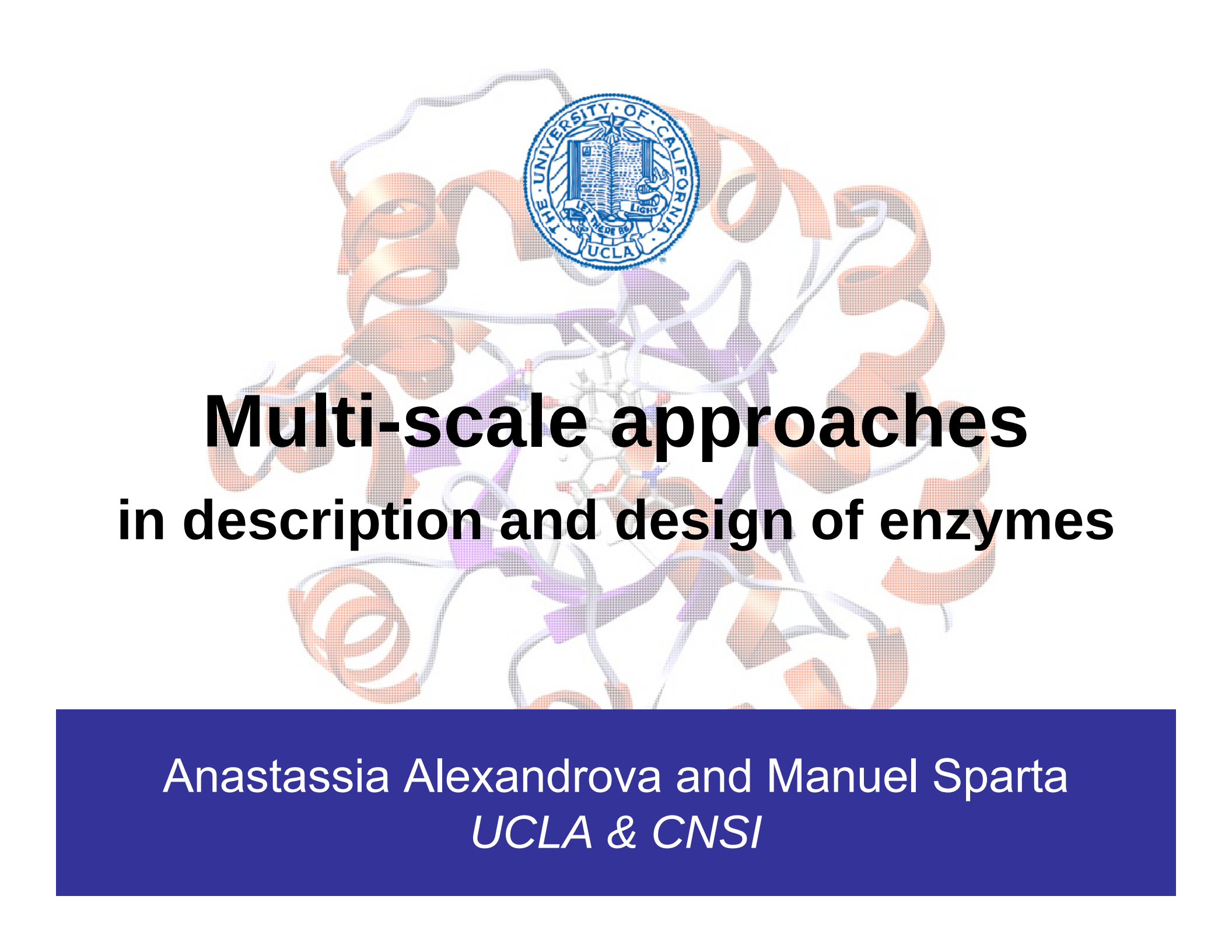


Multi-scale approaches

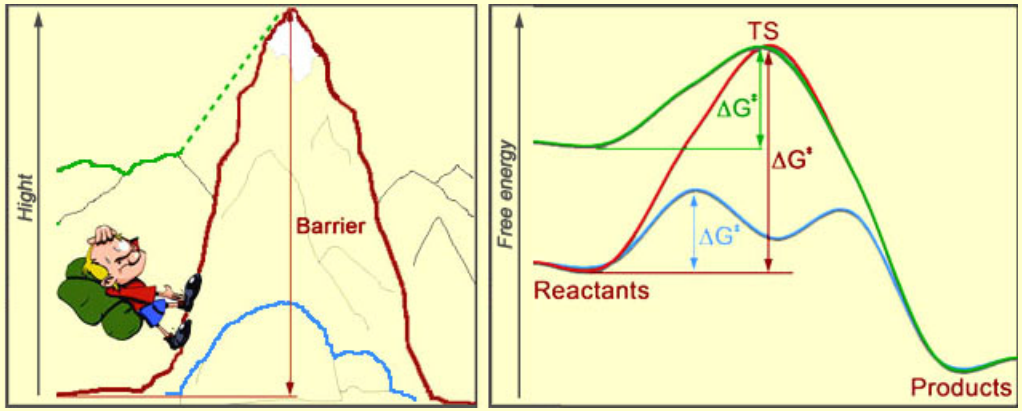
in description and design of enzymes

Anastassia Alexandrova and Manuel Sparta

UCLA & CNSI

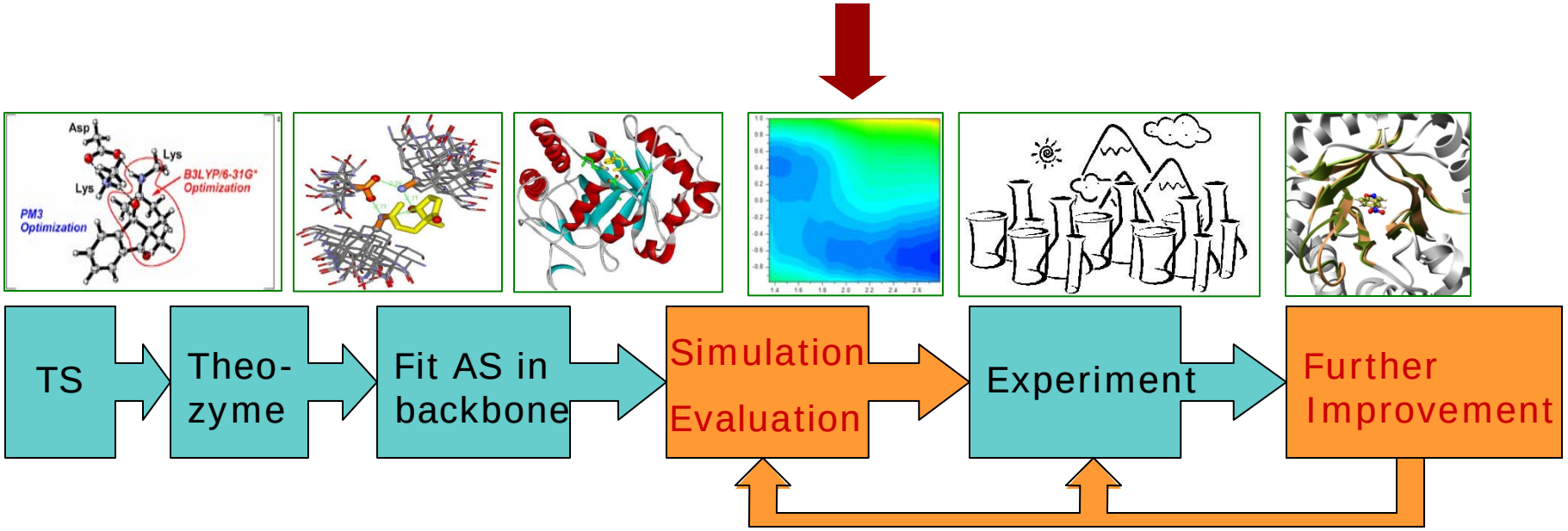
Strategy for enzyme design
Test case: Kemp elimination
Mechanism, performance
Deducing design strategies
Our current push

Catalysis: it is all about the barrier



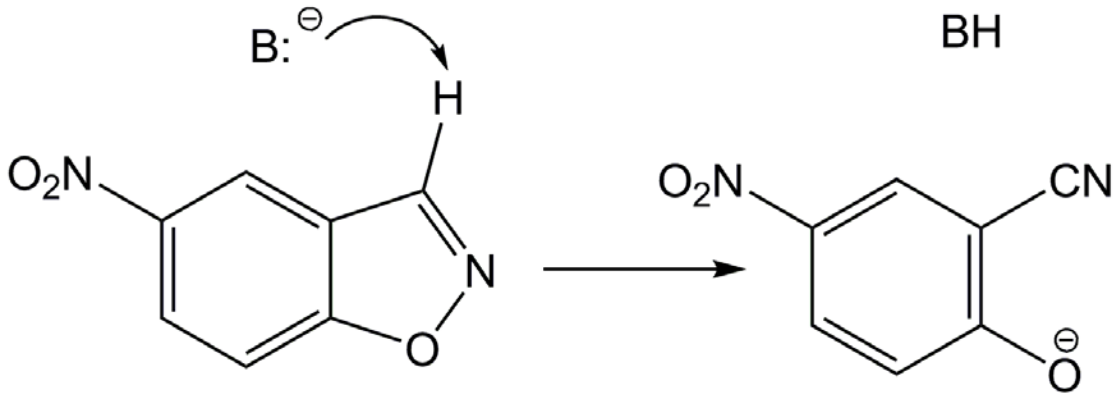
The "inside-out" protocol:

Big Aim: development of an efficient protocol for the design of artificial enzymes catalyzing any reaction of interest



D. Rothlisberger et al. *Nature* 453 (2008), 109-195

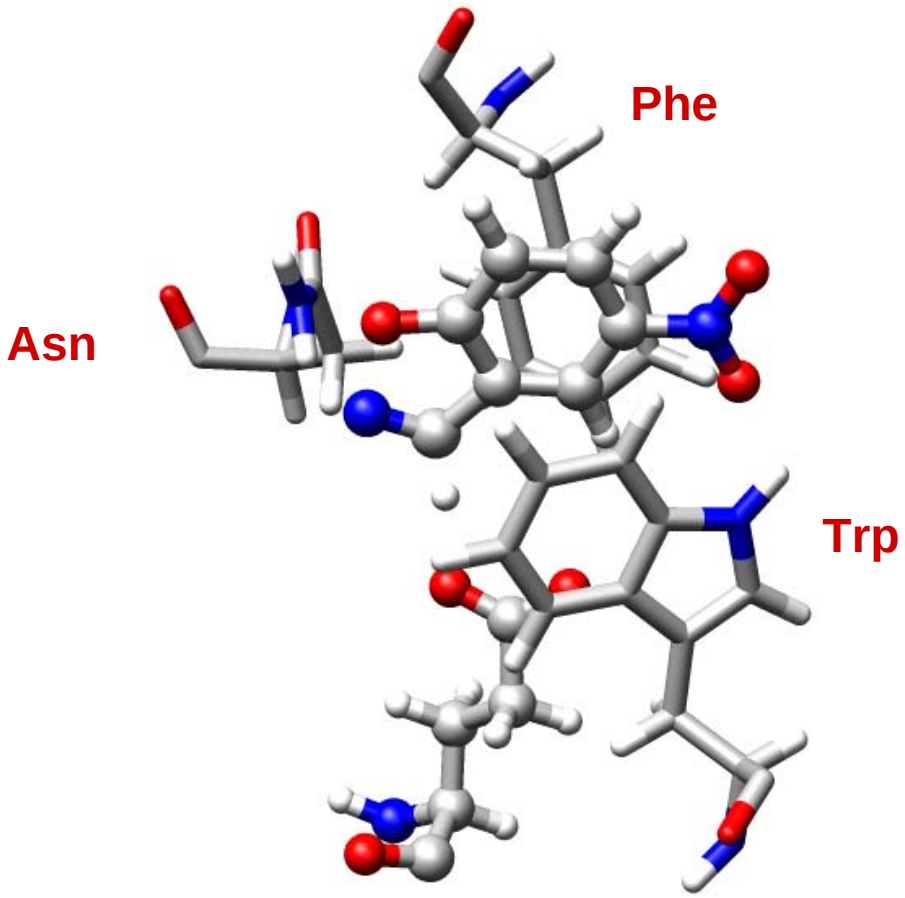
Test reaction: Kemp elimination



D. Rothlisberger et al. *Nature* 453 (2008), 109-195.

Inside-out design of enzymes

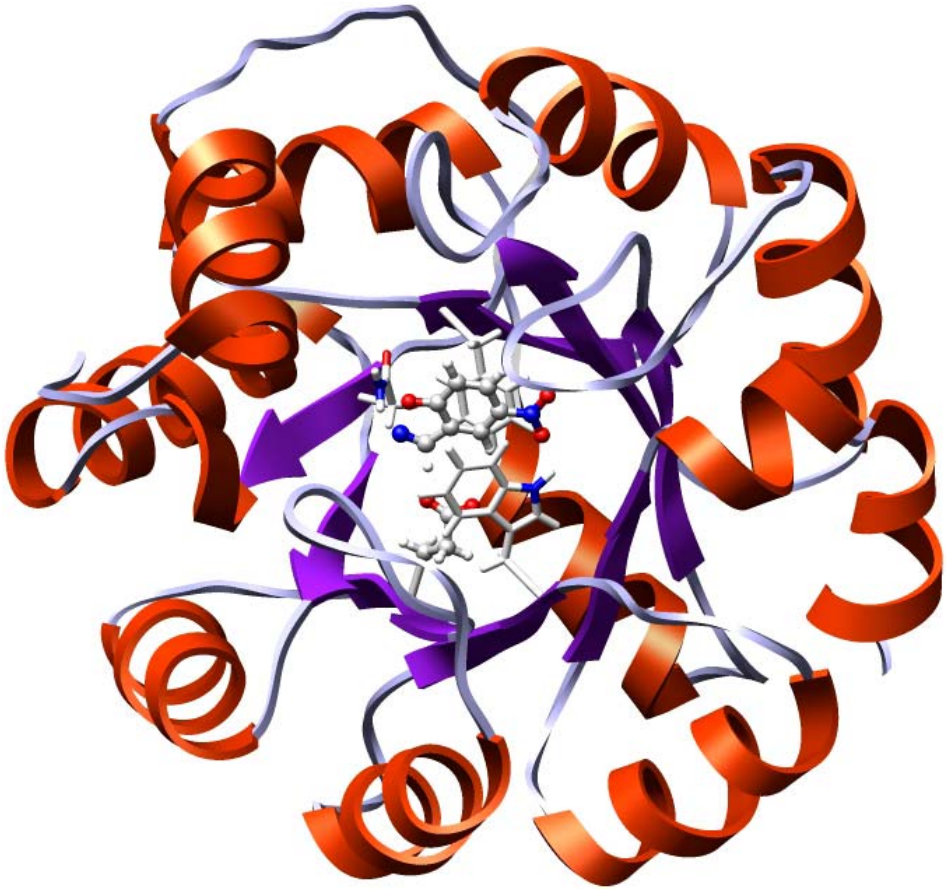
4. Stabilize the TS via hydrogen bond to O^-

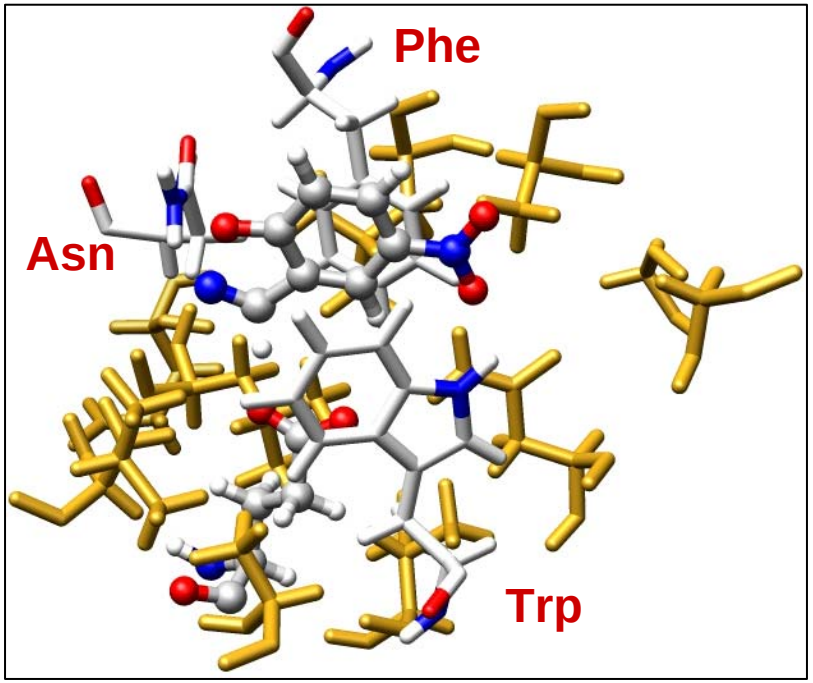


Strategy for enzyme design
Test case: Kemp elimination
Mechanism, performance
Deducing design strategies
Our current push

Inside-out design of enzymes

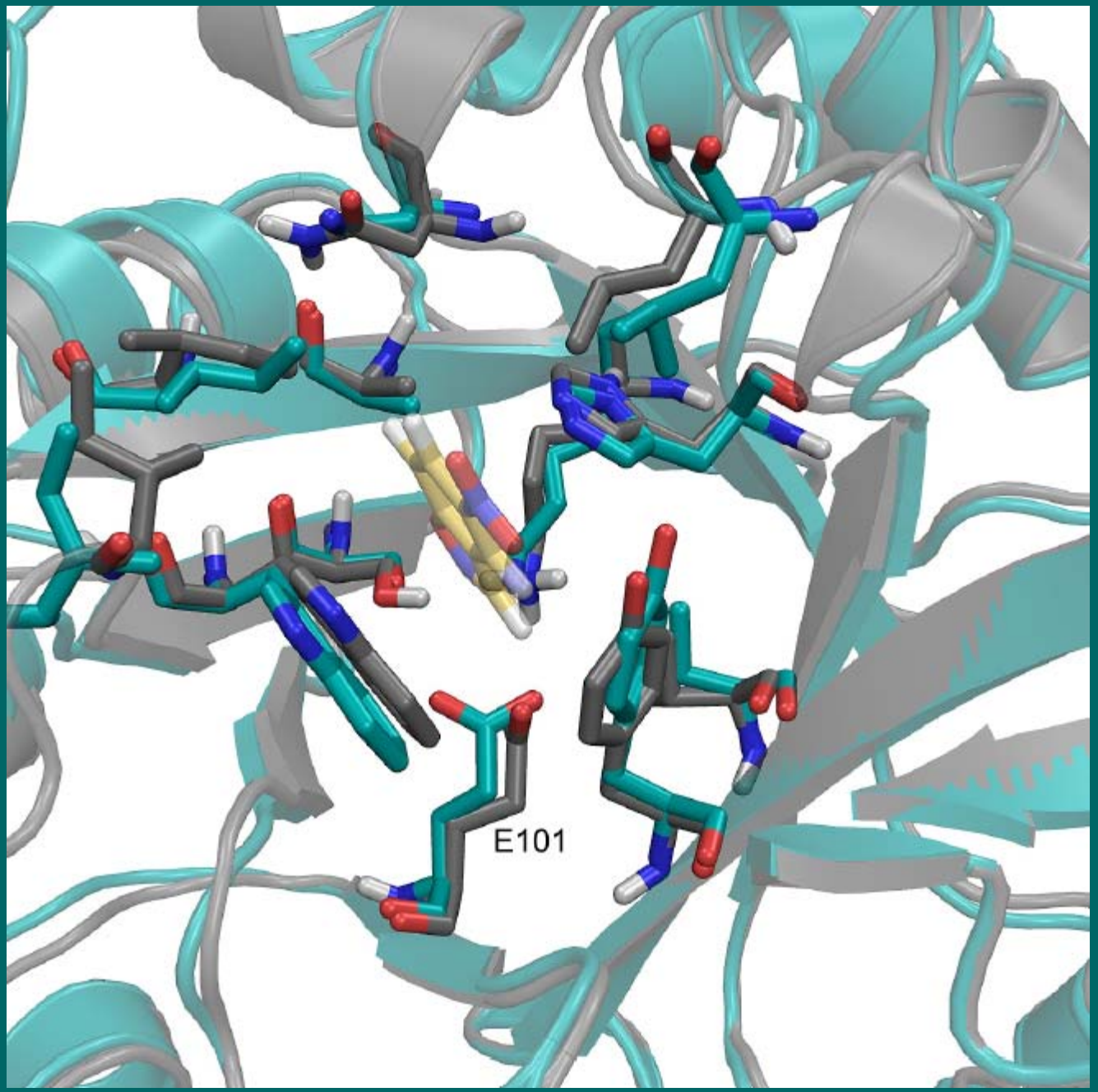
Raw design. Testing, evaluation, and improvement are to follow.





Yellow stuff: Leu, Ile, Val, Ala, Gly

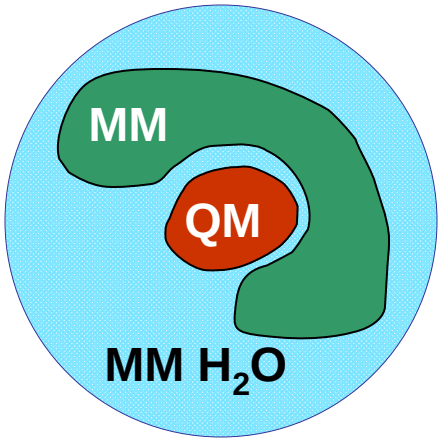
KE07: crystal structure (cyan) vs. design (grey). $RMSD_{backbone}$ = 0.32Å, $RMSD_{overall}$ = 0.95Å



Testing: QM/MM Monte Carlo

System:

chopped protein (ca. 300 residues)
22 Å TIP4P water cap: (400-500 water molecules)
QM-region: the substrate and the base
MM-region: the rest of the protein and solvent



QM/MM Metropolis Monte Carlo at 25˚C and 1 atm:

5 M solvent equilibration, 10 M full eq., 25 M sampling

Degrees of freedom:

QM-region - bond lengths, angles, dihedrals
MM-protein - angles and dihedrals
Solvent - translations and rotations

QM: PDDG-PM3 - Pair-wise Distance Directed Gaussian

(A single small Gaussian function is added to pair-wise core repulsion function)

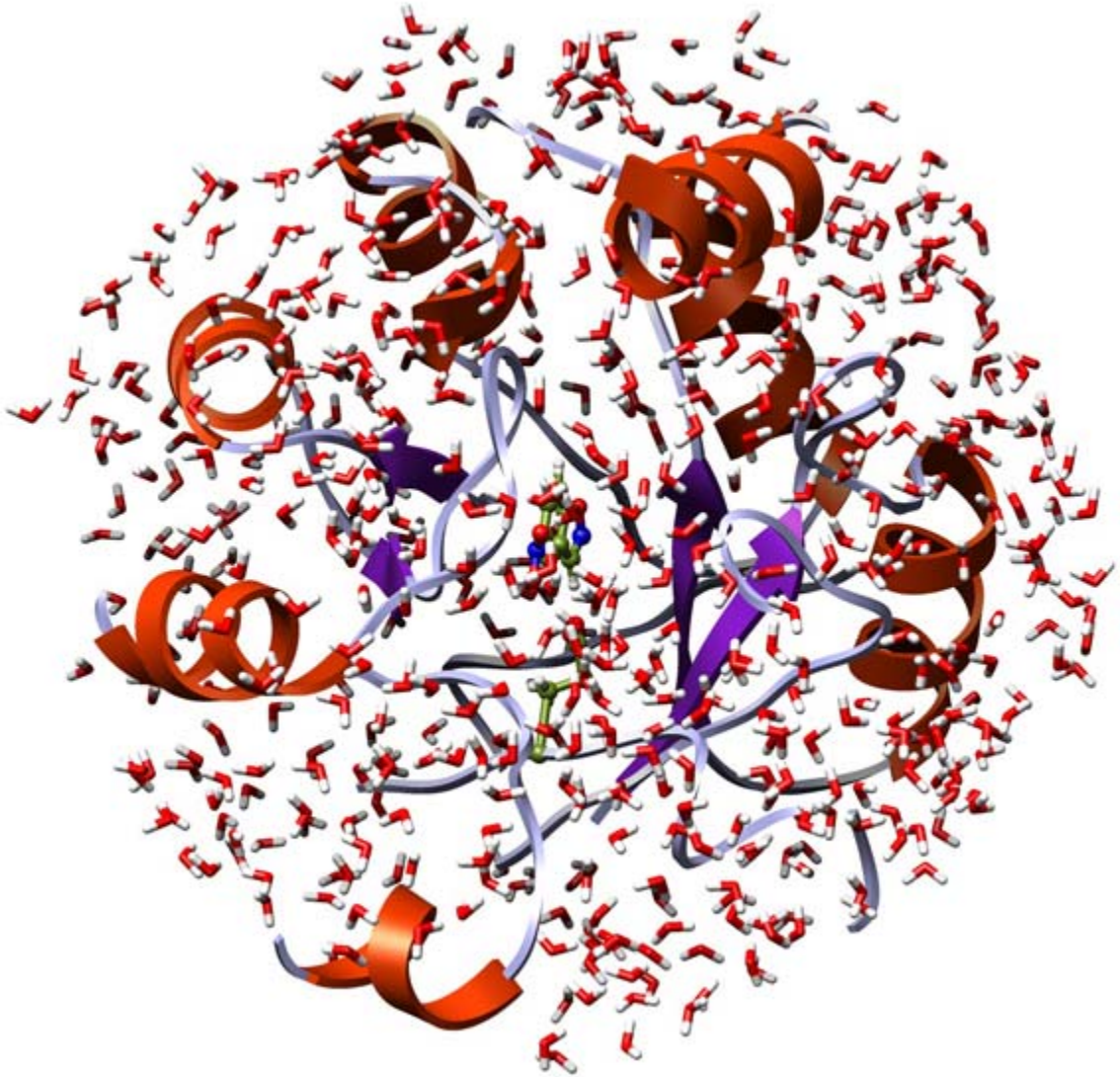
$$U_{ss}, U_{pp}, \beta_s, \beta_p, \zeta_s, \zeta_p, \alpha, a_1, b_1, c_1, a_2, b_2, c_2, P_1, P_2, D_1, D_2$$

MNDO **PM3** **PDDG**

MM: OPLS-force field

QM-MM interface: link atom approach, CM charges for Coulomb, and LJ

System prepared for simulations:



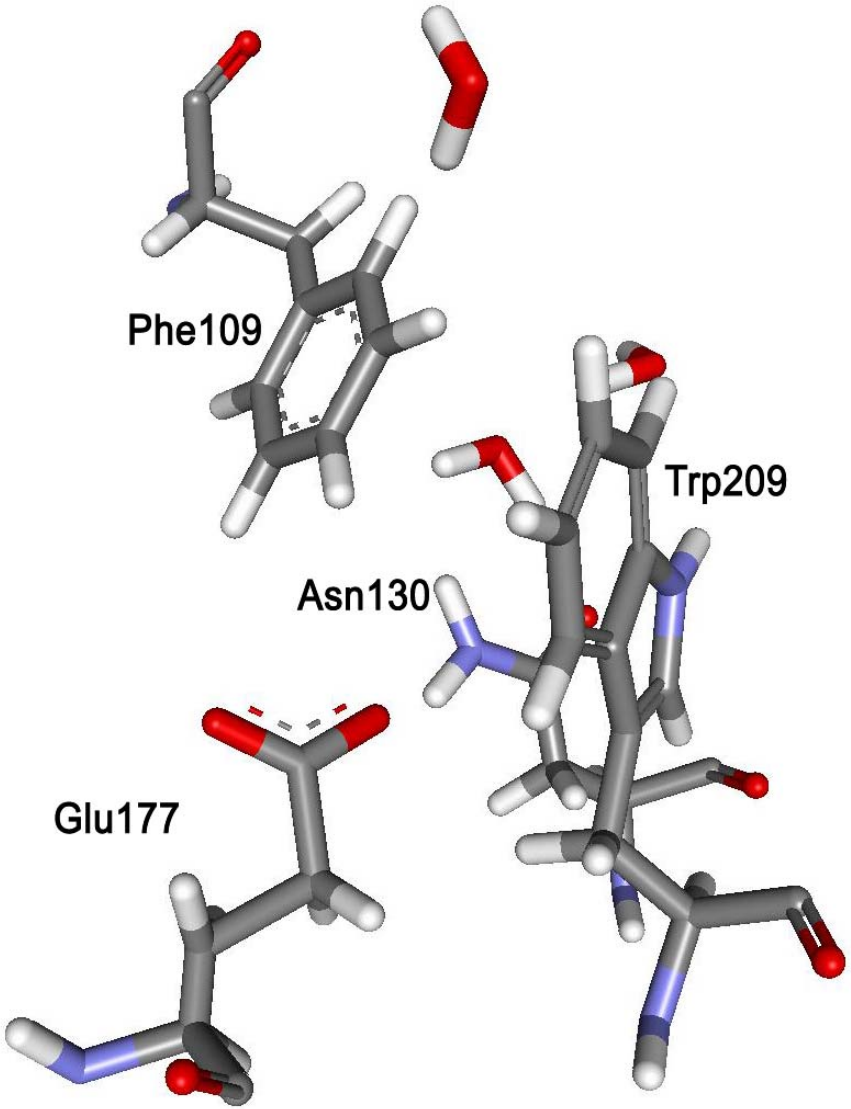
Alexandrova et al. *J. Am. Chem. Soc.* 130 (2008), 15907-15915.

QM/MM MC relaxation without the substrate

1) The base, Glu177 is properly positioned, isolated from the solvent, not forming any salt bridges

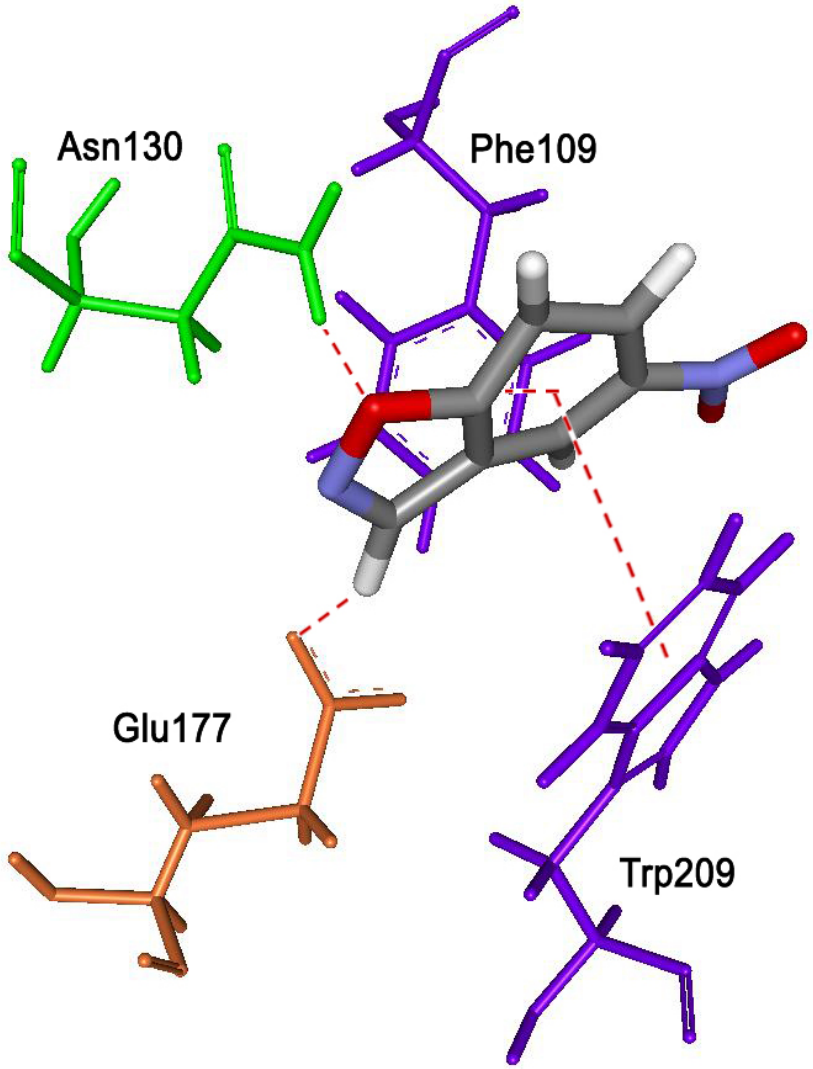
2) There is space for the substrate

3) All other residues are in correct orientations

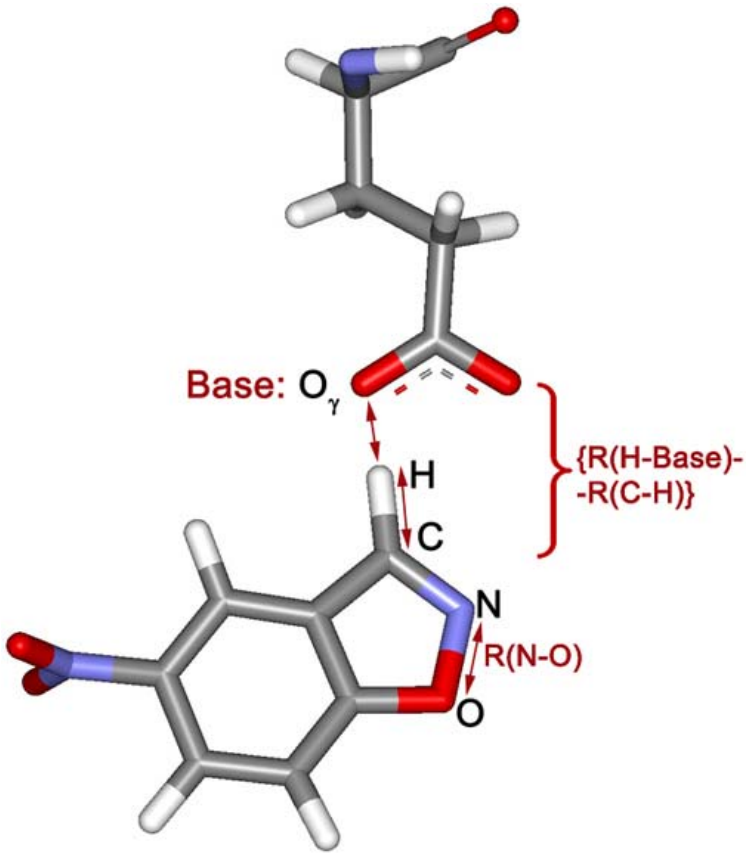


QM/MM MC relaxation with the substrate

1) π-stacking is present
2) Glu177- correct protonation state
3) Asn130 interacts with O
4) No water at the reaction site



Free Energy Perturbation for mechanism and rate



1. Reaction coordinates:

R(N-O), {R(C-H)-(R(O-H)}

2. Drive the reaction along the reaction coordinates: increment of 0.02-0.04 Å

3. Compute ΔG per each step:

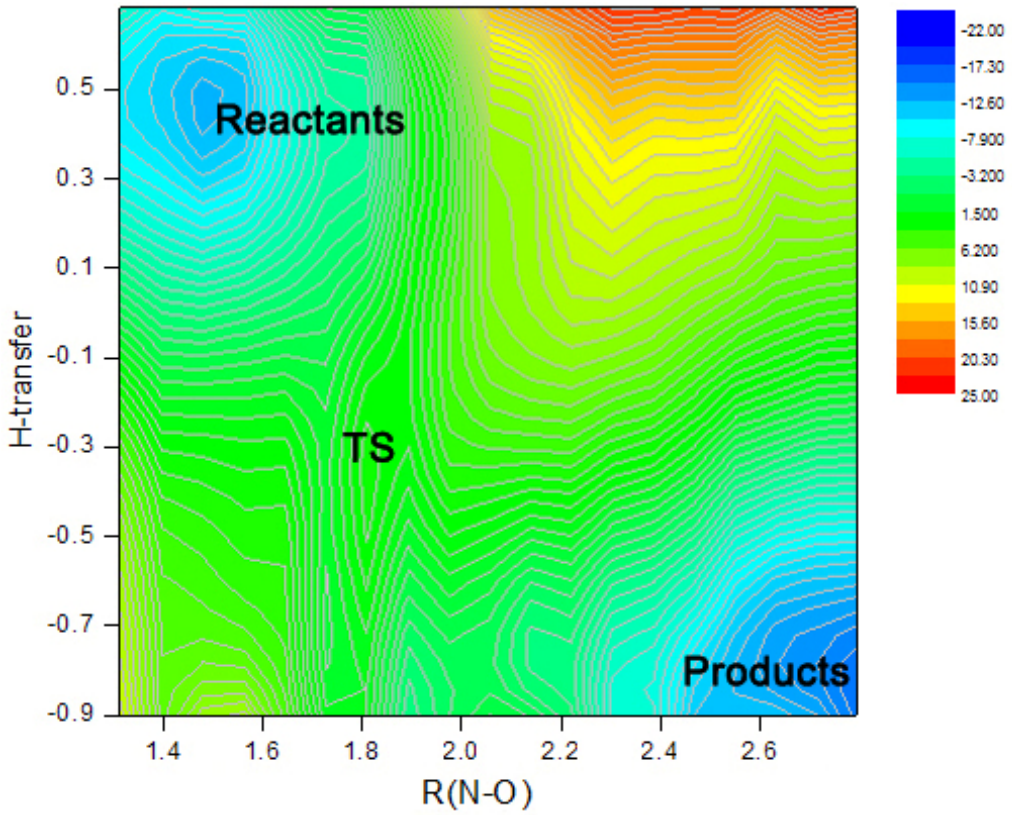
$$\Delta G(AtoB) = G_B - G_A = -k_b T \ln \left\langle \exp\left(-\frac{E_B - E_A}{k_b T} \right) \right\rangle_A$$

50 x 10^6 conf. QM/MM Monte Carlo at each step.

4. construct 1-D or 2-D free energy maps

→ **$\Delta G^{\ddagger}$**

Catalyzed Kemp elimination: FEP results

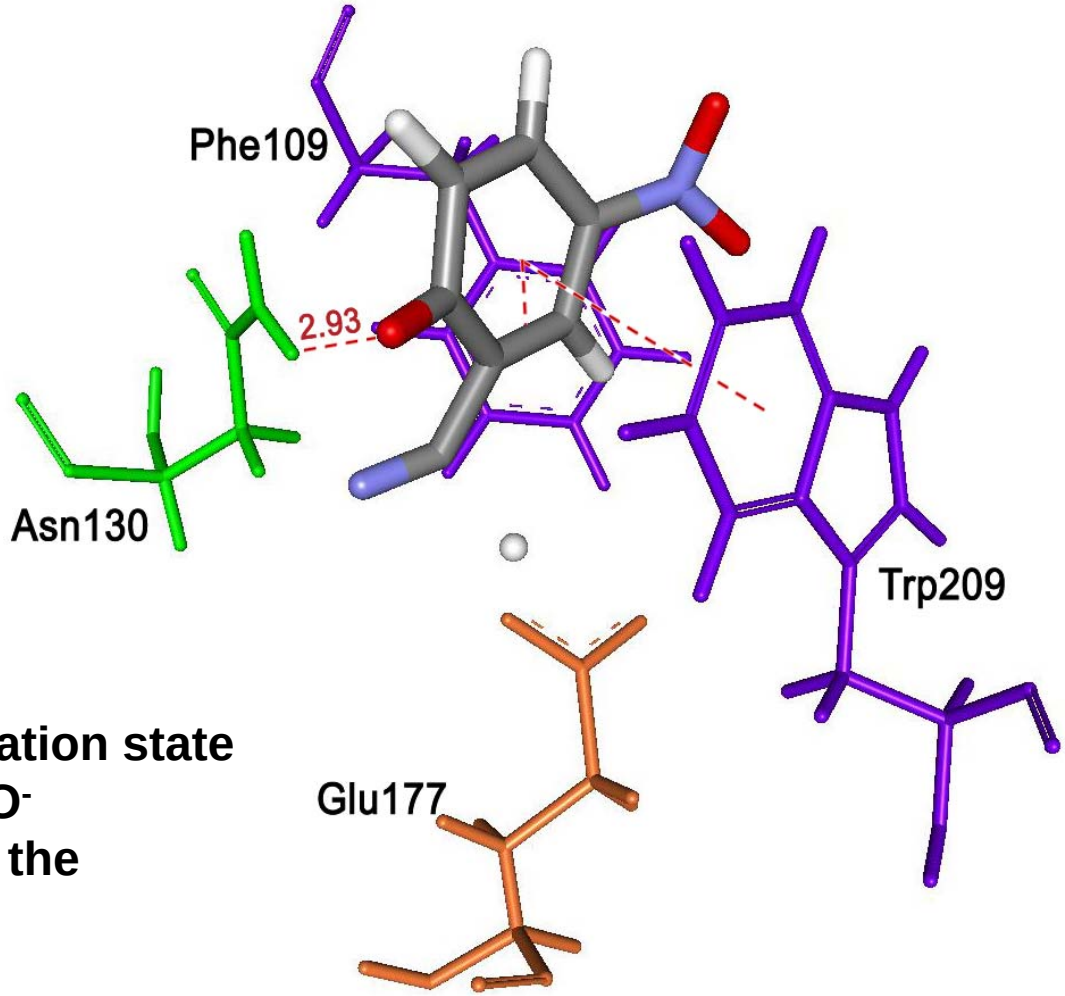


Concerted, single-step mechanism.
Asymmetric TS. H-transfer driven process.
$\Delta G^{\ddagger}$ = 13.5 kcal/mol ΔG = -4.6 kcal/mol

Alexandrova et al. *J. Am. Chem. Soc.* 130 (2008), 15907-15915.

snap-shot from MC: region of the products

We can look at any region of the map to see what the system is doing



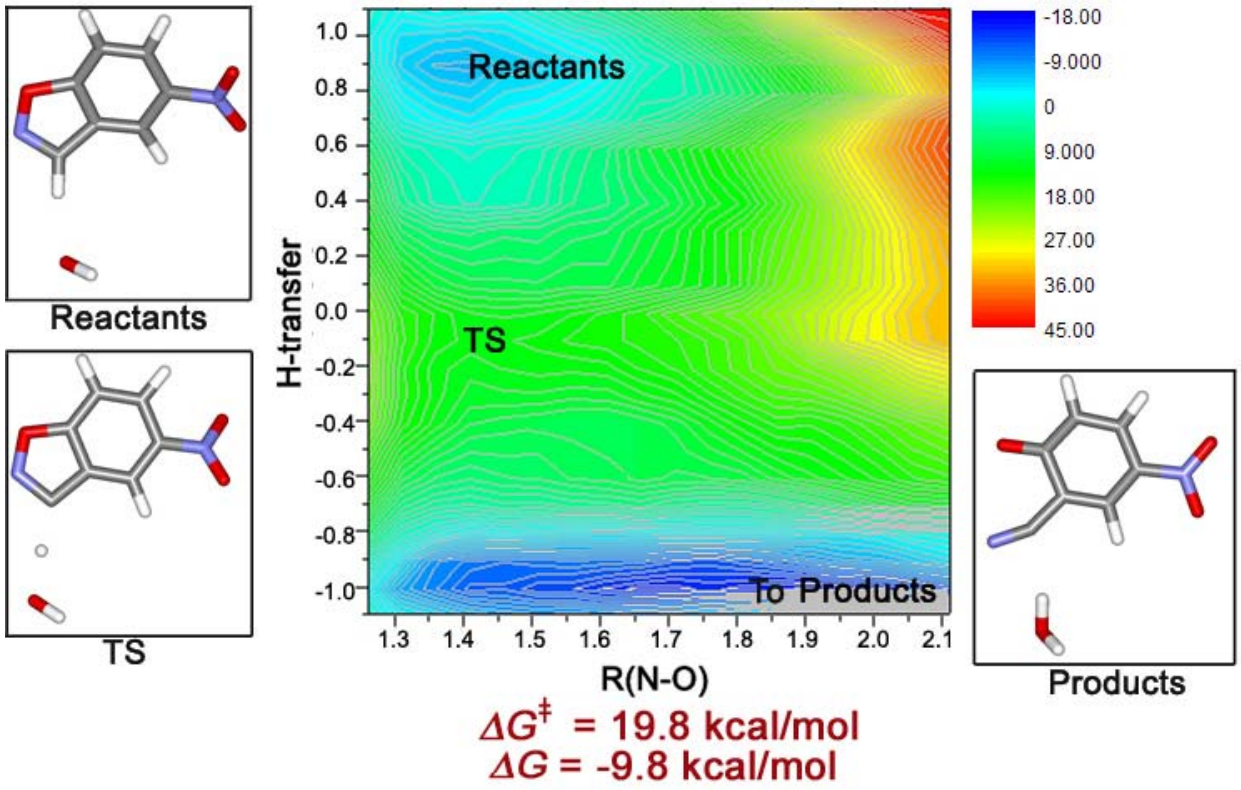
1) π-stacking
2) Glu177- correct protonation state
3) Asn130 interacts with O^- (expected stabilization of the product)

$\Delta G^{\ddagger} = 13.5$ kcal/mol

Is it good or bad?...

Strategy for enzyme design
Test case: Kemp elimination
Mechanism, performance
Deducing design strategies
Our current push

Uncatalyzed reaction in water:

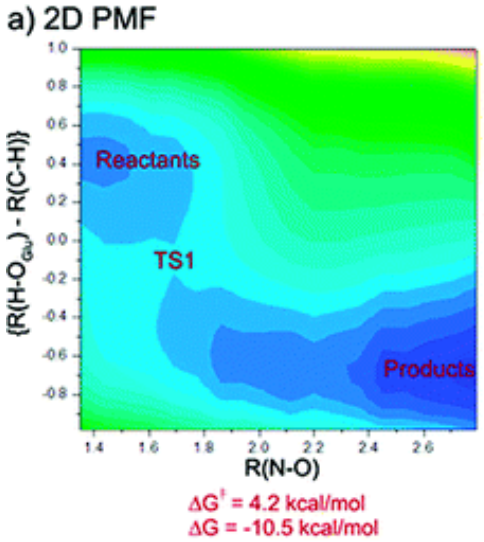


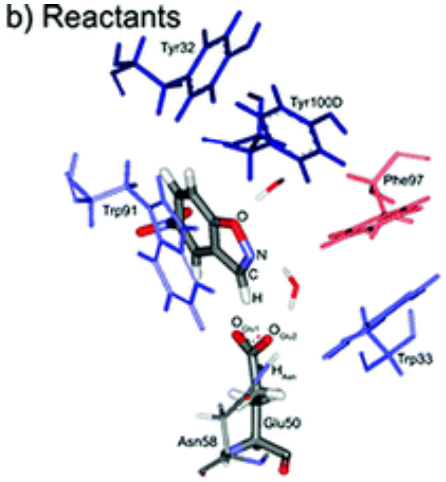
compare to $\Delta G^{\ddagger}$ = 13.5 kcal/mol => 1a53 is an active enzyme

Experiment also showed catalytic activity: k_{cat}/k_{uncat} is 2.5 x 10^5

Enzyme design is a very delicate business:

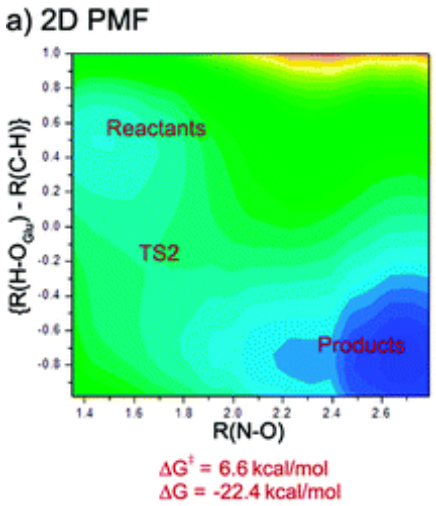
34E4 catalytic antibody:

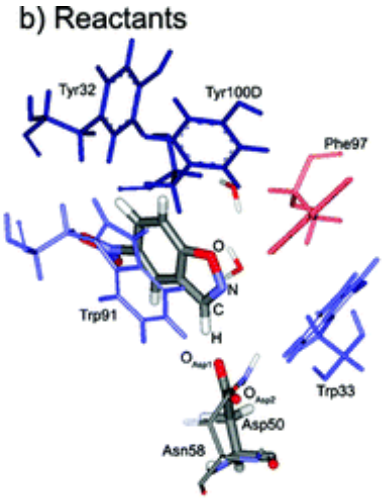




Its E50D variant:

1 small mutation =
30-fold rate drop





S.N. Thorn et al. *Nature* 373 (1995), 228-230
Alexandrova et al. *J. Phys. Chem. B* 113 (2009), 497-504

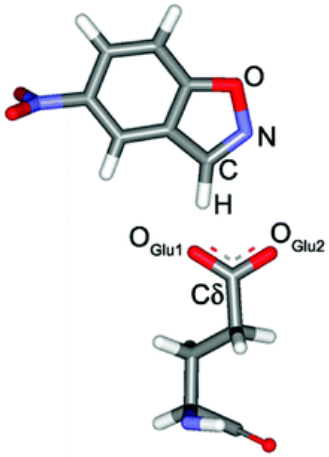
Strategy for enzyme design
Test case: Kemp elimination
Mechanism, performance
Deducing design strategies
Our current push

Enzyme design is a very delicate business:

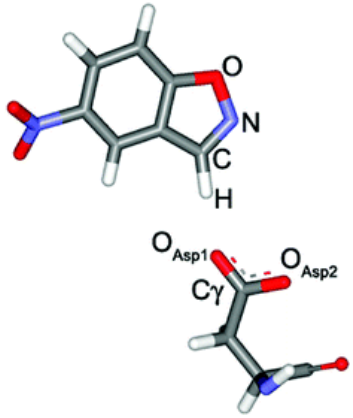
1 small mutation = 30-fold rate drop

Slightly suboptimal contact with the base

34E4

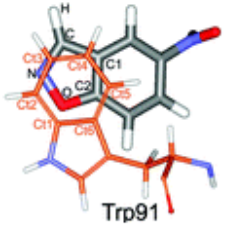


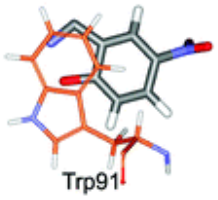
E50D variant



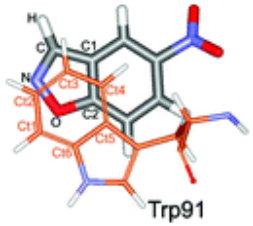
Slightly suboptimal π-stacking

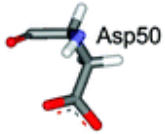
34E4

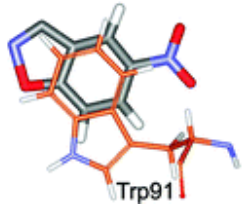




E50D variant

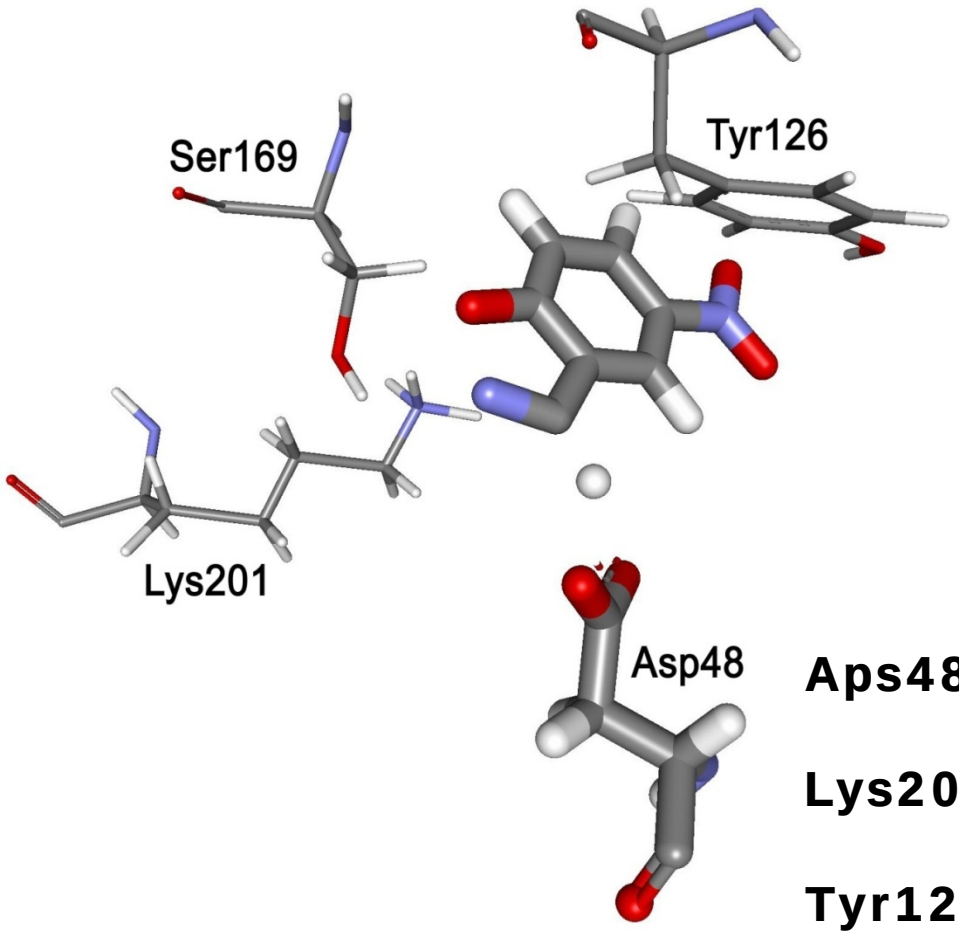


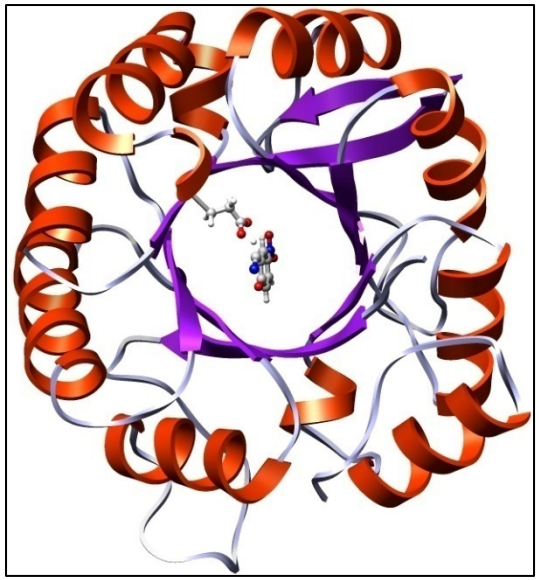




Alexandrova et al. *J. Phys. Chem. B* 113 (2009), 497-504

KE16
An enzyme with a problem





Aps48: general base

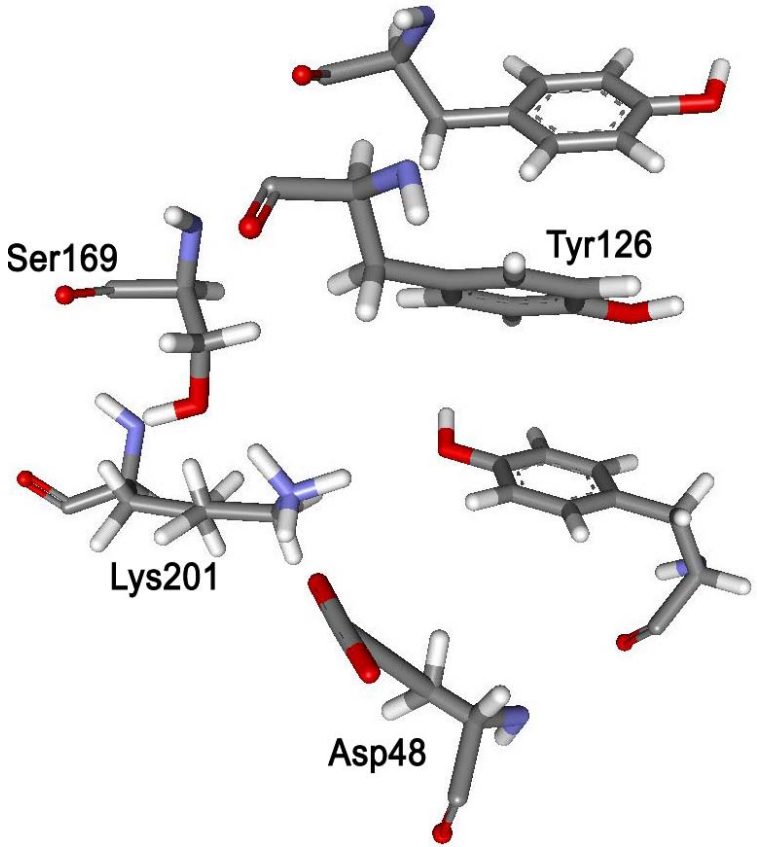
Lys201 or Ser169: stabilization of O^-

Tyr126: weak π-stacking

D. Rothlisberger et al. *Nature* 453 (2008), 109-195.

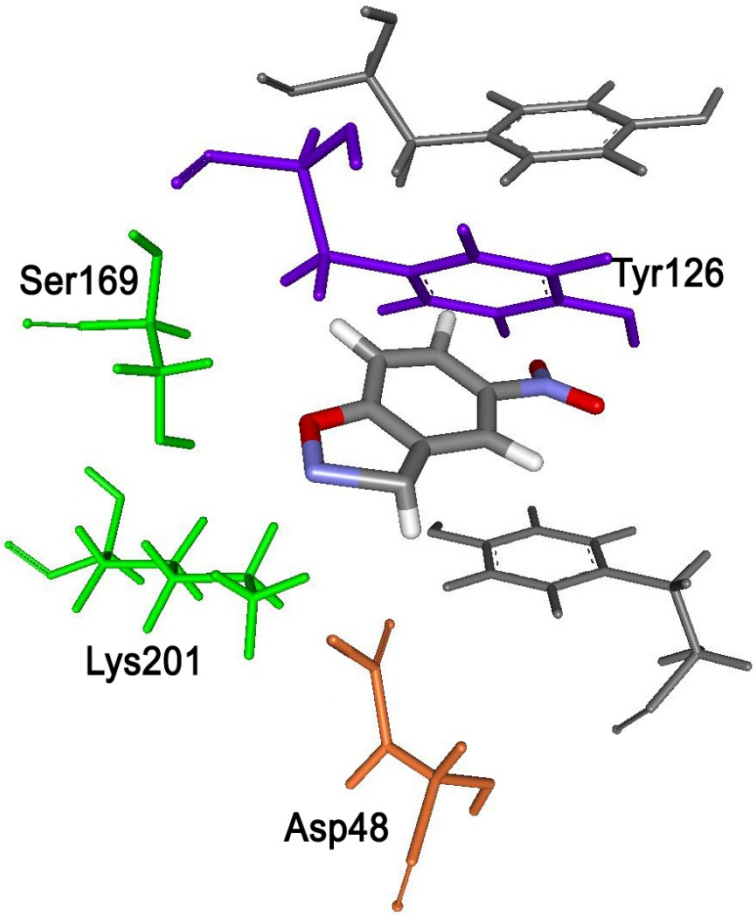
"An enzyme with a problem"

QM/MM MC relaxation without the substrate



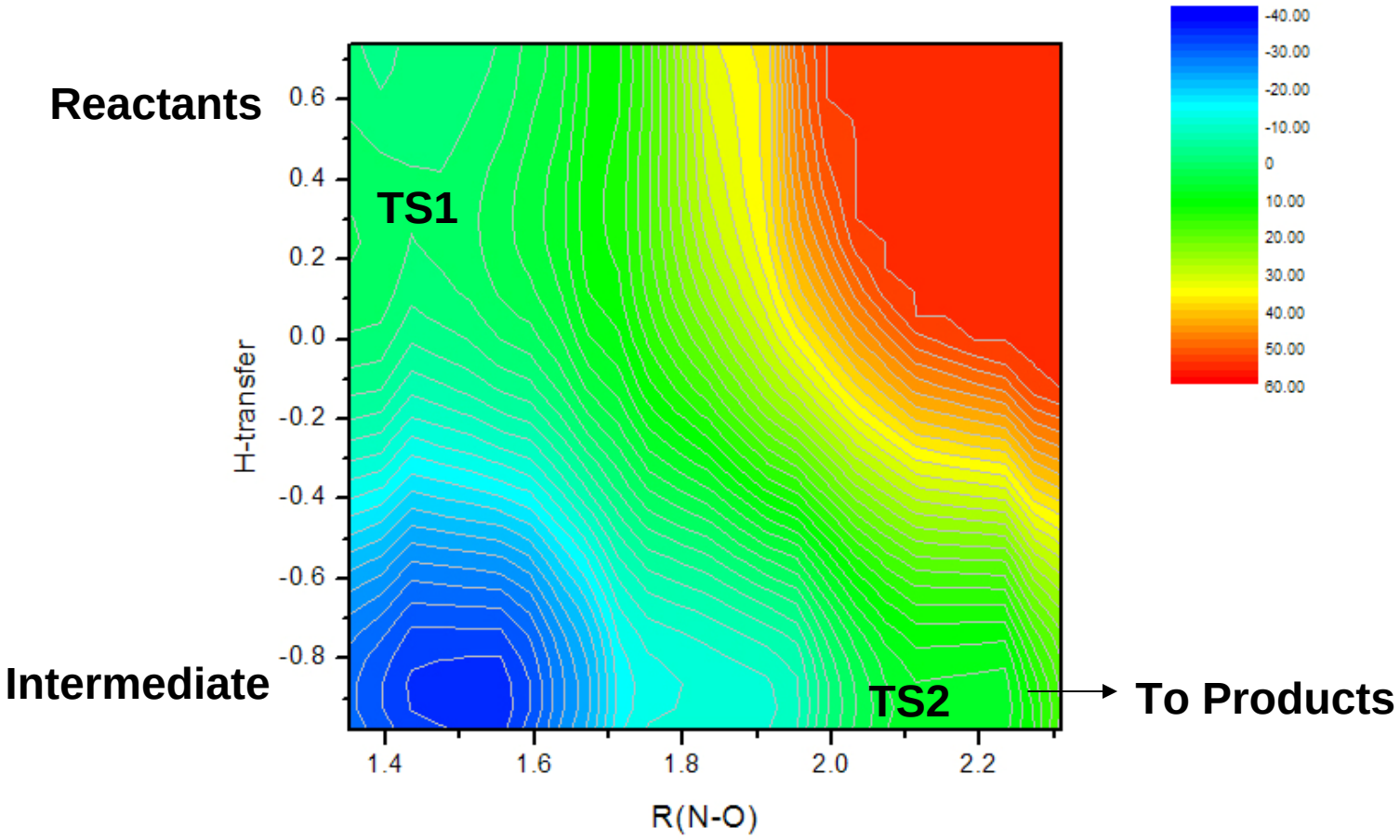
"An enzyme with a problem"

QM/MM MC relaxation with the substrate:



Strategy for enzyme design
Test case: Kemp elimination
Mechanism, performance
Deducing design strategies
Our current push

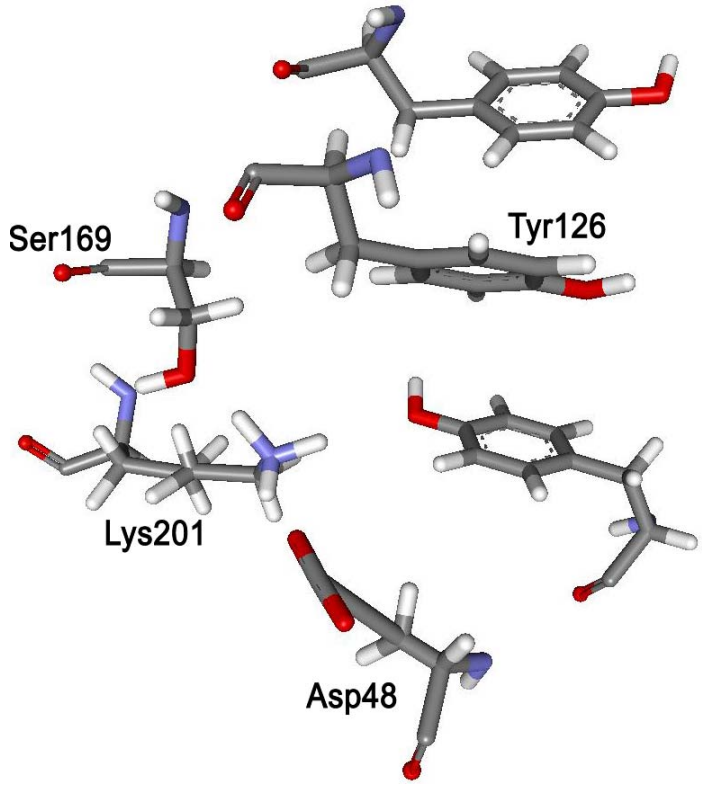
"An enzyme with a problem"



The mechanism in 1thf is step-wise: H-transfer happens first.

Strategy for enzyme design
Test case: Kemp elimination
Mechanism, performance
Deducing design strategies
Our current push

"An enzyme with a problem"



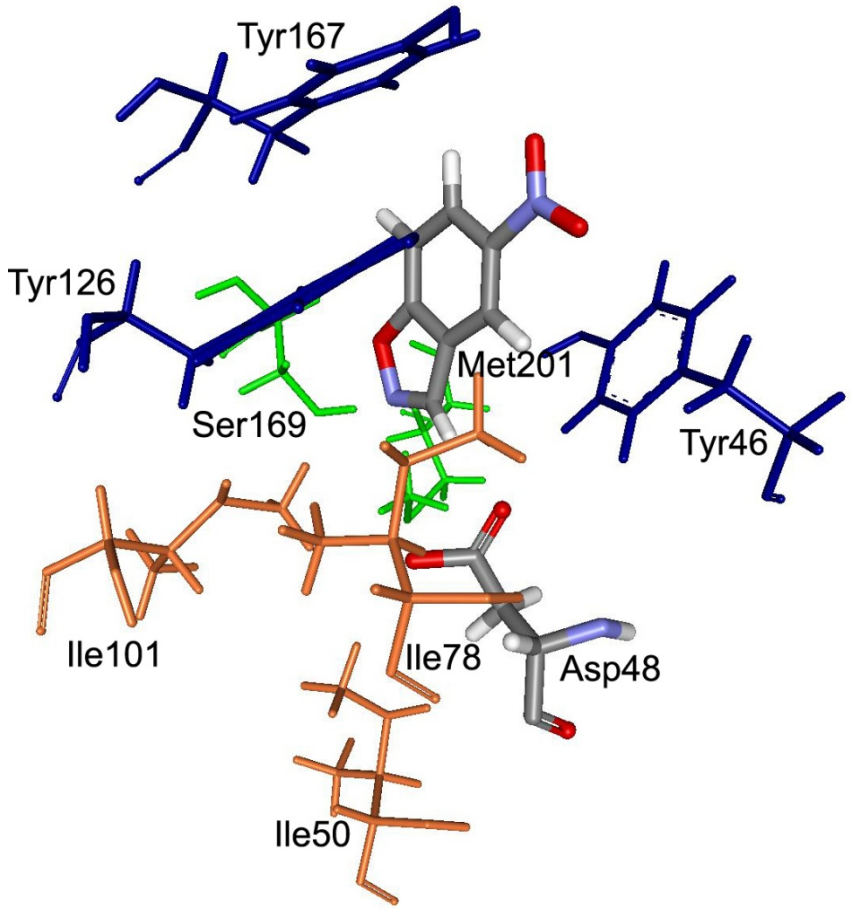
Lys is a bad residue for being in position 201:

1) Tries to protonate the base, and 2) Stabilizes the intermediate too much

Experimentally observed modest activity, k_{cat}/k_{uncat} is 5.2 x 10^3

Proposal for rescue:

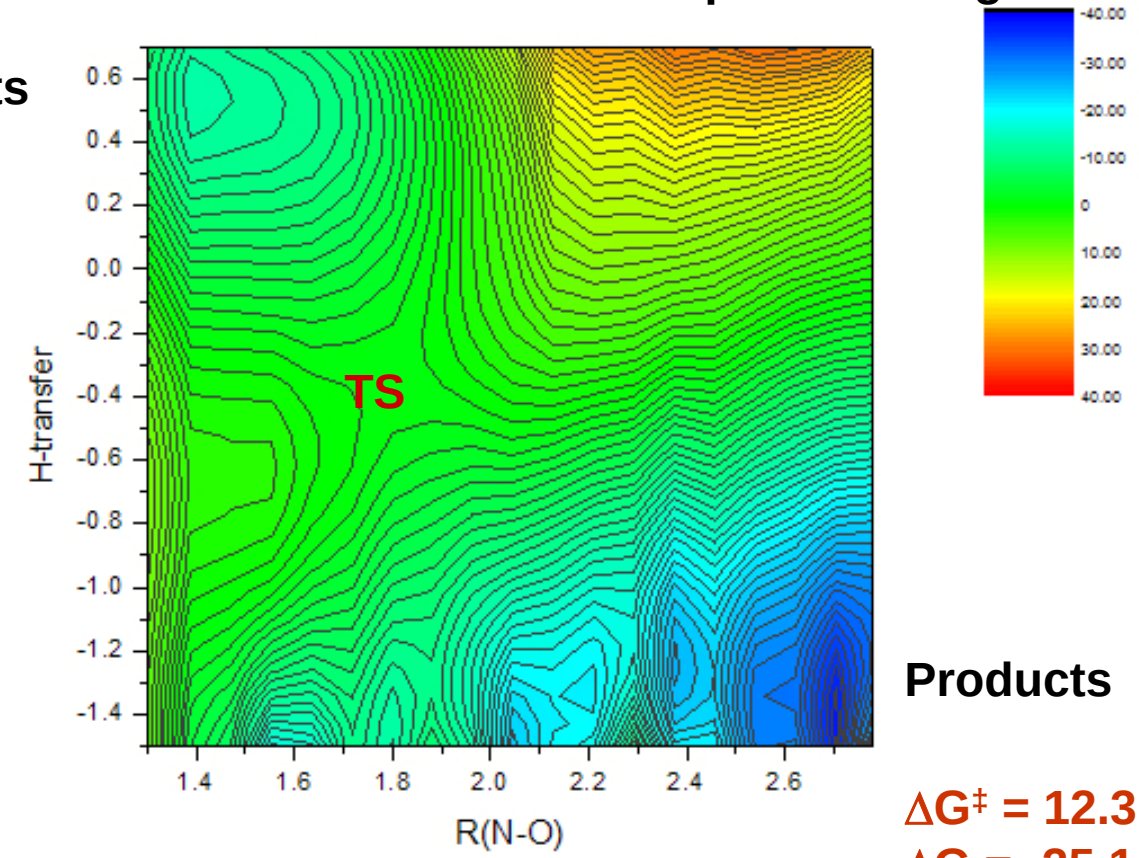
Lys201 ⇒ Met



Proposed mutation helped:

Intermediate is avoided in the improved design

Reactants

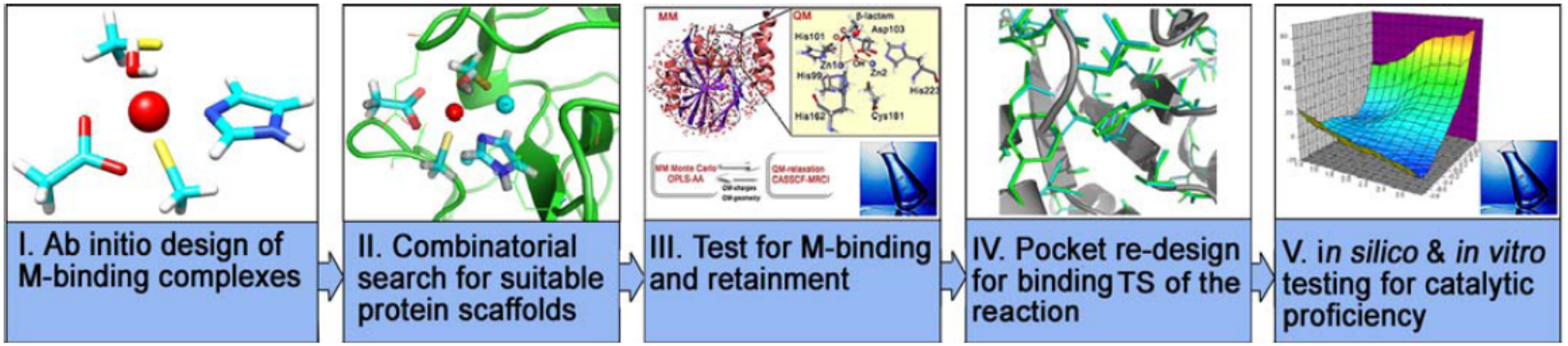


Products

$\Delta G^{\ddagger}$ = 12.3 kcal/mol
ΔG = -25.1 kcal/mol

Strategy for enzyme design
Test case: Kemp elimination
Mechanism, performance
Deducing design strategies
Our current push

Metallo-proteins and metallo-enzymes



Additional dimension:
Metal and its electronic structure

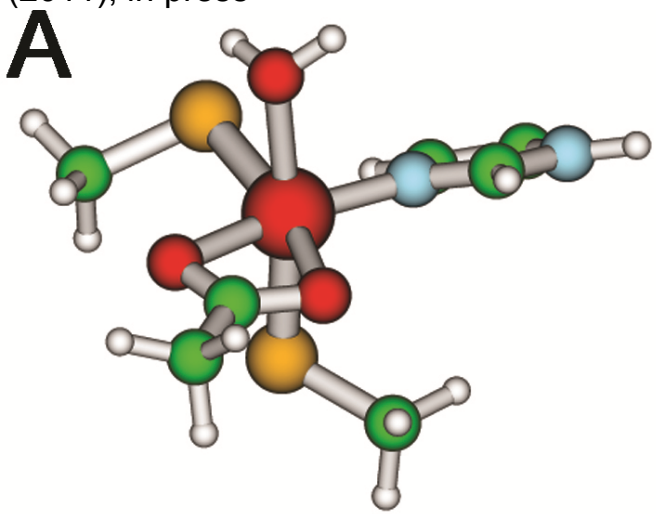
Original inside-out protocol

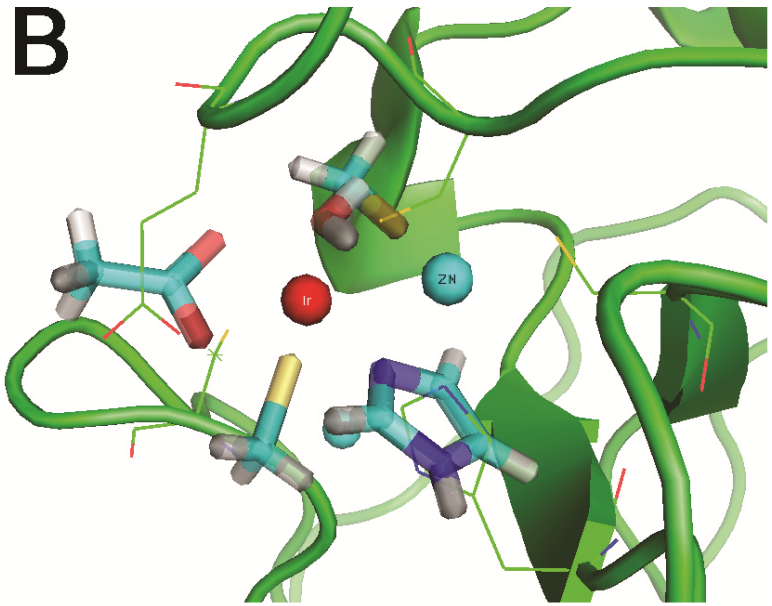
Need for QM/MM stat mech throughout the protocol (starting from the installation of the metal)

Strategy for enzyme design
Test case: Kemp elimination
Mechanism, performance
Deducing design strategies
Our current push

1. Search for host proteins

Bioinformatics 27 (2011), in press





Ab initio complex ***Protein that can host it***

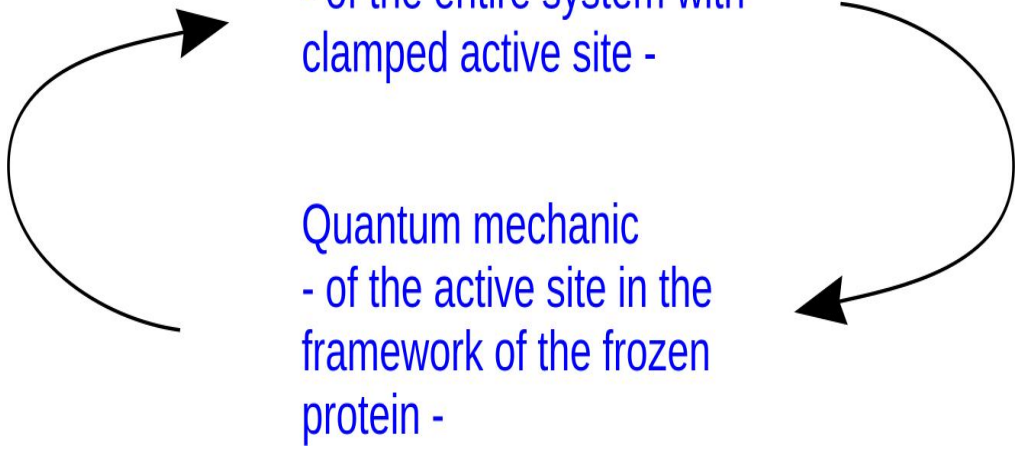
Subgraph isomorphism algorithm with incorporated uncertainty:

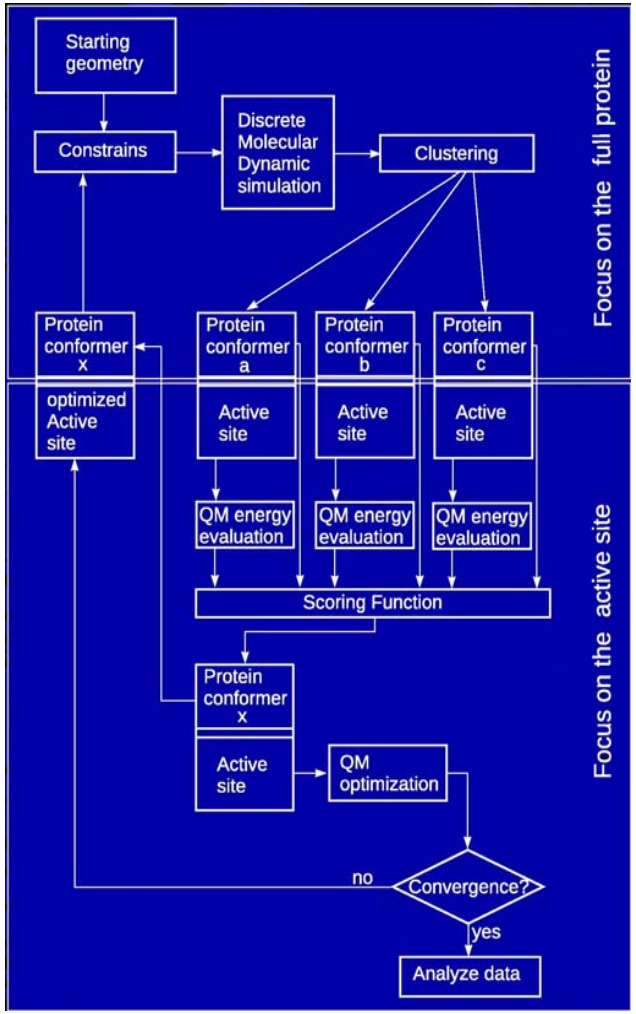
$$W_i = e^{-\frac{\left(\Delta q_i - \Delta t_j\right)^2}{\sigma^2}} \qquad W = \left(\prod_{i=1}^{N} W_i\right)^{1/N} \prod_{j=1}^{M} (1 - w_j)$$

searches through the entire PDB in less than 30 minutes, for a 15-atom query structure

2. QM/DMD: design and modeling

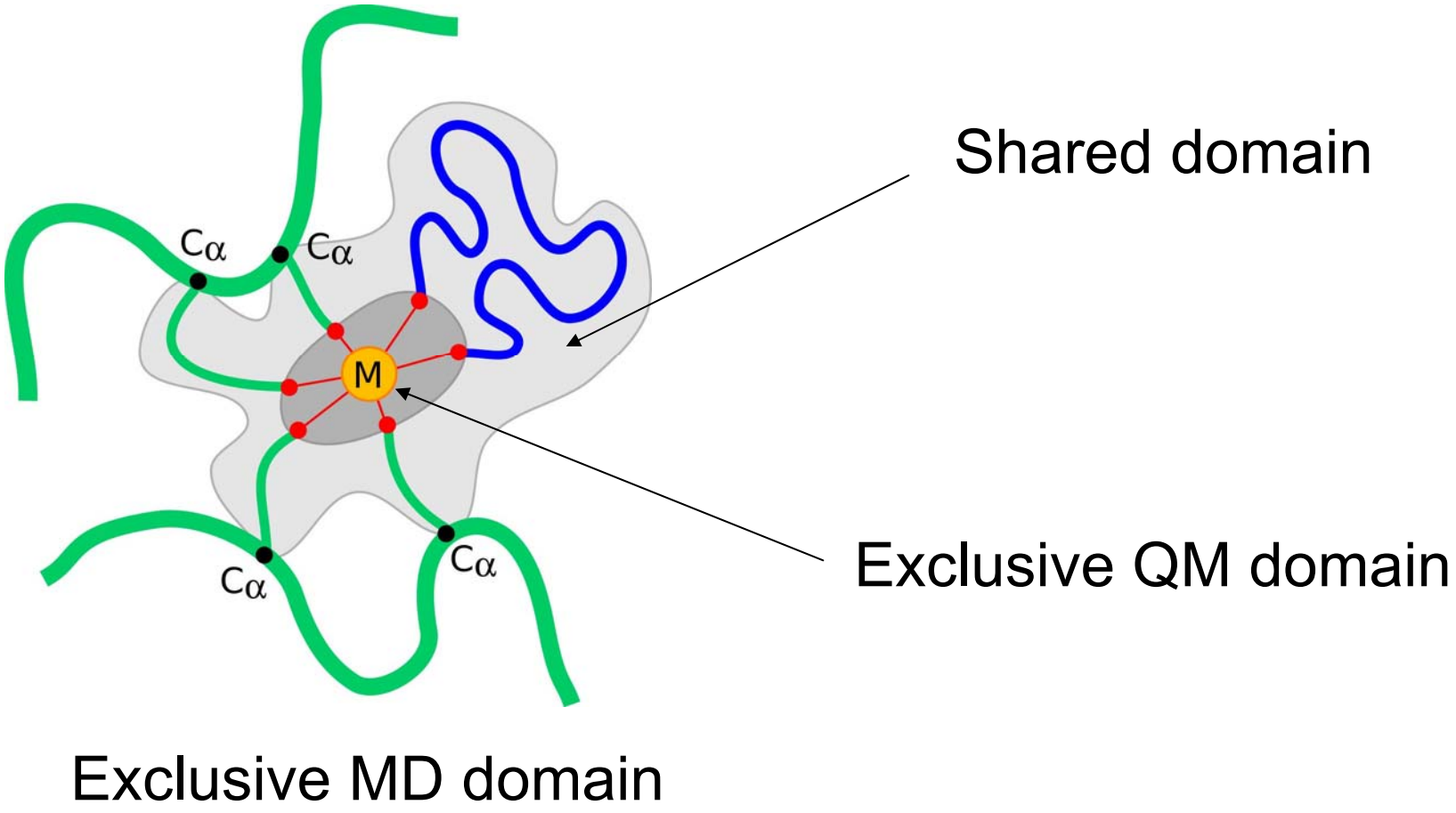
Our strategy:
Coupling an extensive sampling of the whole protein with a quantum mechanical description of the catalytic center.





Strategy for enzyme design
Test case: Kemp elimination
Mechanism, performance
Deducing design strategies
Our current push

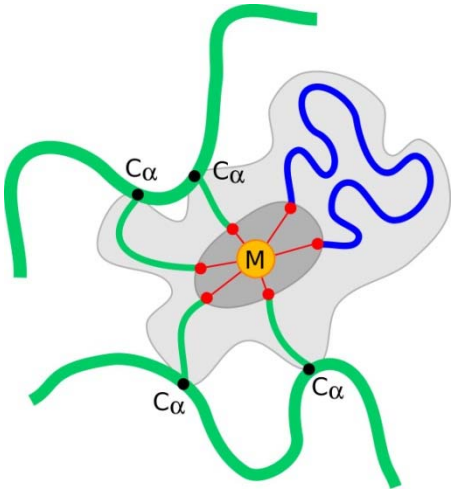
2. QM/DMD: design and modeling



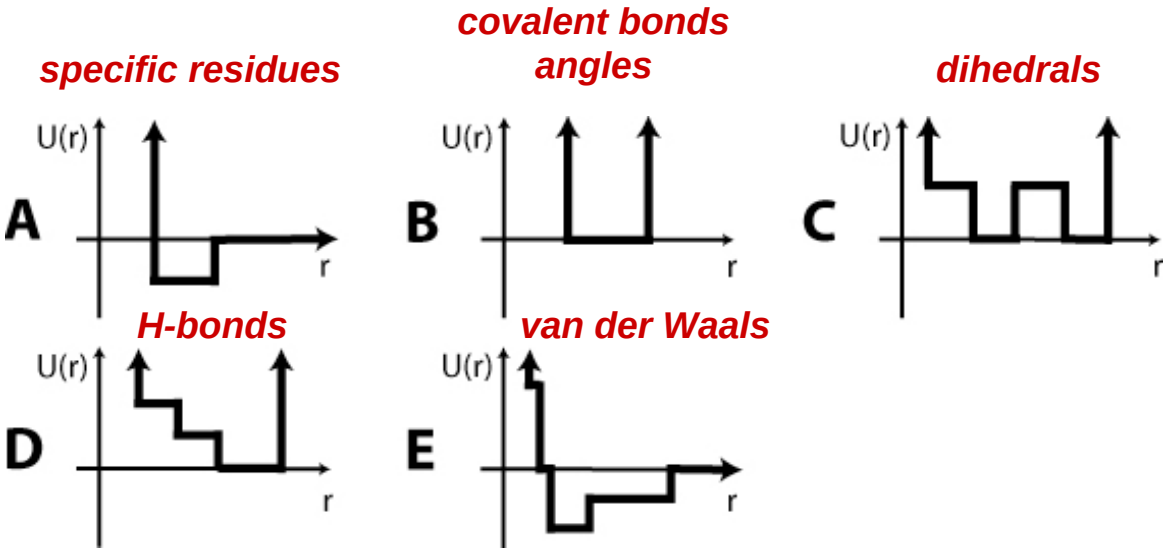
Strategy for enzyme design
Test case: Kemp elimination
Mechanism, performance
Deducing design strategies
Our current push

2. QM/DMD: design and modeling

DMD part: extensive sampling and clustering



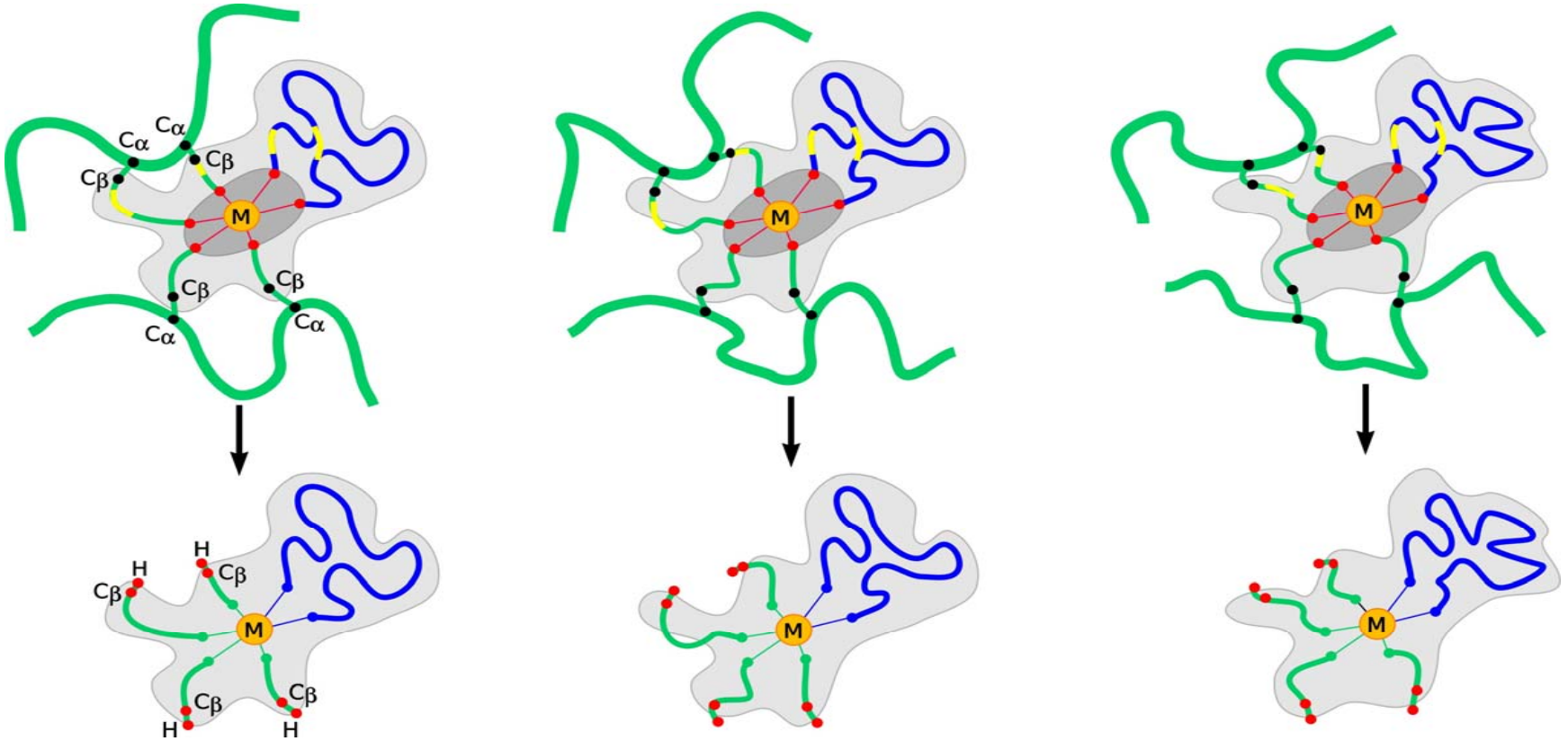
Interaction potentials:



Dokholyan, N.V., Buldyrev, S.V., Stanley, H.E., and Shakhnovich, E.I., Fold. Des. 3 (6), 577 (1998).
Ding F. et al. J. Mol. Biol 350 (5), 1035 (2005). Dokholyan, N. V., Curr. Opin. Struct. Biol. 16 (1), 79 (2006).
Proctor E.A. Ding F. and Dokholyan N.V. Comput. Mol. Sci. 1 80 (2011).

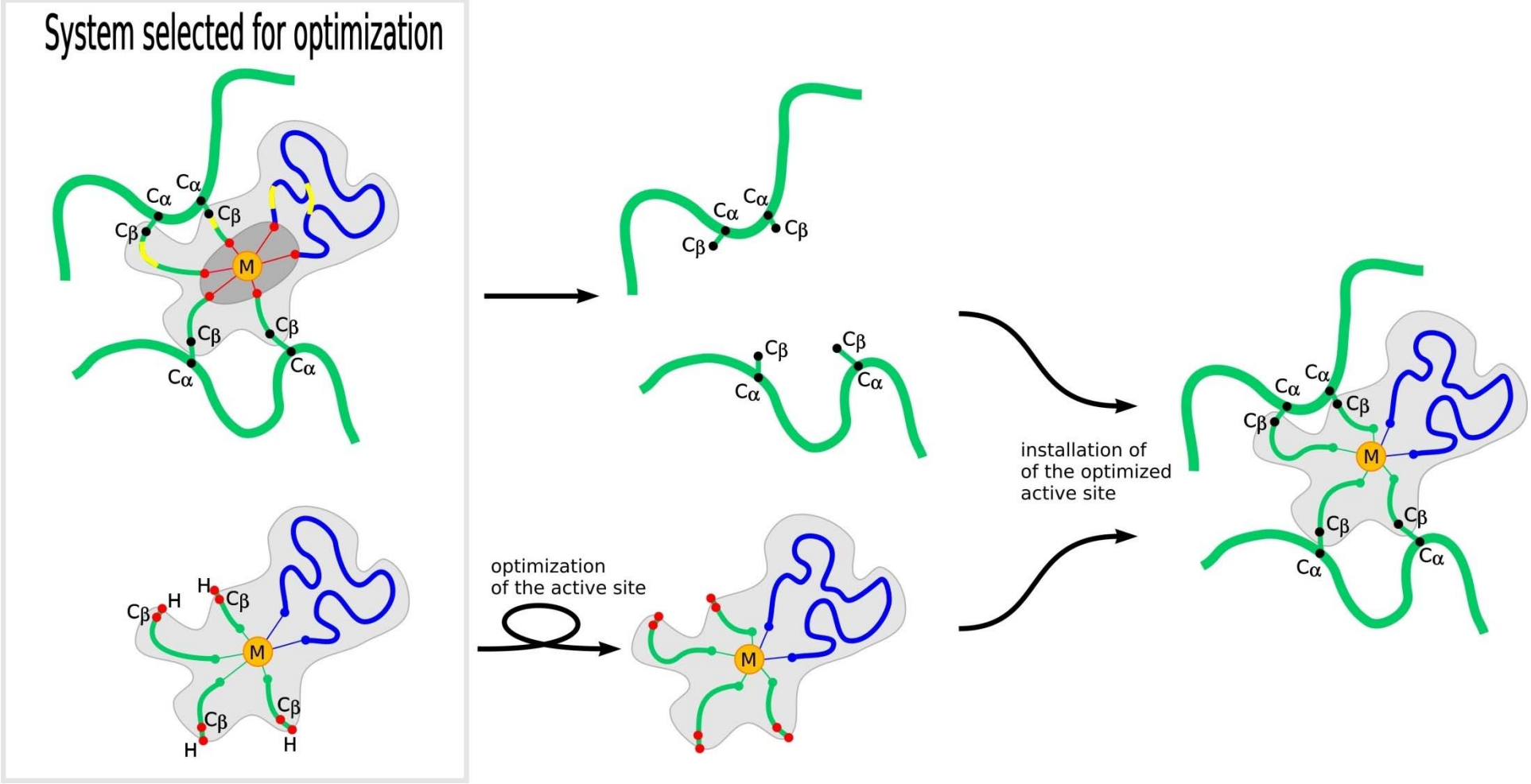
2. QM/DMD: design and modeling

The poses resulting from the simulation are clustered according to a similarity parameter. For each cluster, a representative is selected, its active site QM energy is computed.



Strategy for enzyme design
Test case: Kemp elimination
Mechanism, performance
Deducing design strategies
Our current push

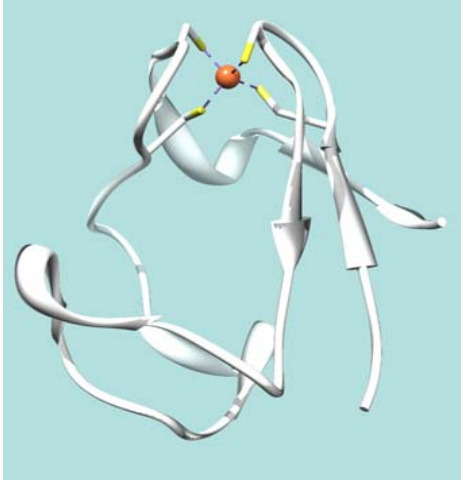
2. QM/DMD: design and modeling

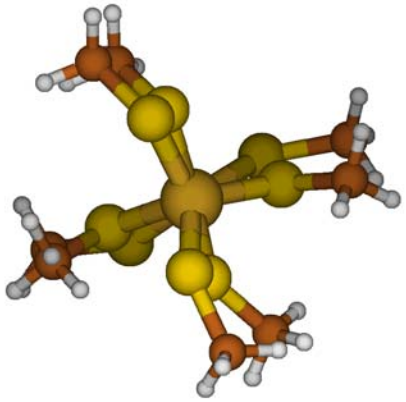


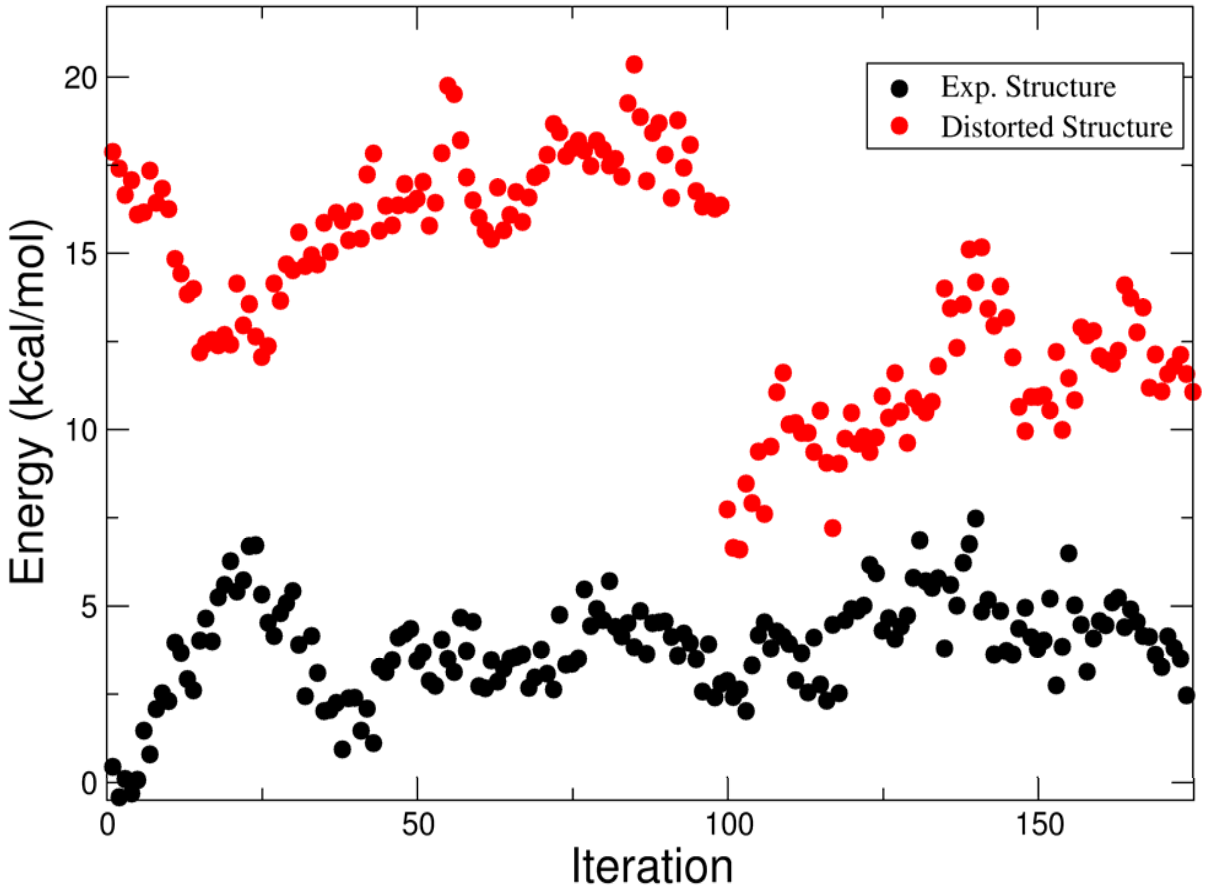
Hu L.H., Söderhjelm P. and Ryde U. J. Chem. Theory Comput. 7 (3), 761 (2011)

Strategy for enzyme design
Test case: Kemp elimination
Mechanism, performance
Deducing design strategies
Our current push

2. QM/DMD: Test case: Rubredoxin

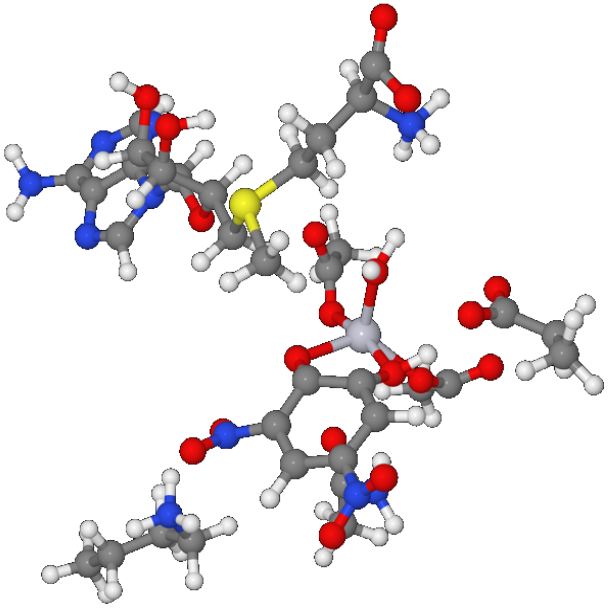




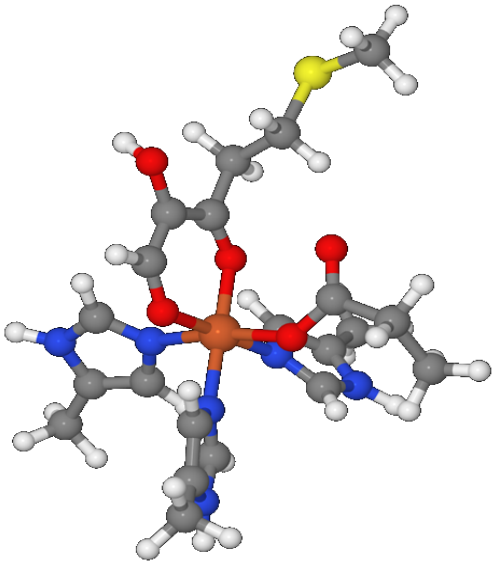


QM/DMD: current applications

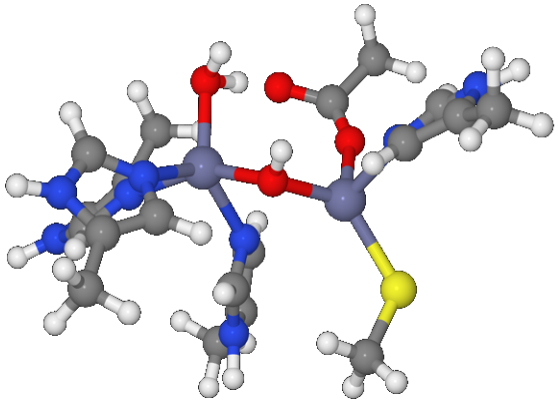
1) Proteins that exhibit radically different structure or function w/different metals



Catechol methyl-transferase



Acireductone dioxygenase



Lactamases and Ureases

2) Design of novel metallo-proteins

Summary

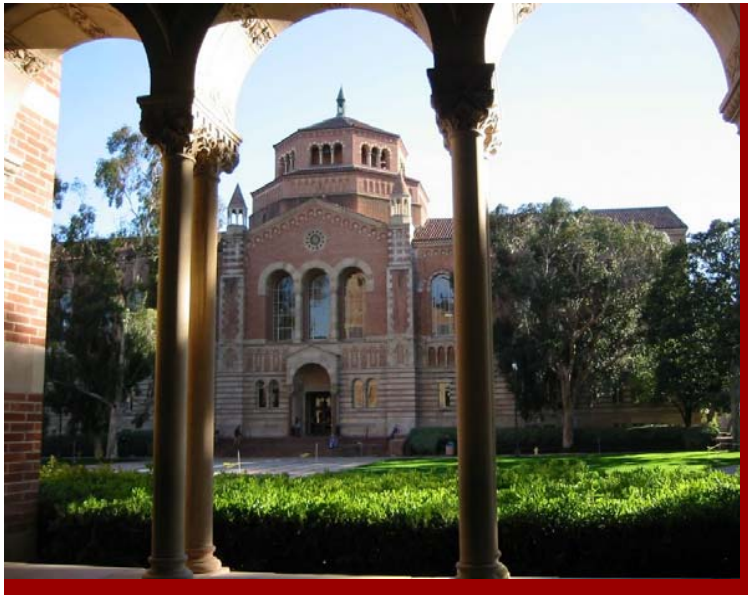
1. An efficient “inside-out” enzyme-design protocol has been developed

2. Multi-scale methodological framework is put together

3. Numerous active Kemp eliminazes have been designed

4. Theory successfully predicts (in)activity, relative rates, and mechanisms, and helps to rescue inactive enzymes.

5. We now got armed to model and design metallo-proteins too.



Acknowledgements:

Dr. William Jorgesen (Yale)
Dr. David Baker (U. Wash.)

Dr. Nikolay Dokholyan (UNC)
Dr. Wolfgang Peti (Brown U.)
Dr. Richard Wolfenden (UNC)

$$ DARPA
$$ UCLA

http://www.chem.ucla.edu/~ana/


Dr. Manuel Sparta

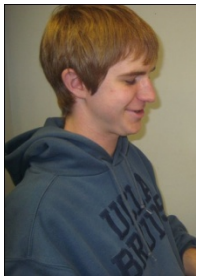

Dr. Jin Zhang


Crystal Valdez


Luan Vu


Mioy Hyunh


Diana Yu


Harry Wilfong


Ms. Sophia