Exploring Potential Energy Surfaces for Enzymatic Reactions Using QM/MM Calculations

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Dr. Jia Zhou
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Dr. Jie (Jessy) Li
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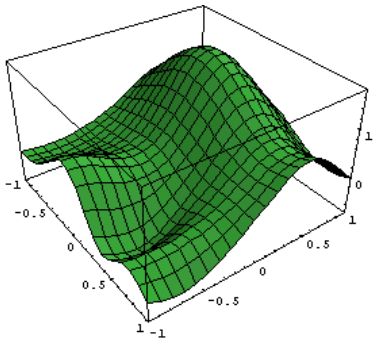
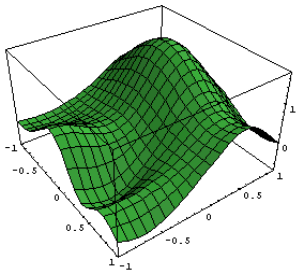
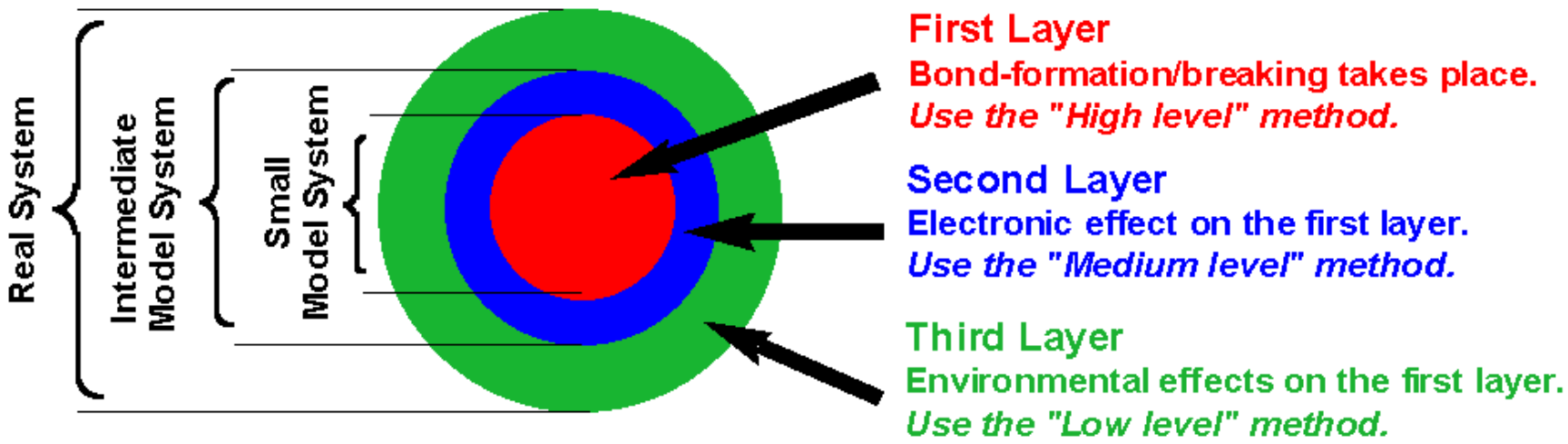
Michael Cato



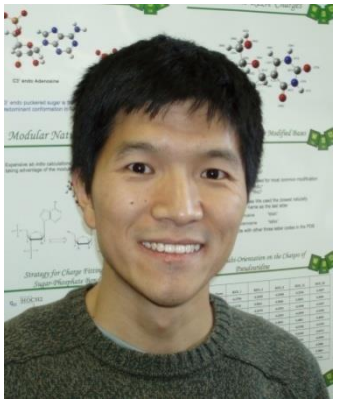


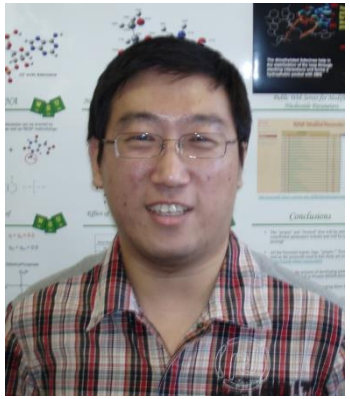
QM/MM Calculations

- The active site region is treated using high-level molecular orbital theory, while the most distant parts of the system are treated using low-cost molecular mechanics.



Inhibition of Matrix Metalloproteinase 2 by SB-3CT

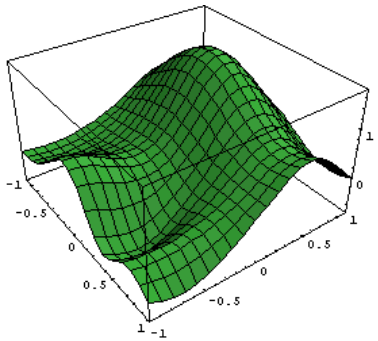


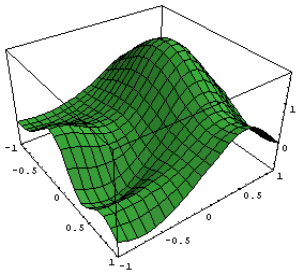


Prof. Shahriar Mobashery — University of Notre Dame

Dr. Peng Tao — Wayne State University

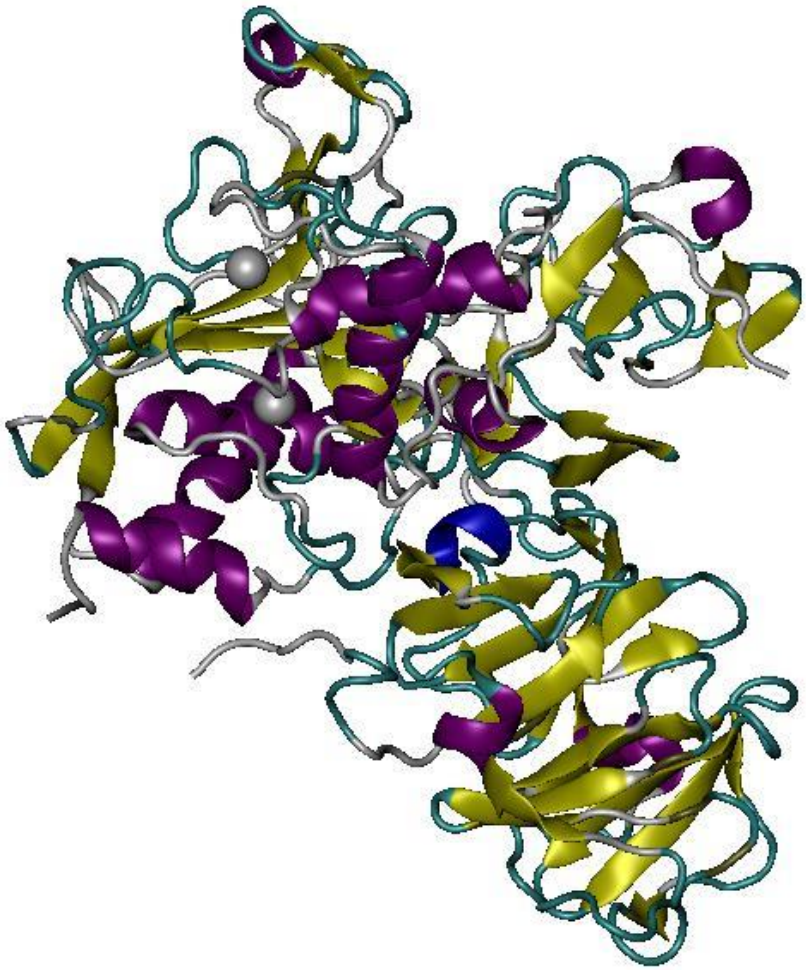
Dr. Jia Zhou — Wayne State University

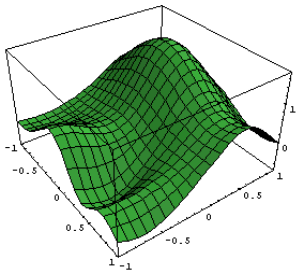




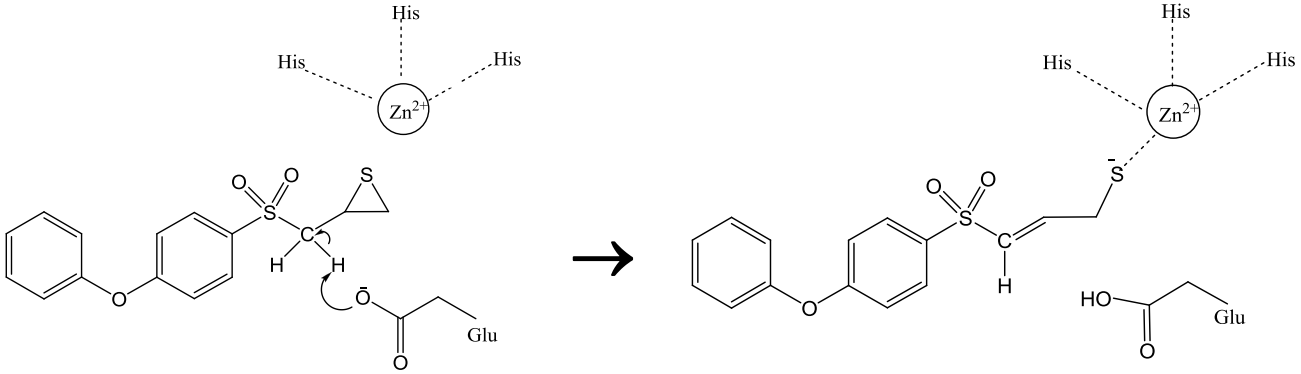
Matrix MetalloProteinase 2 (MMP2)

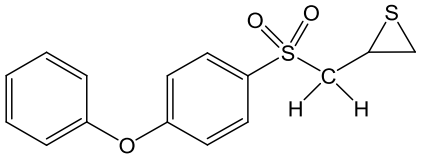
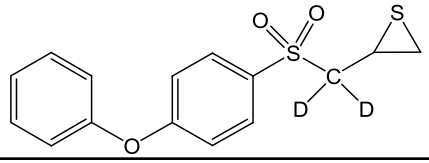
- Involved in organ development, tissue remodeling, and wound healing
- Related to cancer, inflammation and connective tissue diseases
- Zinc dependent



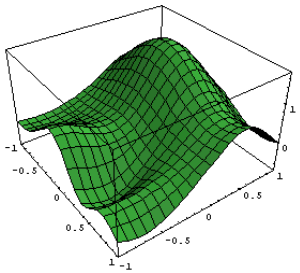


MMP2 Inhibition by SB-3CT: Kinetic Isotope Effect



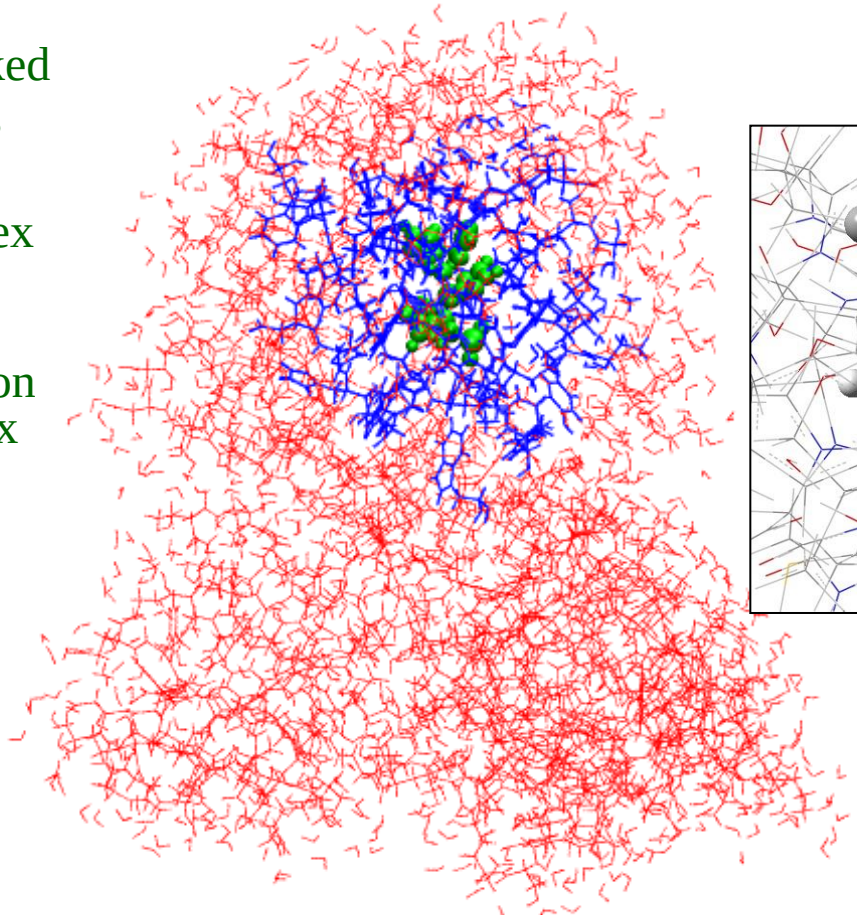
Structure	$K_{on}(M^{-1}S^{-1})$	$K_{off}(S^{-1})$	K_i(nM)
	$(22\pm1)\times10^3$	$(1.4\pm0.1)\times10^3$	64±6
	$(4.4\pm0.5)\times10^3$	$(1.4\pm0.1)\times10^3$	320±45

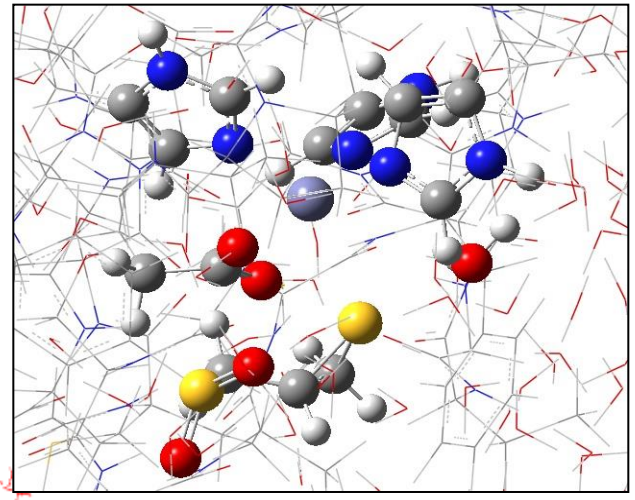
Forbes, C.; Shi, Q. C.; Fisher, J. F.; Lee, M.; Hesek, D.; Llarrull, L. I.; Toth, M.; Gossing, M.; Fridman, R.; Mobashery, S. *Chem. Biol. Drug. Des.* **2009**, *74*, 527–534.



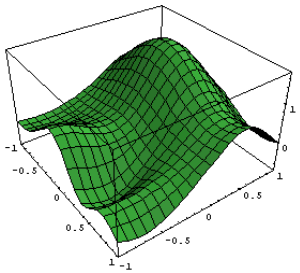
QM/MM Model of MMP2-SB3CT

- Substrate SB-3CT docked into the MMP2 binding pocket.
- MMP2 SB-3CT complex immersed in a TIP3P water box
- Constrained optimization of complex in water box
- Protein complex and water molecules within 3 Å selected for the QM/MM calculations
- B3LYP for QM part
  AMBER for MM part





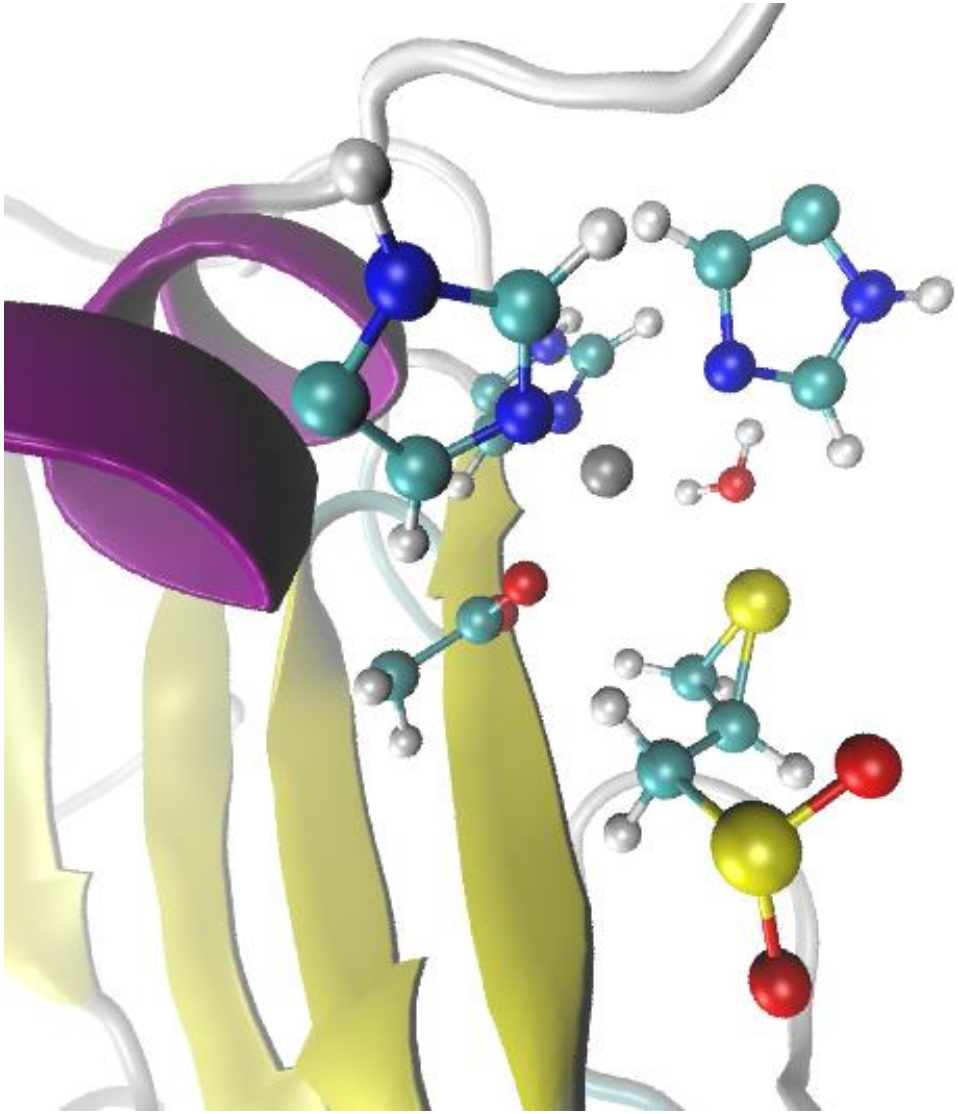
***Peng Tao et al. Biochem.* 2009, *48*, 9839-47, *J. Phys. Chem. B.* 2010, *114*, 1030-7**
***J. Chem. Theory Comput.* 2010, *6*, 3580-3587**

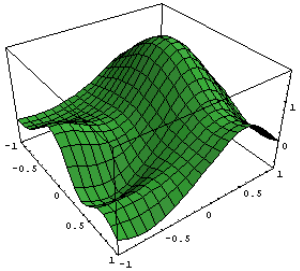


Protocol for QM/MM calculations on MMP2

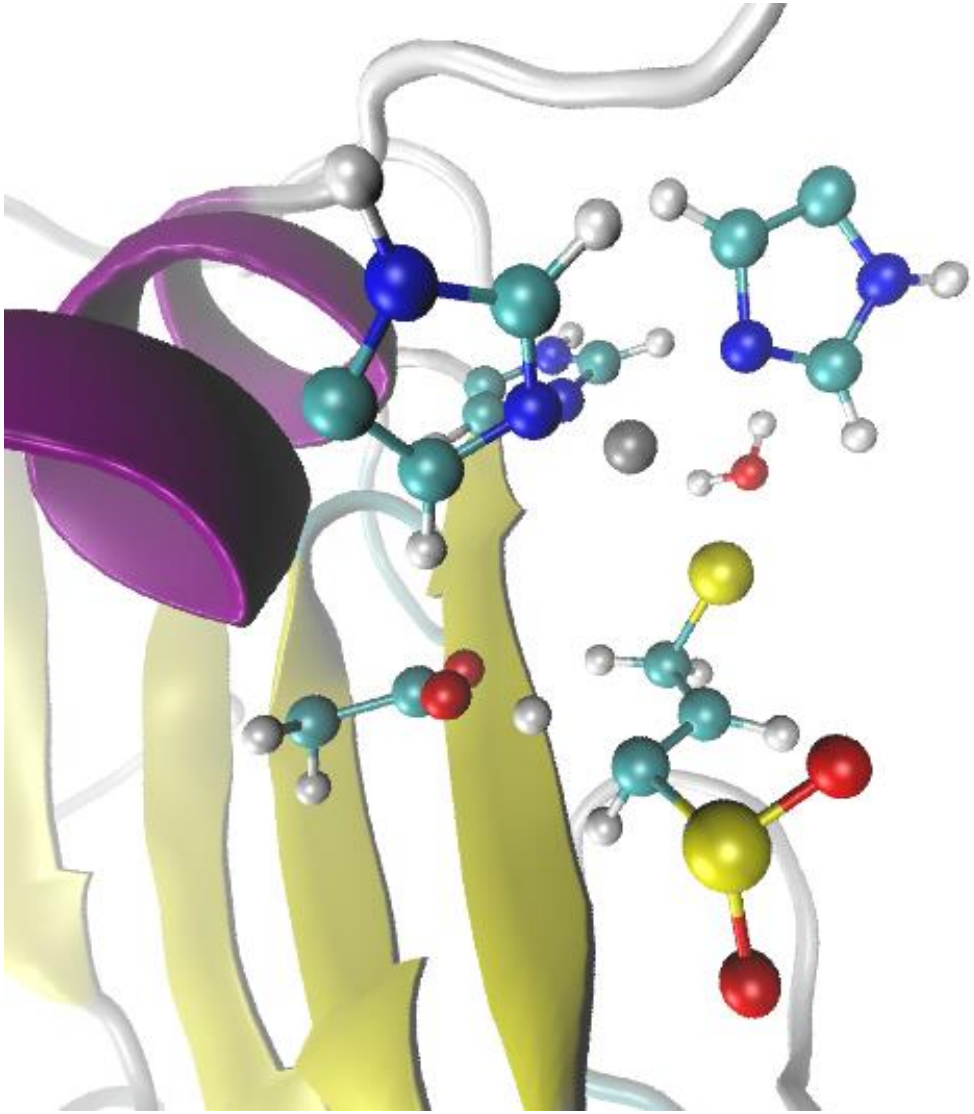
- Initial QM/MM optimization
- Restrained ElectroStatic Potential (RESP) method to fit charges for QM region with capping hydrogens
- QM/MM optimization with new RESP charges using ONIOM(B3LYP/6-31G(d):AMBER)
- Repeat previous two steps until converged (within 0.1 kcal/mol)
- Single point calculation with larger basis set and electronic embedding using ONIOM(B3LYP/6-311+G(d,p):AMBER)

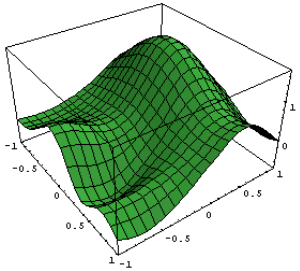
SB-3CT Reactant Complex



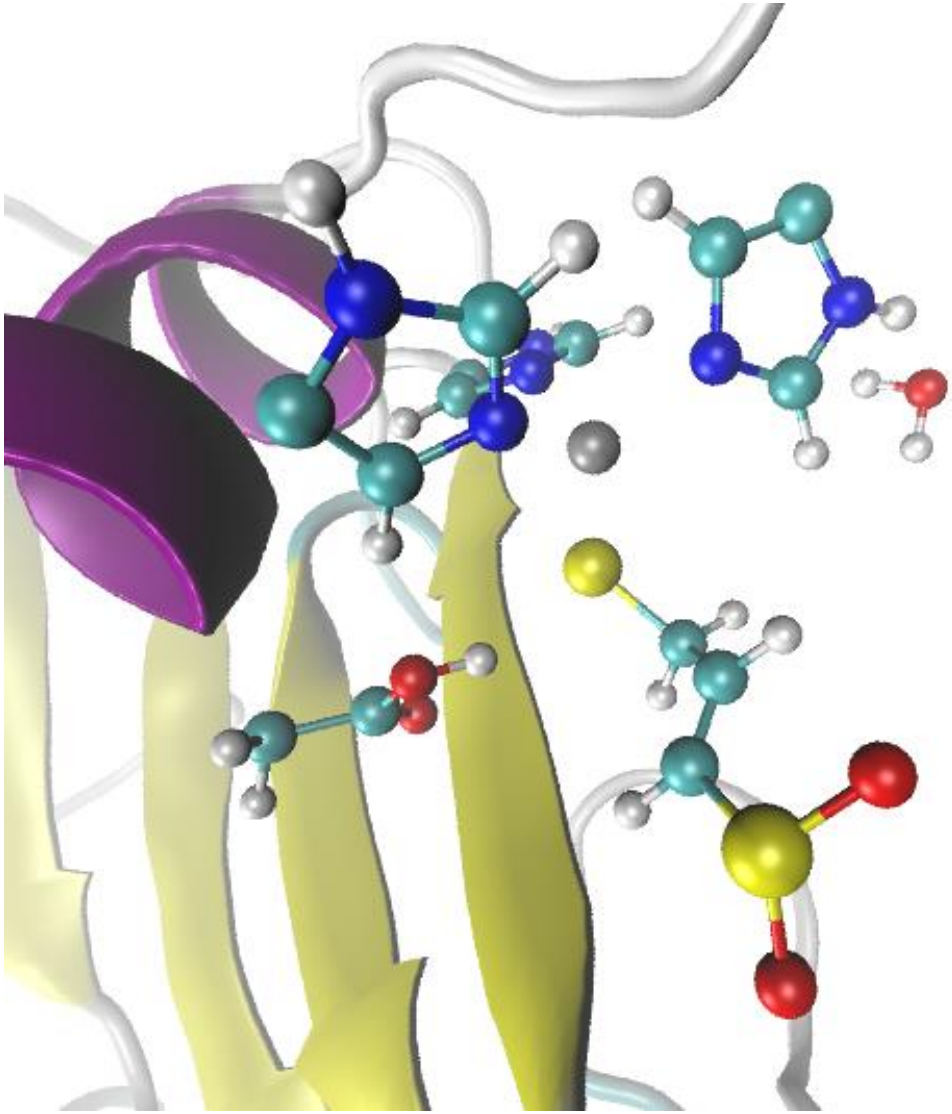


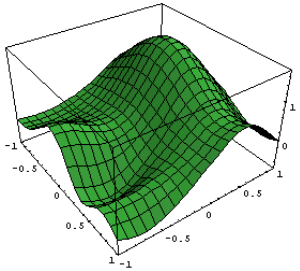
SB-3CT Transition State



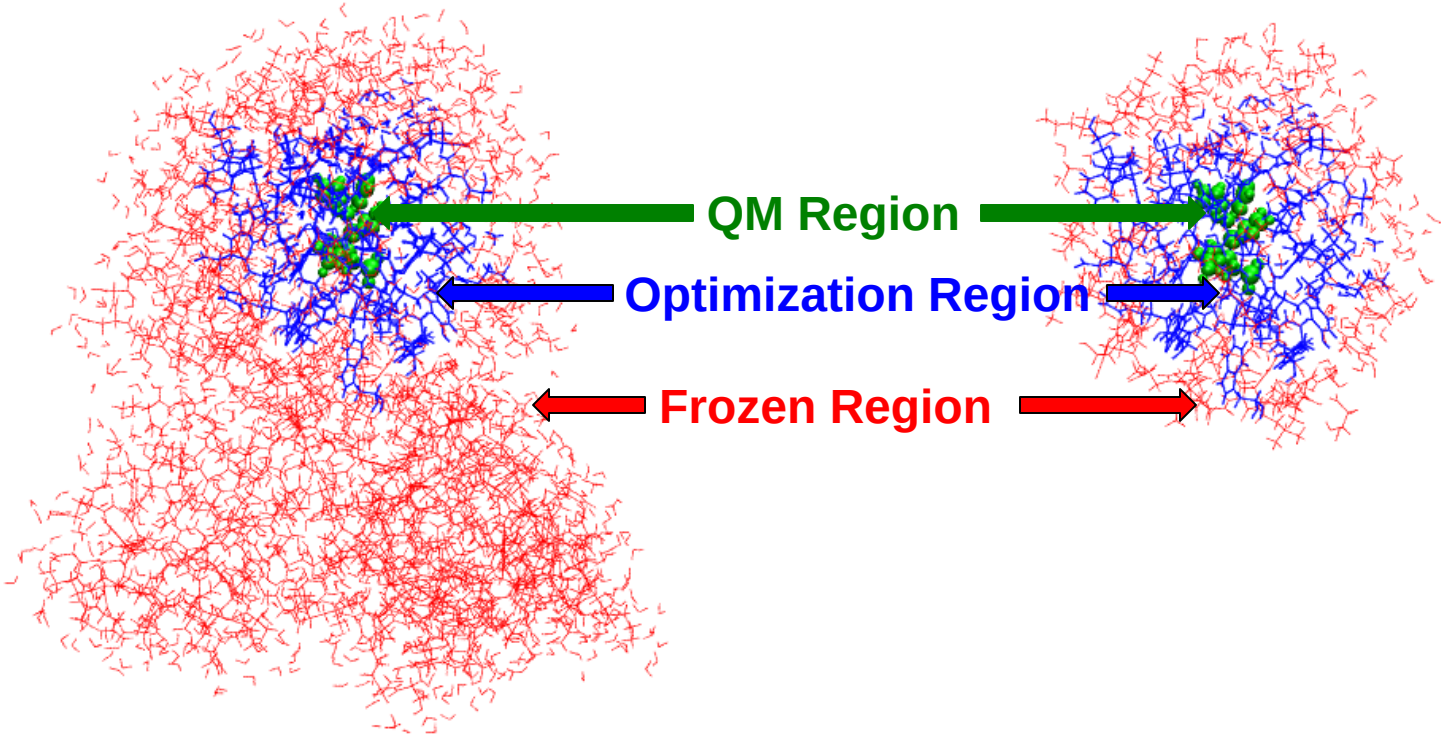


SB-3CT Product Complex



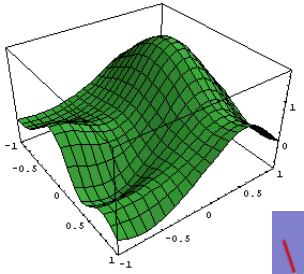


QM/MM Frequency Calculation for MMP2

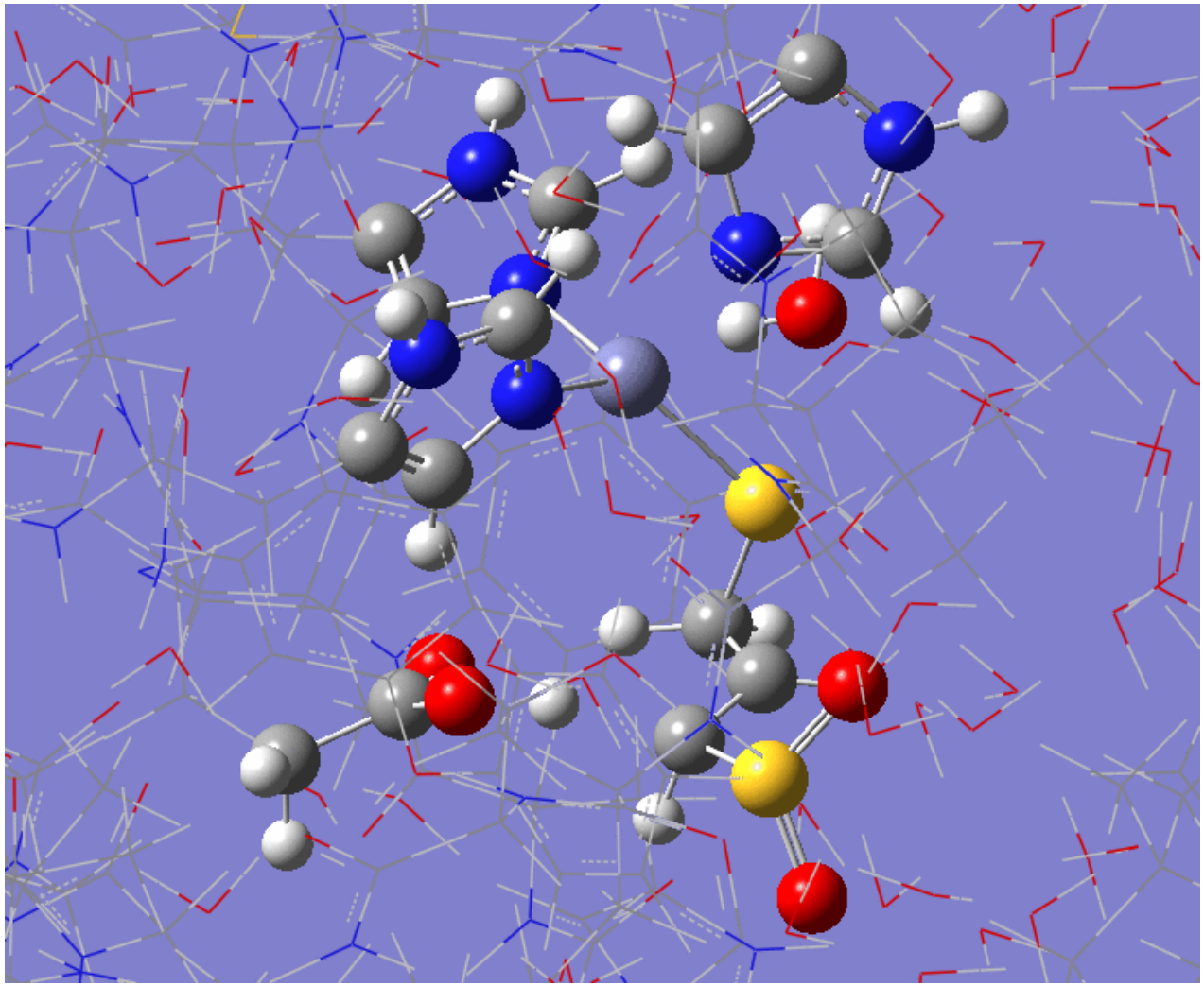


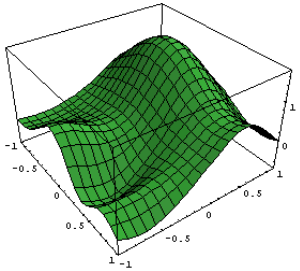
Full Protein Model
767i, 21 cm^{-1}

Partial Protein Model
642i, 18 cm^{-1}



Vibrational Mode for Transition State



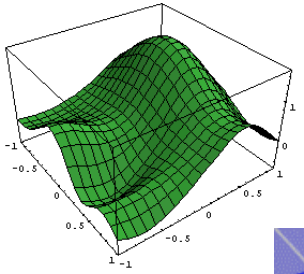


Kinetic Isotope Effect (k_H/k_D) SB-3CT Inhibition of MMP2

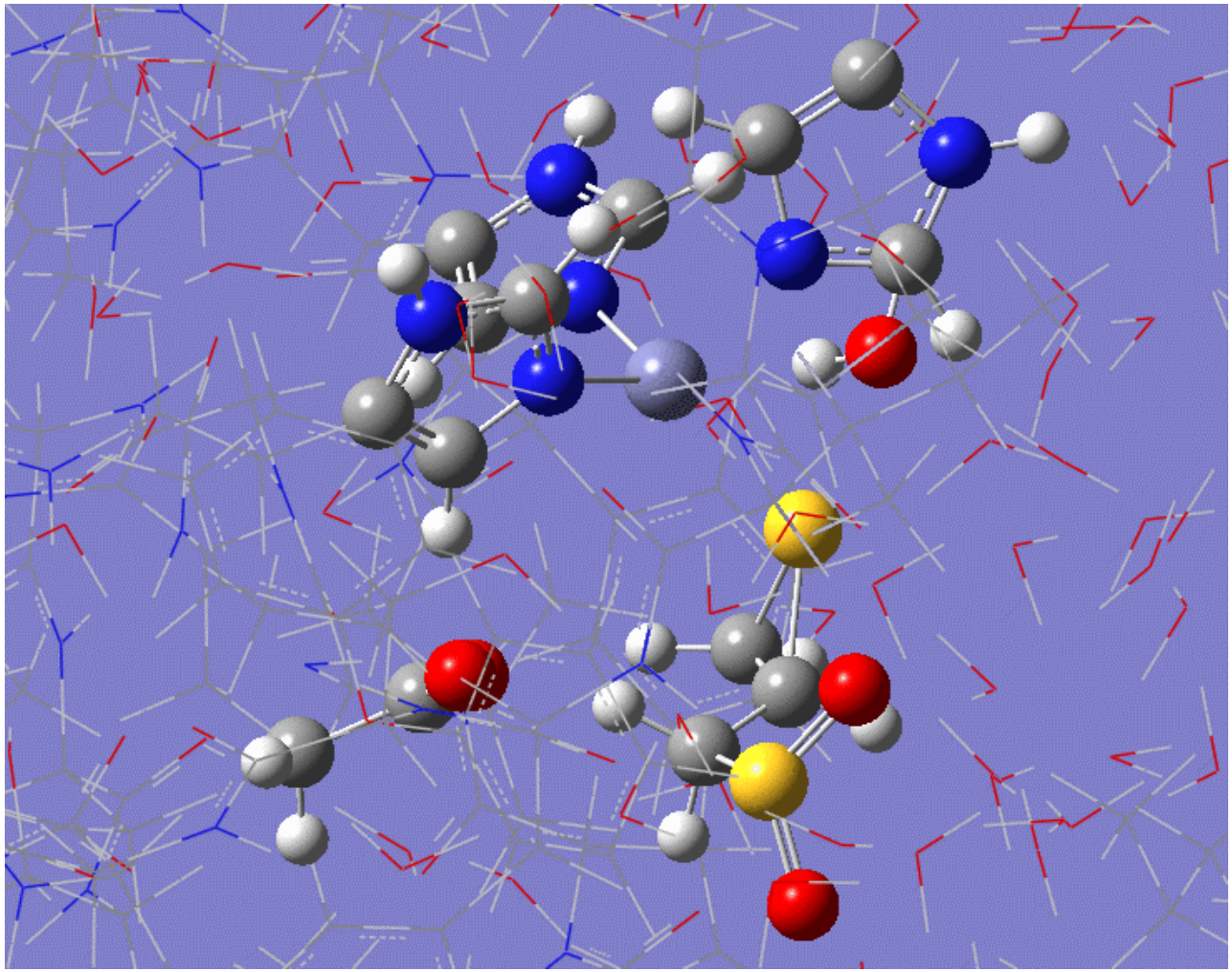
Experiment	QM/MM Model	
± SB-3CT	*R*	*S*
5.0	5.9	6.7

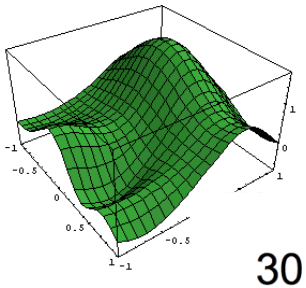
Chem. Biol. Drug Des. 2009, *74*, 527.

With tunneling correction using Wigner model

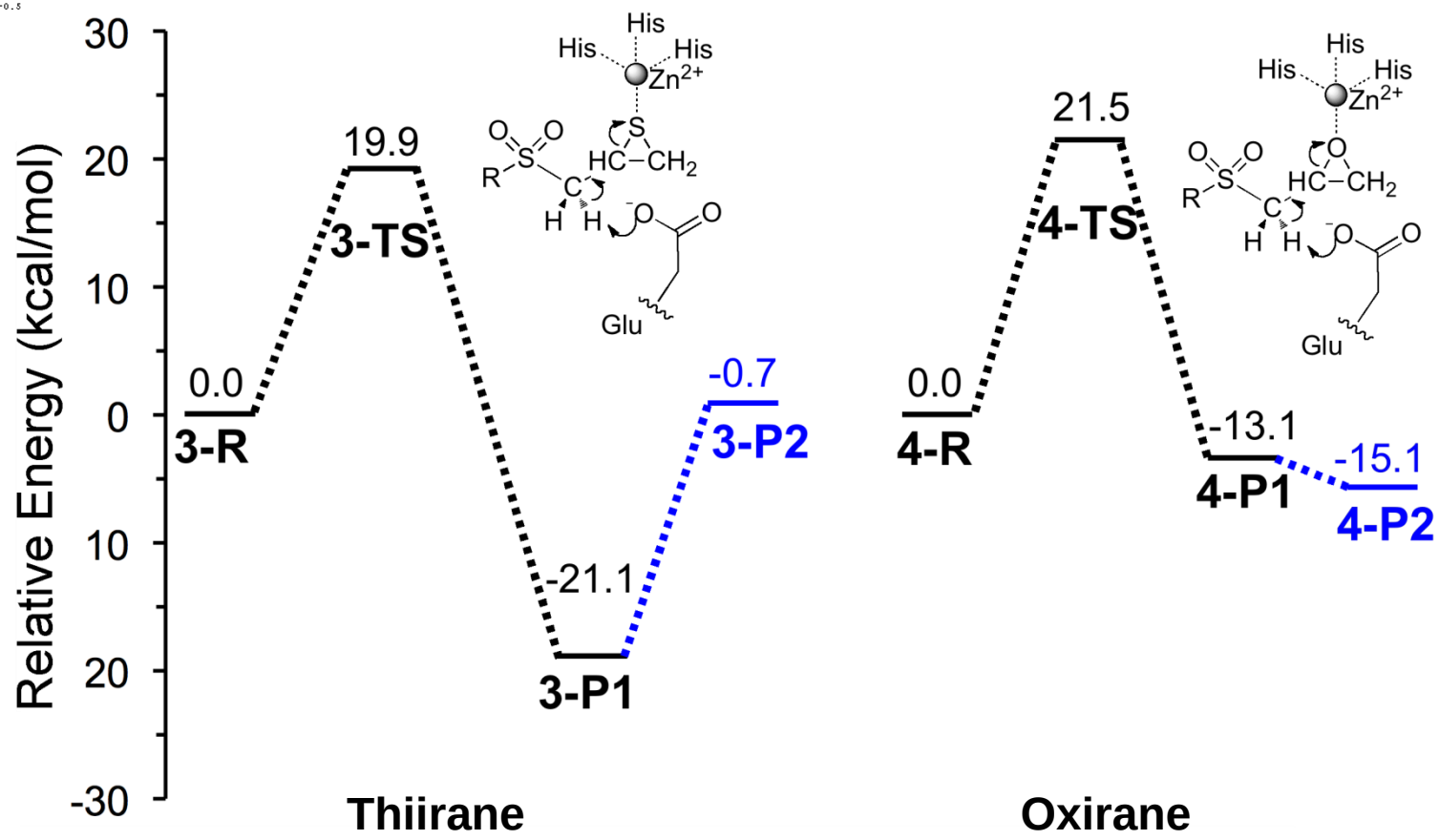


Intrinsic Reaction Coordinate (IRC)



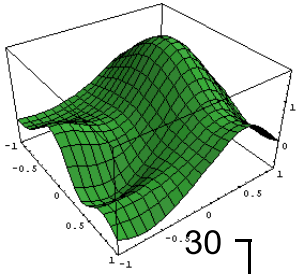


Energetics of (*R*)-SB-3CT + MMP2

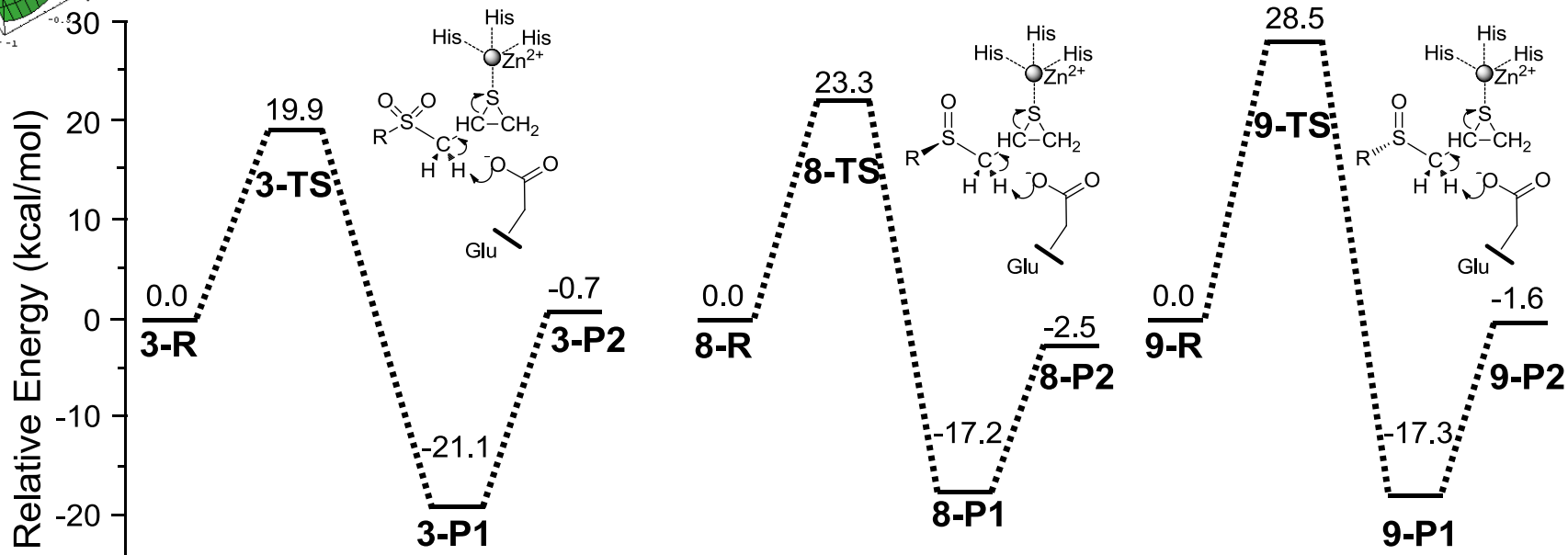


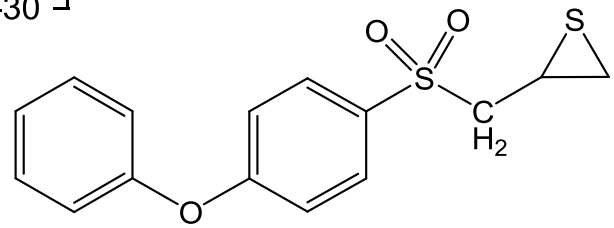
ONIOM(B3LYP/6-311+G(d,p):AMBER,EE)//ONIOM(B3LYP/6-31G(d):AMBER)

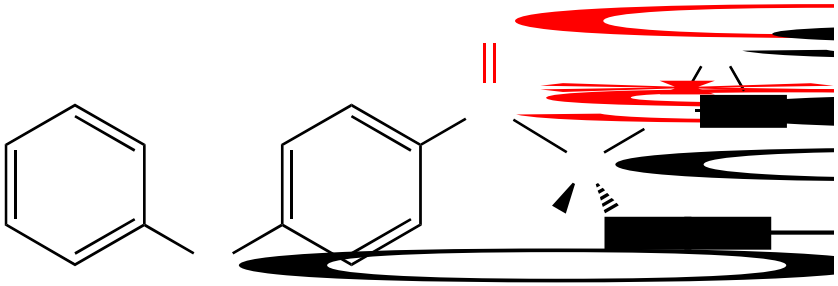
P. Tao *et al*. *Biochemistry* 2009, *48*, 9839



Sulfoxide Analog of (*R*)-SB-3CT



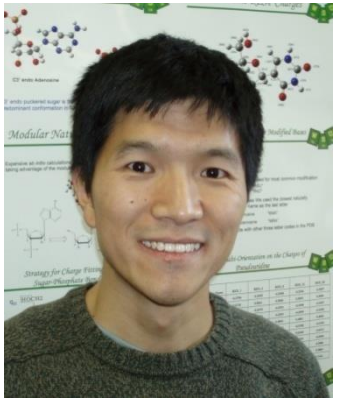




ONIOM(B3LYP/6-311+G(d,p):amber)//ONIOM(B3LYP/6-31G(d):AMBER)

P. Tao, *et al*. *J. Phys. Chem. B* 2010, *114*, 1030

Exploring the Potential Energy Surface for KDO8P Synthase



Dr. Peng Tao

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Prof. Dominico Gatti

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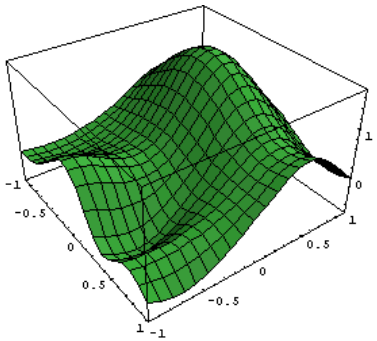
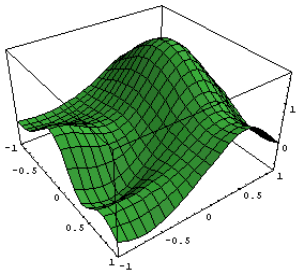
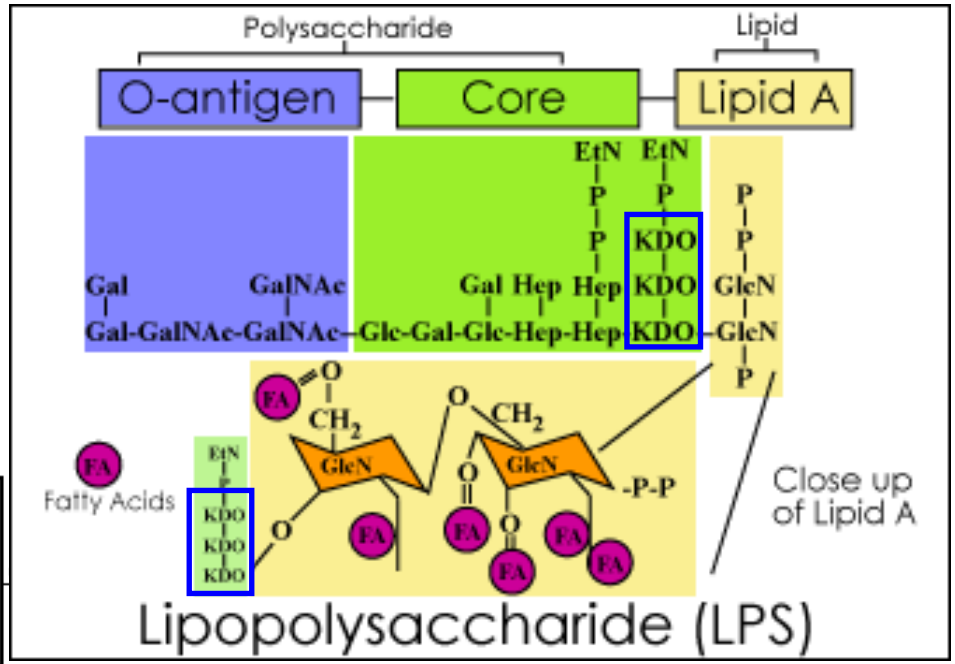
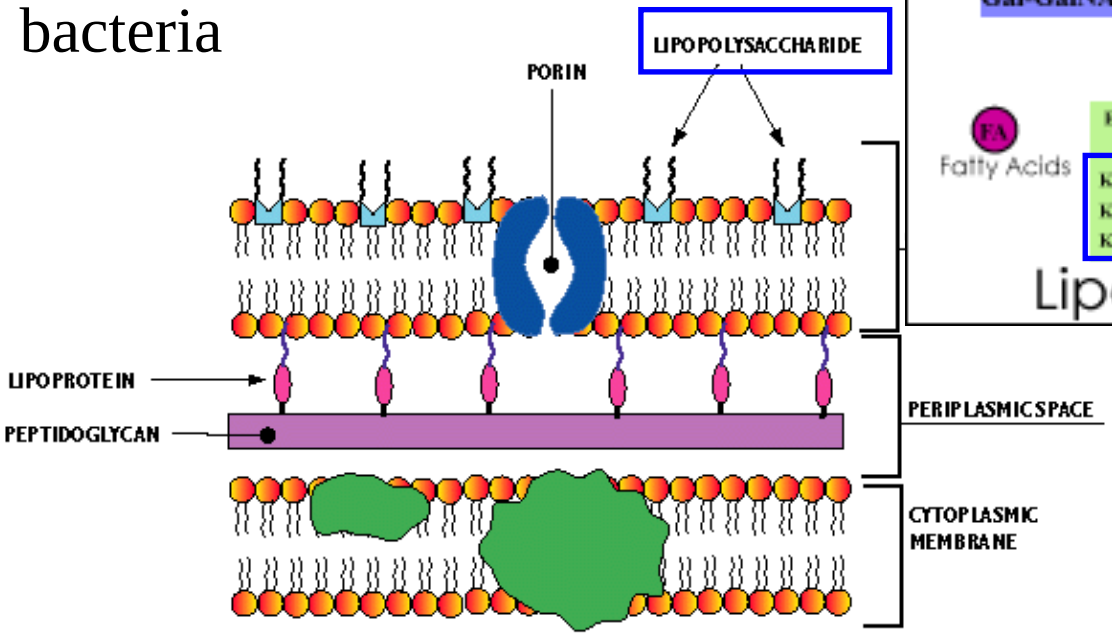




Gram-negative Bacteria Cell Wall Structure

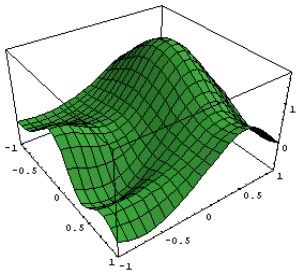
3-Deoxy-D-manno-octulosonate (KDO) is an essential constituent of the lipopolysaccharide in the cell wall of all Gram-negative bacteria

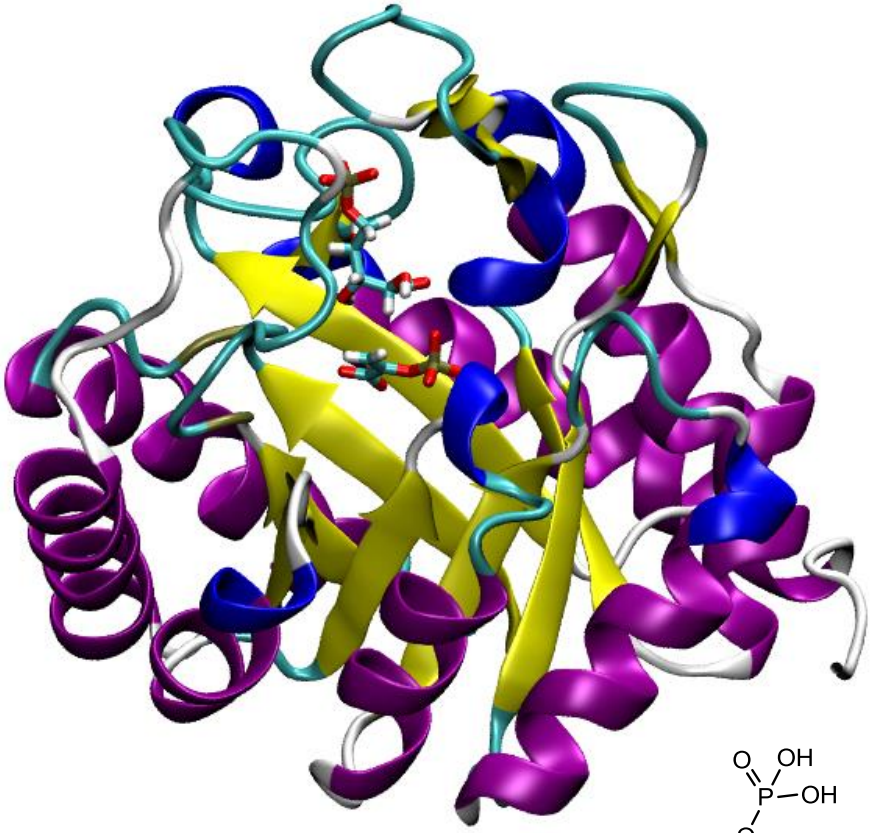




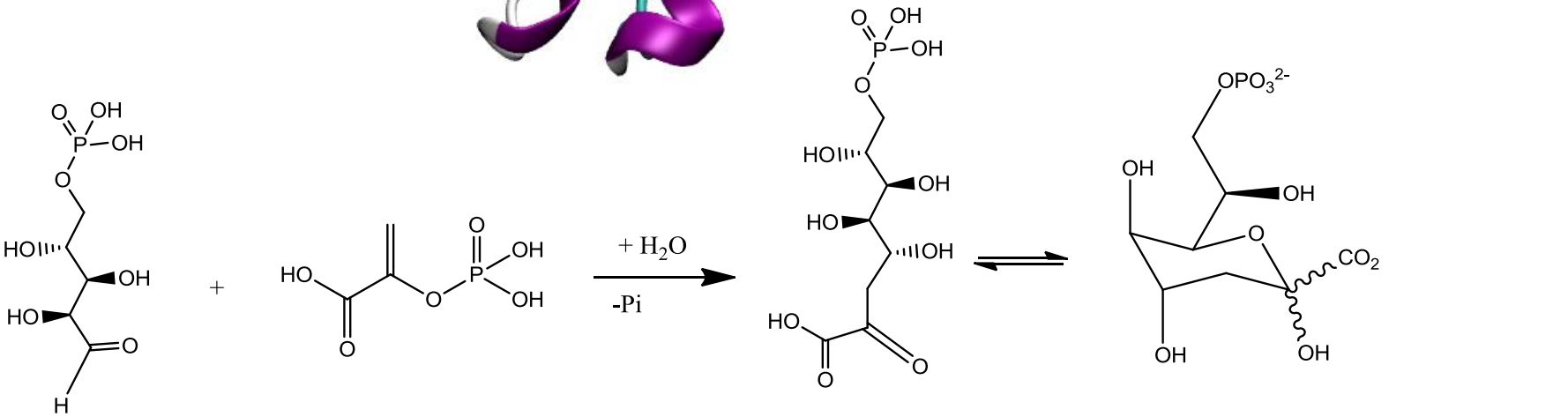
http://www.hhmi.org/biointeractive/Antibiotics_Attack/bb_2a.html

http://dlg.myweb.uga.edu/gm-ve.gif





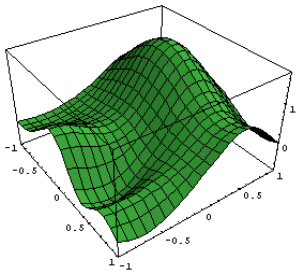
KDO8P synthase



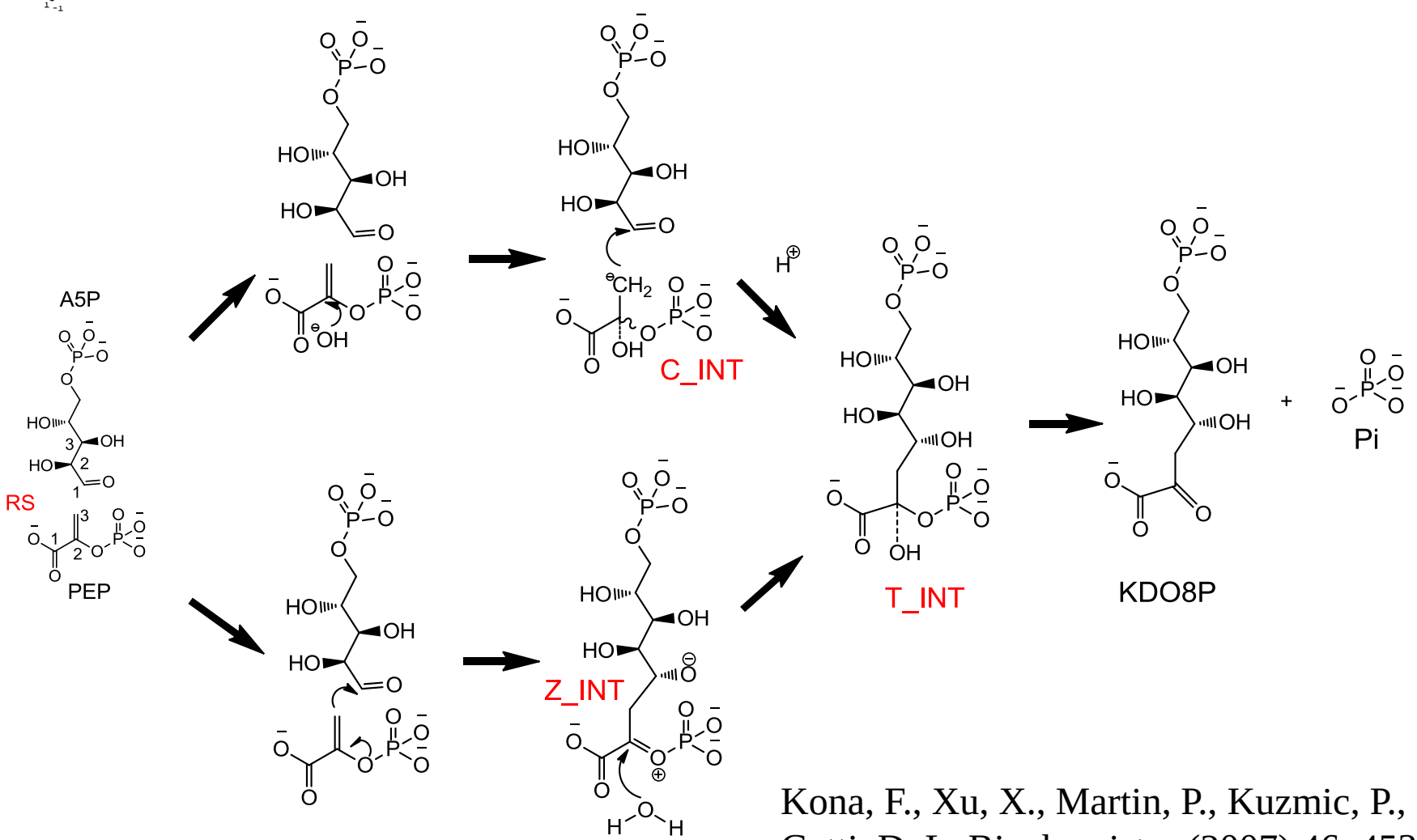
Arabinose 5-phosphate (A5P)

Phosphoenolpyruvate (PEP)

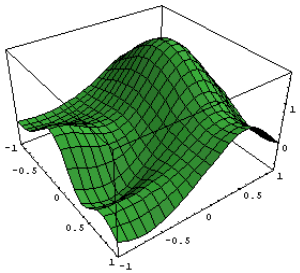
3-Deoxy-D-manno-octulosonate-8-phosphate (KDO8P)



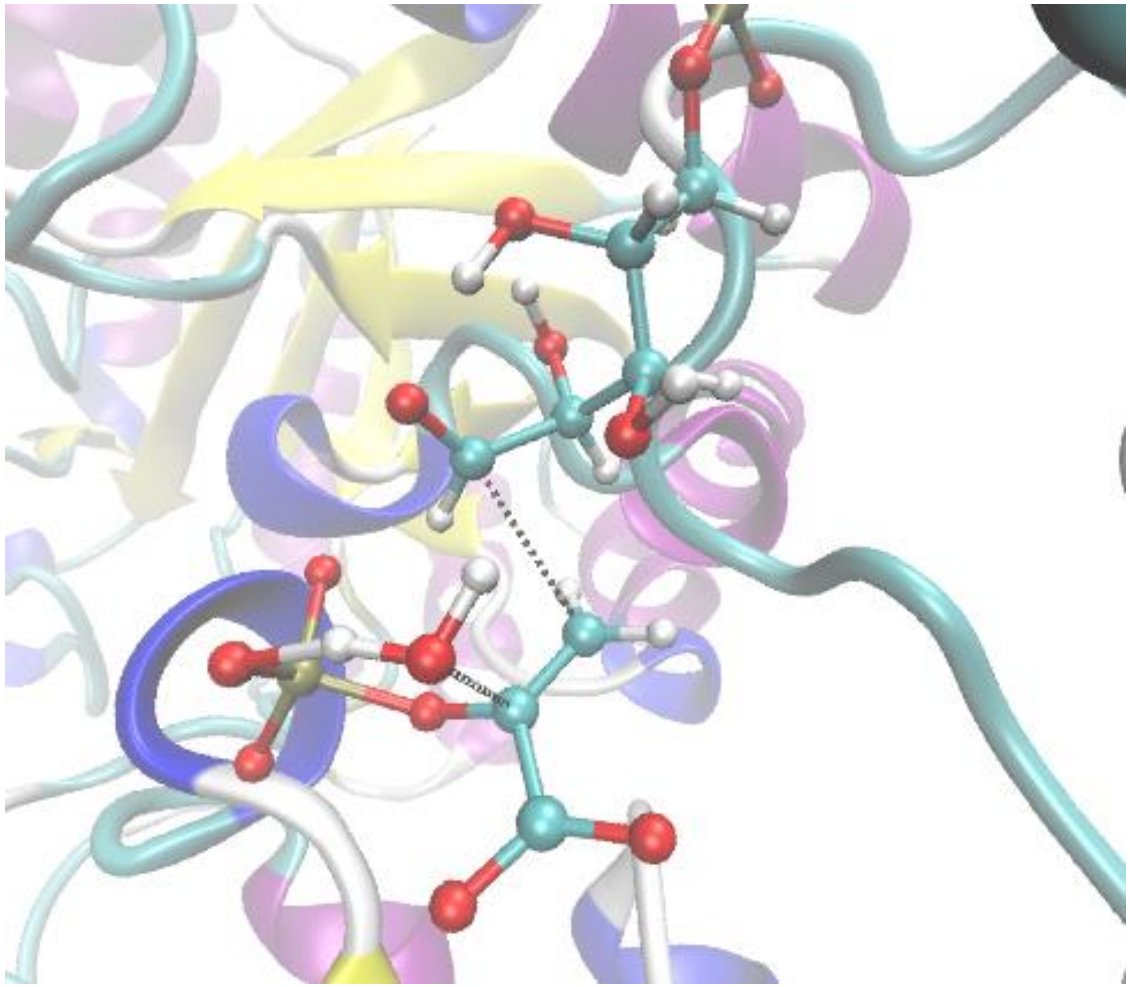
KDO8PS Proposed Mechanisms



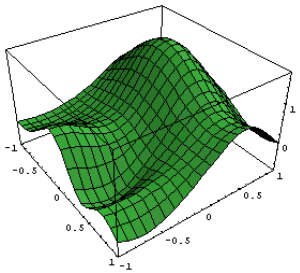
Kona, F., Xu, X., Martin, P., Kuzmic, P., Gatti, D. L. Biochemistry (2007) 46, 4532



Exploring the KDO8PS PES

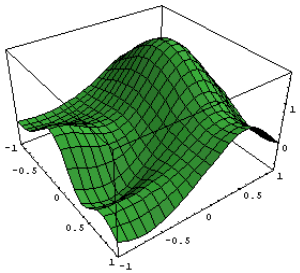


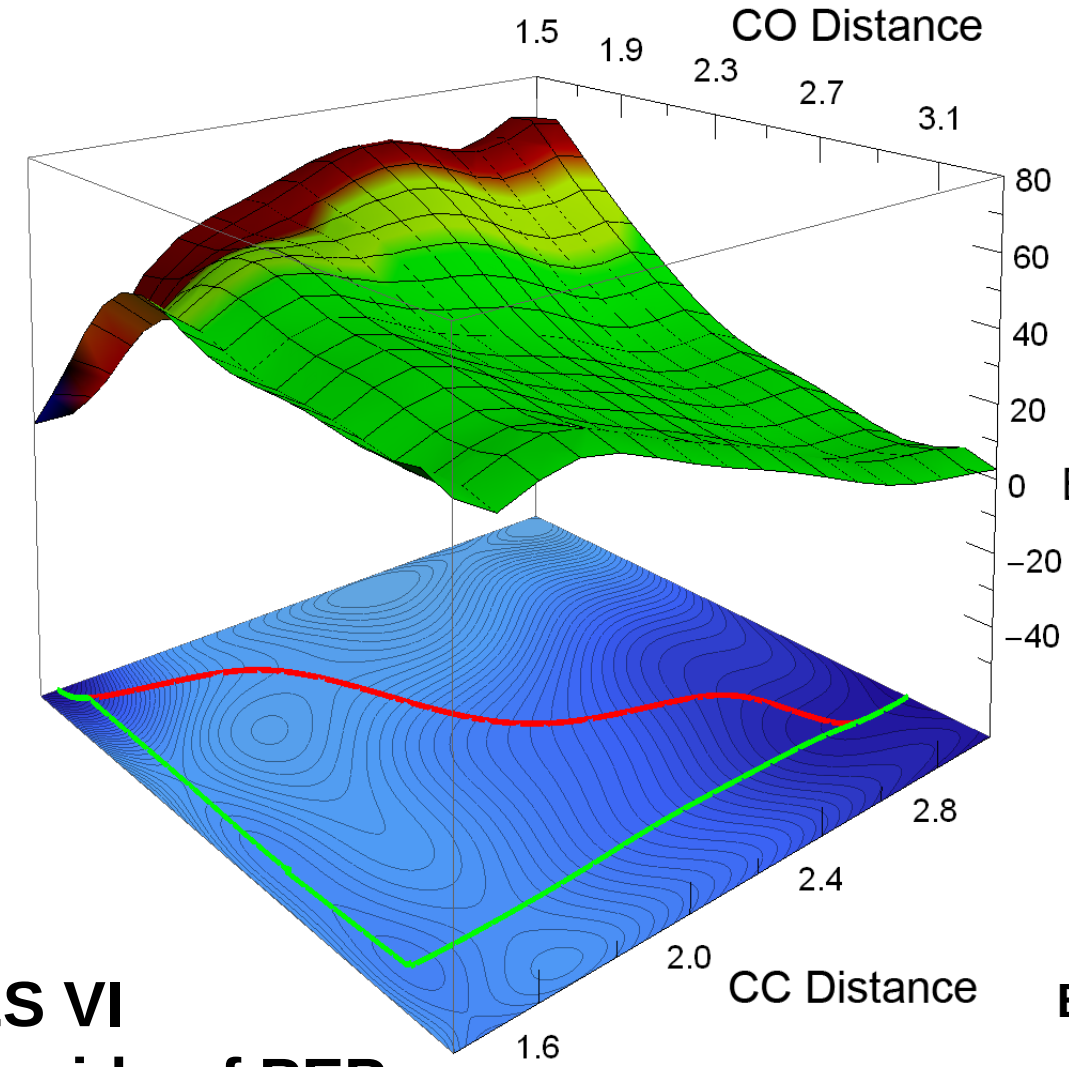
Tao, P.; Gatti, D. L.; Schlegel, H. B.
***Biochem*. 2009, *48*, 11706–11714 (10.1021/bi901341h)**

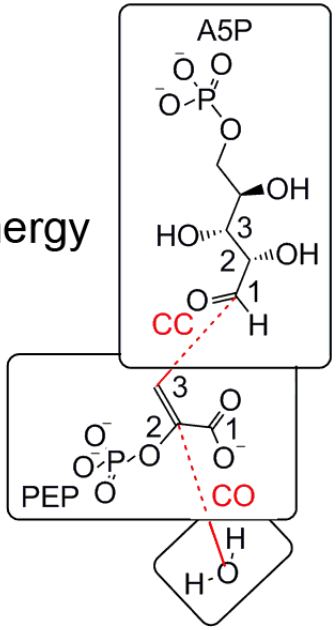


Protocol for QM/MM calculations on KDO8Pase

- Crystal structure of KDO8Pase from *A. aeolicus* (PDB entry 2NWS)
- Simulated annealing and MD to prepare complex
- 2 dimensional reduced potential energy surface constructed by constraining the forming C-C and C-O bonds and optimizing all the remaining parameters
- QM/MM optimization using ONIOM(B3LYP/3-21G:AMBER) followed by more accurate energy calculation using ONIOM(B3LYP/6-31+G(d,p):AMBER) with electronic embedding
- ca. 200 points for each reduced potential energy surface
- Transition states and reaction paths obtained from a polynomial fit to the reduced surface

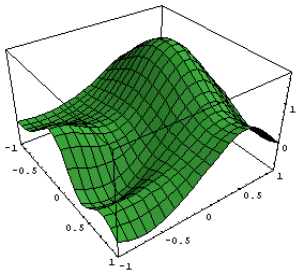


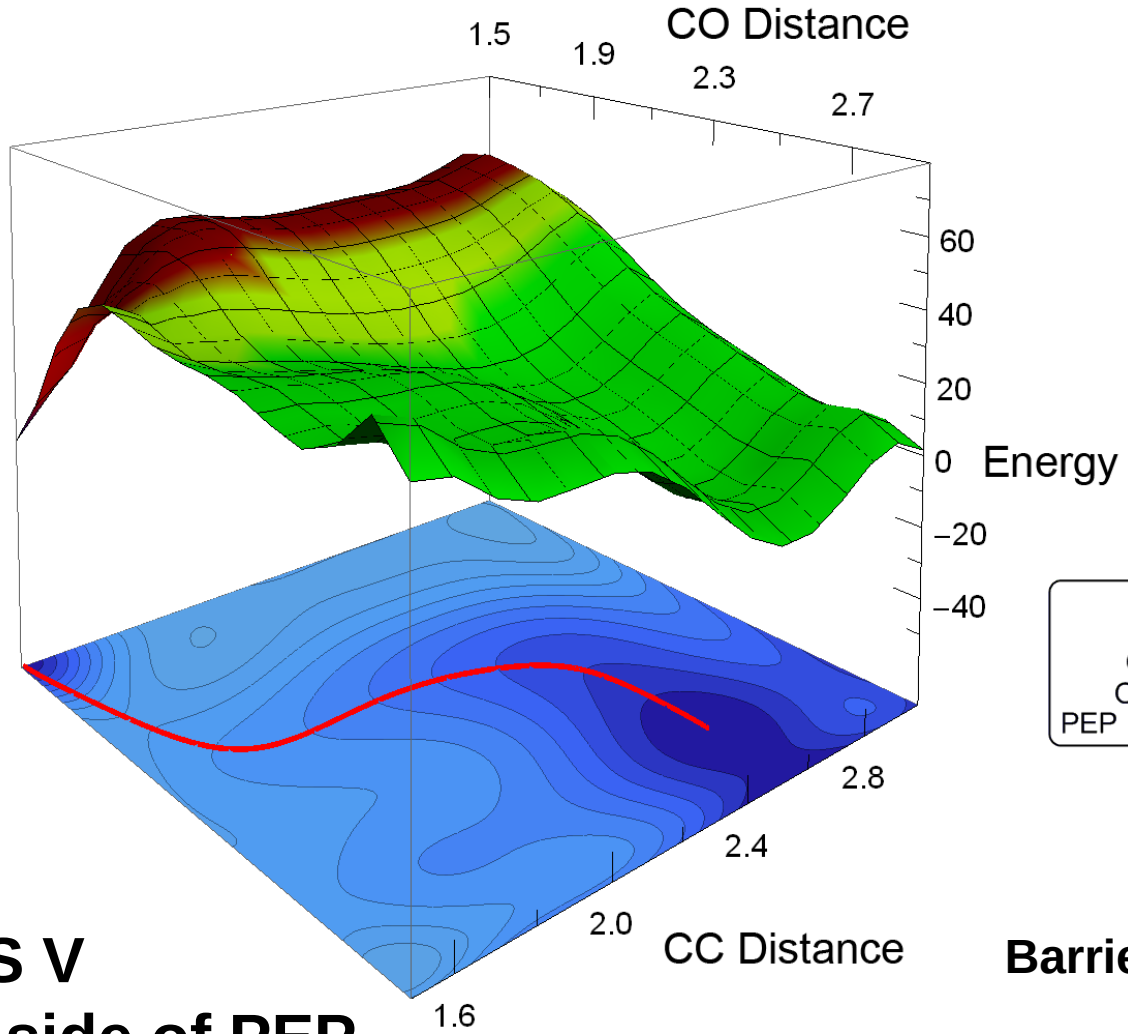


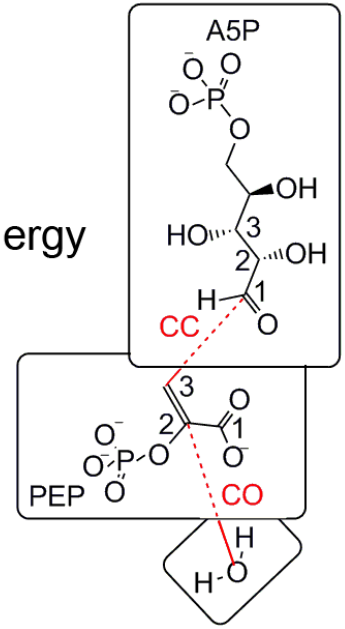


PES VI
(water on *re* side of PEP
PEP on *si* side of A5P*)*

Barrier: 44 kcal/mol

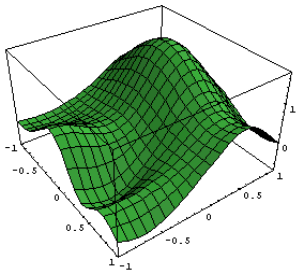


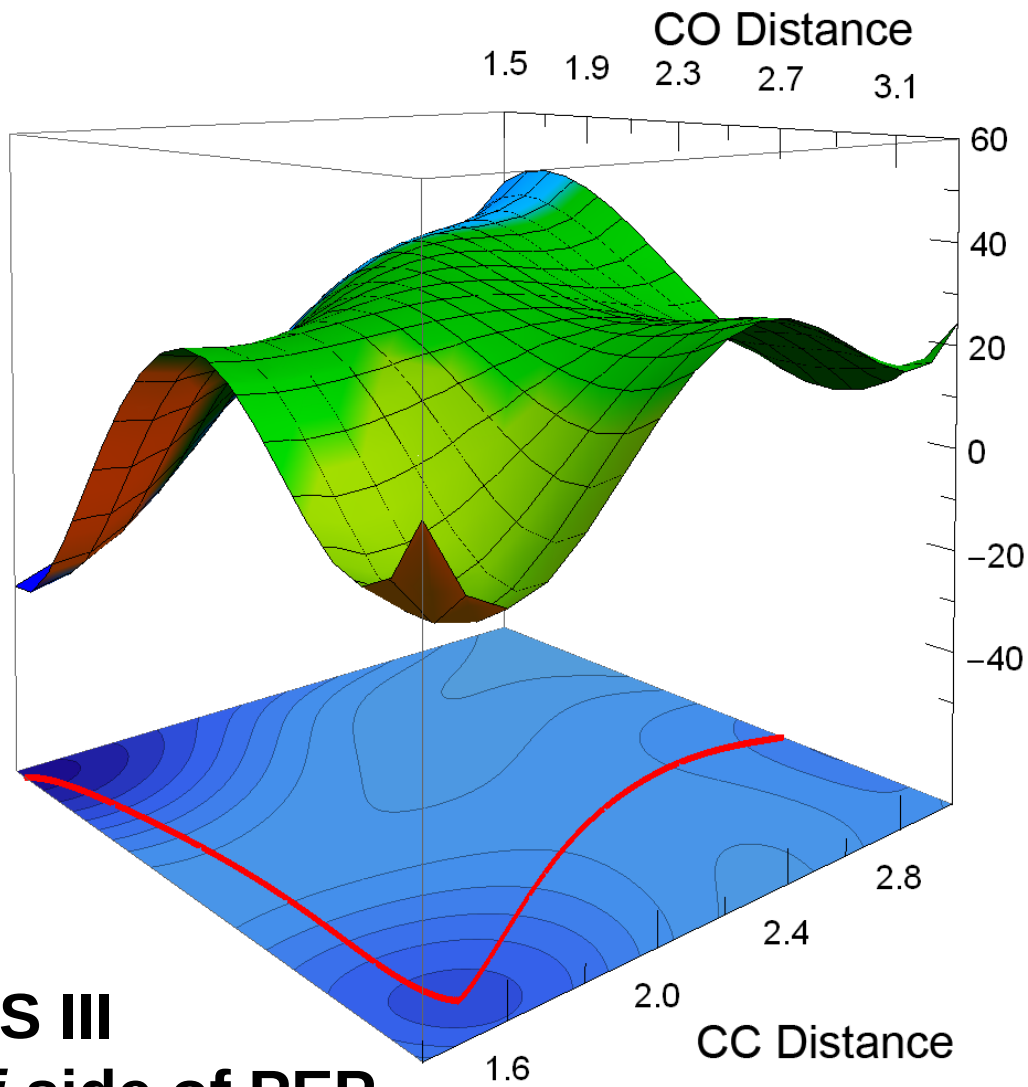


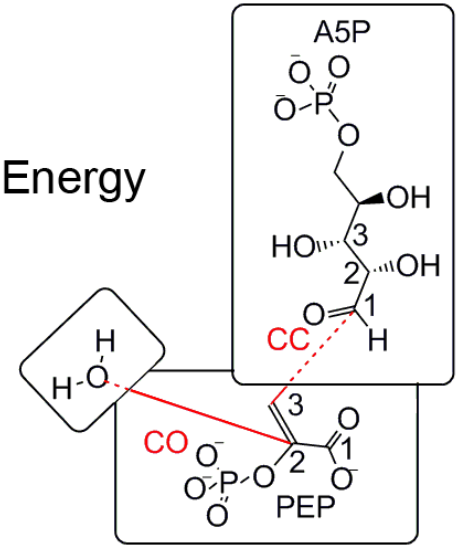


PES V
(water on *re* side of PEP
PEP on *re* side of A5P*)*

Barrier: 44 kcal/mol

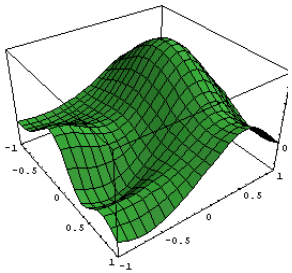


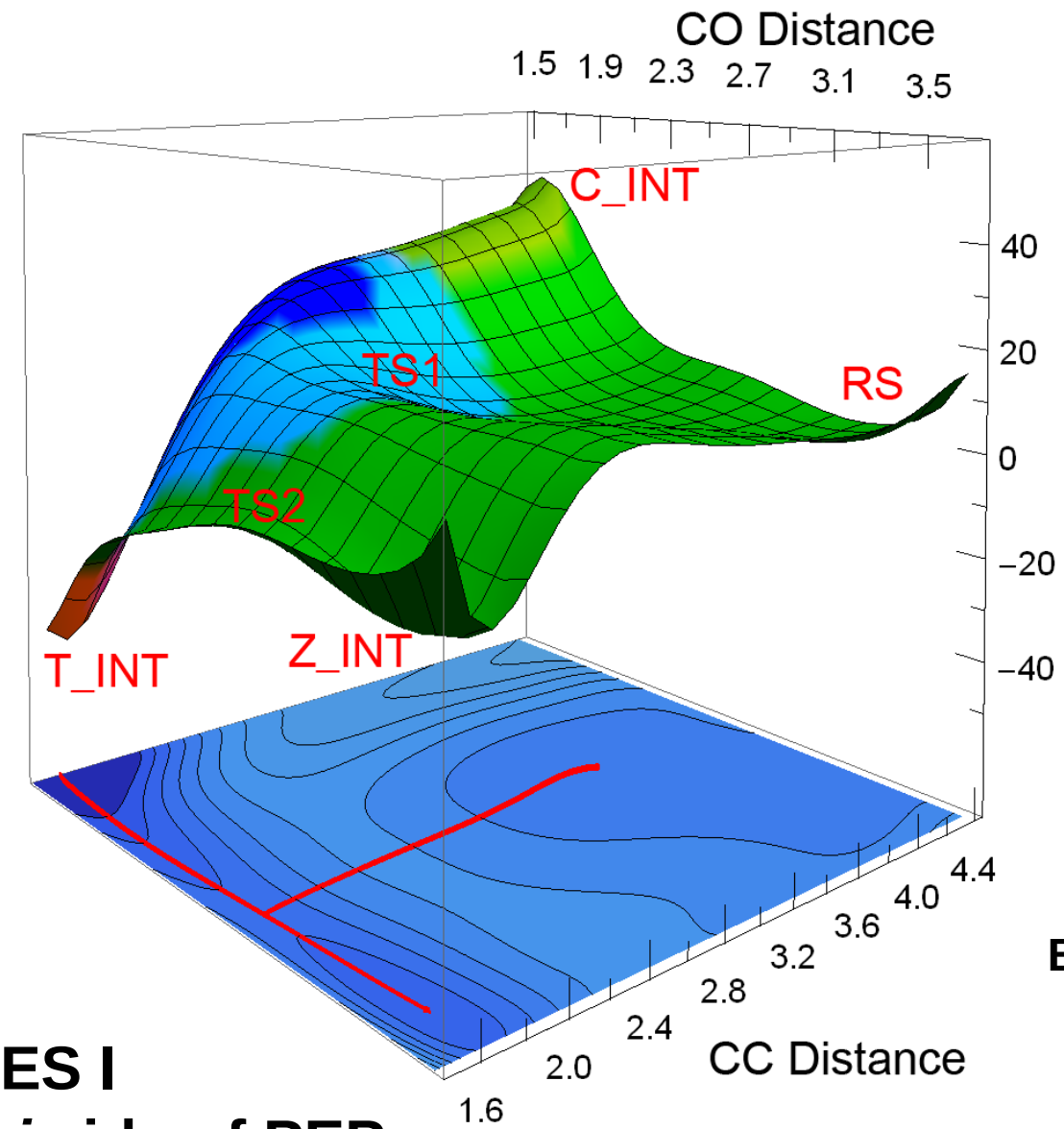


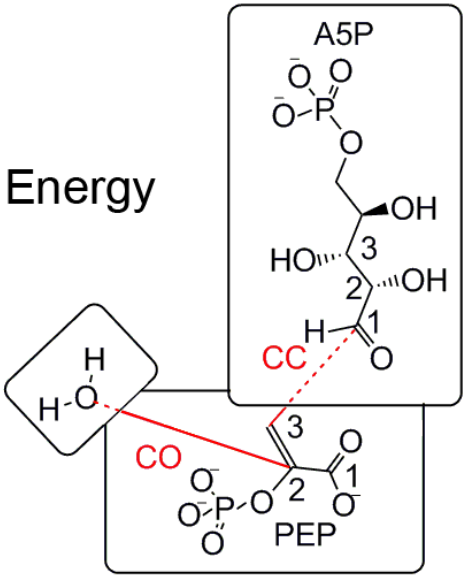


PES III
(water on *si* side of PEP
PEP on *si* side of A5P*)*

Barrier: 25 kcal/mol



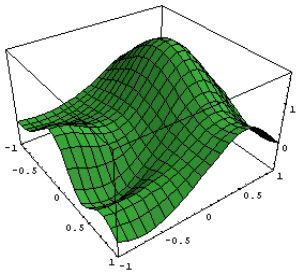




Barrier: 14 kcal/mol

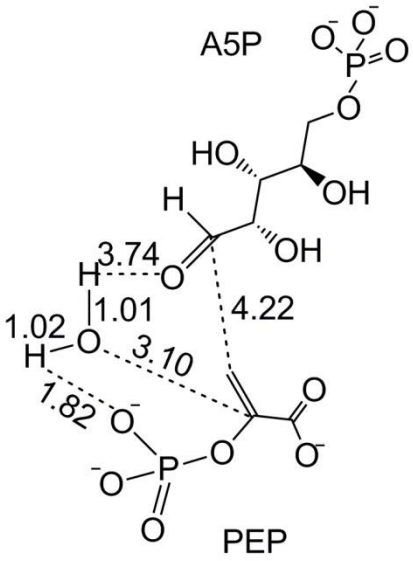
PES I
(water on *si* side of PEP
PEP on *re* side of A5P*)*

Summary

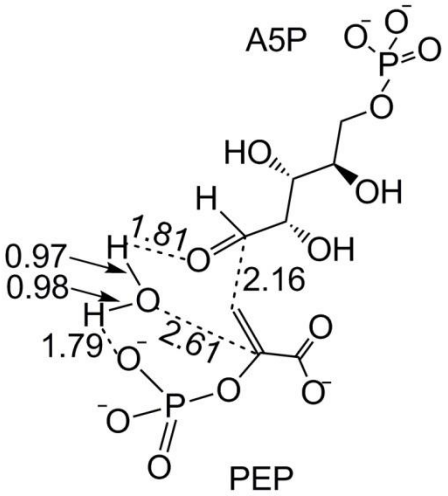


PES 1

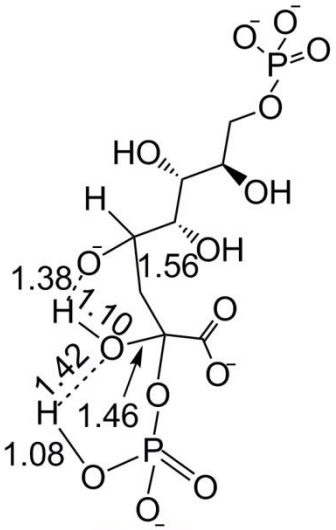
- **14 kcal/mol barrier**
- **water and PEP attacks concerted**
- **water from *si* side**
- **PEP from *re* side**



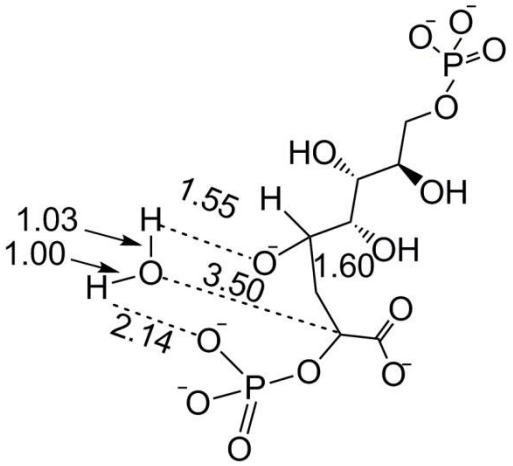
RS



TS1



T_INT



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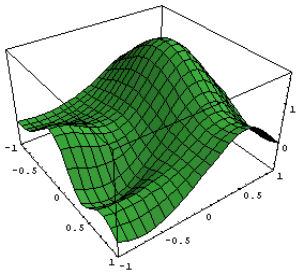

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