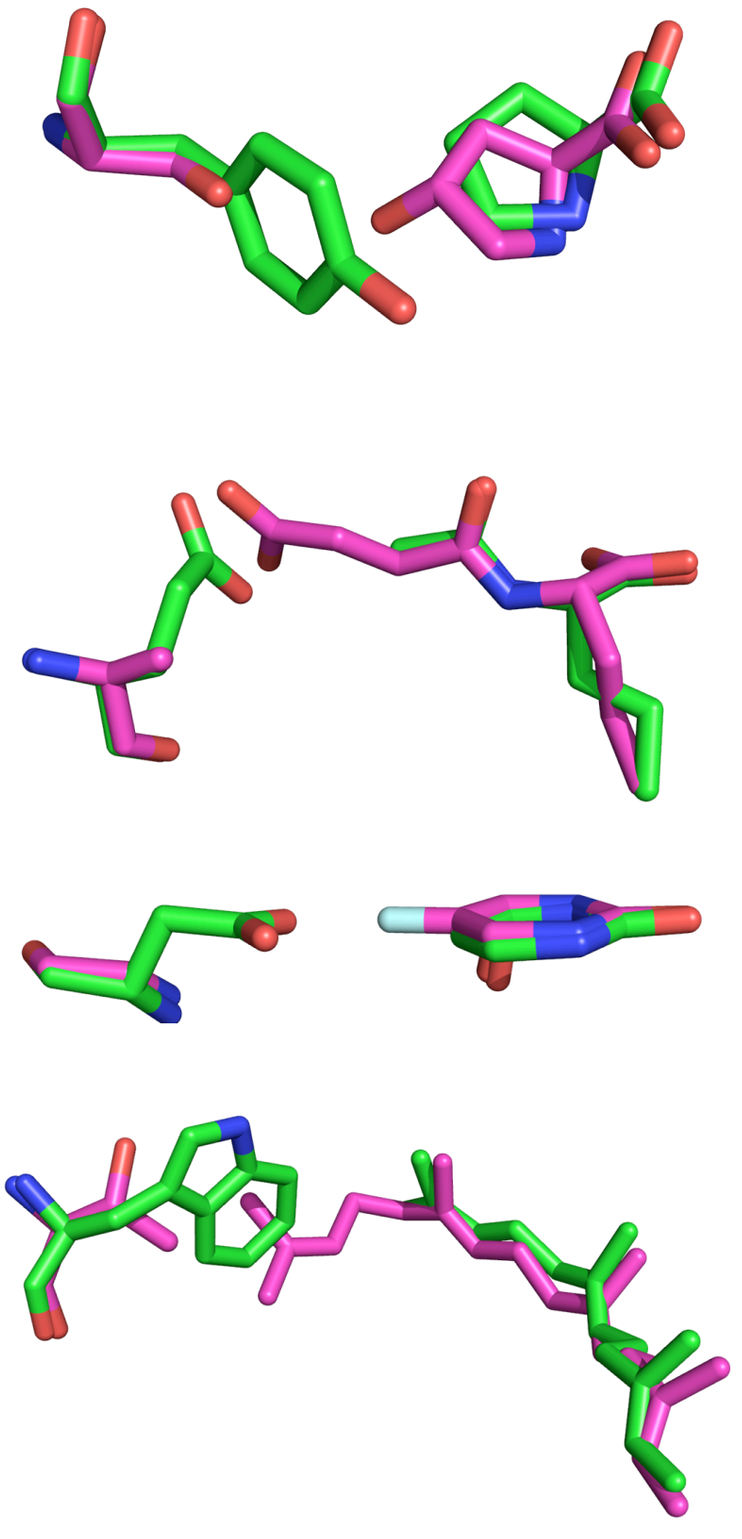


Current challenges

Run benchmark in opposite direction (start with mutant and try to predict wild-type) and with different protocol parameters and score functions.

Determine exactly why coupled moves side-chain / backbone outperform fixed backbone design.

Check in code and write documentation.



Acknowledgements

DSM
Rene de Jong
Jan van Leeuwen
Jan-Metske van der Laan

UCSF
Kortemme lab
Tanja Kortemme