Computational Enzyme Design

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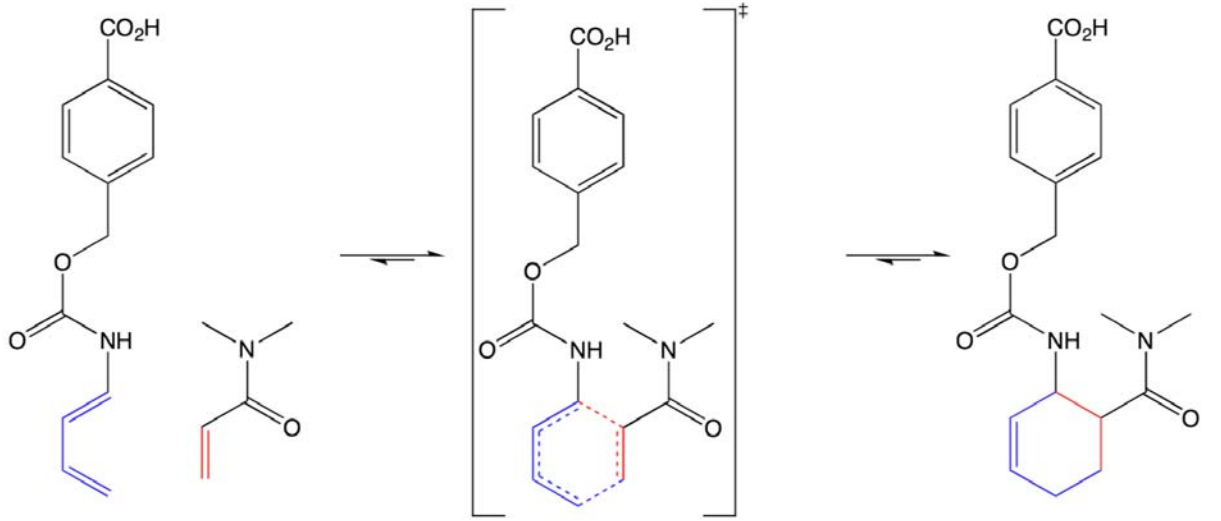
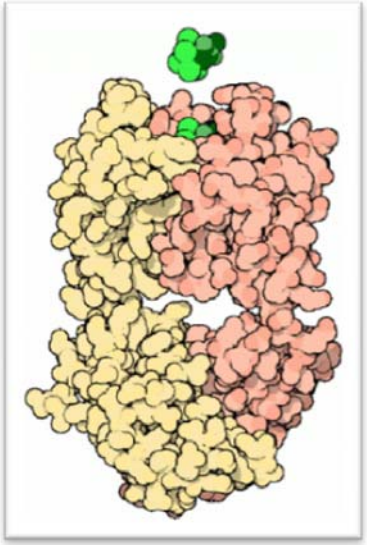
RosettaCon August 4th 2009

de novo Computational Enzyme Design: Engineering a Stereoselective Bimolecular Catalyst

The Diels-Alder Reaction





Science 1993, 262:204-208.

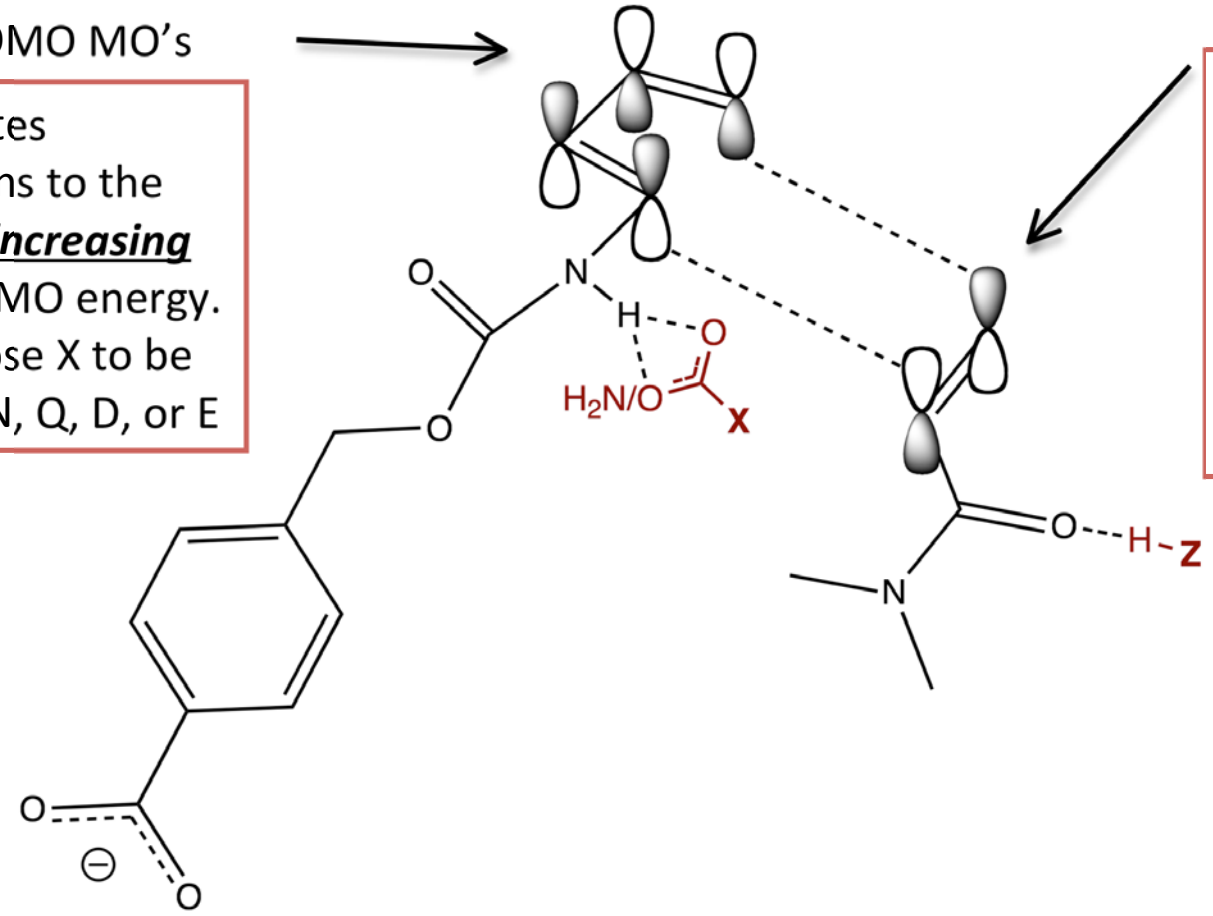
A Minimal "Diels-Alderase" Active Site

HOMO MO's

X donates electrons to the diene, ***increasing*** the HOMO energy. We chose X to be either N, Q, D, or E

LUMO MO's

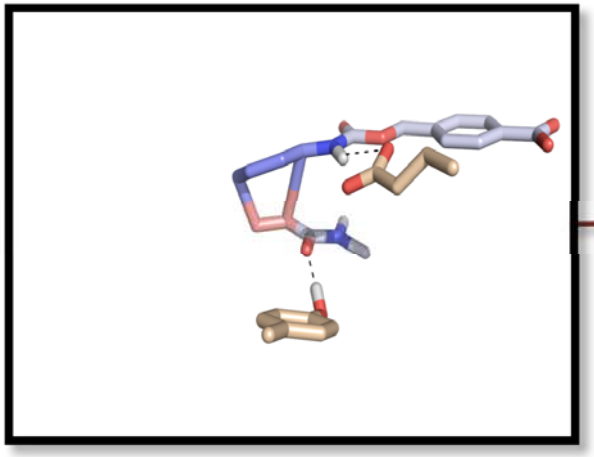
Z pulls electrons from the dienophile, ***decreasing*** the LUMO energy. We chose Z to be either Y, S, or T.



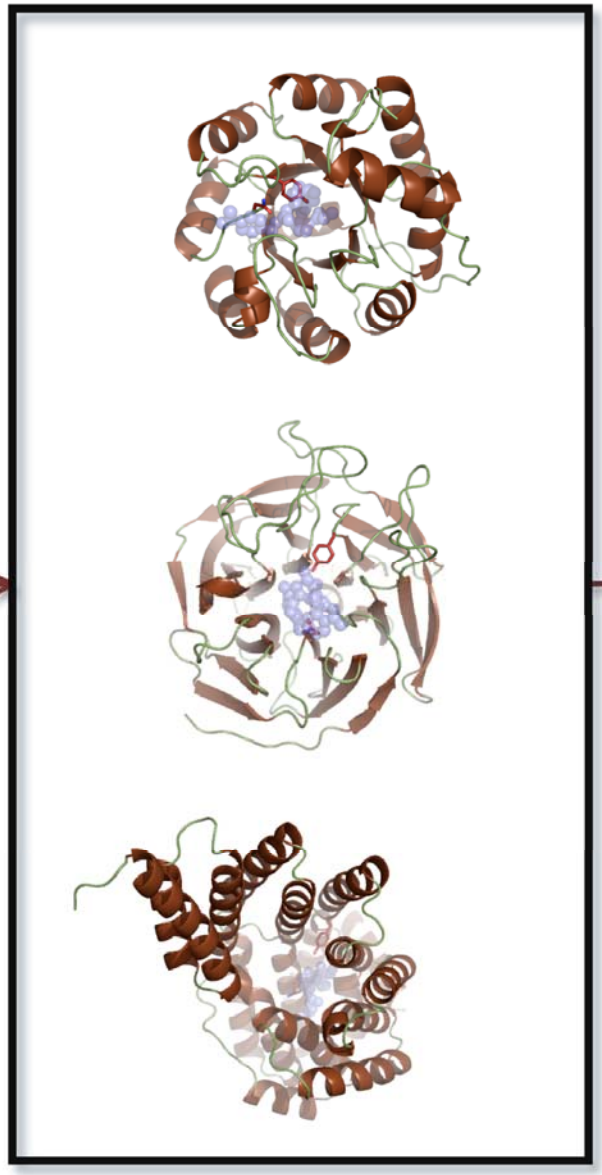
Science 1993, 262:204-208.
Theozymes from Gert Kiss in the Houk Group, UCLA

de novo Design Methodology using Rosetta

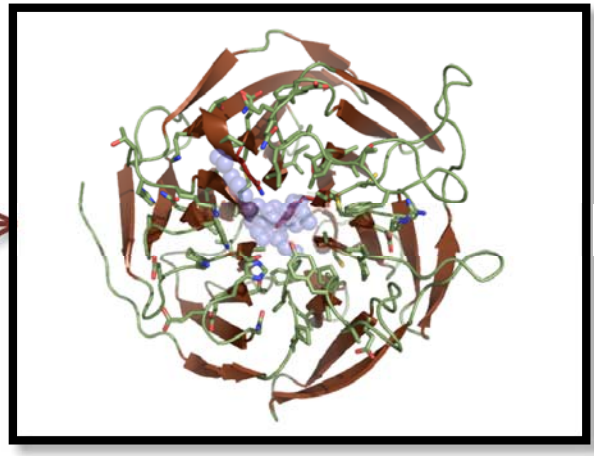
Build A Theozyme
Using QM/MM
Techniques



Search A Scaffold Library
for ones that fit the Theozyme



Iterative Optimization
Via RosettaDesign
and Manual Analysis



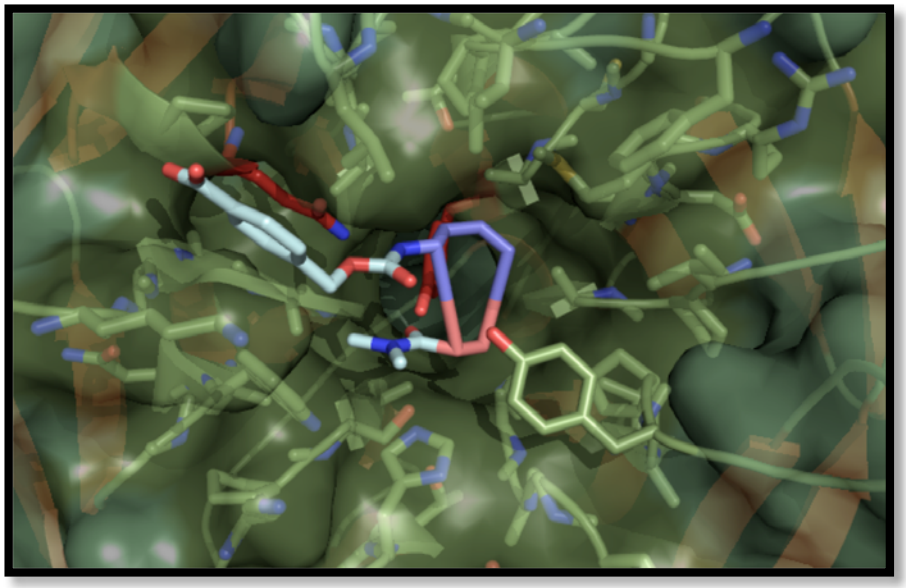
Protein Sci 2006, 15:2785-2794.
Nature 2008, 453:190-U194.
Science 2008, 319:1387-1391.

Experimental Characterization of *de novo* "Diels-Alderases"

54 Designs Were Tested

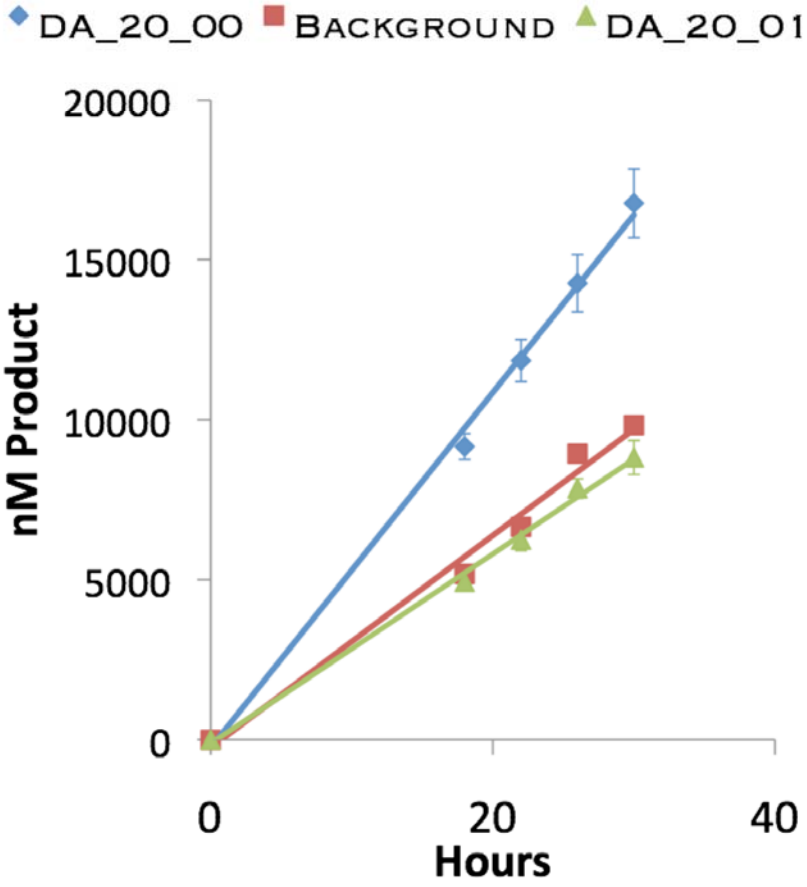
28 Expressed as Soluble Protein

1 showed activity...



Progress Curve of The Diels-Alder Reaction

(200μM Protein, 1mM Diene, 1mM Dienophile)

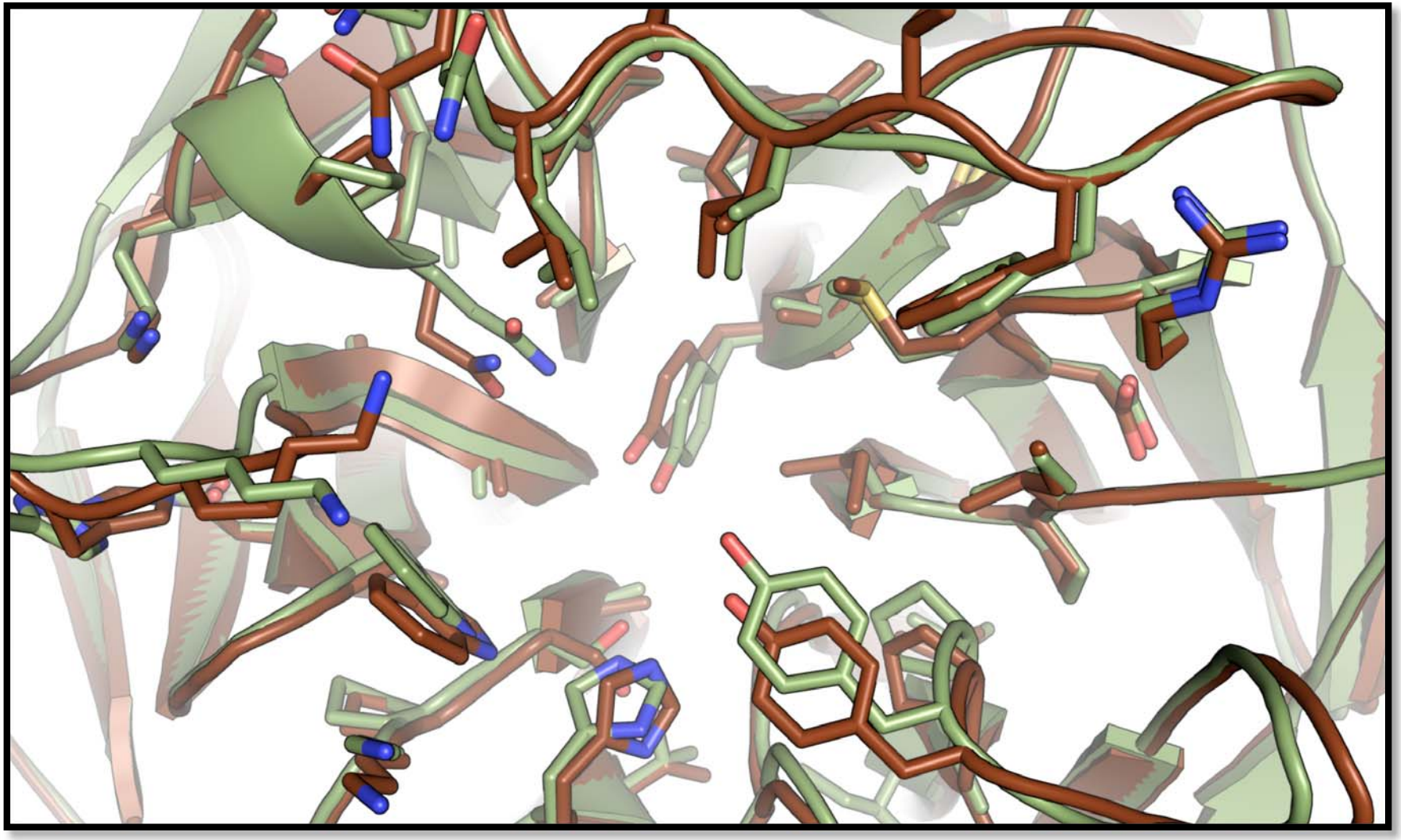


Crystal Structure of a "Diels-Alderase"

DESIGN (GREEN) vs. CRYSTAL STRUCTURE (BROWN) ALL ATOM RMSD: 0.5Å

Resolution: 2.2Å, R-factor: 18.5%, R- free: 24.8% refined by molecular replacement with WT 1e1a

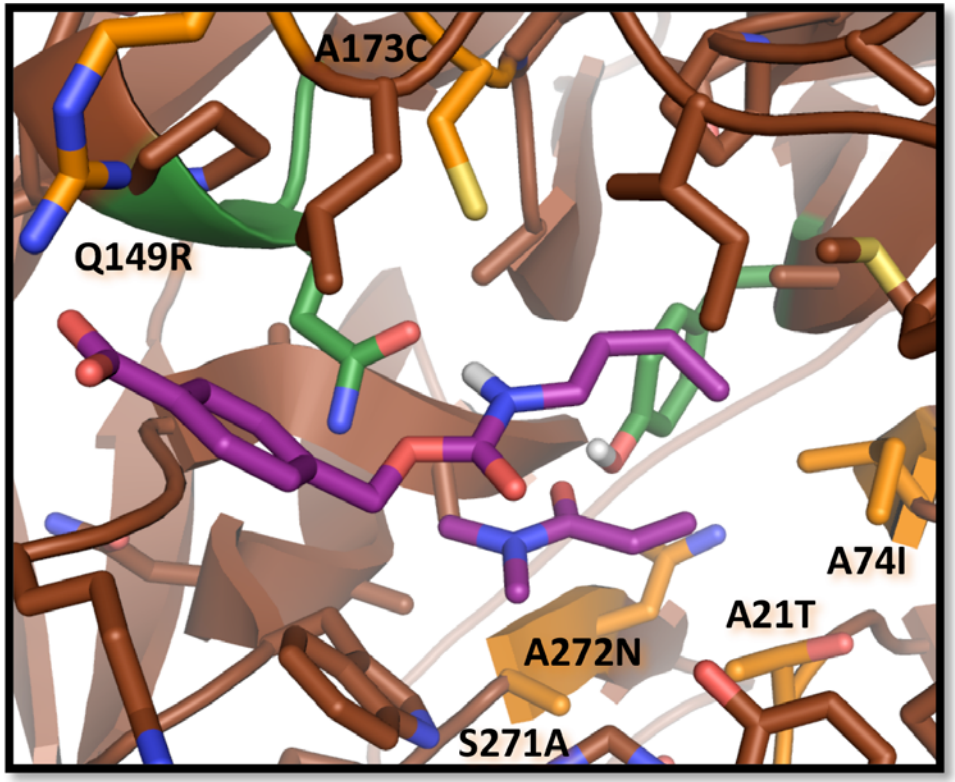
Crystallization and refinement by Abigail Lambert from Barry Stoddard's lab, FHCRC



Active Site Optimization

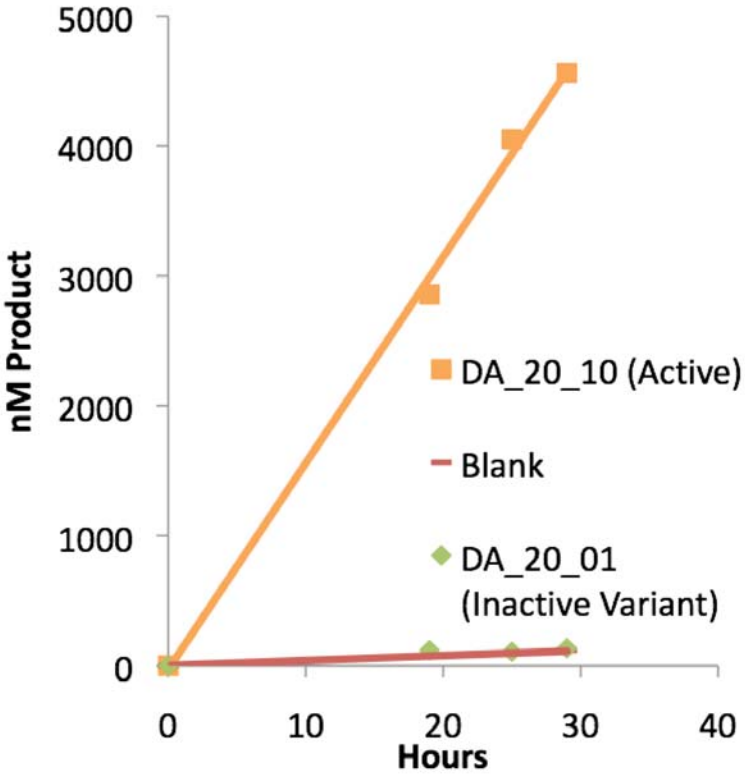
DA_20_10 Active Site View

Beneficial Mutations, catalytic residues



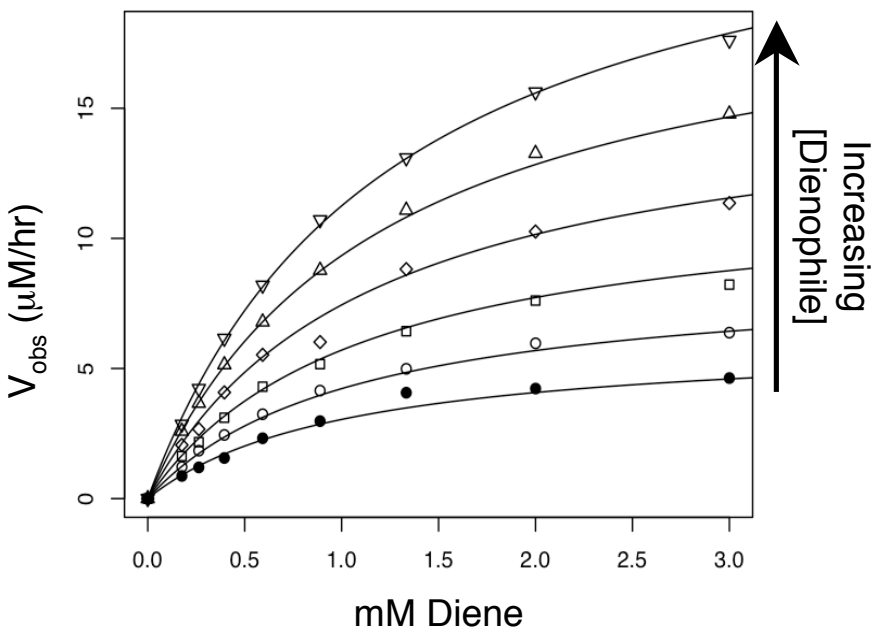
Progress Curve of The Diels-Alder Reaction

0.1mM Diene, 3mM Dienophile, 20μM Protein

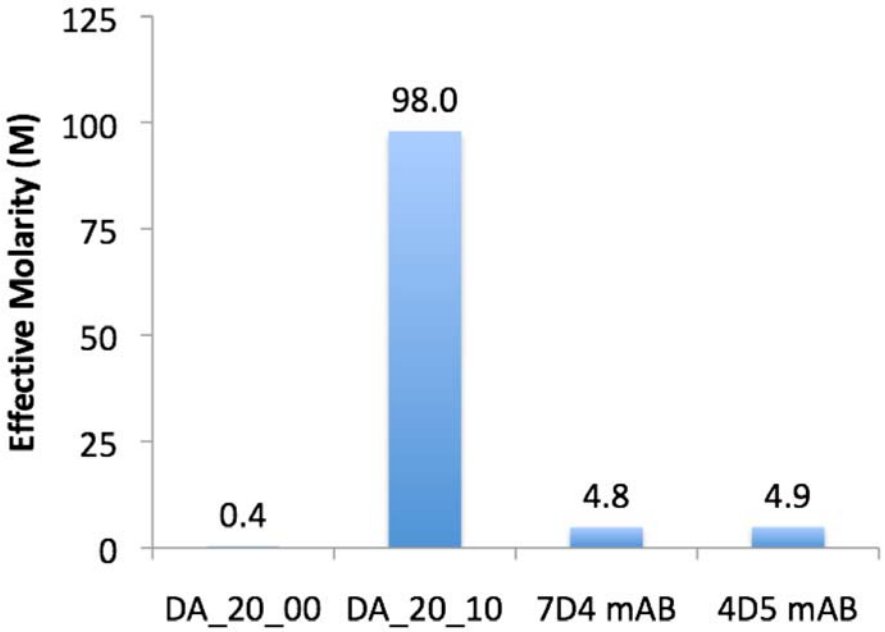


A *de novo* "Diels-Alderase" Compared to Catalytic Antibodies

Substrate vs. Velocity Curves
298K, PBS, 20μM Protein.
Varied Diene at Various Dienophile Concentrations.



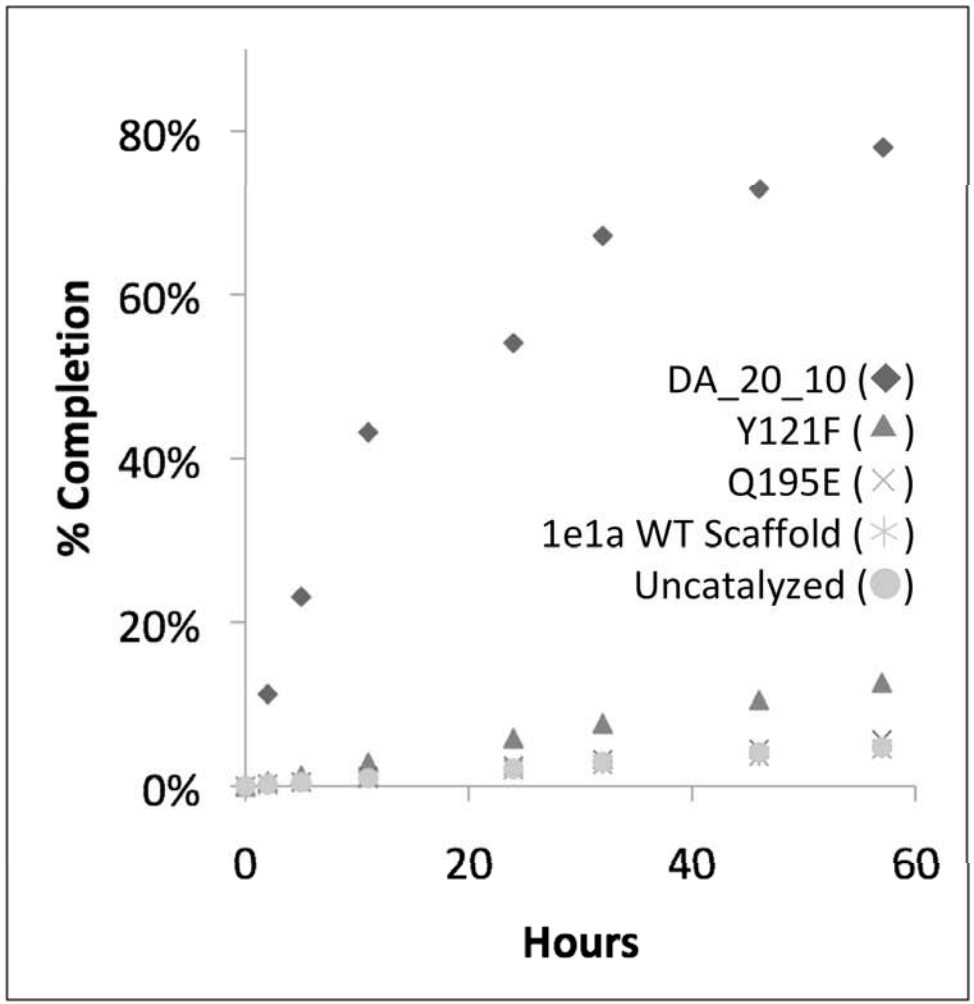
Effective Molarity Comparison
(k_{cat}/k_{uncat} = M)



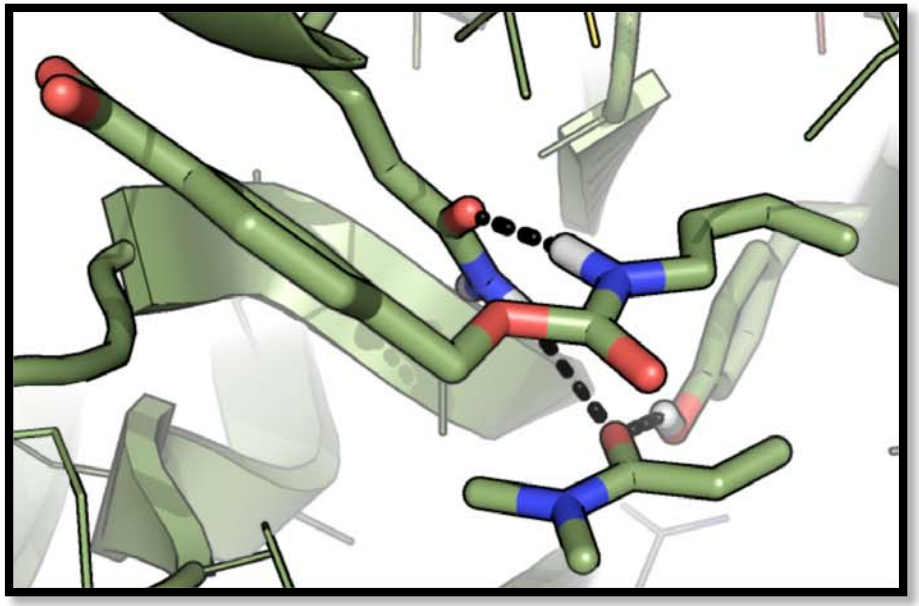
Catalyst	k_{cat} (hr^{-1})	$K_{M\text{-diene}}$ (mM)	$K_{M\text{-dienophile}}$ (mM)	α
DA_20_00 (298K)	0.01 ± 0.002	3.53 ± 1.5	146 ± 2.5	-
DA_20_10 (298K)	2.44 ± 0.3	0.98 ± 0.2	58.1 ± 14	1.7 ± 0.7
mAb 7D4 (310K)	0.21	0.96	1.70	1
mAB 4D5 (310K)	0.21	1.60	5.90	1

Catalytic Residue Contributions and Production Capability

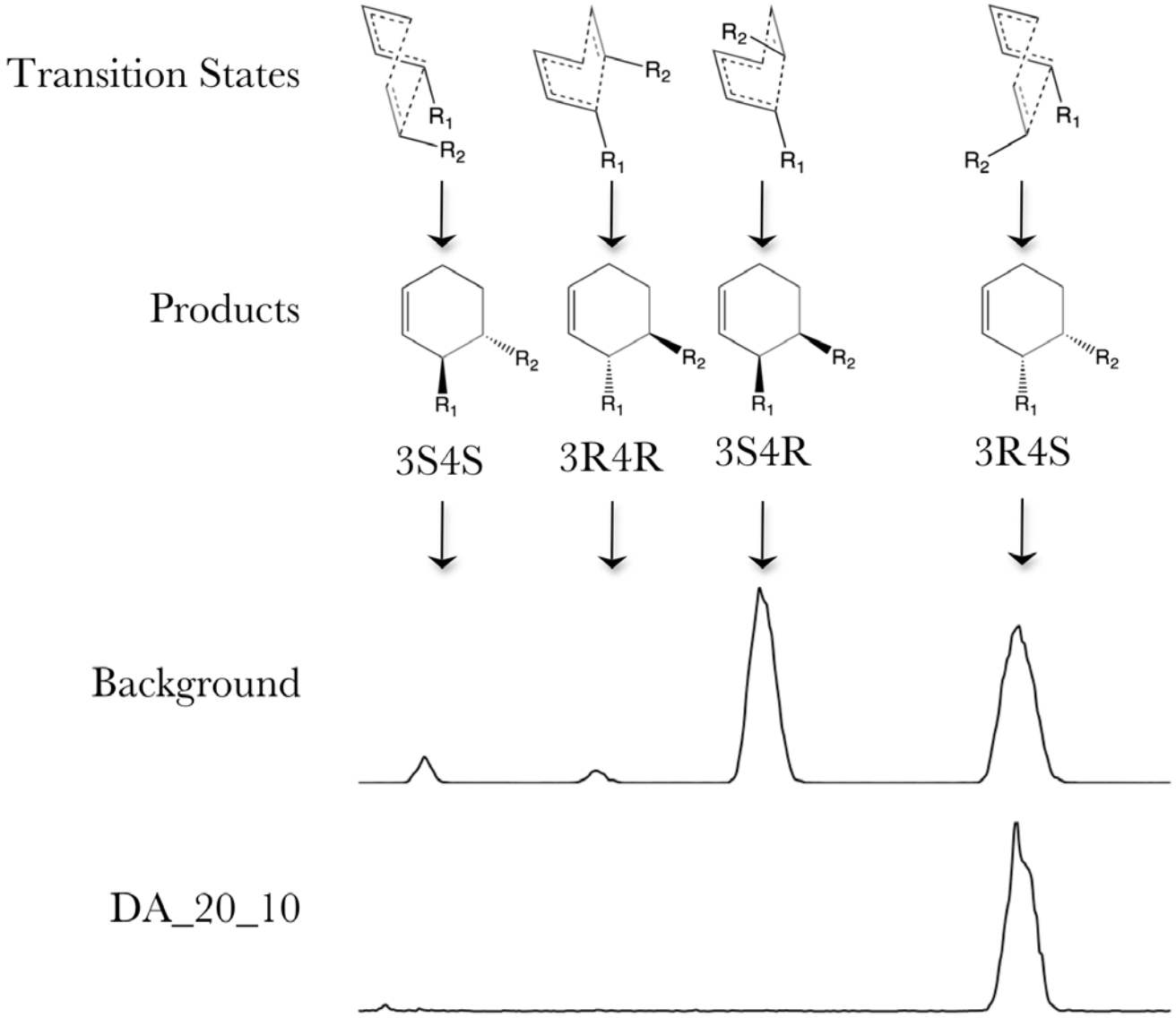
1 mM diene, 50 mM dienophile, 200 μM enzyme, in PBS at 298K.



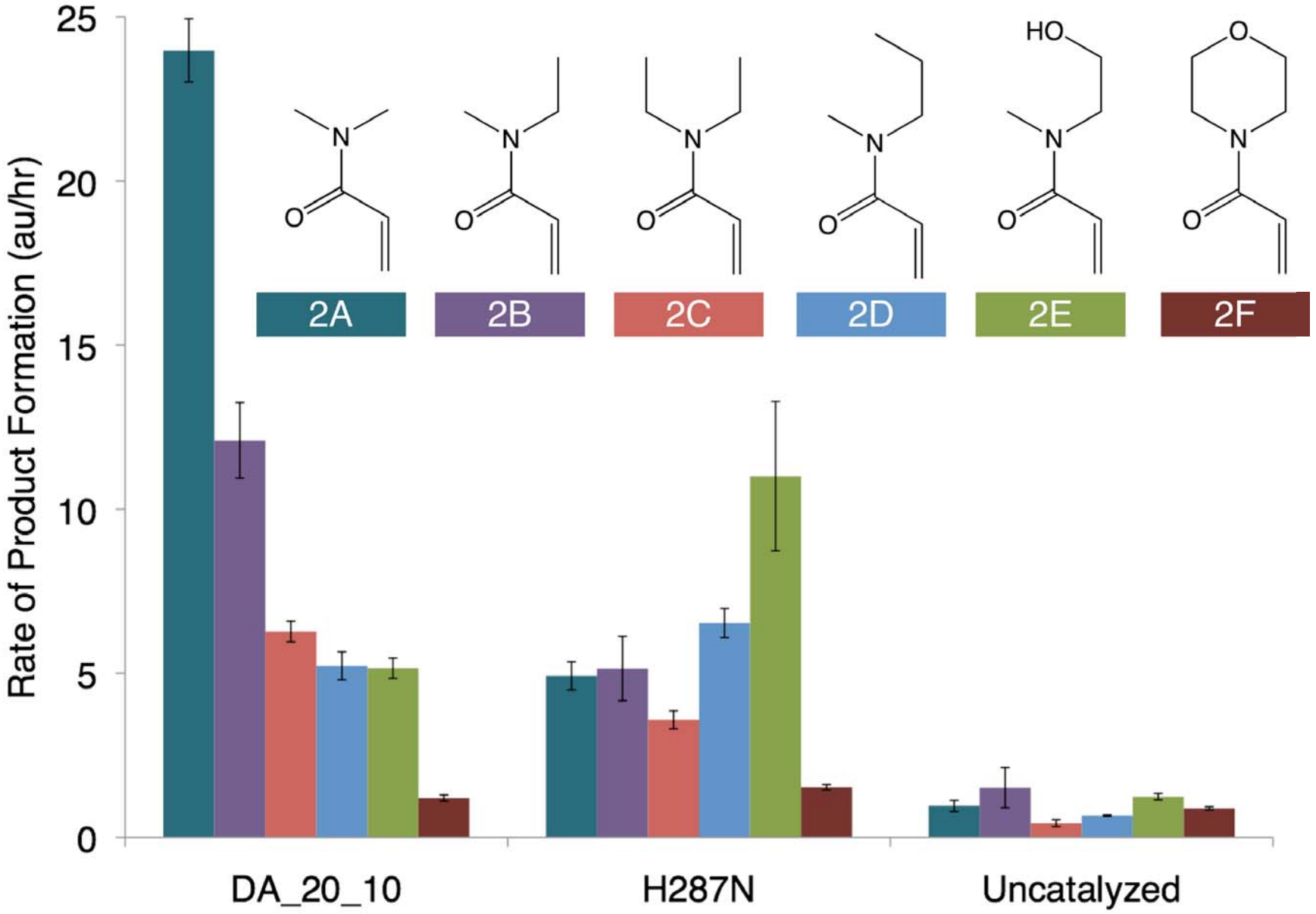
Active Site of DA_20_10
Q195 and Y121 Highlighted



Absolute Enantio- and Diastereo- selectivity of DA_20_10

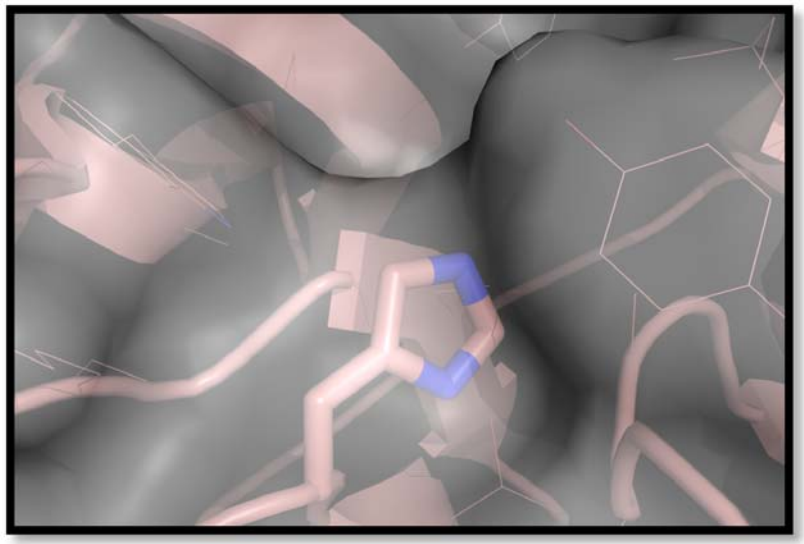


Modulation of DA_20_10 Substrate Specificity

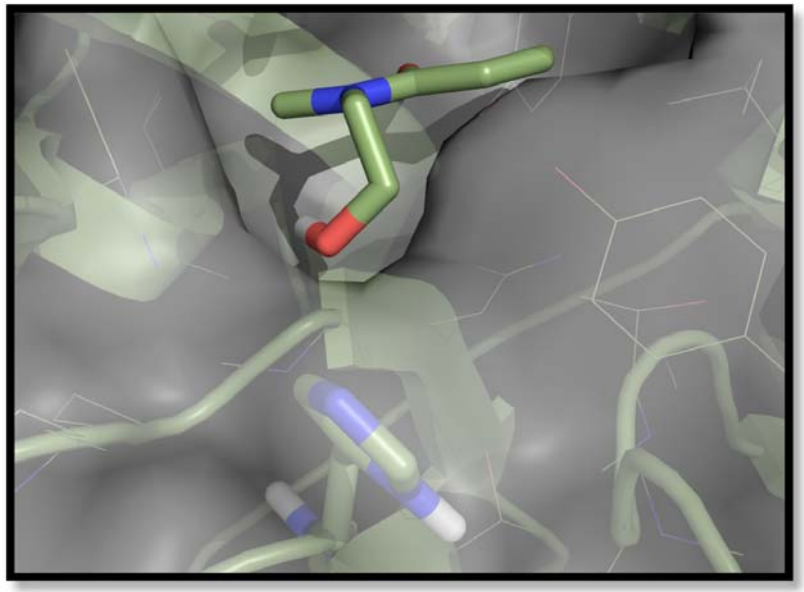


Model of H287N Mutation with Alternate Ligand

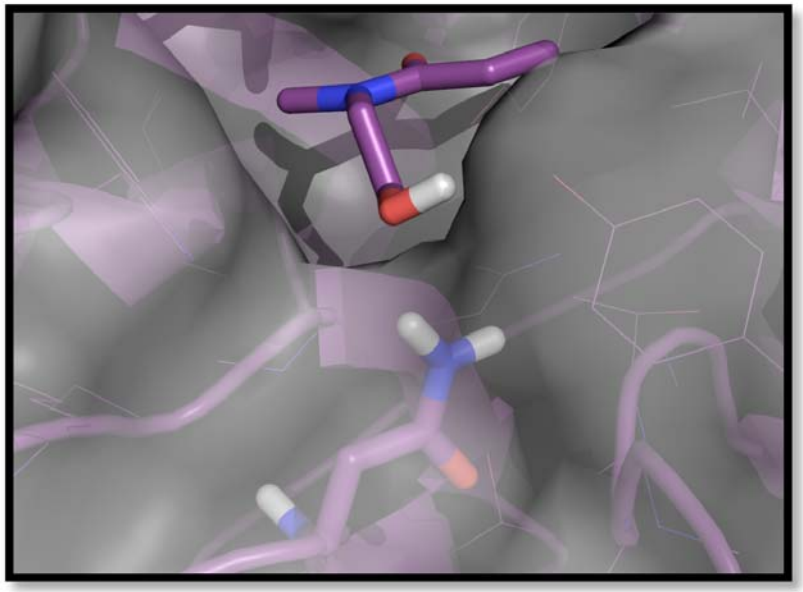
Crystal Structure



DA_20_10



H287N



Future Plans for the "Diels-Alderase"

Match into New Scaffolds

Loop Remodeling

Design Towards
New Stereoisomers

Redesign Substrate
Specificity

This work (and future work) could not have been done without:

MY ADVISORS
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EXTRA SLIDES