

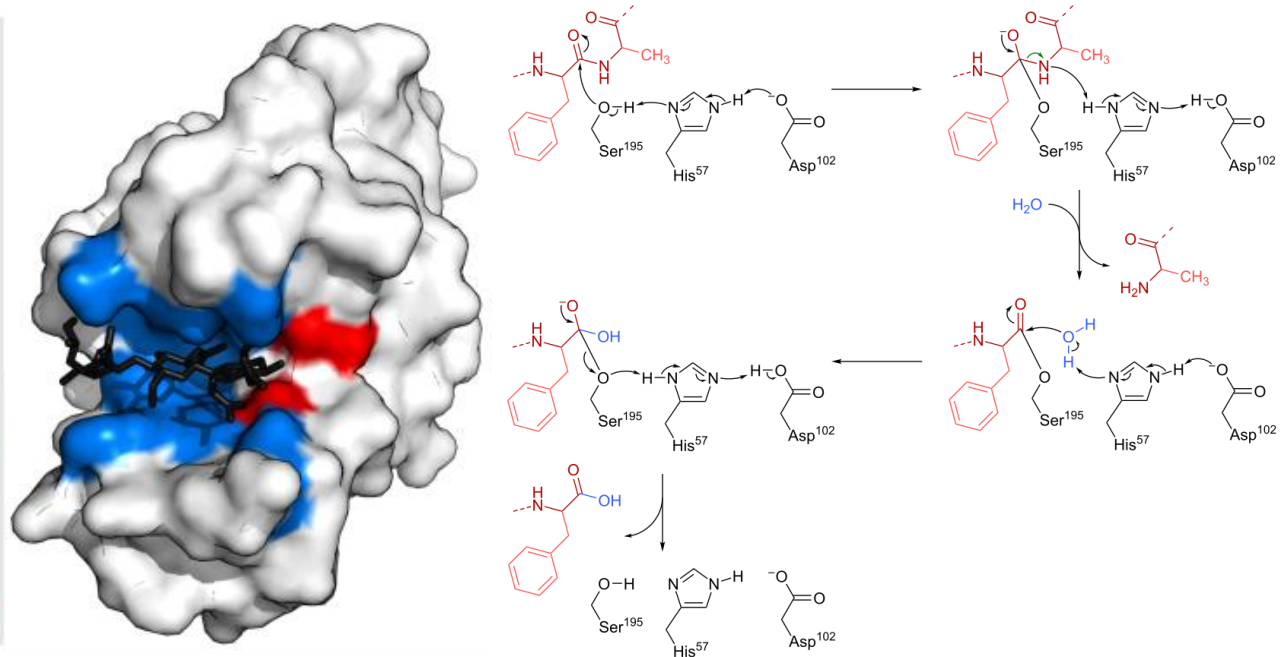
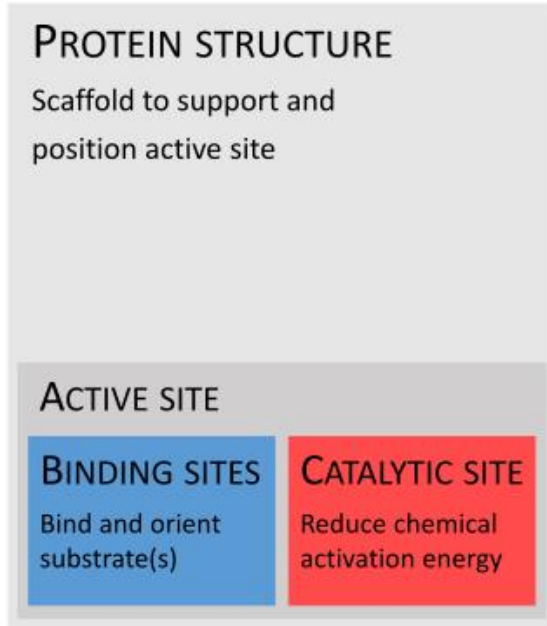
Enzyme Design with RFdiffusionAA and LigandMPNN



Rocco Moretti
Rosetta Workshop November 2025

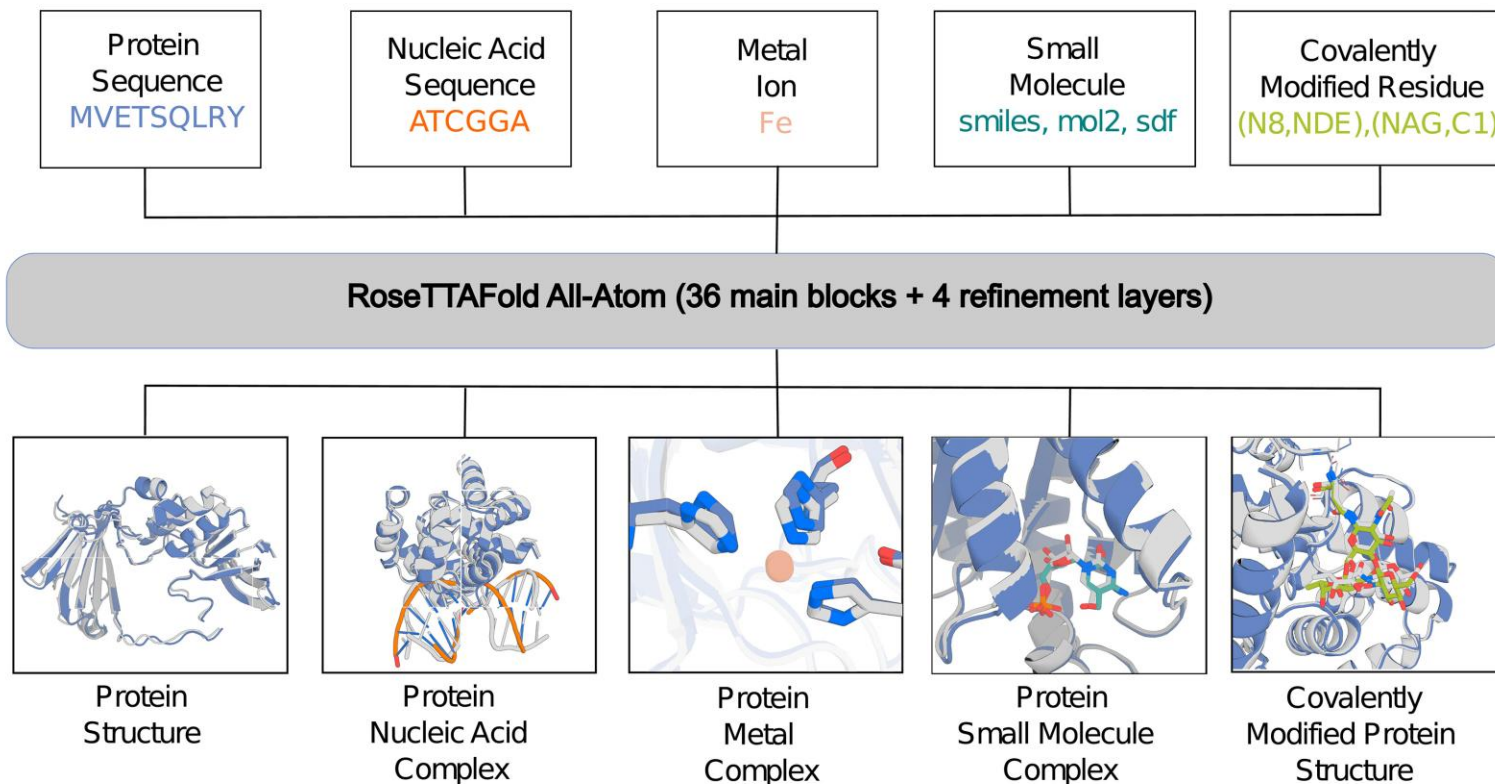
Enzyme Design

- Protein only versus Protein + Small Molecules

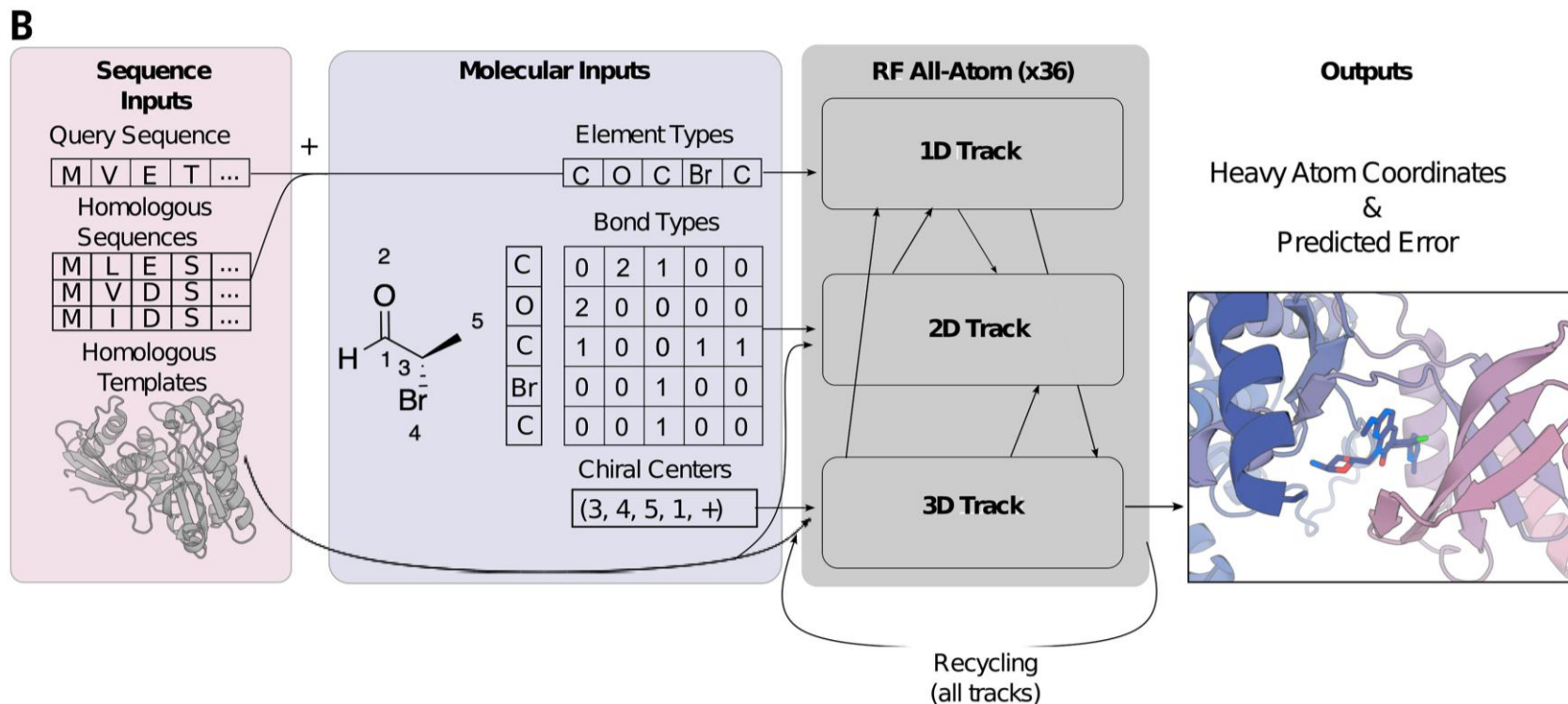


RoseTTAFold All Atom

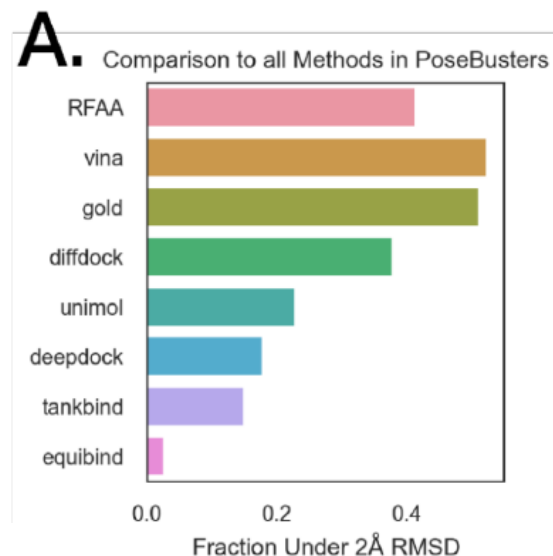
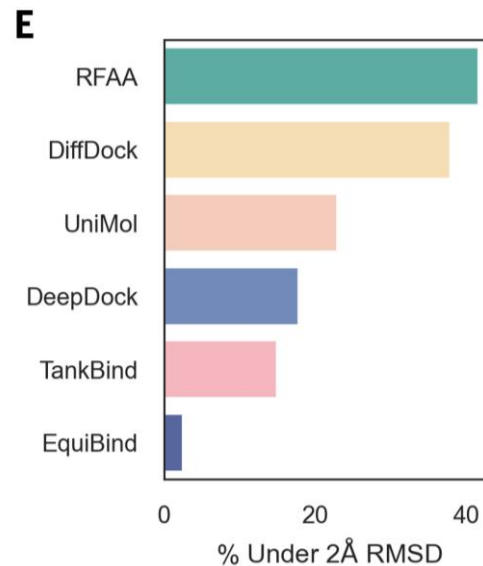
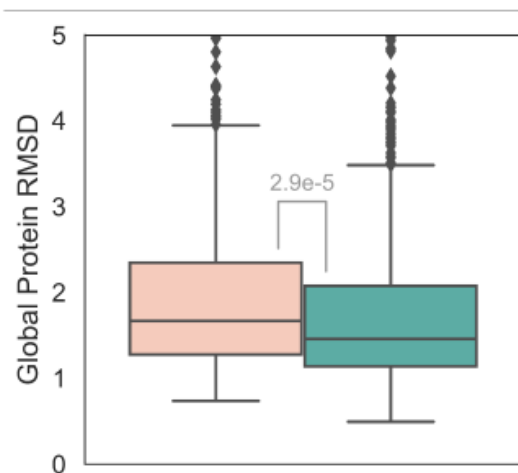
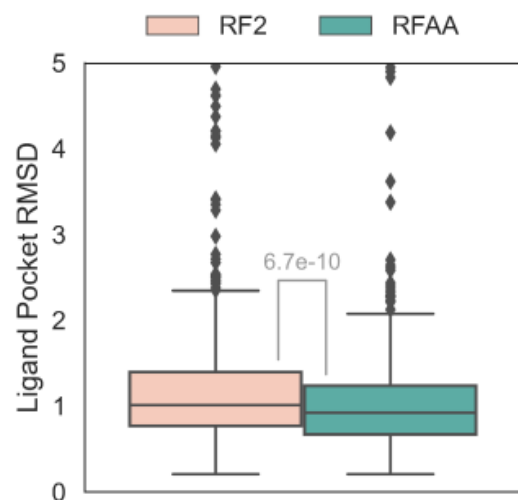
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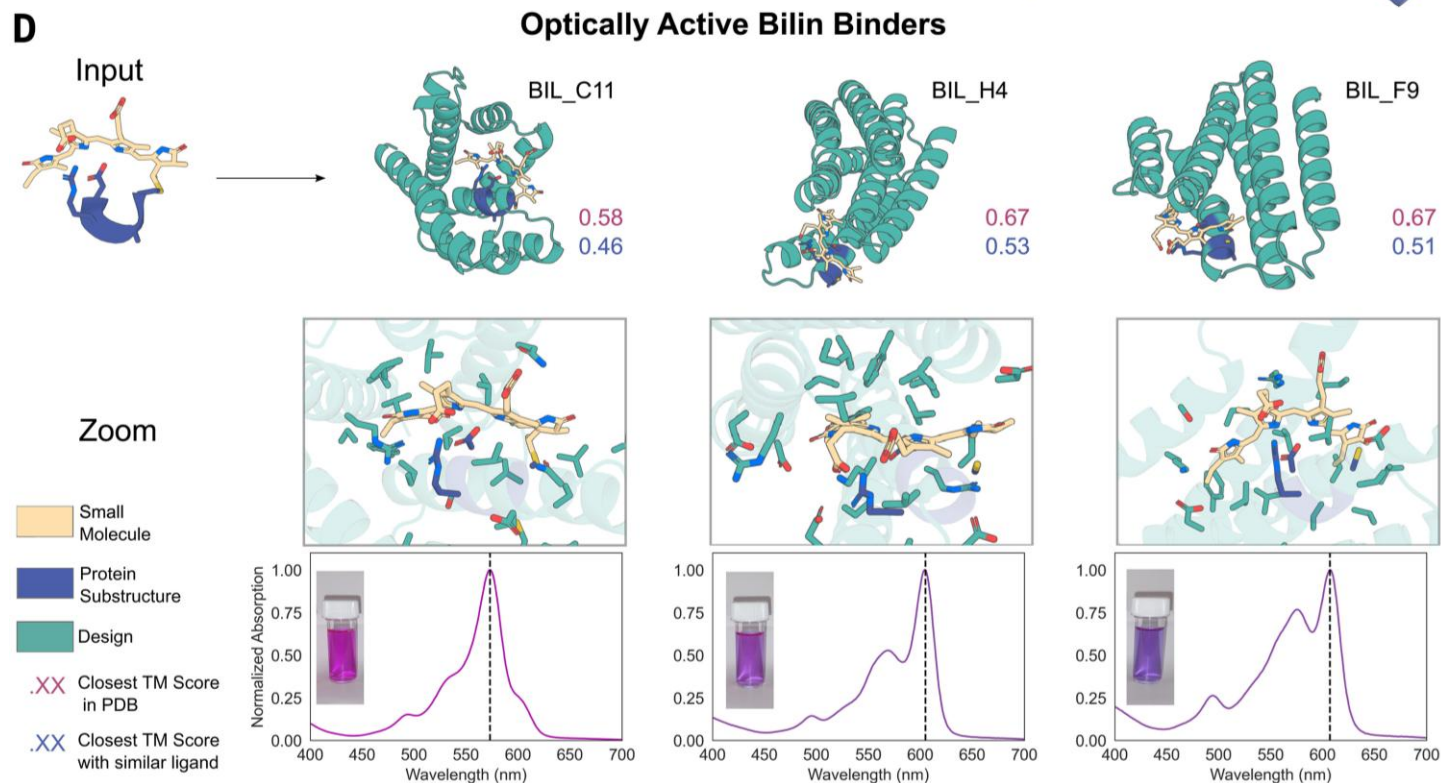
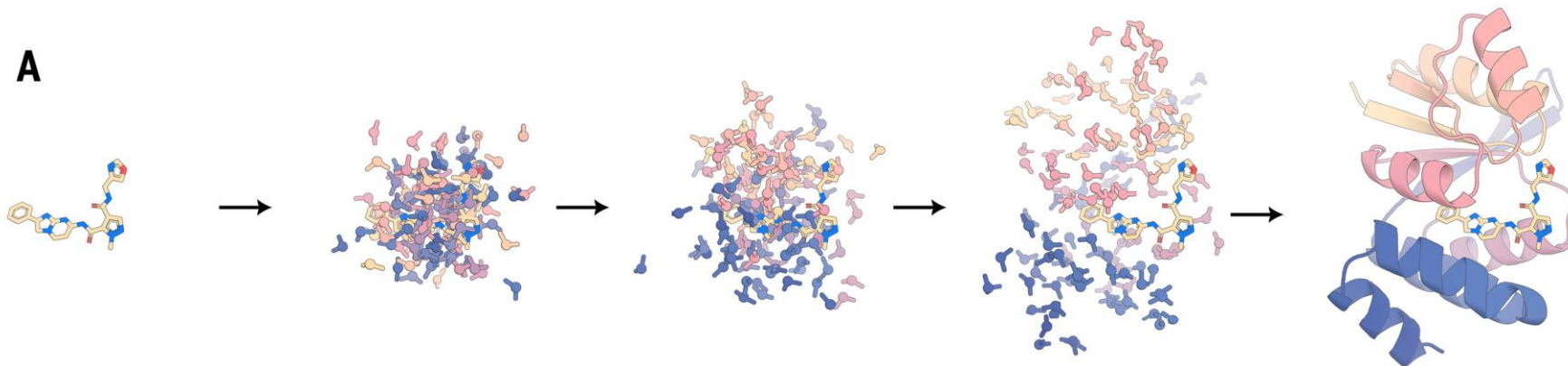
RoseTTAFold All Atom



RoseTTAFold All Atom

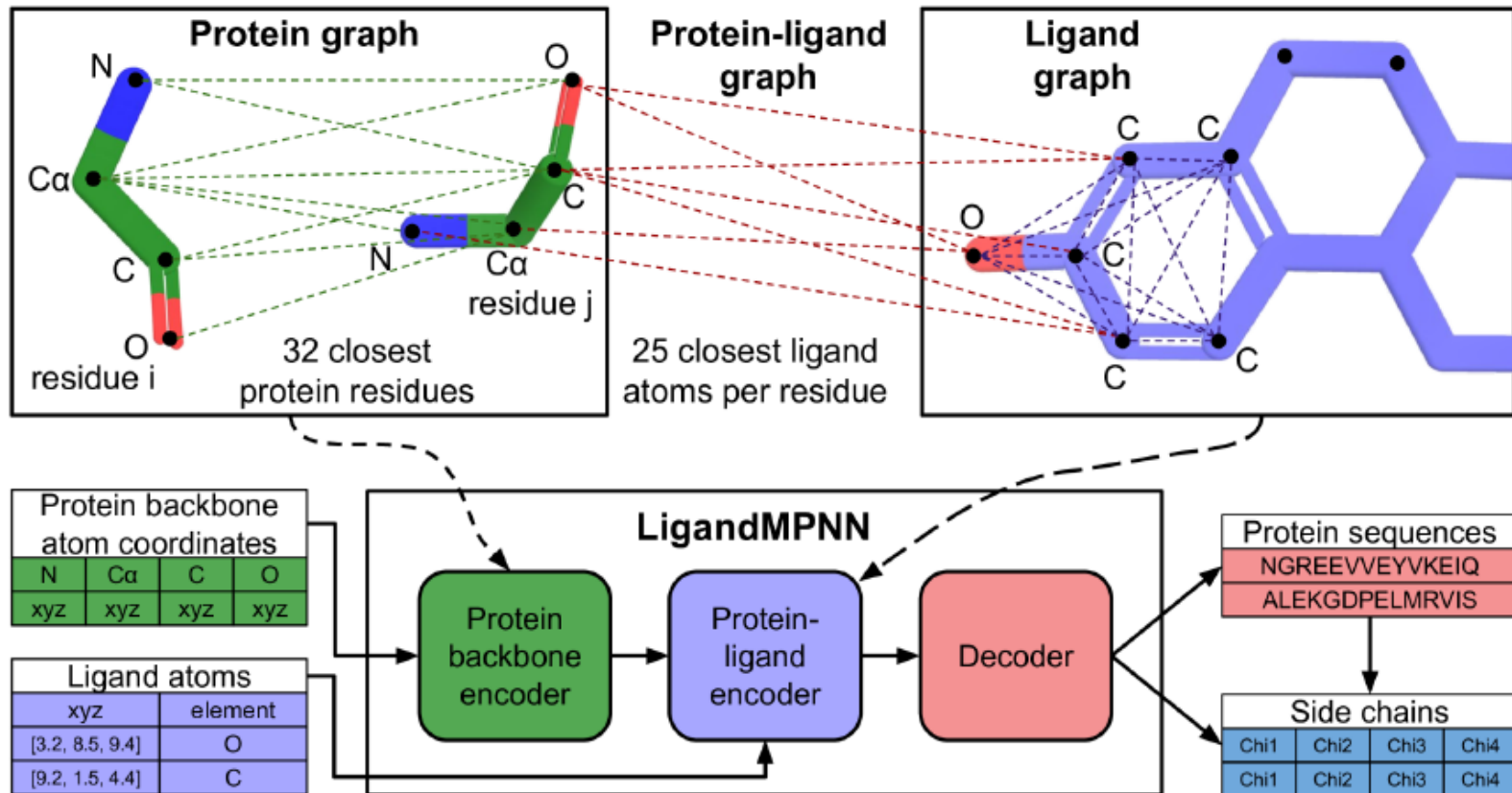


RFDiffusion All Atom



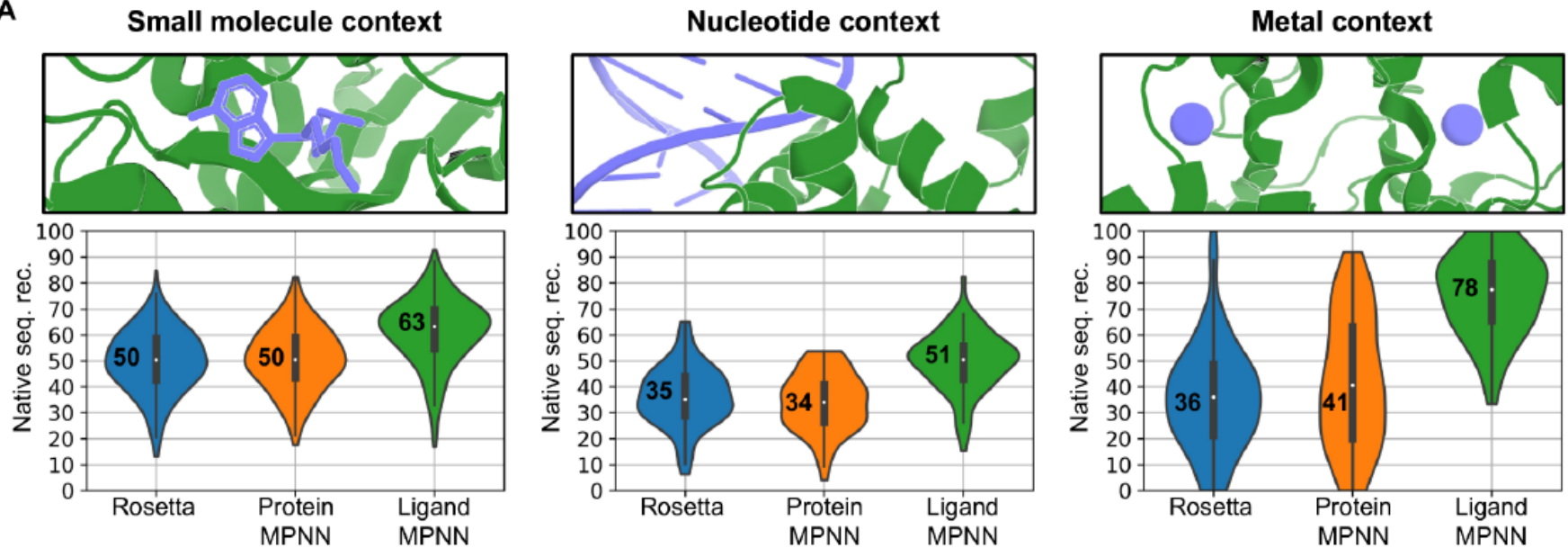
LigandMPNN

- ProteinMPNN ... but with Ligands

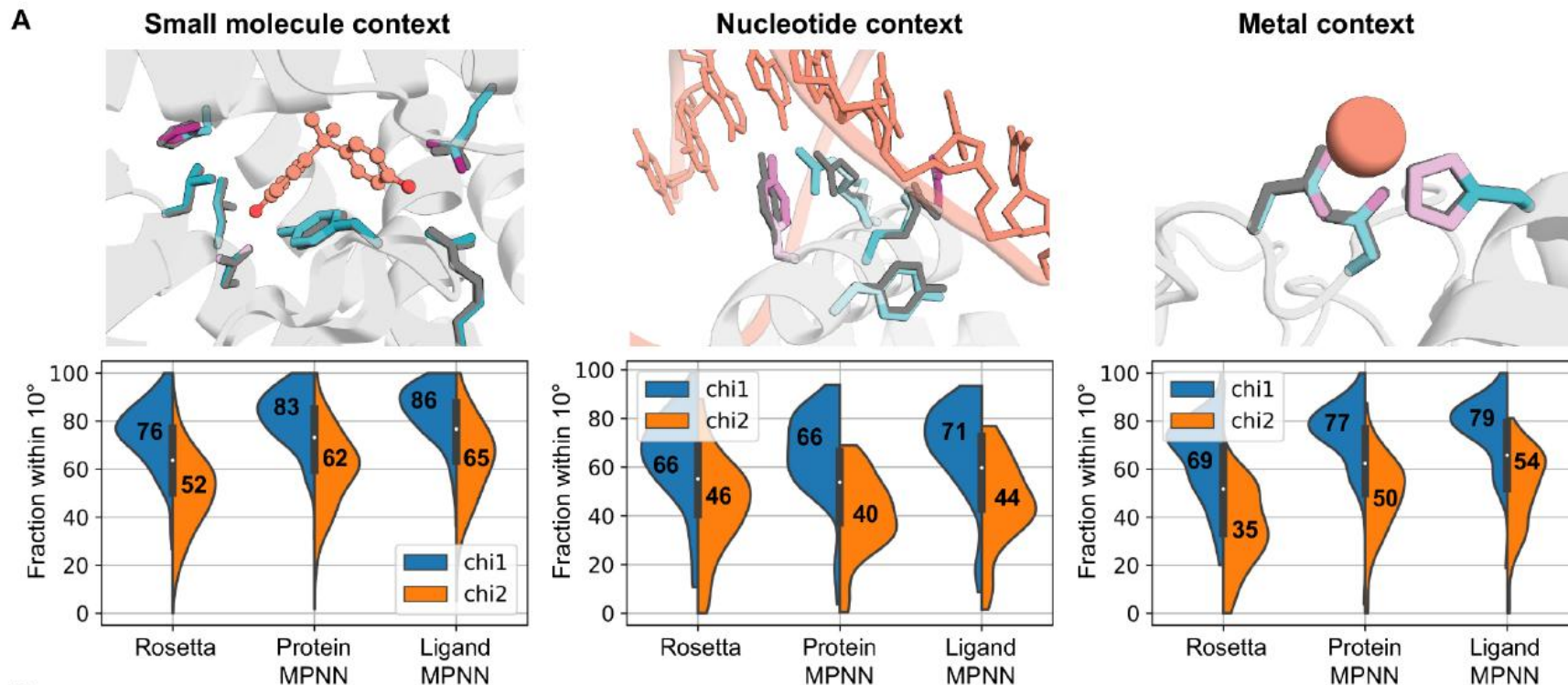


LigandMPNN

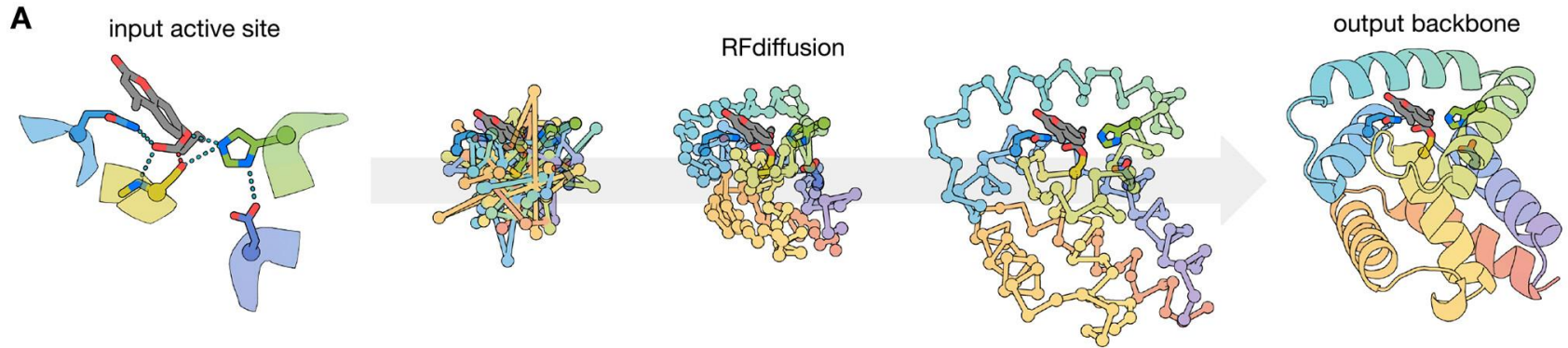
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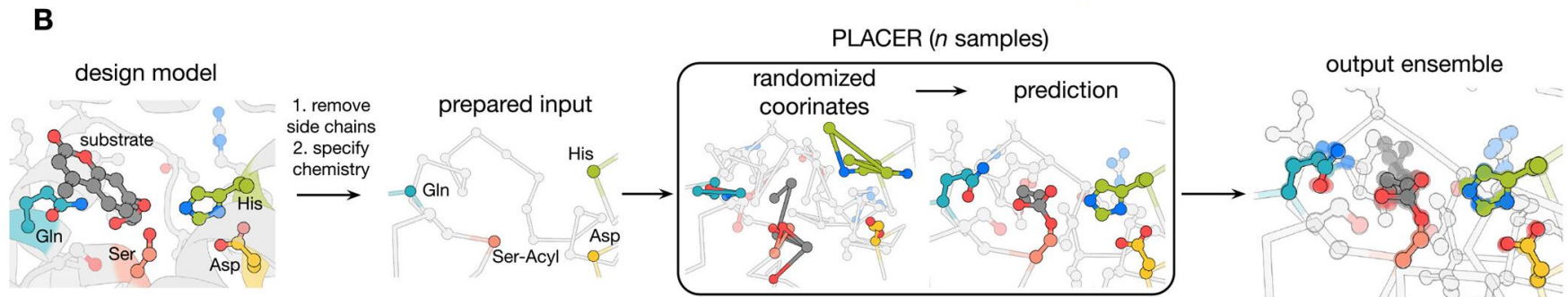
LigandMPNN can also pack sidechains



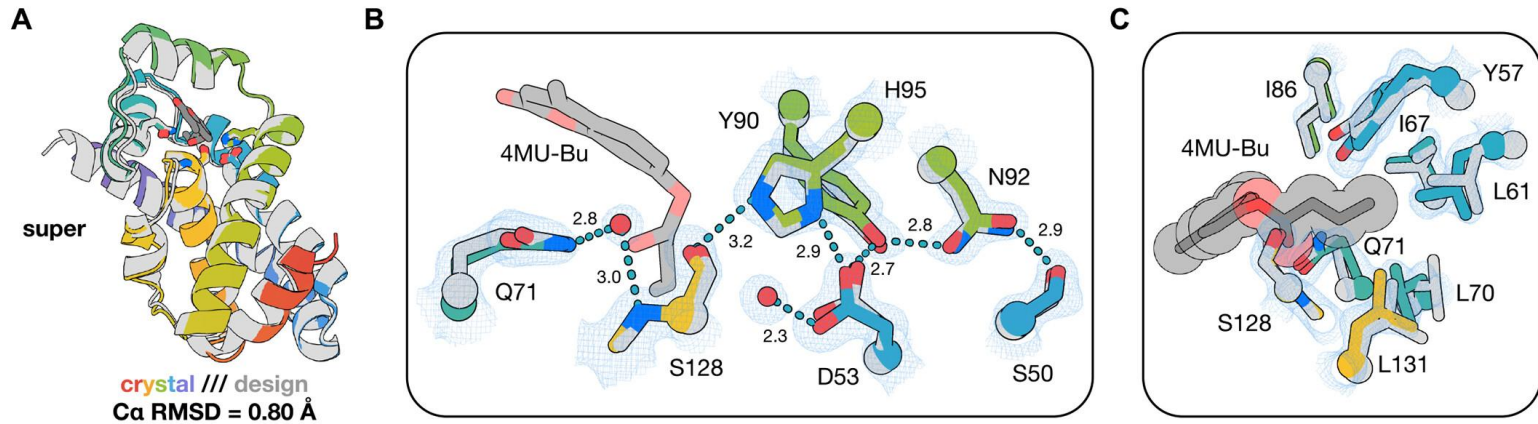
Computational design of serine hydrolases



RFdiffusion $\Rightarrow$ LigandMPNN $\Rightarrow$ FastRelax $\Rightarrow$ AlphaFold $\Rightarrow$ PLACER



Computational design of serine hydrolases



TM-score to known: 0.52

- Best k_{cat}/K_m : $2.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$
- Previously: $210 \text{ M}^{-1} \text{ s}^{-1}$ ($3190 \text{ M}^{-1} \text{ s}^{-1}$ after directed evolution)



Tutorial Example:

Computational design of an enzyme catalyst for a stereoselective bimolecular Diels-Alder reaction. Siegel et al (2010) <https://doi.org/10.1126/science.1190239>

- “De novo” enzyme design.

- Theozyme:

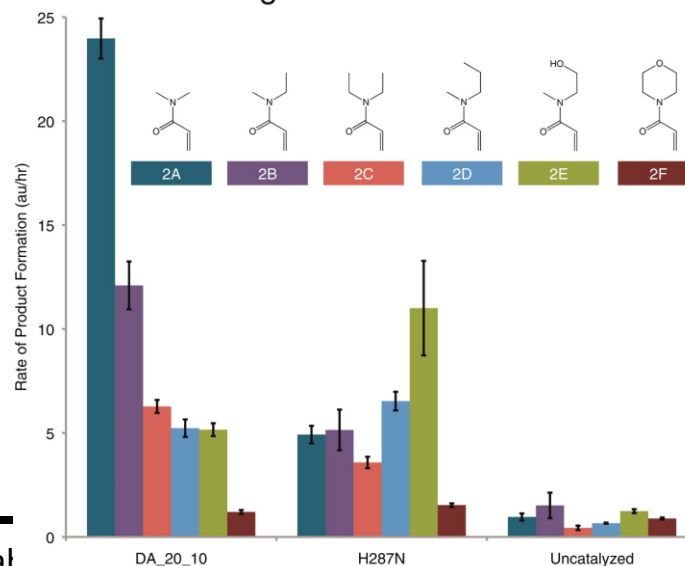
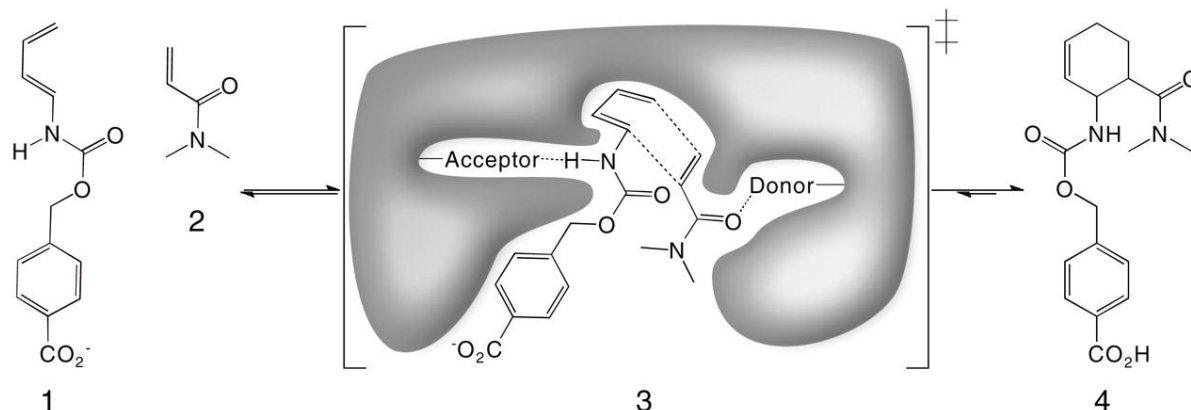
- QM

- Grafting

- Rosetta Matcher

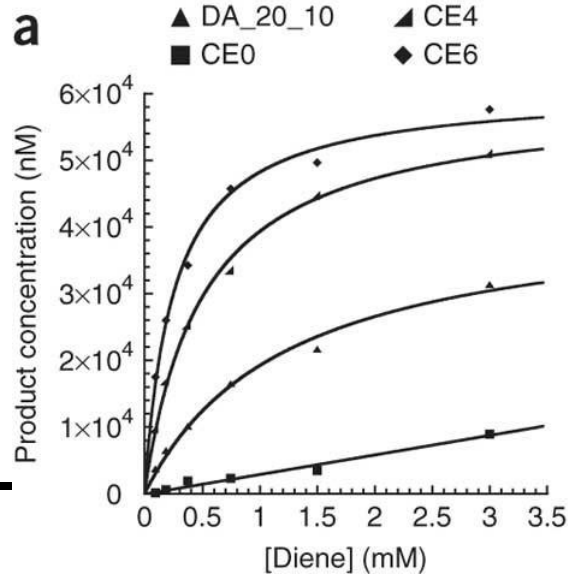
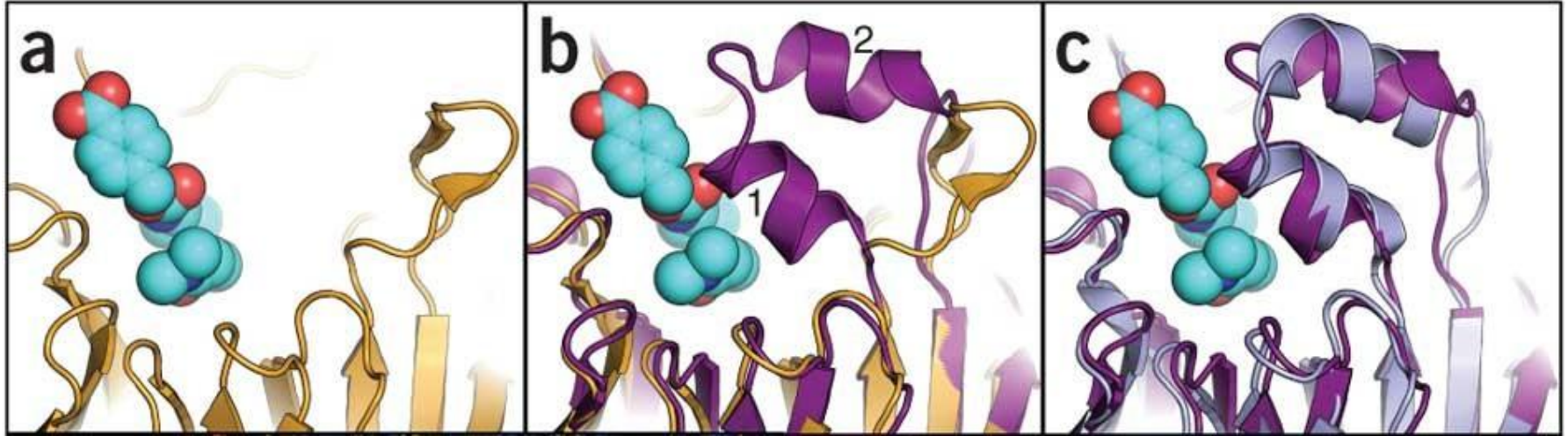
- Redesign

- Rosetta



Tutorial Example:

Increased Diels-Alderase activity through backbone remodeling guided by Foldit players. Eiben et al (2012) <https://doi.org/10.1038/nbt.2109>



Broader Applicability

- Any protein-small molecule interaction
 - Enzymes
 - Receptors
 - Sensors
 - Binders
- Protein/Nucleic Acid interactions
 - DNA/RNA binding proteins
 - Transcription factors
- Protein Glycan interactions
 - Glycan binders
 - Proteins with glycans attached
- Proteins with modified residues
 - Post-translational modifications
 - Noncanonical amino acid containing proteins

