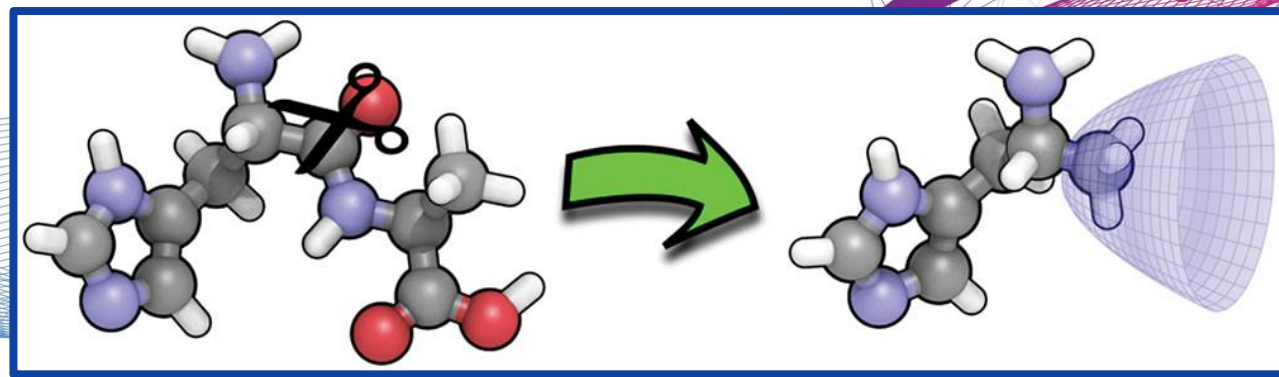


Towards More Physical Models of Enzyme Active Sites Using Quantum Mechanics

Alex Platt

Department of Chemistry, Colorado State University

Literature Seminar – November 16th, 2023



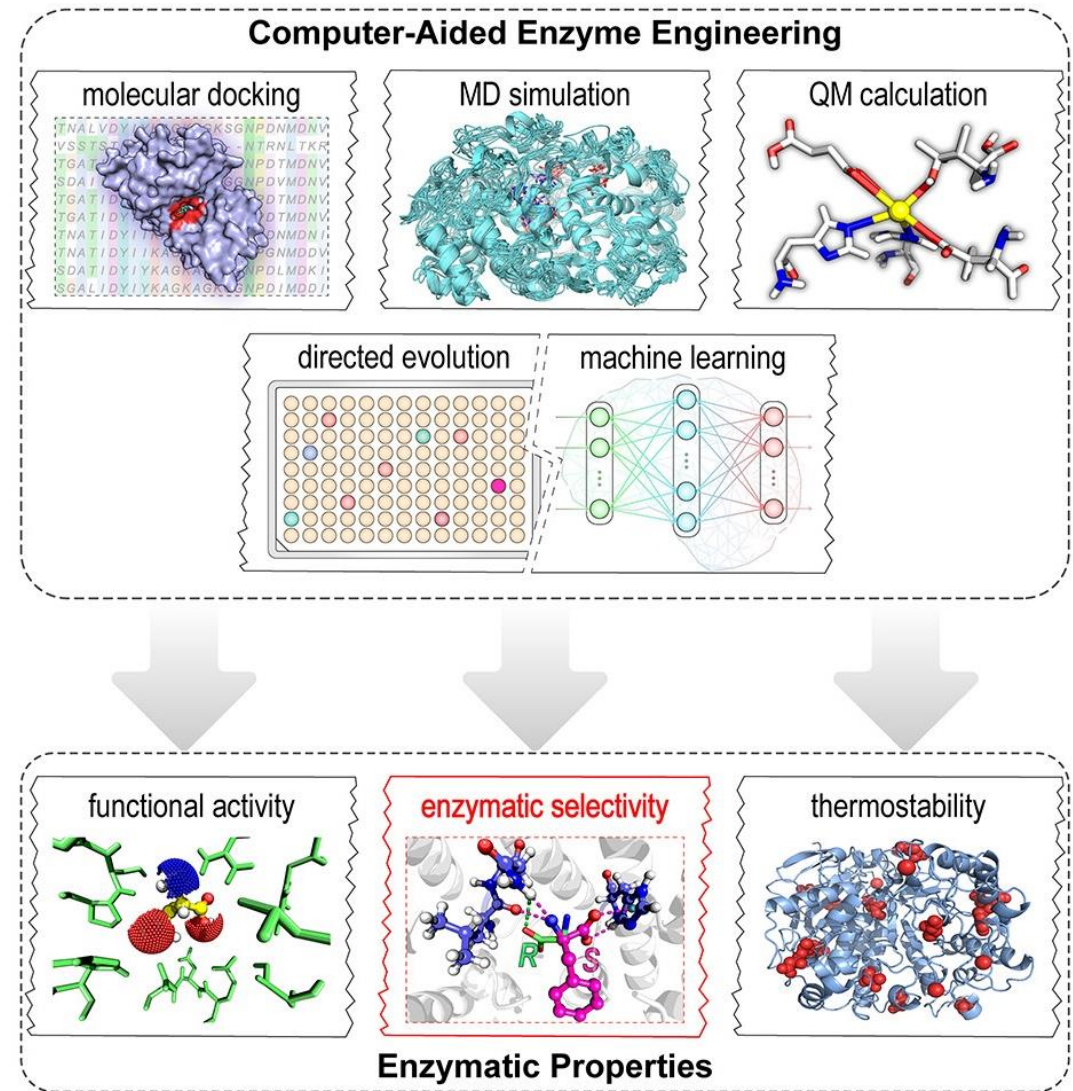
Why Study Enzymes Computationally?

Enzymatic Research

- Enzymes are highly efficient catalysts
 - Increase reaction rates up to 26 orders of magnitude
- Cell-free biocatalysis
 - Synthesize industrial molecules using enzymes
 - Pharmaceuticals, biofuels, etc.

Computational Advantage

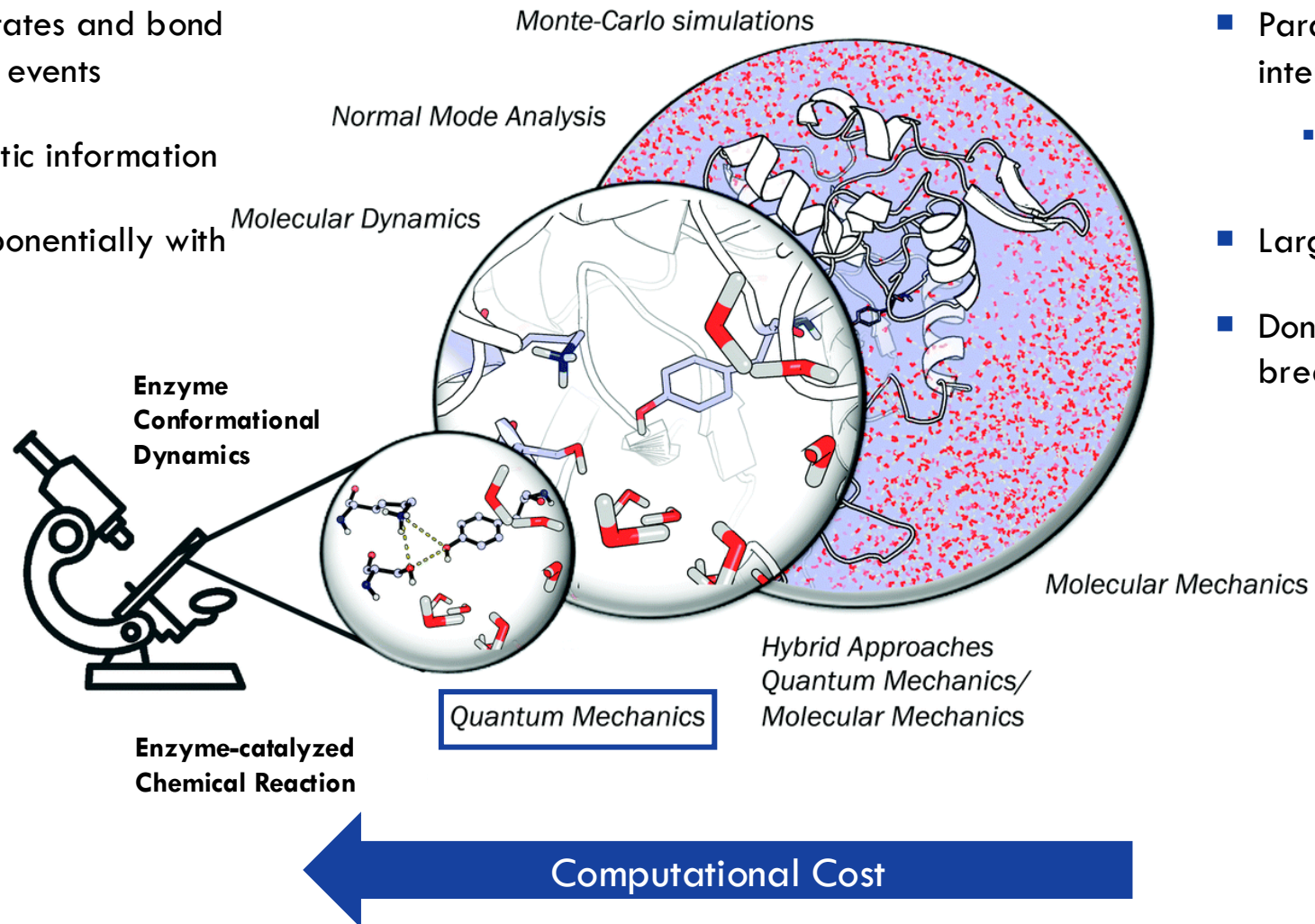
- No experimental waste or purification steps
- Computational enzyme engineering
 - Tune enzymatic properties
- Elucidate enzymatic reaction details
 - Informs design of molecular catalysts



Classical Force Fields vs. Quantum Mechanics (QM) Calculations

QM Calculations

- Model transition states and bond breaking/forming events
- Detailed mechanistic information
- Cost increases exponentially with size of system
 - 2x size, cost scales by a factor of 2^4



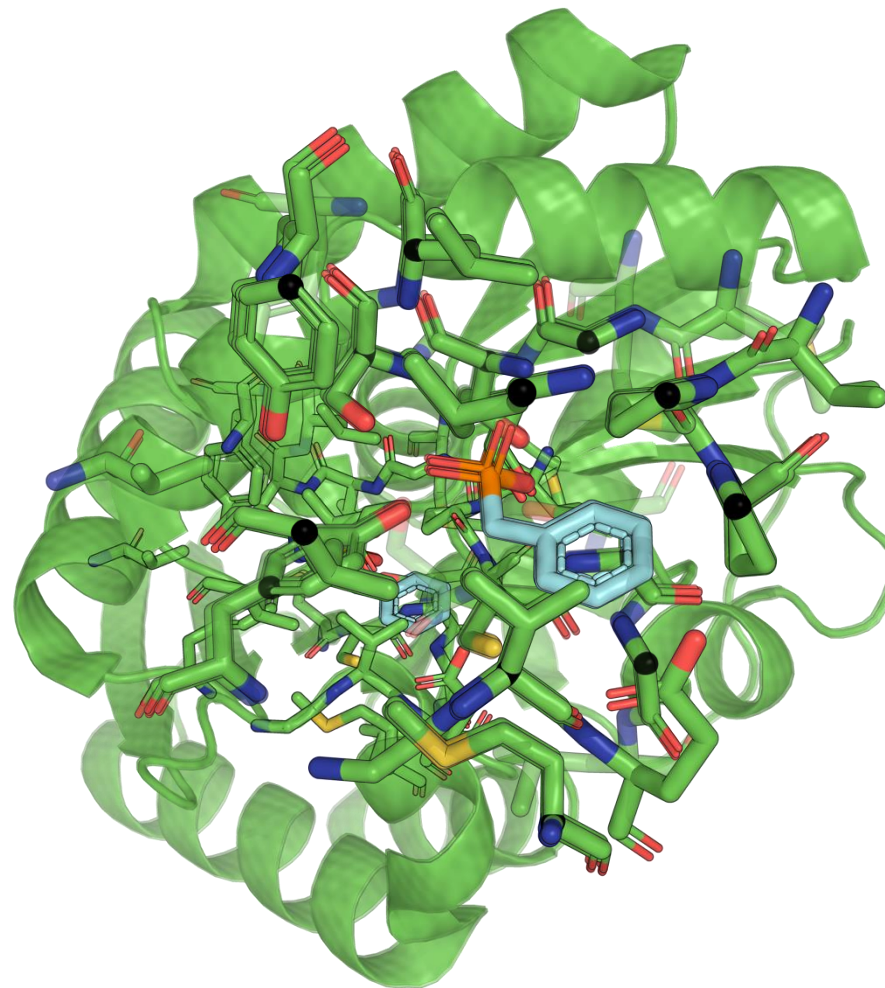
Classical Force Fields

- Parameterized to describe interactions
 - Electrostatic interactions, dispersion forces, etc.
- Large systems
- Don't model bond breaking/forming events

Computational Enzyme Modeling

Quantum Mechanical Clusters (QM Cluster)

1. Obtain starting protein structure
2. Identify active site region
3. Extract active site region from surrounding enzyme
4. Select key residues for reaction modeling and environment
5. Truncate active site system
 - Methyl capping at C α
6. Constrain select atoms to hold active site shape
 - Anchor atoms
 - Typically fix crystallographic positions
7. Run QM calculations on enzyme active site with constraints



PDB: 3IP8



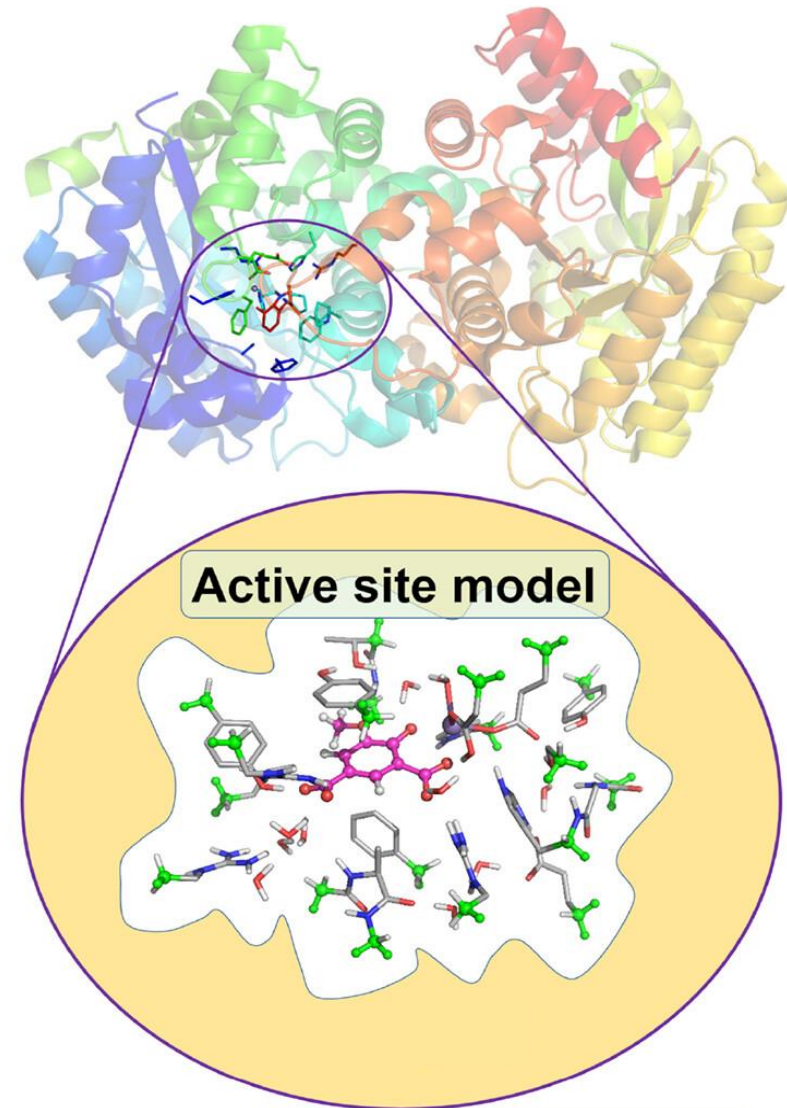
QM Cluster Models

Advantages

- Allows for QM calculations in enzymatic systems
 - Model chemical reactions
 - Gain mechanistic insight
- Small, simple models for enzyme reaction modeling

Disadvantages

- Difficult to select important residues
 - Only reactive species? Surrounding environment? Backbone?
- Difficult to model allosteric effects
- ***Need constraints to prevent excessive movement without surrounding protein***



Problems with QM Cluster Models: Fixed-Atom Constraints

Zero out atomic contributions to the gradient

Geometry optimization in Cartesian coordinates (inefficient)

Constrained atoms given infinite mass to calculate vibrational frequencies

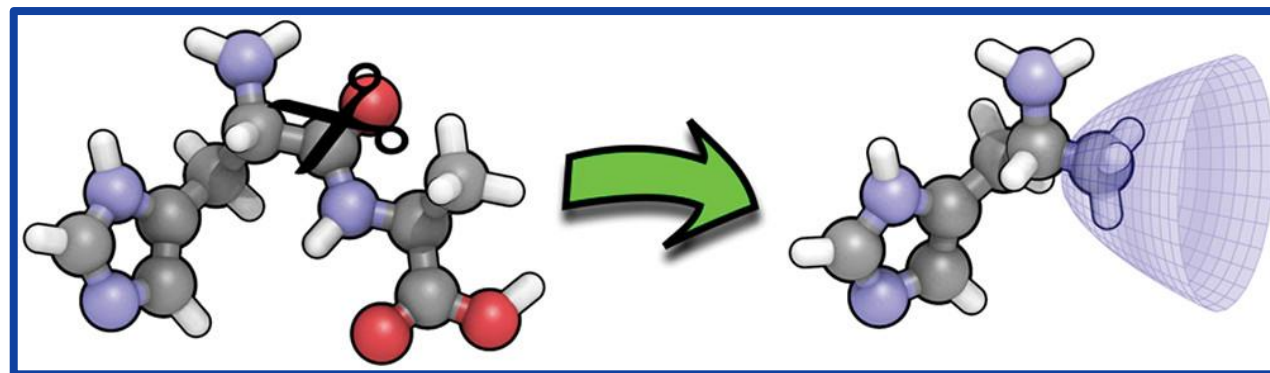
Do not contribute to zero-point energy or normal vibrational modes

Cannot reliably include entropic contributions

Key for enzymes which release-consume gaseous species (e.g. CO_2) or change molecularity

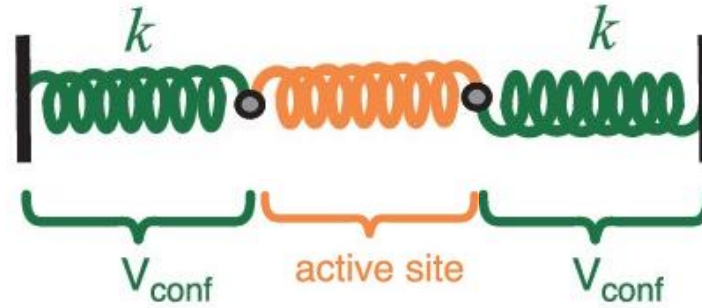
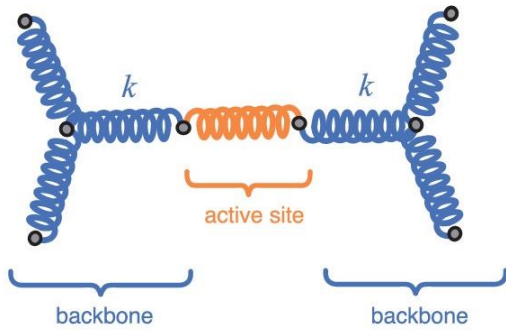
Using Atomic Confining Potentials for Geometry Optimization and Vibrational Frequency Calculations in Quantum-Chemical Models of Enzyme Active Sites

Saswata Dasgupta & John M. Herbert
J. Phys. Chem. B., **2020**



THE OHIO STATE UNIVERSITY

Harmonic Confining Potentials for Anchor Atoms



Harmonic Confining Potentials for Anchor Atoms

Kinetic Energy

$$E_{\text{electronic}} = E_T + E_V + E_J + E_{XC}$$

Exchange-Correlation

Nuclear-Electron Attraction

Coulomb Self-Interaction of Electron Density

- Additional classical term:

$$V_{\text{conf}}(\mathbf{r}_1, \mathbf{r}_2, \dots) = \frac{1}{2} \sum_i k \|\mathbf{r}_i - \mathbf{r}_i^0\|^2$$

- $k = 450 \text{ N/m}$ (close to C–C bond)

$$E_{\text{electronic}} = E_T + E_V + E_J + E_{XC} + V_{\text{conf}}$$

- First derivative of V_{conf} added to gradient of electronic energy

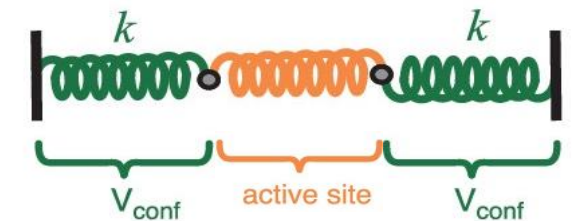
- Geometry optimization performed without constraints

$$\nabla_{\text{conf}} = \nabla_{\text{electronic}} + \frac{dV_{\text{conf}}}{dr_i}$$

- Second derivative of V_{conf} added to Hessian of electronic energy

- Vibrational frequencies calculated without modifying atomic masses

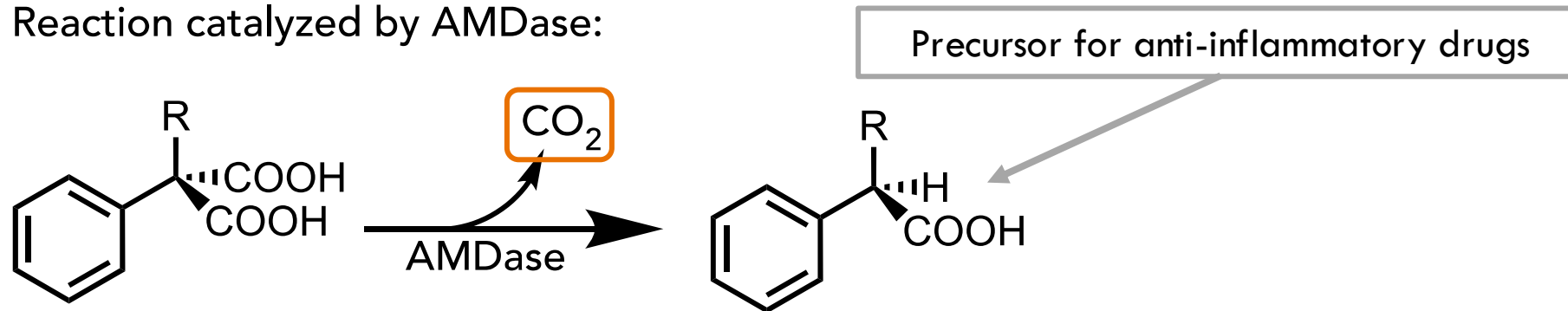
$$\nabla_{\text{conf}}^2 = \nabla_{\text{electronic}}^2 + \frac{d^2 V_{\text{conf}}}{dr_i^2}$$



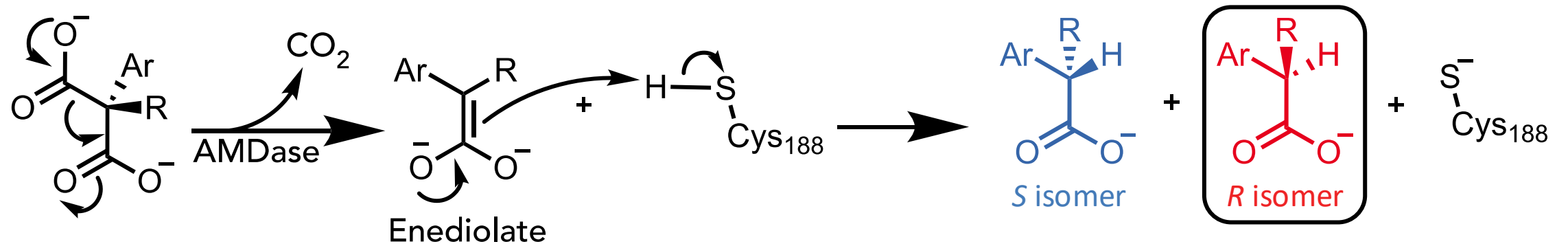
Harmonic Confining Test Case

ArylMalonate Decarboxylase (AMDase)

Reaction catalyzed by AMDase:

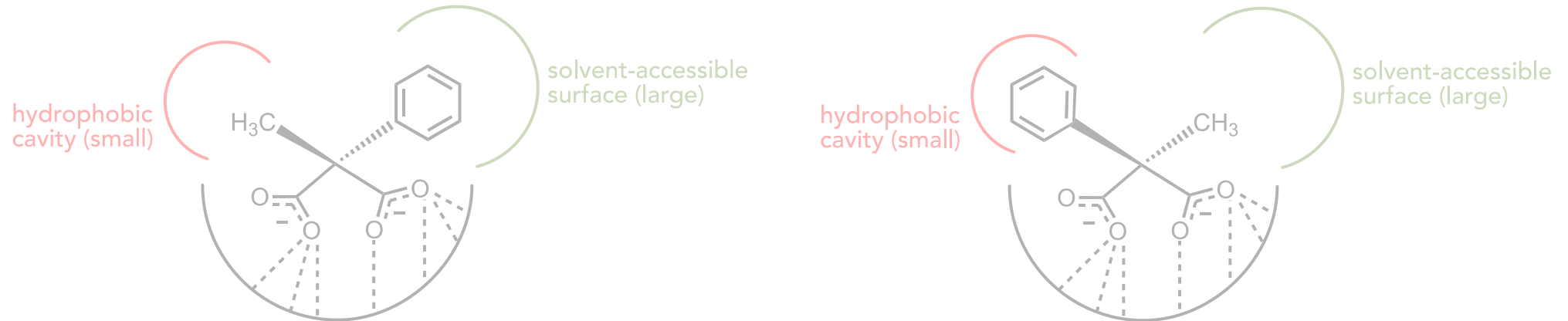


Two-step mechanism:

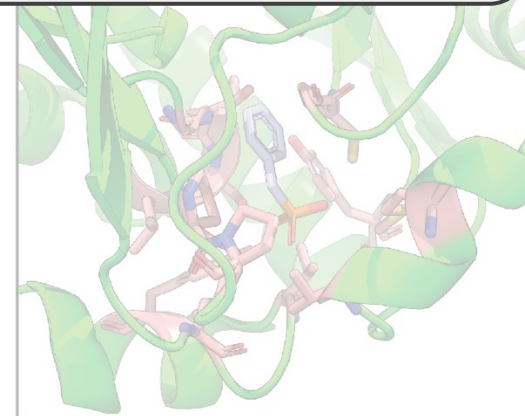
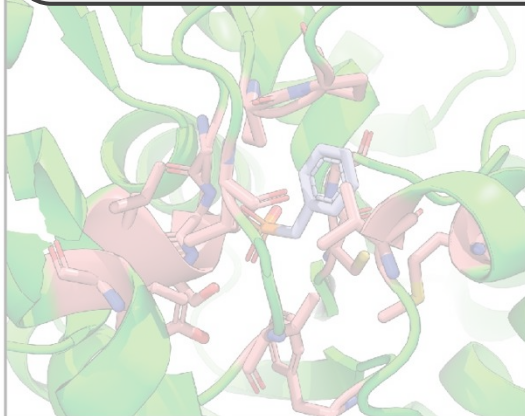


Harmonic Confining Test Case

ArylMalonate Decarboxylase (AMDase)



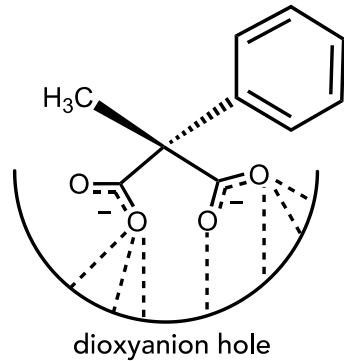
R isomer is favored due to steric clashes between the aryl group and protein



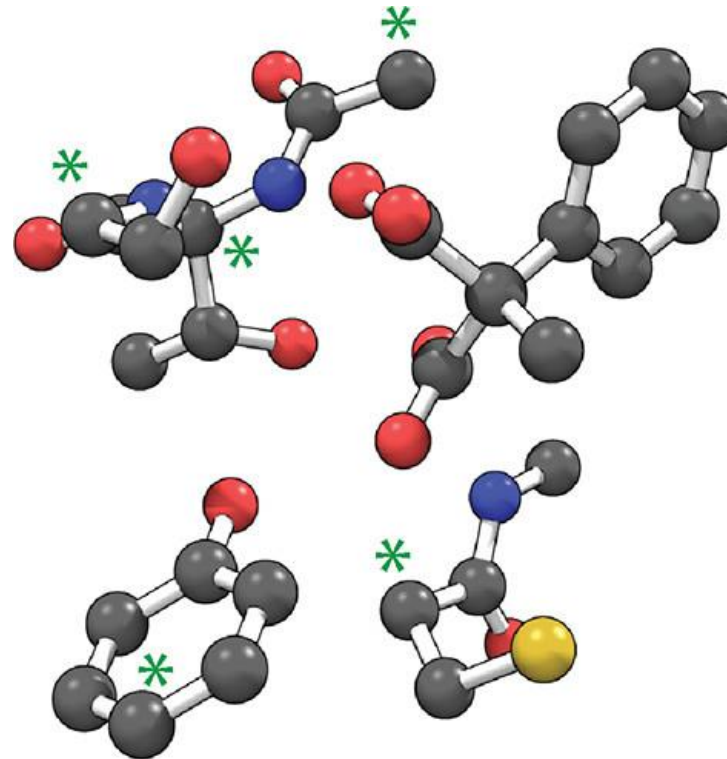
Harmonic Confining Test Case - AMDase

Model I

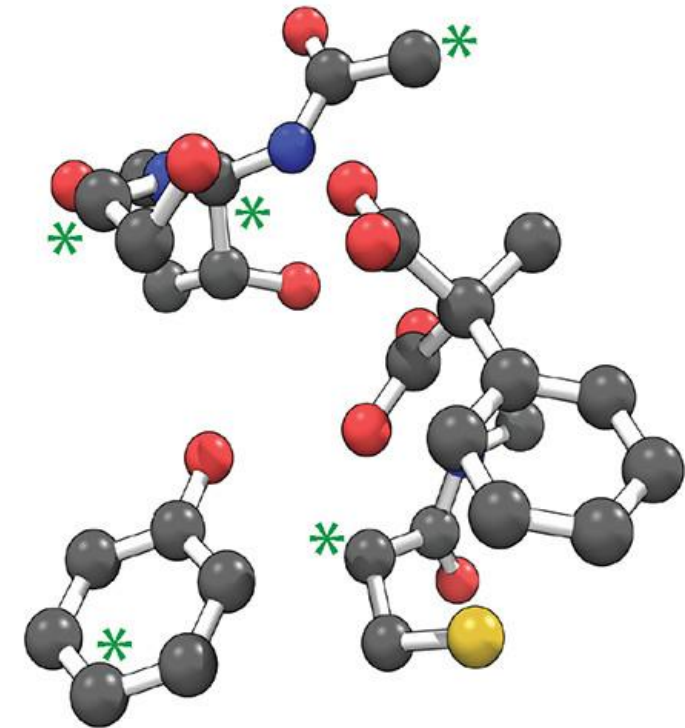
- Complex of methyl(phenyl)malonate and AMDase
- 81 atoms
 - Dioxyanion hole (reaction center)
 - Lacks solvent-exposed & hydrophobic cavities



- Truncated at Cd
 - Tyr126 replaced with phenol
- Net charge: -2
- Constrained 5 anchor atoms () *



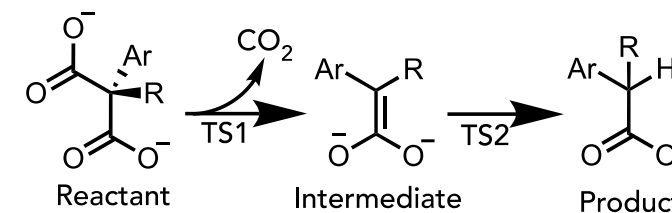
R isomer (model I)



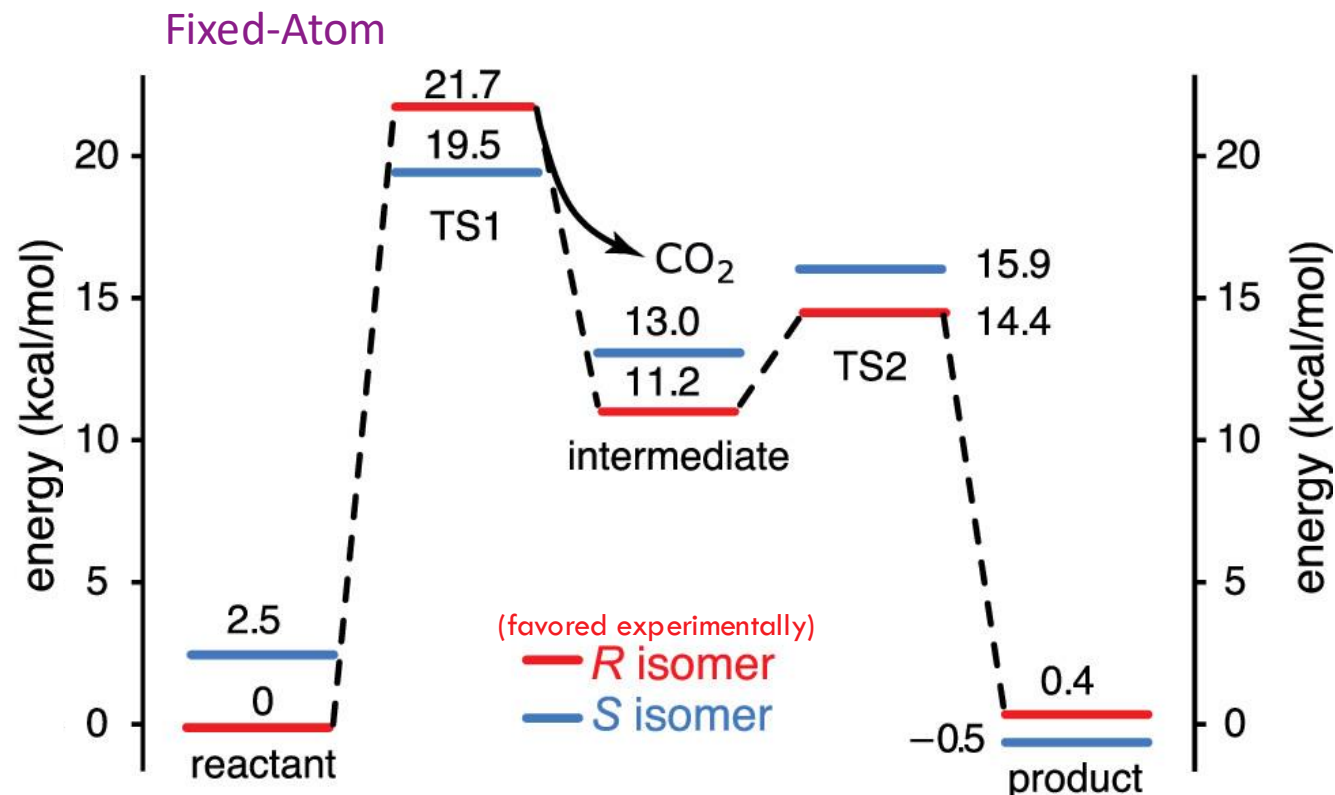
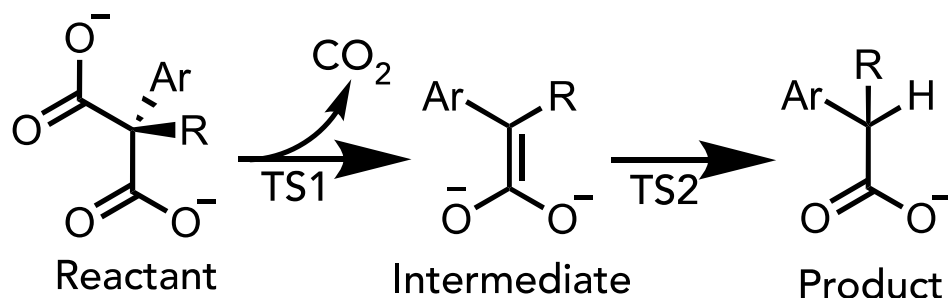
S isomer (model I)

Harmonic Confining Test Case - AMDase

Model I Results



B3LYP+D3(BJ)/6-311+G(2d,2p)//B3LYP+D3(BJ)/6-31G(d,p) CPCM, $\epsilon=4$



Harmonic confiners produce similar results to current methods

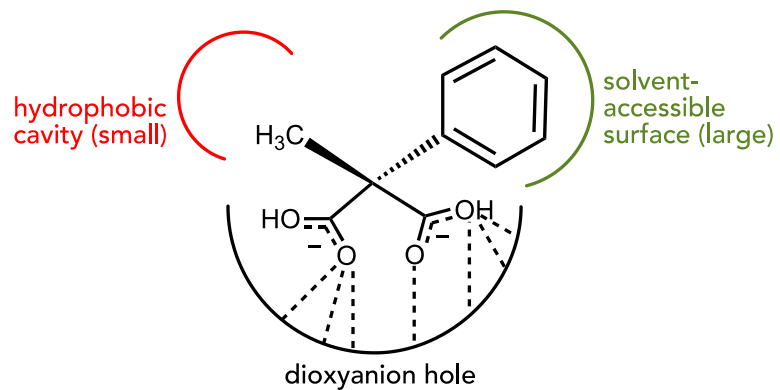
Harmonic Confining Test Case - AMDase

Model II

- Complex of methyl(phenyl)malonate and AMDase

- 223 atoms

- Includes solvent-exposed & hydrophobic cavities

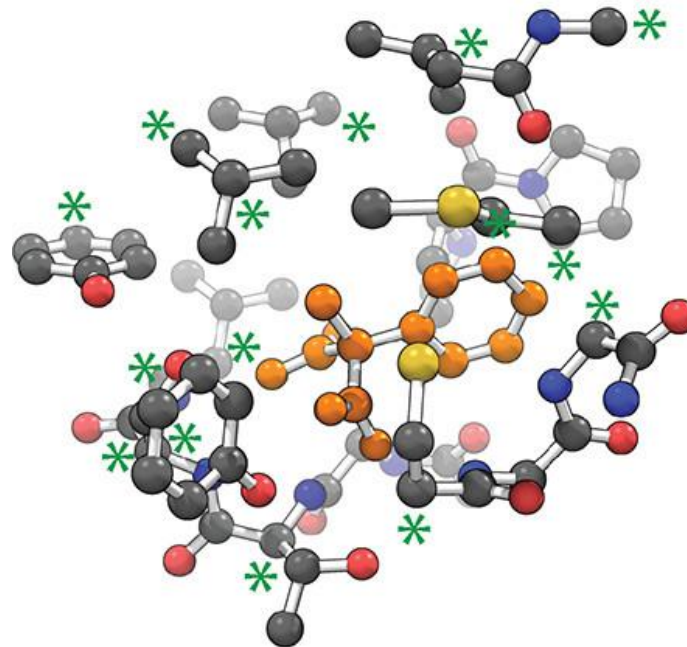


- Truncated at Ca

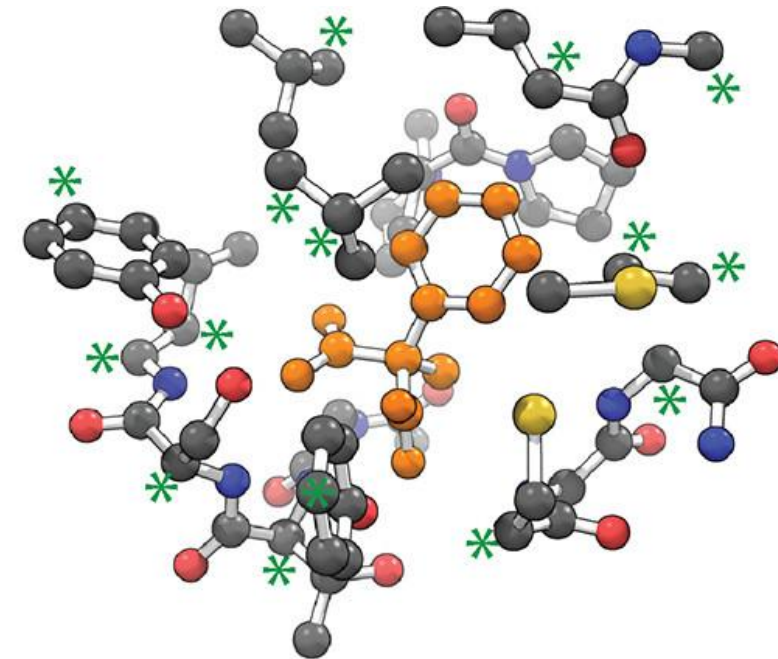
- Tyr126 & Tyr48 replaced with phenol

- Net charge: -2

- Constrained 17 anchor atoms () *



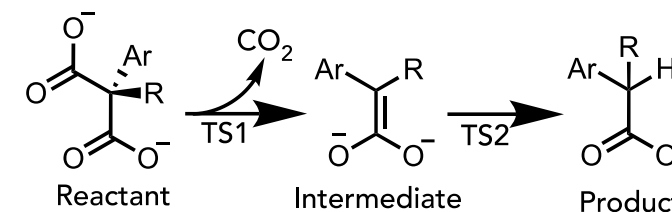
R isomer (model II)



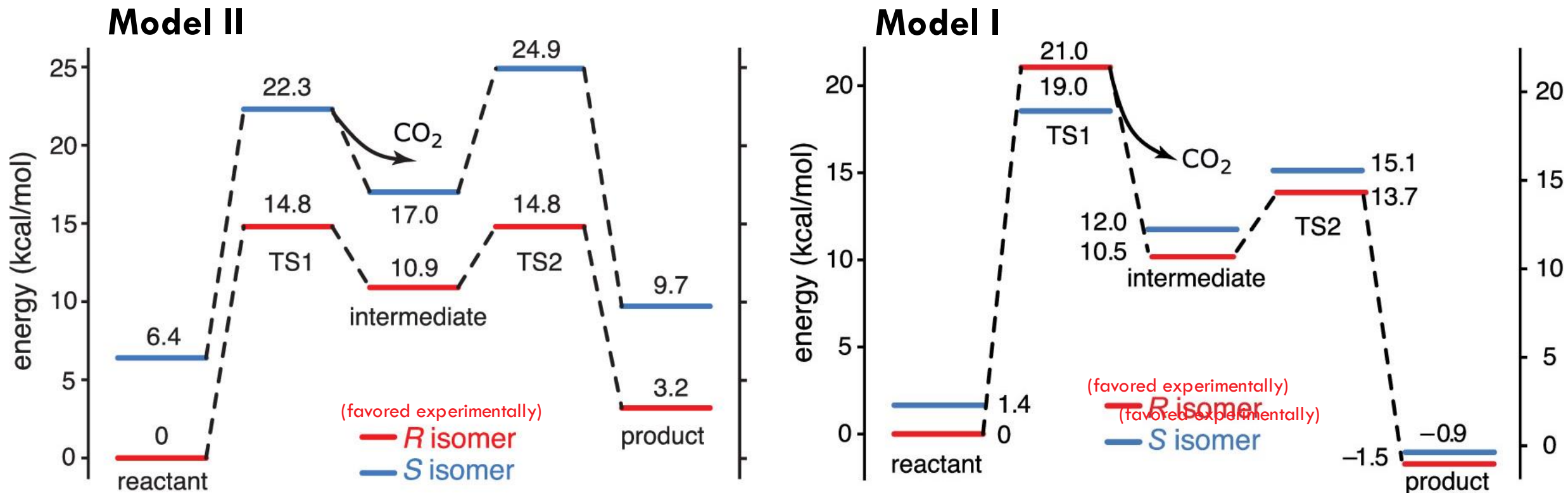
S isomer (model II)

Harmonic Confining Test Case - AMDase

Model II Results



B3LYP+D3(BJ)/6-311+G(2d,2p)//B3LYP+D3(BJ)/6-31G(d,p) CPCM, $(\epsilon=4)$



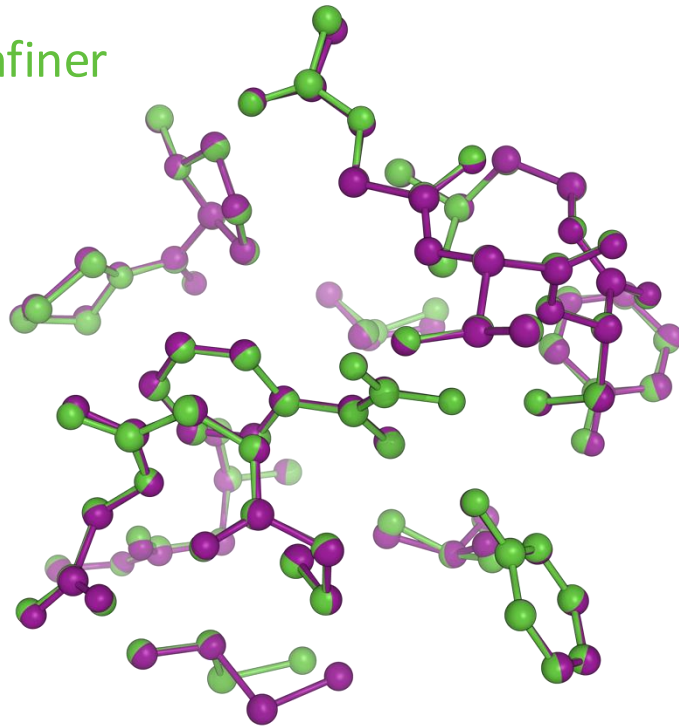
Larger Model II is more accurate than Model I
Harmonic confining potential relieves artificial strain of fixed-atom constraints

Harmonic Confining Test Case - AMDase

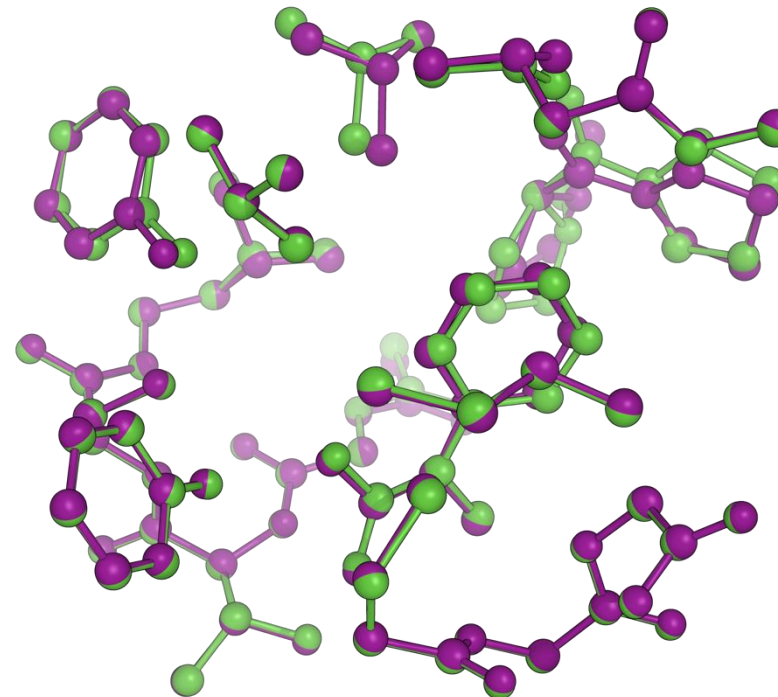
Model II Optimized Structures

Fixed-Atom

Harmonic Confiner



R intermediate
RMSD = 0.35 Å

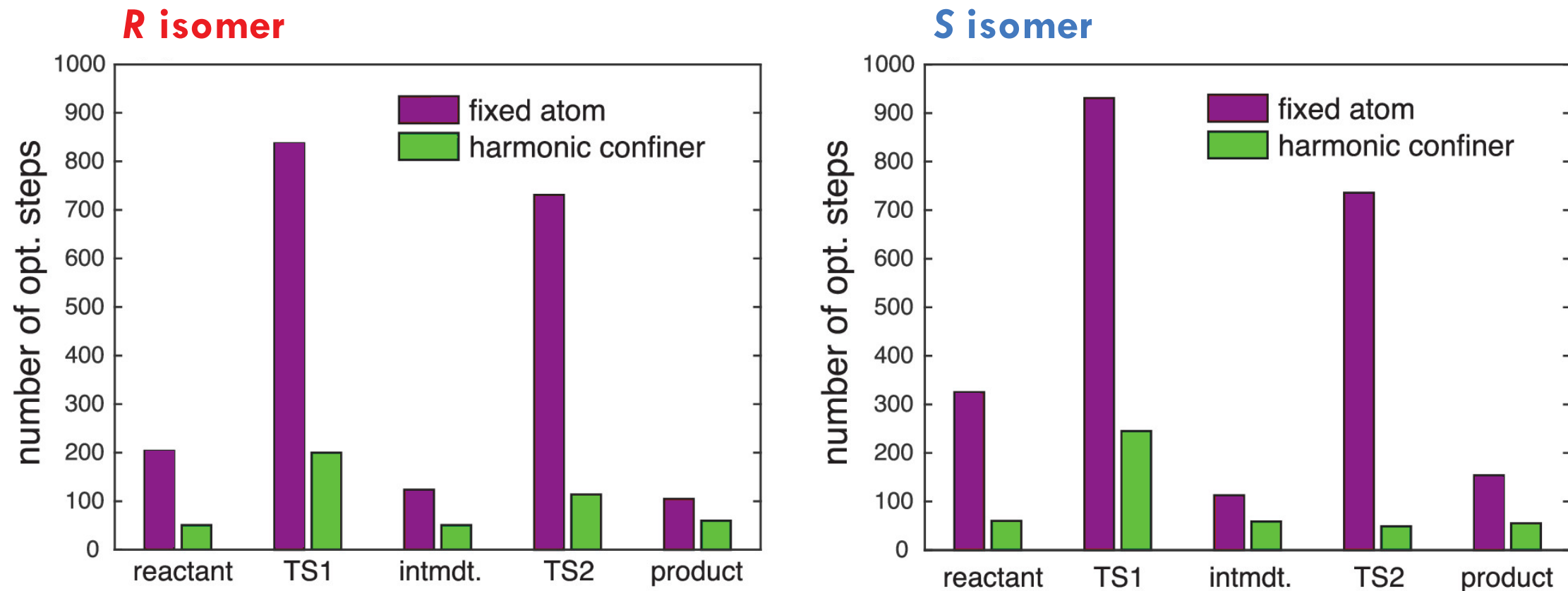


S intermediate
RMSD = 0.33 Å

Harmonic confining potentials have same effect as fixed-atom constraints on the structure

Harmonic Confining Test Case - AMDase

Improved Computational Efficiency – Model I



Harmonic confining potentials are far more computationally efficient than fixed-atom constraints

Implications of Harmonic Confining Potentials

Problems Solved

- Harmonic confining potentials are easy to implement
 - One term in energy expression
 - Implemented in QChem software
- Number of optimization steps can be dramatically reduced
- Allows modeling of entropic contributions
 - Atomic masses do not change
- **More realistic method of QM cluster modeling**

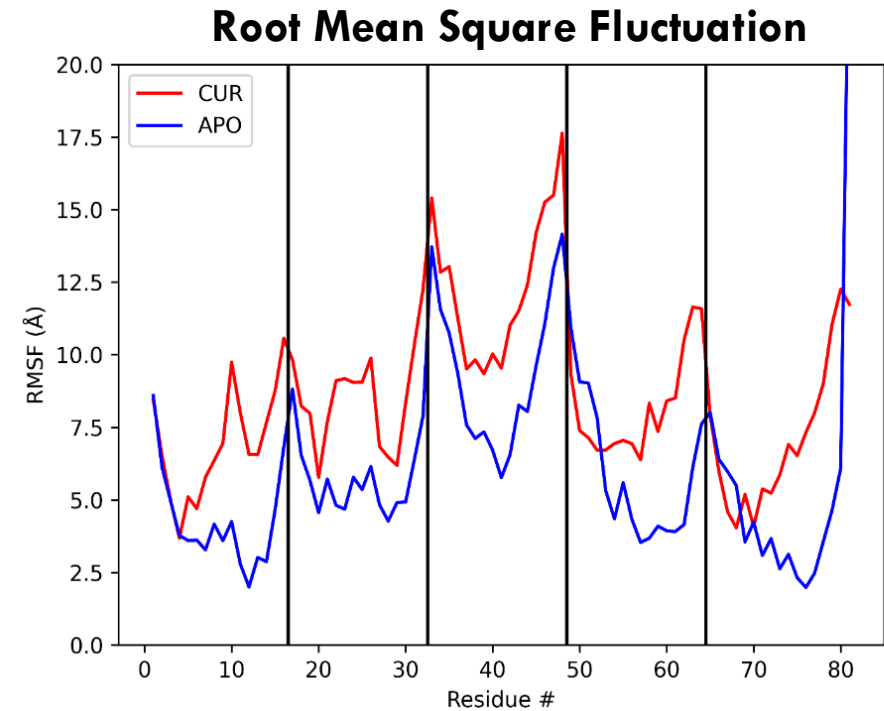
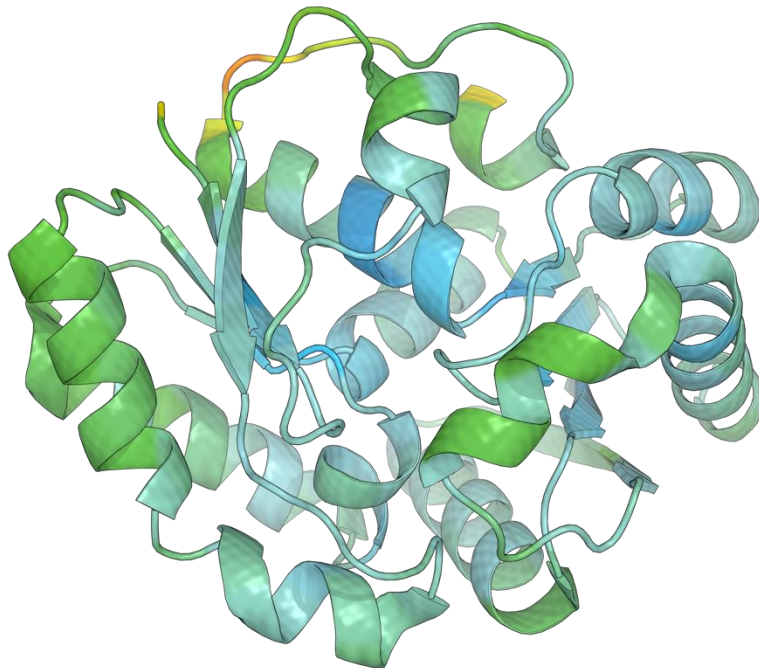
Unanswered Questions

- Don't calculate free energies or show anything regarding vibrational frequencies
- Atomic positions are only constraints
 - Angles, torsions, etc.
- Reaction profiles still sensitive to model size
 - Possibly use smaller models to reduce artificial strain with harmonic confining potentials

My Proposed Future Directions

How Can We Further Improve QM Cluster Calculations?

- Enzyme-specific force constants from structural uncertainty
 - Atom-specific force constants
- Allow asymmetric movement/constraints from molecular dynamics simulation
 - How would the protein actually move?



Acknowledgements

- Rob Paton
- Seonah Kim
- Nancy Levinger
- CSU Theory Suite
- Addison Bralick



Questions?

