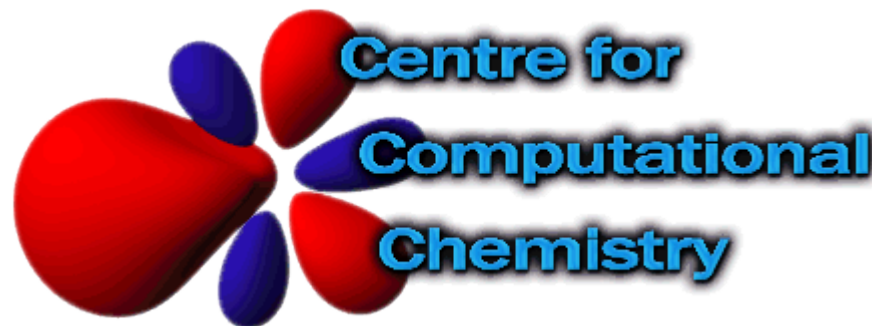


Towards chemical accuracy in QM/MM modelling of enzyme catalytic mechanisms and protein-ligand binding



Adrian Mulholland

Centre for Computational Chemistry
School of Chemistry, University of Bristol

BioExcel Virtual Workshop on Best Practices in QM/MM Simulation of Biomolecular Systems, 14th December 2020

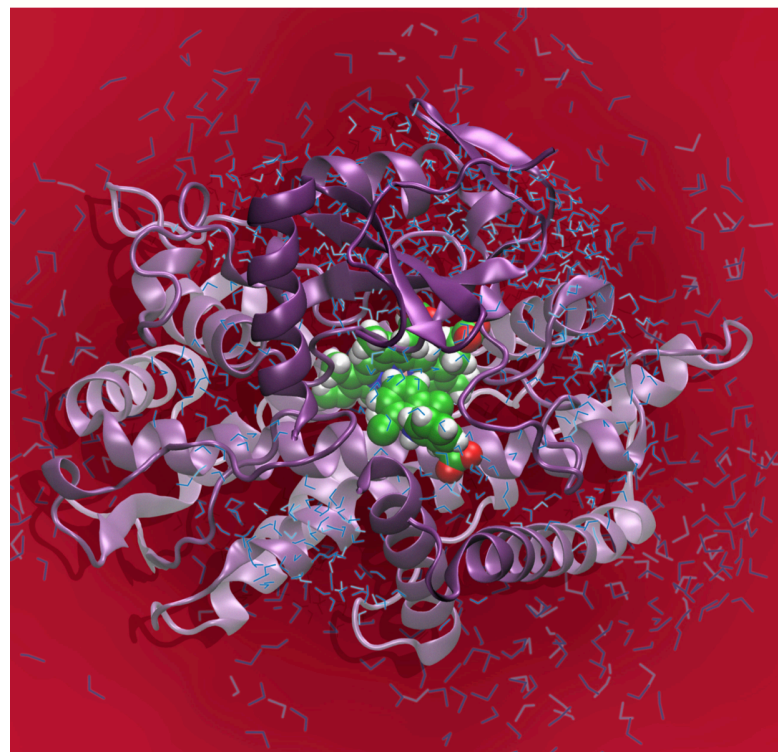
🔥 Multiscale modelling for chemical biology

- Many problems in biology are inherently multiscale
- E.g. drugs are typically small molecules
- Chemical change has macroscopic effects
- Multiscale methods couple together different levels of theory

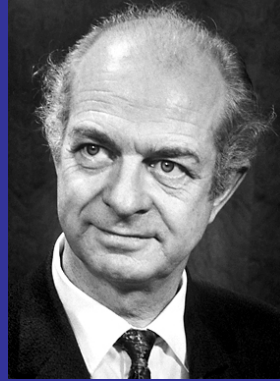
nature
REVIEWS

April 2018 volume 2 no. 4
www.nature.com/reviews

CHEMISTRY



🔥 Transition state analogues as enzyme inhibitors

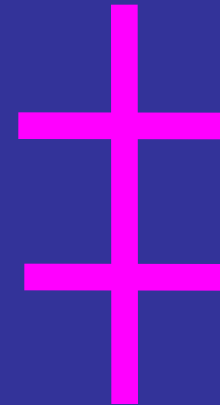
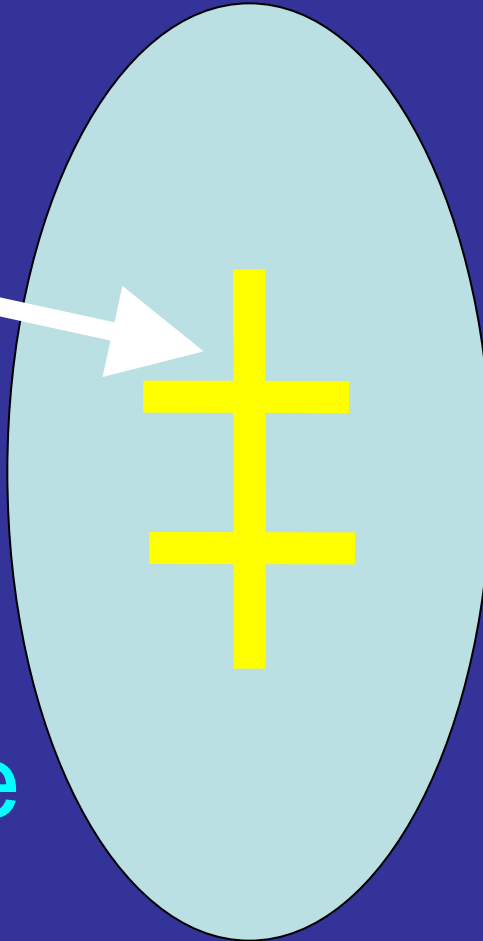


Linus Pauling

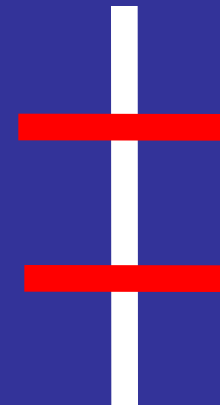
Enzyme
active
site



Enzyme



Transition
state



Transition
state
analogue
drug

A METHOD FOR DETERMINING REACTION PATHS IN LARGE MOLECULES: APPLICATION TO MYOGLOBIN ☆

R. ELBER and M. KARPLUS

Department of Chemistry, Harvard University, 12 Oxford Street, Cambridge, MA 02138, USA

Received 8 July 1987



Graham Richards,
PhD supervisor

An algorithm is described for determining reaction paths between two known structures with many degrees of freedom. The method uses first-derivative techniques to optimize the entire path between the two end forms subject to certain constraints. The stability and convergence properties of the method are illustrated by applications to structural transitions in two test systems (cyclohexane and dialanine) and to a conformational change involving all degrees of freedom in the protein, myoglobin, with 1531 atoms.

1. Introduction

The problem of finding the transition state or, more generally, a reaction path between two known local minimum-energy structures is of considerable interest [1–4]; methods currently in use have been reviewed recently [1]. For small and intermediate sized molecules with less than 100 degrees of freedom, search methods involving local propagation by means of the second derivative of the potential (modified Newton–Raphson algorithms) have been shown to be effective [2,3]. However, they are difficult to extend to large systems, such as proteins with more than a thousand degrees of freedom, because

dialanine) and to the protein myoglobin are given in section 3.

2. Method

We consider the general problem of minimizing the line integral, $S(\mathbf{R}_i, \mathbf{R}_f)_L$

$$S(\mathbf{R}_i, \mathbf{R}_f)_L = \frac{1}{L} \int_{\mathbf{R}_i}^{\mathbf{R}_f} [\mathbf{G}(\mathbf{R}) \cdot d\mathbf{I}(\mathbf{R})]_L, \quad (1)$$

where $\mathbf{G}(\mathbf{R})$ is a function of the N -dimensional position vector \mathbf{R} representing the coordinates of the sys-

Computer Simulation and Analysis of the Reaction Pathway of Triosephosphate Isomerase[†]

P. A. Bash,^{‡,§} M. J. Field,^{‡,||} R. C. Davenport,^{⊥,○} G. A. Petsko,^{⊥,#} D. Ringe,^{⊥,#} and M. Karplus^{*,‡}

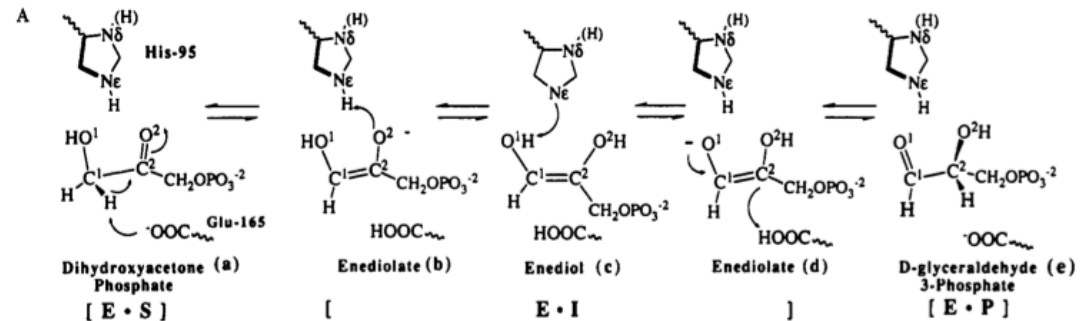
Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138, and Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received February 12, 1991; Revised Manuscript Received March 22, 1991

Accelerated Publications

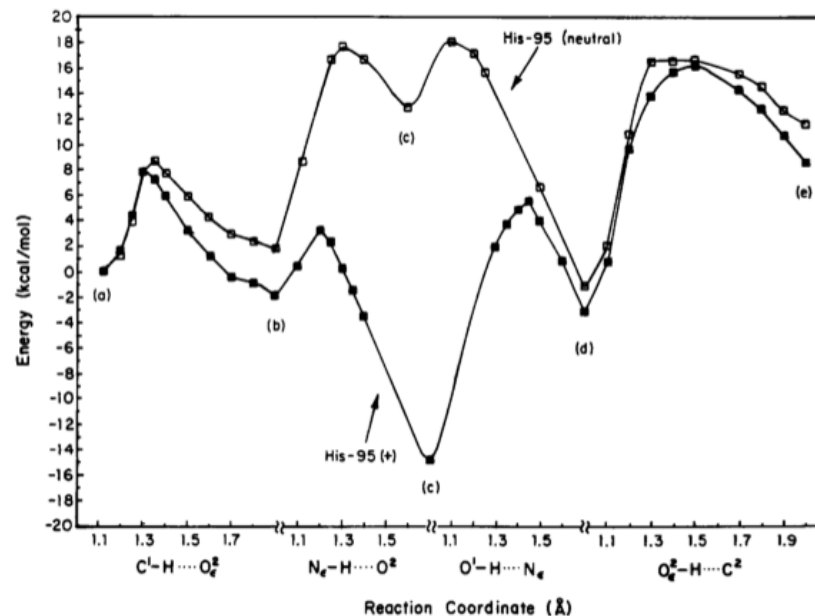
Biochemistry, Vol. 30, No. 24, 1991 5827

- AM1/ CHARMM
- QM/MM
- Suggests neutral histidine



B

TIM Reaction Energy Profile



Bash *et al.* Biochemistry 1991

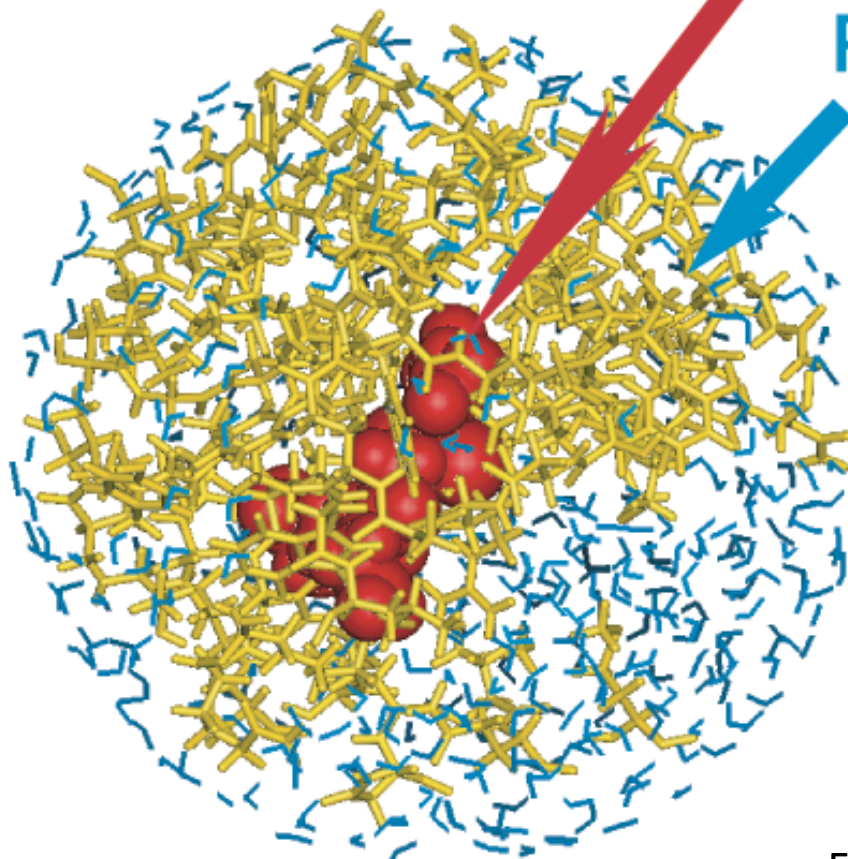


Martin Karplus

QM/MM methods

Substrate, catalytic residues
treated by QM

Protein, solvent treated
MM; interacts with QM



Modelling enzyme catalytic
mechanisms with QM/MM
methods

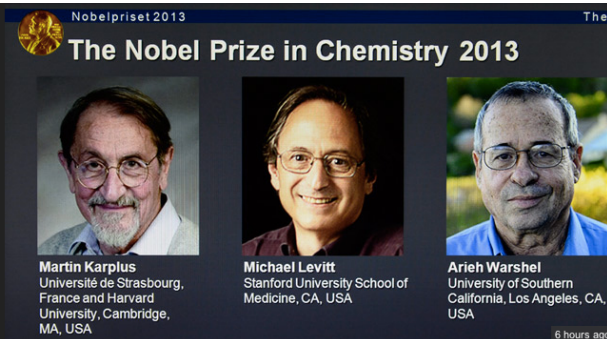
Warshel & Karplus *JACS* 1972

Warshel & Levitt *J. Mol. Biol.* 1976

Field, Bash & Karplus *J. Comp. Chem.* 1990

Nobel Prize for Chemistry 2013:

Karplus, Levitt & Warshel

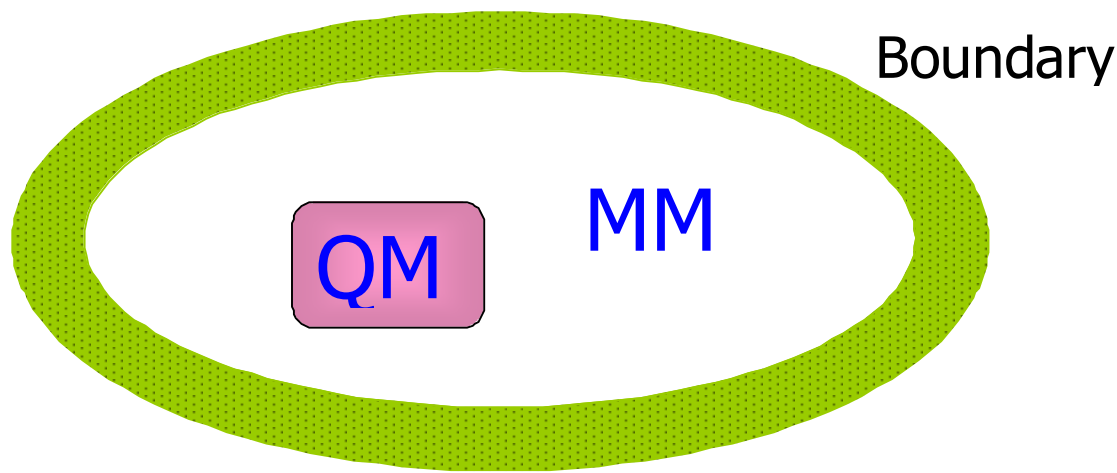


Senn & Thiel *Angewandte Chemie* 2009

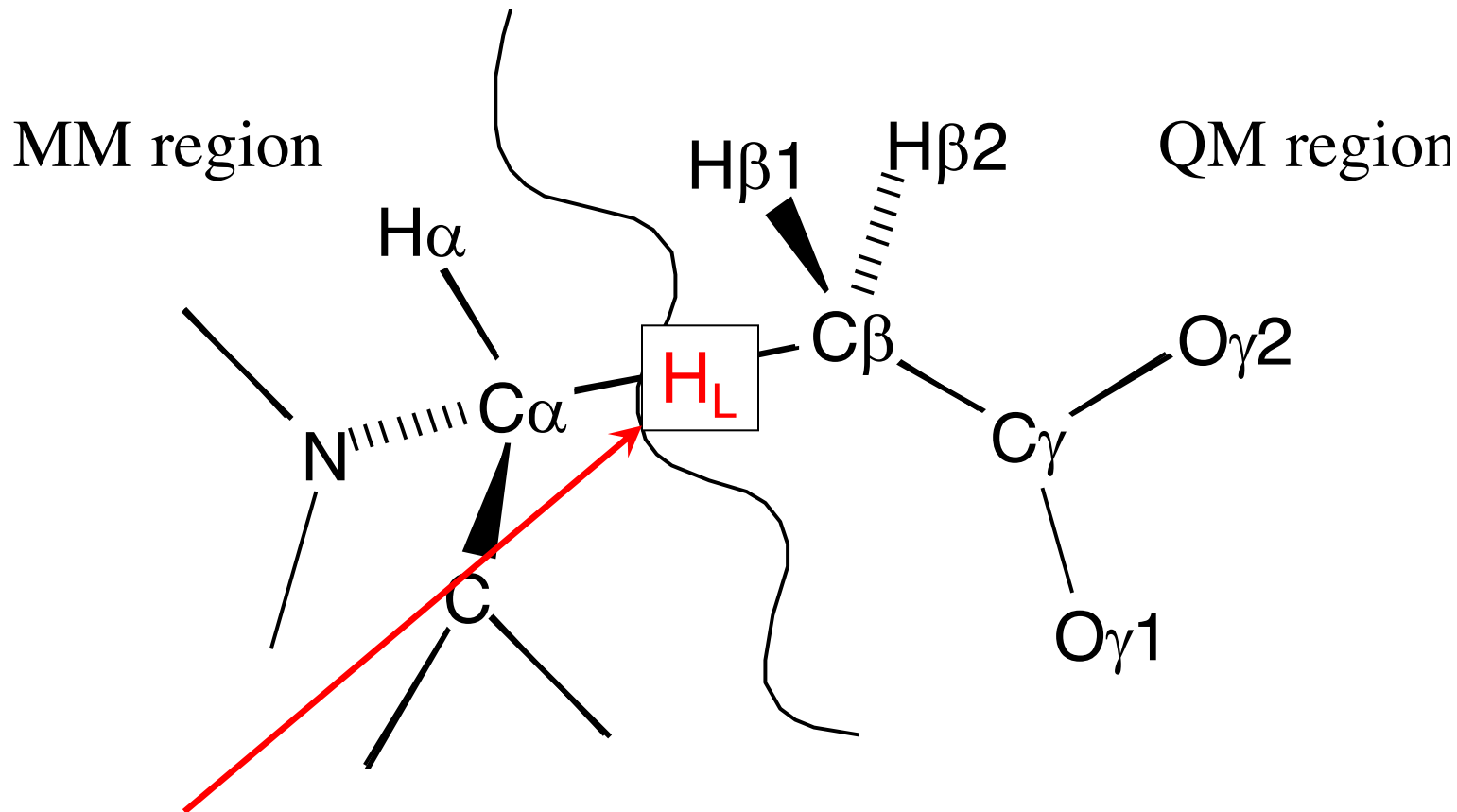
van der Kamp & Mulholland *Biochemistry* 2013

Combined 'quantum mechanics/molecular mechanics' (QM/MM) methods

- **Small QM region (active site)**
- **MM for surrounding protein, solvent**
- **QM & MM regions interact – (van der Waals and electrostatic)**
- **QM atoms 'feel' MM atomic charges: i.e. the QM region is polarized by them**
- **'Ab initio', DFT or more approximate semiempirical QM**



QM/MM partitioning



- ‘Link’ H-atom added to satisfy QM atom valence when QM/MM border is across a bond
- MM bonded energy terms (e.g. bond stretching etc.) included when at least 1 MM atom is involved



Some early applications of QM/MM in CHARMM

- **Citrate synthase** (Mulholland & Karplus, *Biochem Soc Trans* 1996; Mulholland & Richards, *Proteins* 1997; Mulholland, Lyne and Karplus, *JACS* 2000)
- **Chorismate mutase** (Lyne *et al.*, *JACS* 1995)
- **DNA cross-linking** (Elcock *et al.*, *JACS* 1995)
- ***para*-hydroxybenzoate hydroxylase** (Ridder *et al.*, *JACS* 1998)

J. Am. Chem. Soc. **1998**, *120*, 7641–7642

7641

Correlation of Calculated Activation Energies with Experimental Rate Constants for an Enzyme Catalyzed Aromatic Hydroxylation

Lars Ridder,^{*,†} Adrian J. Mulholland,^{‡,§} Jacques Vervoort,[†] and Ivonne M. C. M. Rietjens[†]

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Cambridge, Massachusetts 02138*

Received February 26, 1998

Revised Manuscript Received April 28, 1998

p-Hydroxybenzoate hydroxylase (PHBH, EC 1.14.13.2) is involved in the microbial degradation of aromatic compounds. It has become the model enzyme for the family of external flavoprotein monooxygenases.¹ In the present study, a combined quantum mechanical (QM) and molecular mechanical (MM) method is applied to calculate energy barriers for the hydroxy-

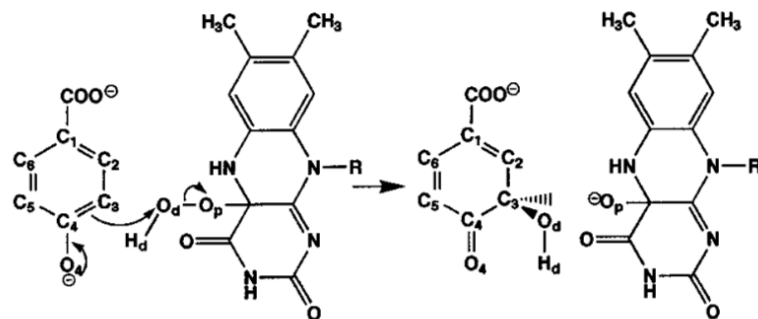
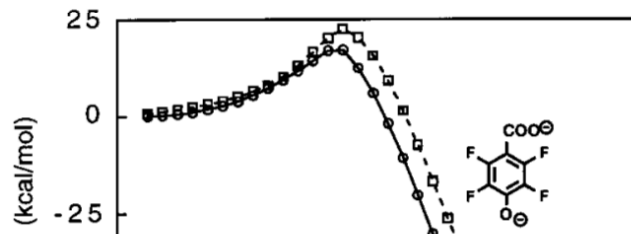


Figure 1. The electrophilic attack of the distal oxygen of the flavin cofactor on the substrate according to an electrophilic aromatic substitution mechanism. The cyclohexadienone formed converts into the final product 3,4-dihydroxybenzoate via keto–enol tautomerization.



Comparison with experiment

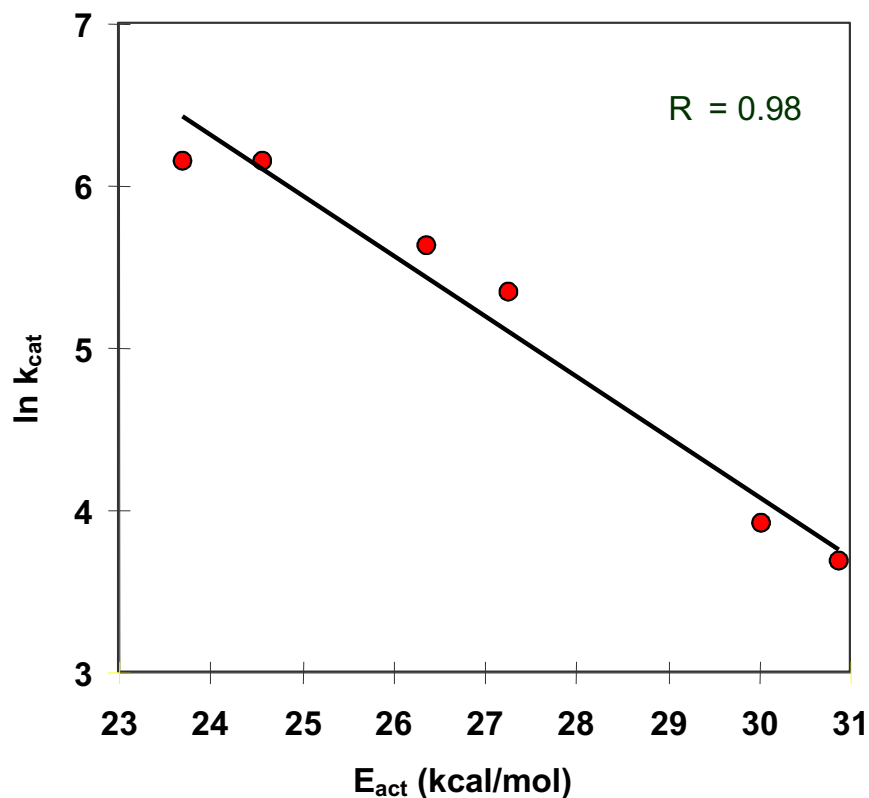
- Can we discriminate between alternative possible mechanisms?
- Test by comparison with experiment
- E.g. simple transition state theory, dynamical contributions to rate contained in transmission coefficient, κ

$$k_{TST} = \kappa \left(\frac{kT}{h} \right) e^{-\Delta^\ddagger G / RT}$$

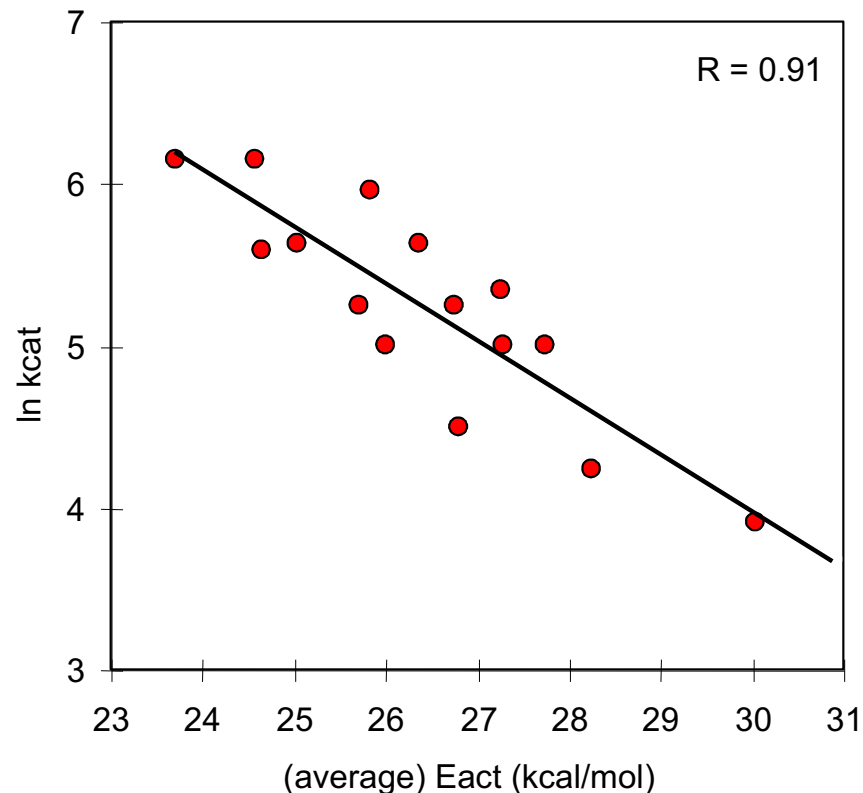
Consistency with experimental rates (e.g. agree within 10kcal/mol? 5?)

🔥 Correlation of QM/MM energy barriers with experimental k_{cat} for phenol hydroxylase

- Symmetrical phenols

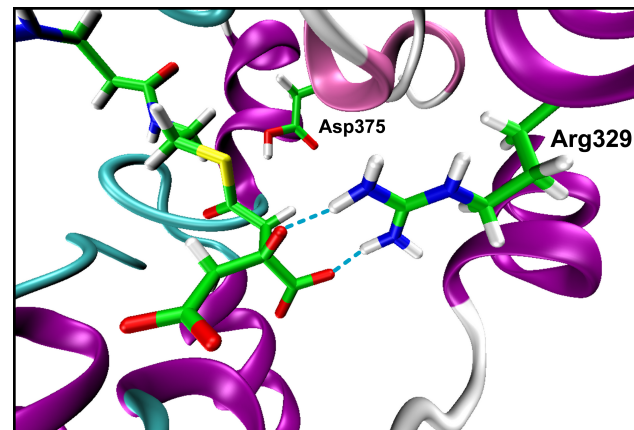


- All phenols



AM1/CHARMM; Ridder *et al.* JACS 1998, 2000

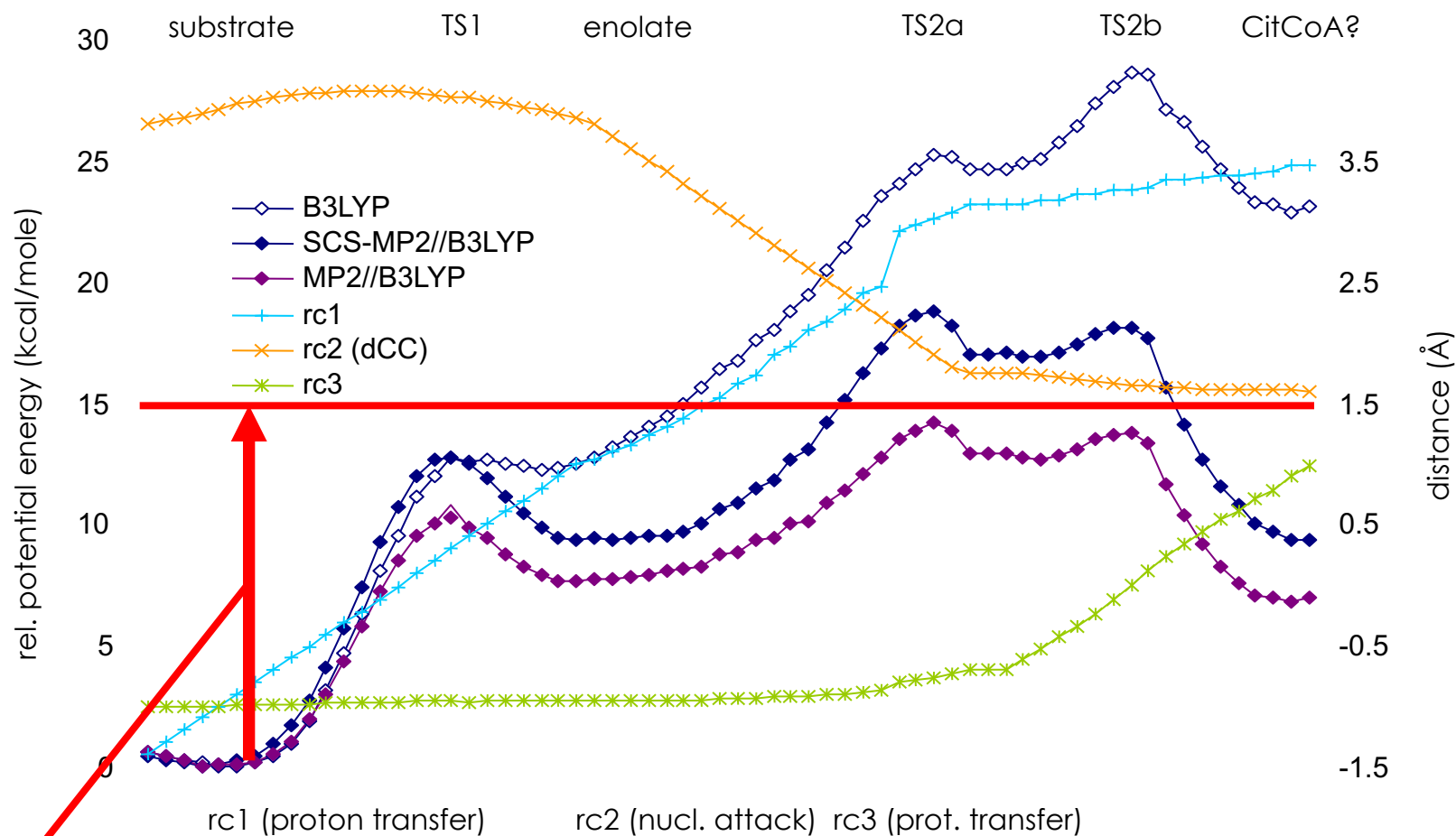
Ab initio QM/MM calculations
predict arginine as an acid



van der Kamp *et al.* *Chem Commun.* 1874-6 (2008)
doi: 10.1039/b800496j



Arginine as the acid for citryl-CoA formation in citrate synthase?

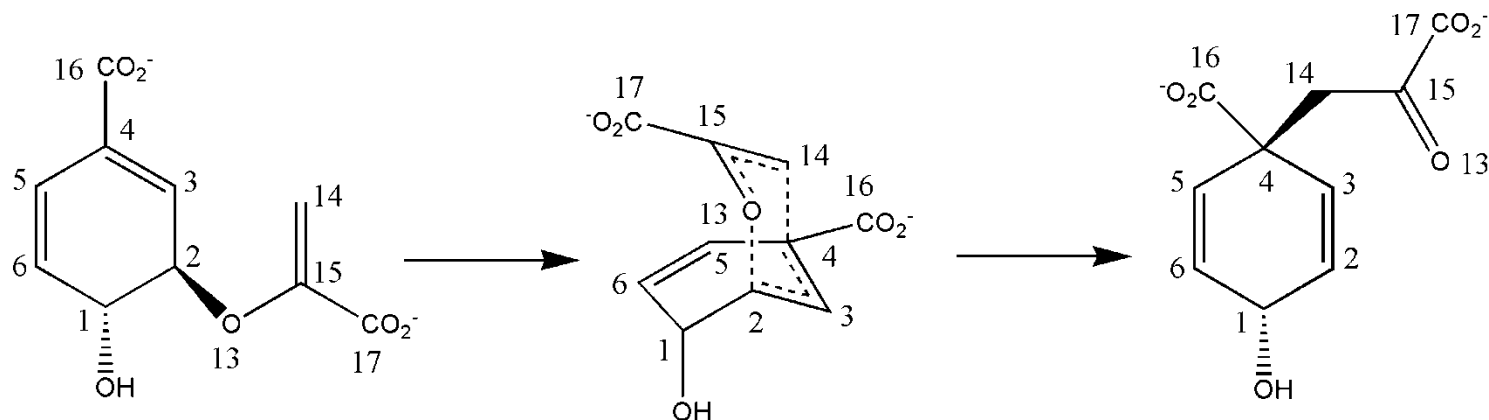


- Experimental $\Delta^\ddagger G \approx 15$ kcal/mol)

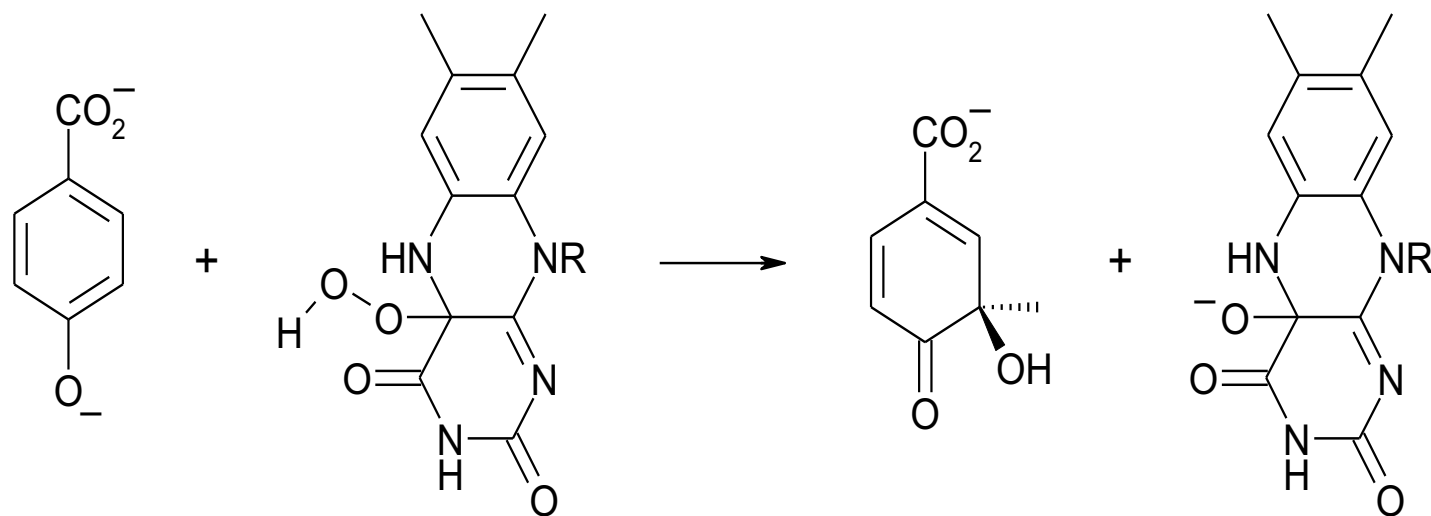
*van der Kamp et al.,
Chem. Commun. 2008*

Testing QM/MM: chorismate mutase (CM) and *p*-hydroxybenzoate hydroxylase (PHBH)

CM



PHBH



QM/MM activation enthalpies $\Delta^\ddagger H$ for CM and PHBH (kcal/mol)

Method	CM		PHBH	
Hartree-Fock	28.3	(2.1)	36.7	(2.6)
B3LYP	10.2	(1.8)	8.4	(1.4)
LMP2	9.5	(1.0)	10.7	(1.2)
LCCSD(T0)	13.1	(1.1)	13.3	(1.5)
Experiment	12.7		12.0	

Activation enthalpies from averages of energy differences from single-point QM/MM calculations for the reactant complex and the TS on different adiabatic pathways. aug-cc-pVTZ basis used on oxygen and cc-pVTZ on all other atoms, and a point-charge representation of the MM environment was included in the QM calculations

Calculating activation free energies

- Molecular dynamics simulations at low QM/MM levels (e.g. AM1, SCC-DFTB)
- Entropic contributions from difference between activation free energy and mean activation enthalpy at low QM/MM level
- For CM, entropic contribution (300K) is 2.5 kcal/mol (experiment gives 2.7 kcal/mol)
- PHBH activation free energy = 13.7 kcal/mol (experiment gives 14-15 kcal/mol)

Implications

- **High-level QM/MM gives near-quantitative activation enthalpies and free energies for CM and PHBH**
- **‘Chemically accurate’ QM/MM calculations of activation energies in best cases**
- **Agreement with experiment shows transition state theory works well**
- **Allows reliable predictions of reaction mechanisms in enzymes**

Transition state stabilization in enzyme catalysis

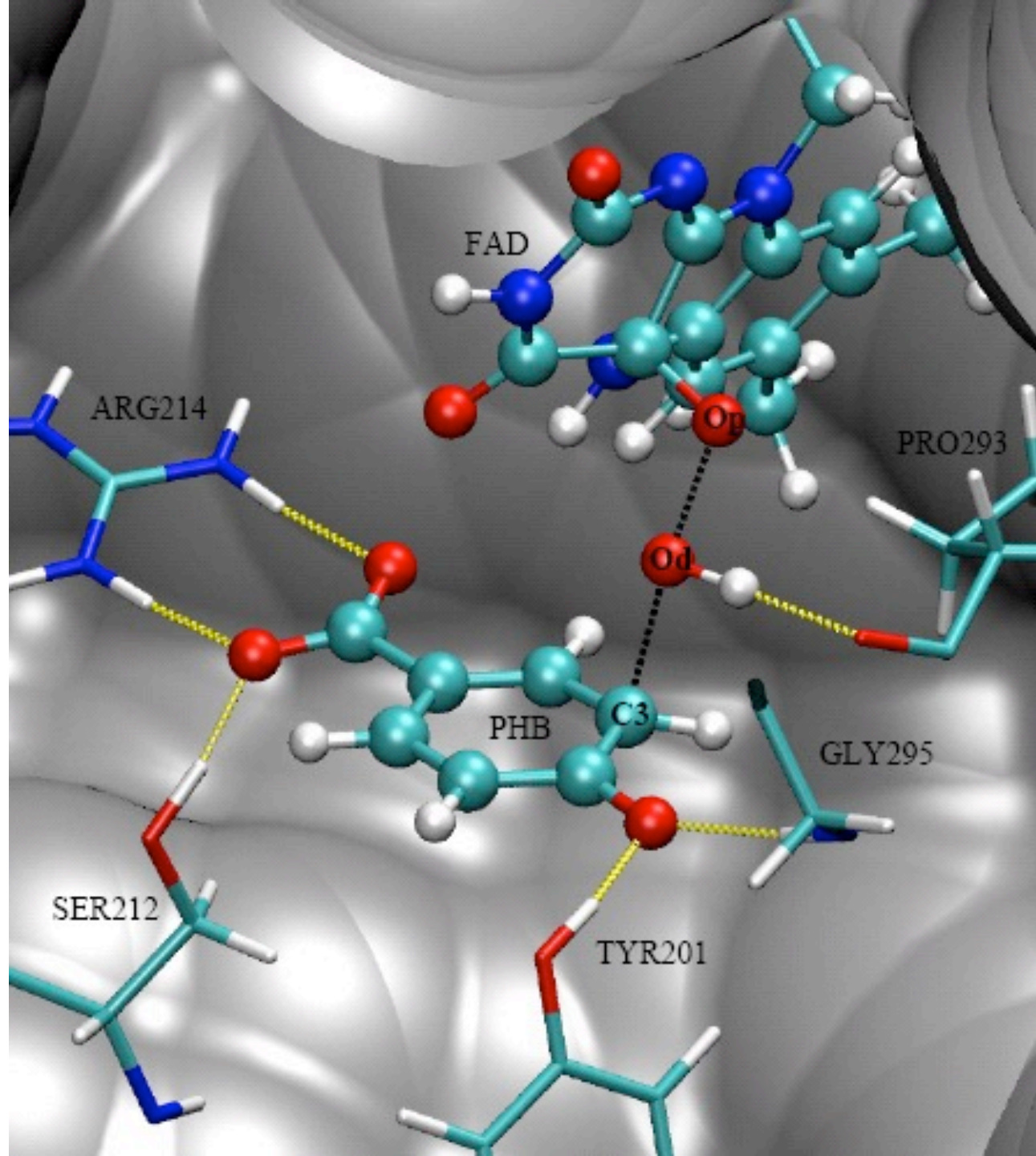
[Ridder et al. 1999](#)

[Ridder et al. 2003](#)

[Claeyssens et al.](#)

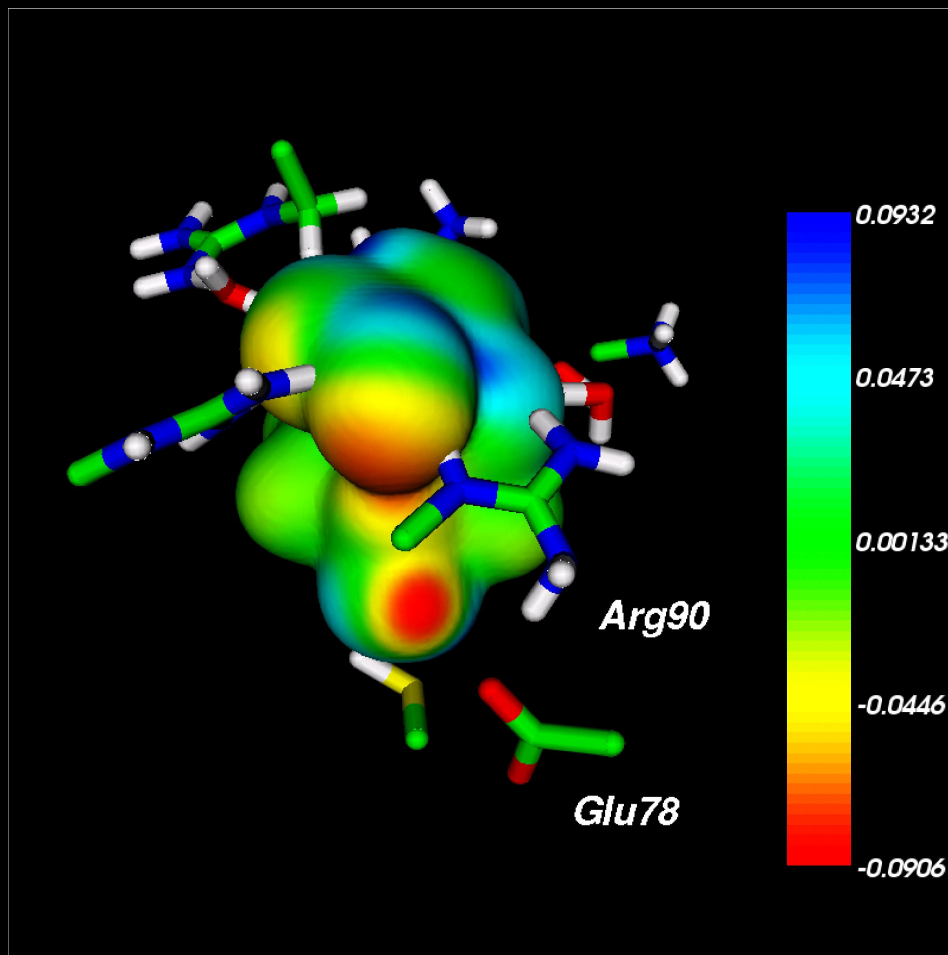
[Angewandte 2006](#)

(TS in *para*-
hydroxybenzoate
hydroxylase)



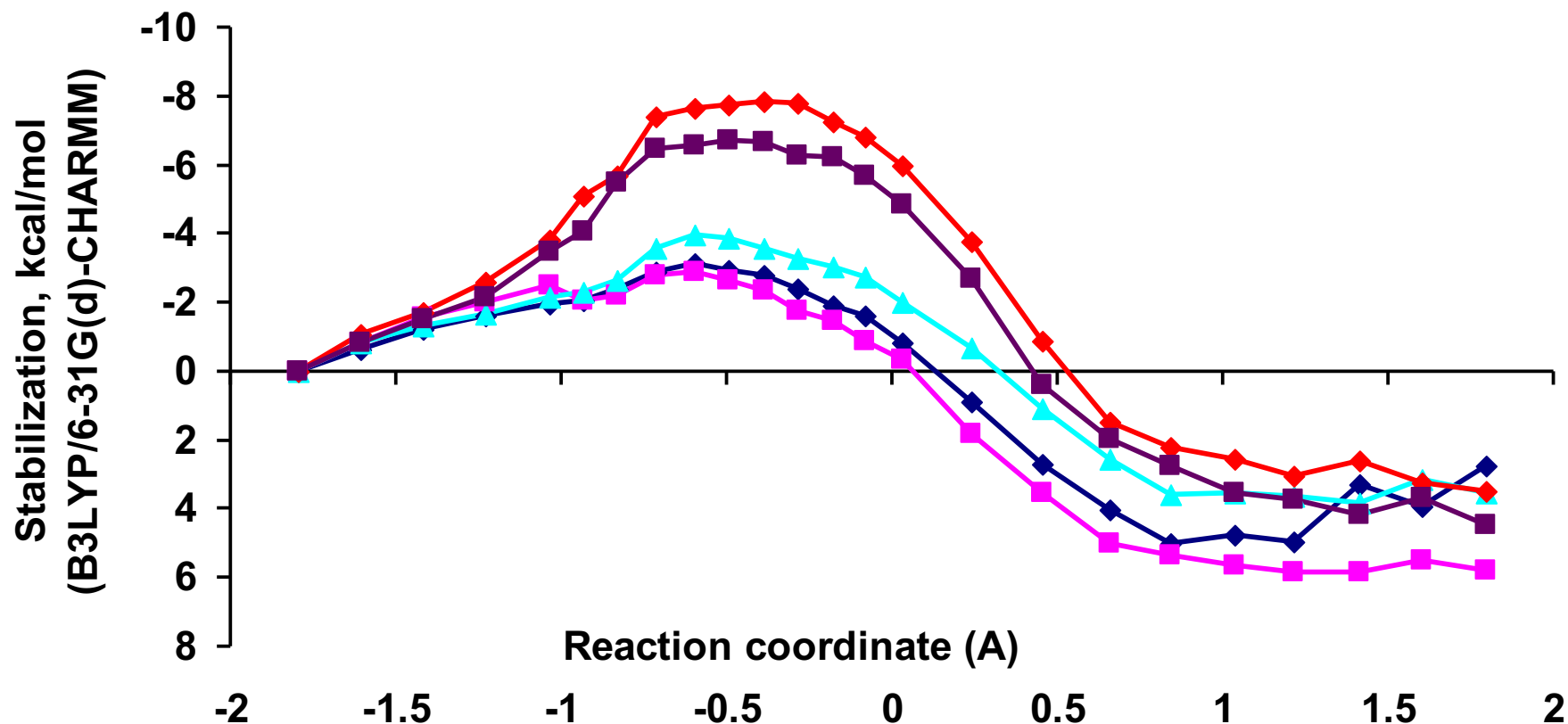
Chorismate mutase: catalytic field

Szefczyk et al.
JACS 2004

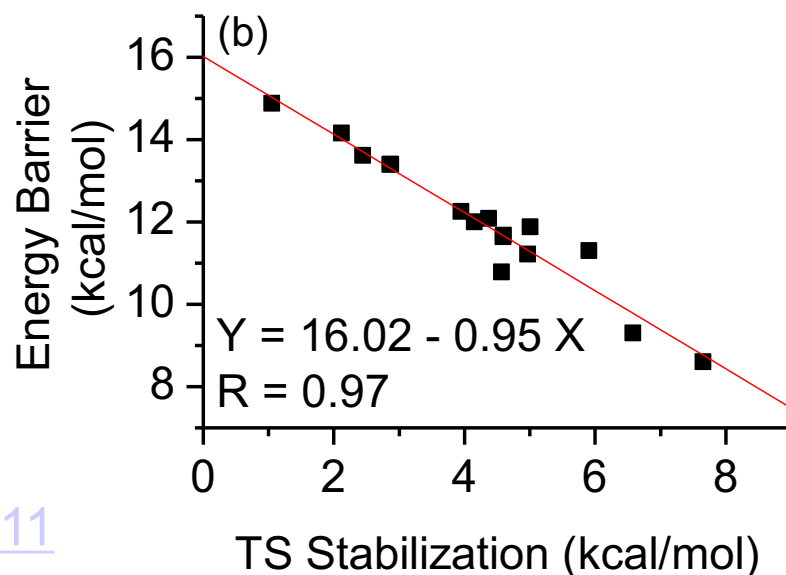
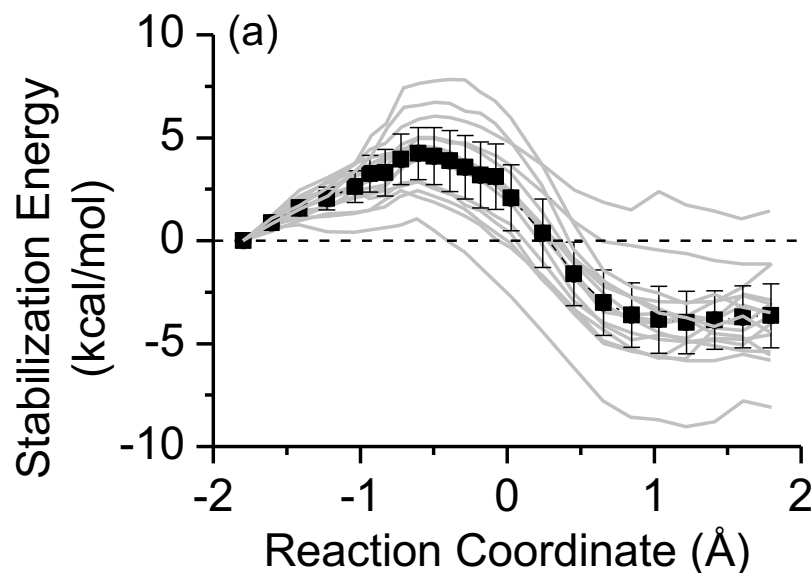


- Electrostatic interactions stabilize TS
- Charged residues positioned to stabilize TS

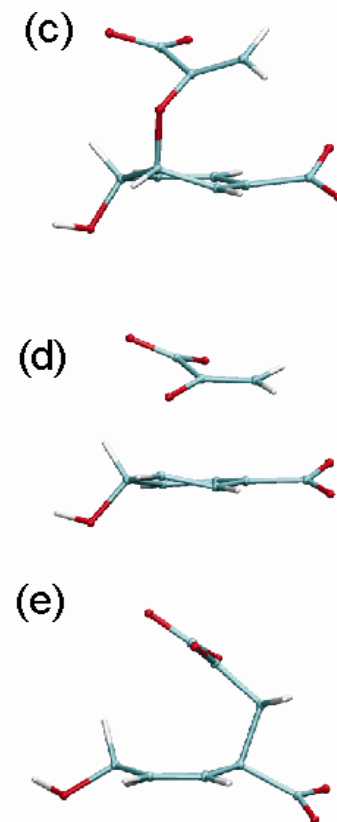
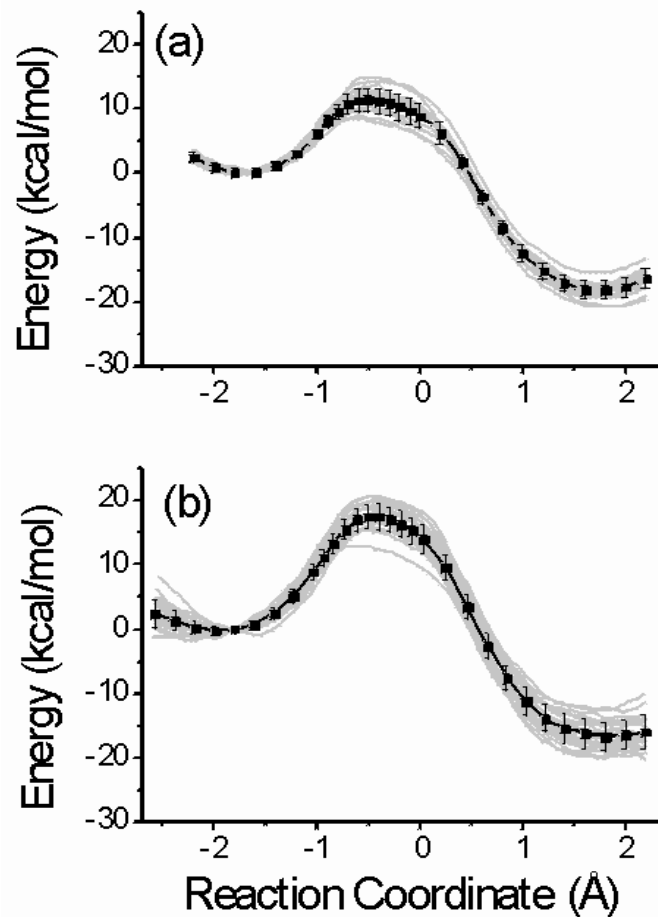
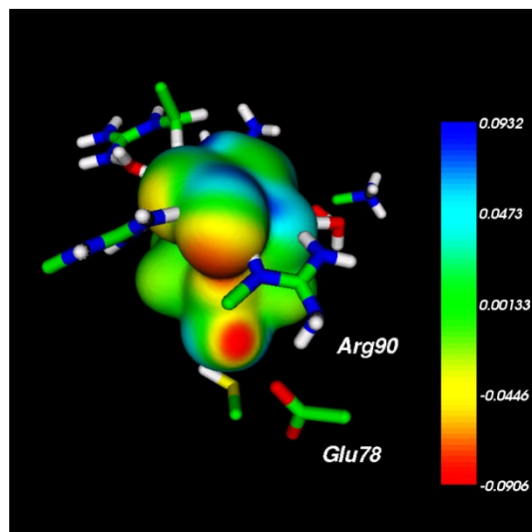
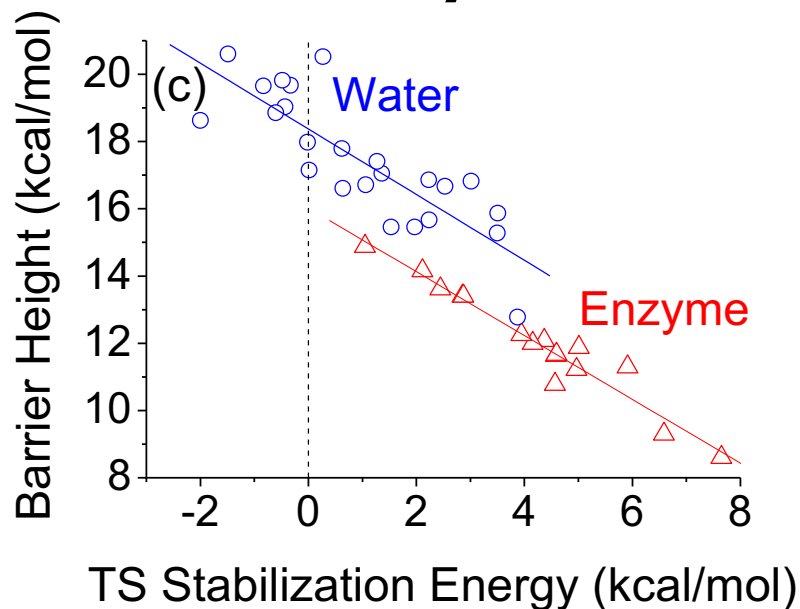
Chorismate mutase TS stabilization: B3LYP/6-31G(d)-CHARMM (for multiple pathways sampled from AM1/MM MD)



Correlation of barrier with TS stabilization



QM/MM calculations account for catalysis in chorismate mutase



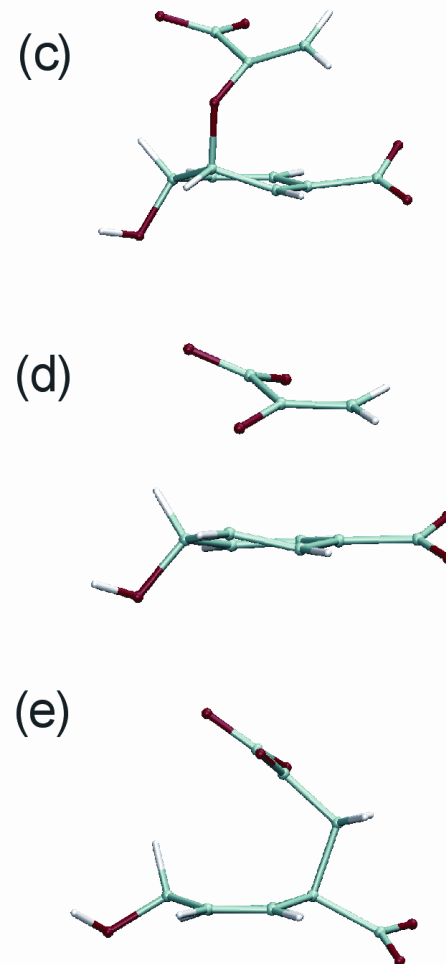
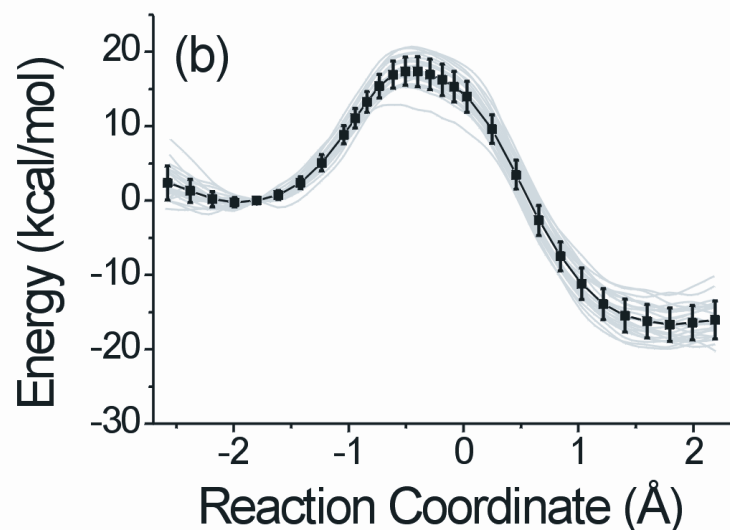
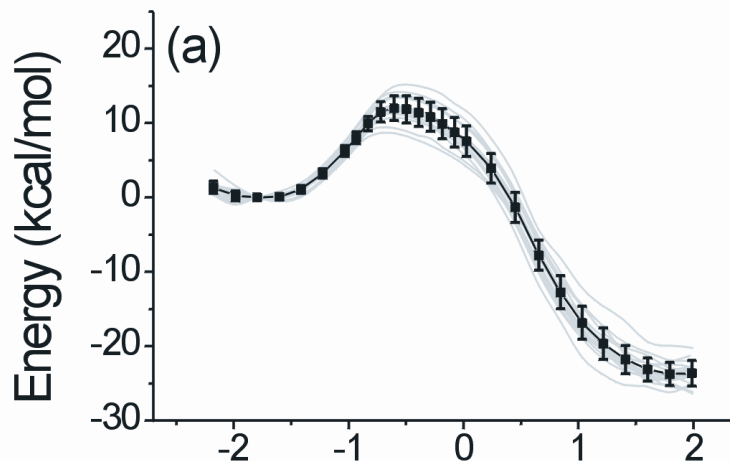
Analysing catalysis: reaction in enzyme versus same reaction in water

Reaction in chorismate mutase enzyme: barrier = 12.0 ± 1.7 kcal/mol

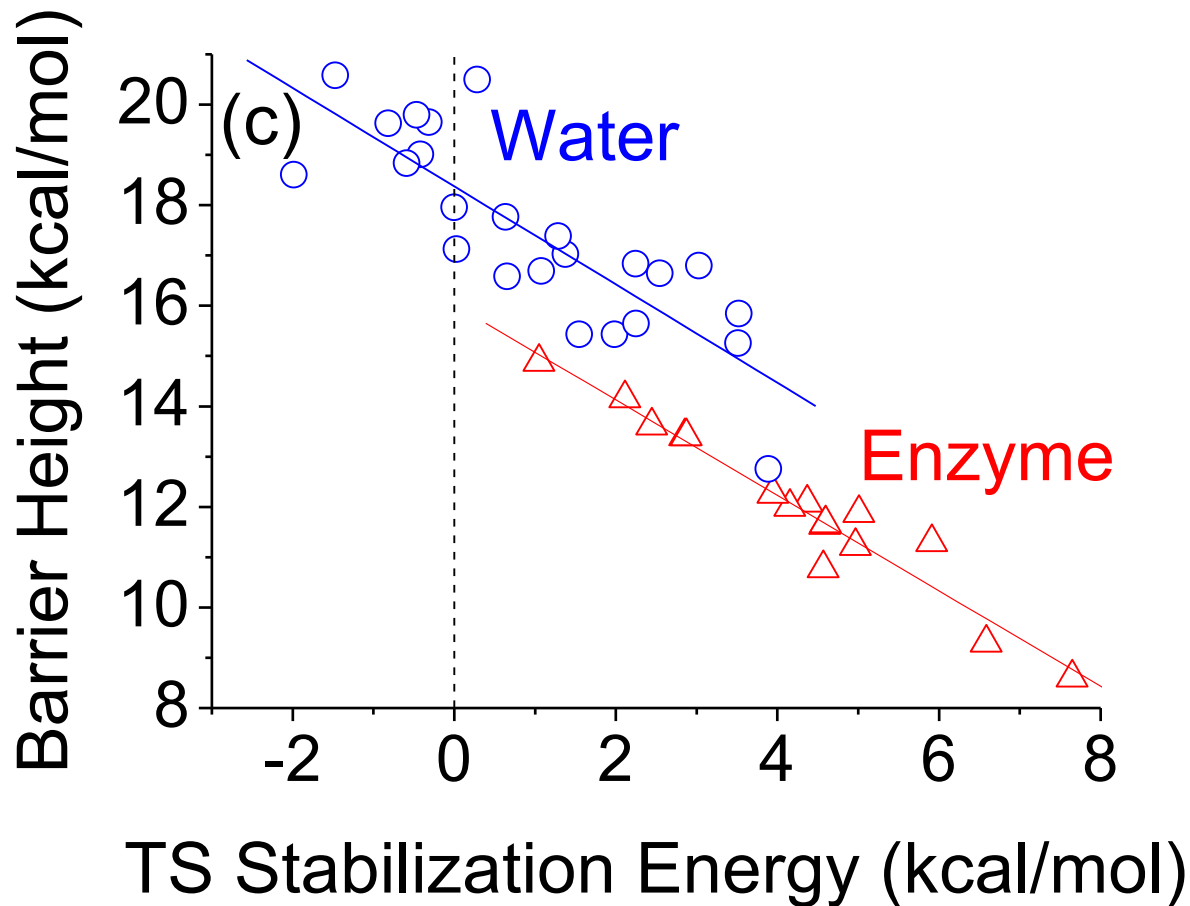
Reaction in water: barrier = 17.7 ± 1.9 kcal/mol

(B3LYP/MM QM/MM potential energy barriers)

Experimental $\Delta\Delta^\ddagger G = 9.1$ ($\Delta\Delta^\ddagger H = 8$) kcal/mol



TS stabilization: reaction in enzyme versus reaction in water



Chorismate mutase: catalysis is due to a combination of TS stabilization and conformational effects

- Experimental $\Delta\Delta^\ddagger G = 9.1$ ($\Delta\Delta^\ddagger H = 8$) kcal/mol
- Better TS stabilization in enzyme than in water (4.2 kcal/mol vs 1.3 kcal/mol) = 2.9 kcal/mol
- Substrate compression/strain contributes 2.2 kcal/mol (positioning of carboxylates); + 0.6 kcal/mol (shorter C-C distance)
- Binding of reactive pseudo-diaxial conformation contributes ~1.5-3 kcal/mol (from QM/MM MD FEP)
- All these effects are probably due to TS complementarity

Large scale QM calculations on chorismate mutase: excellent agreement of QM and QM/MM

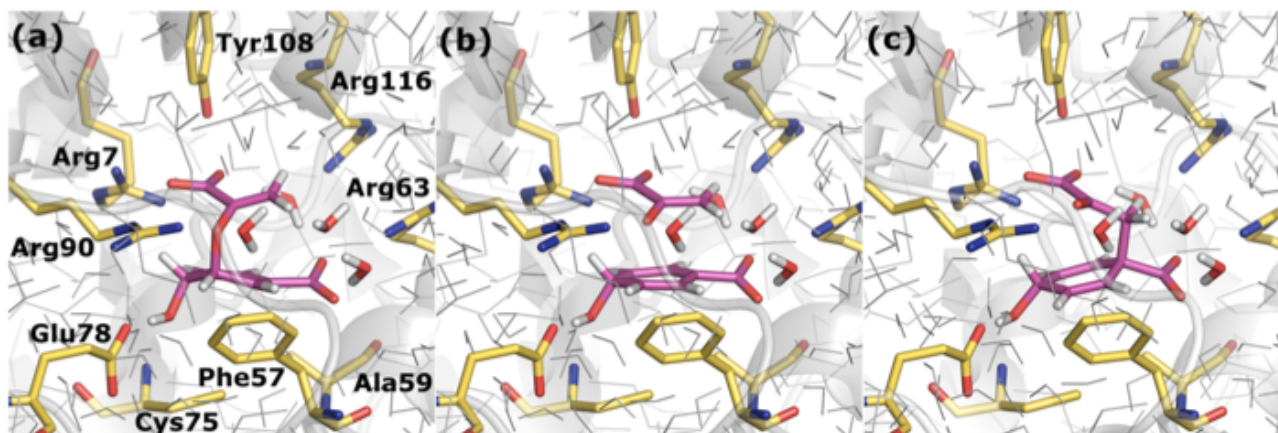
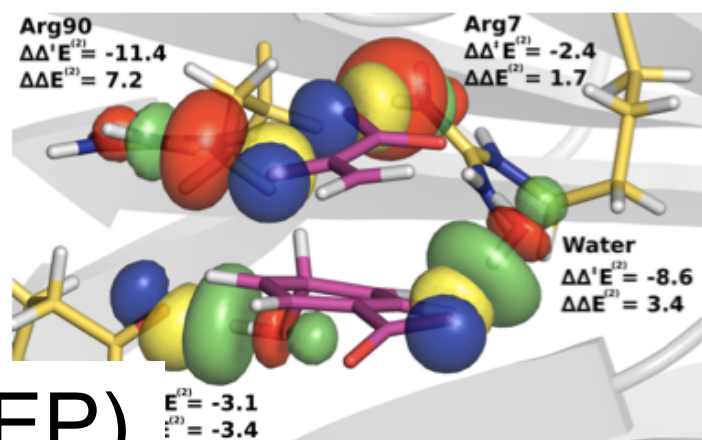


Figure 2. Rearrangement of the substrate (magenta) from chorismate to prephenate within the CM active site (yellow) and surrounding protein (gray). (a) RS, (b) TS, and (c) PS conformations obtained from LS-DFT structural optimization.

Table 2. Energy Difference Comparison^a

		$\Delta^\ddagger E$	ΔE
enzyme	ONETEP	13.6 ± 1.3	-7.8 ± 0.5
	experiment ²⁴	12.7 ± 0.4	
water	ONETEP	24.1 ± 1.1	-9.4 ± 2.2
	experiment ²³	20.7 ± 0.4	-13.2 ± 0.5

^aComparison of averaged activation ($\Delta^\ddagger E$) and reaction (ΔE) energies (kcal mol⁻¹) in CM and water with experiment.

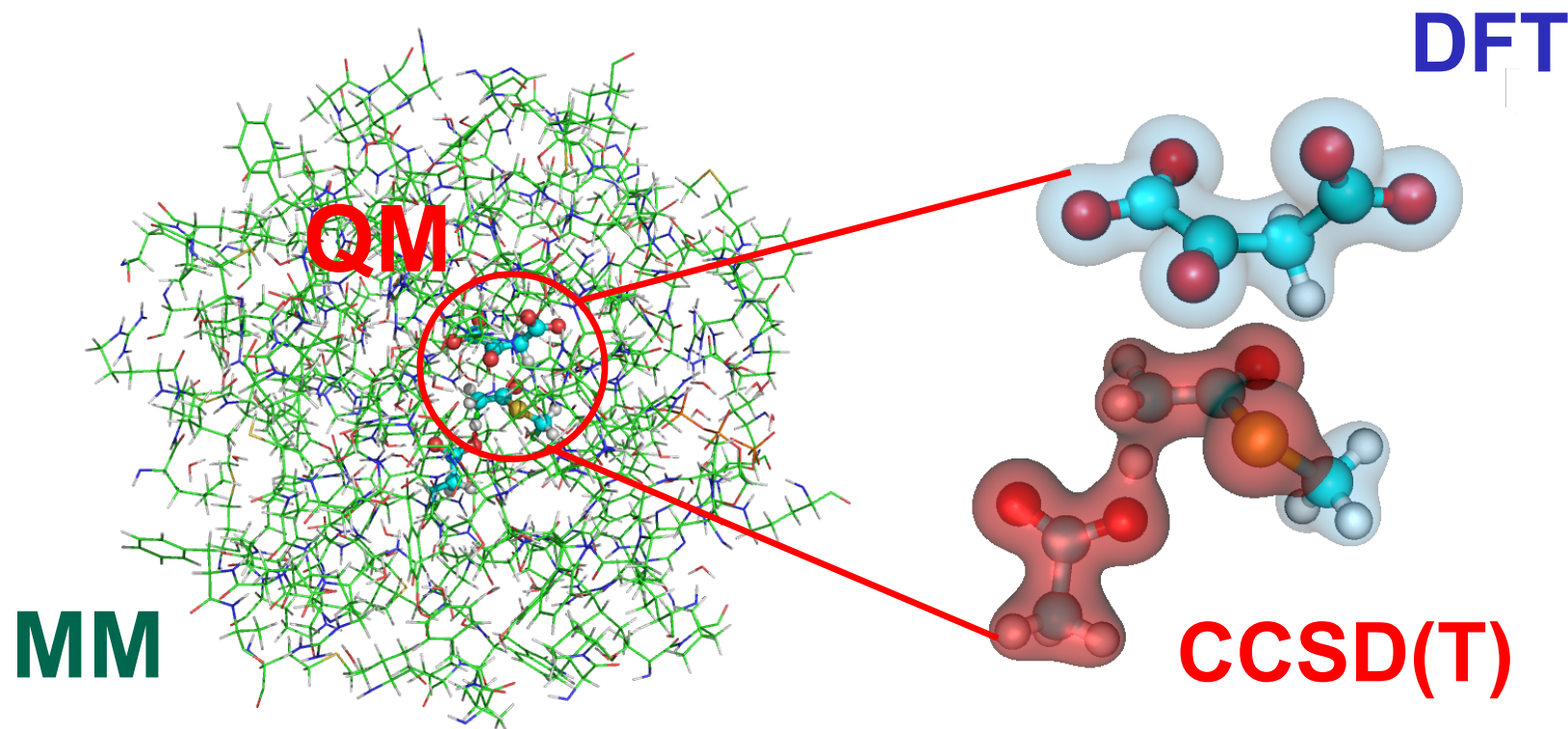


participating in enzyme–substrate interactions at the (n) are shown as blue/yellow isosurfaces, while (n) orbitals are in red/green. Electronic delocalization contributions (kcal mol⁻¹) to the stabilization of the TS and the destabilization of the product ($\Delta\Delta E^{(2)}$), relative to the reactant, are also shown.

- 2000 QM atoms (ONETEP)
- Full TS optimization

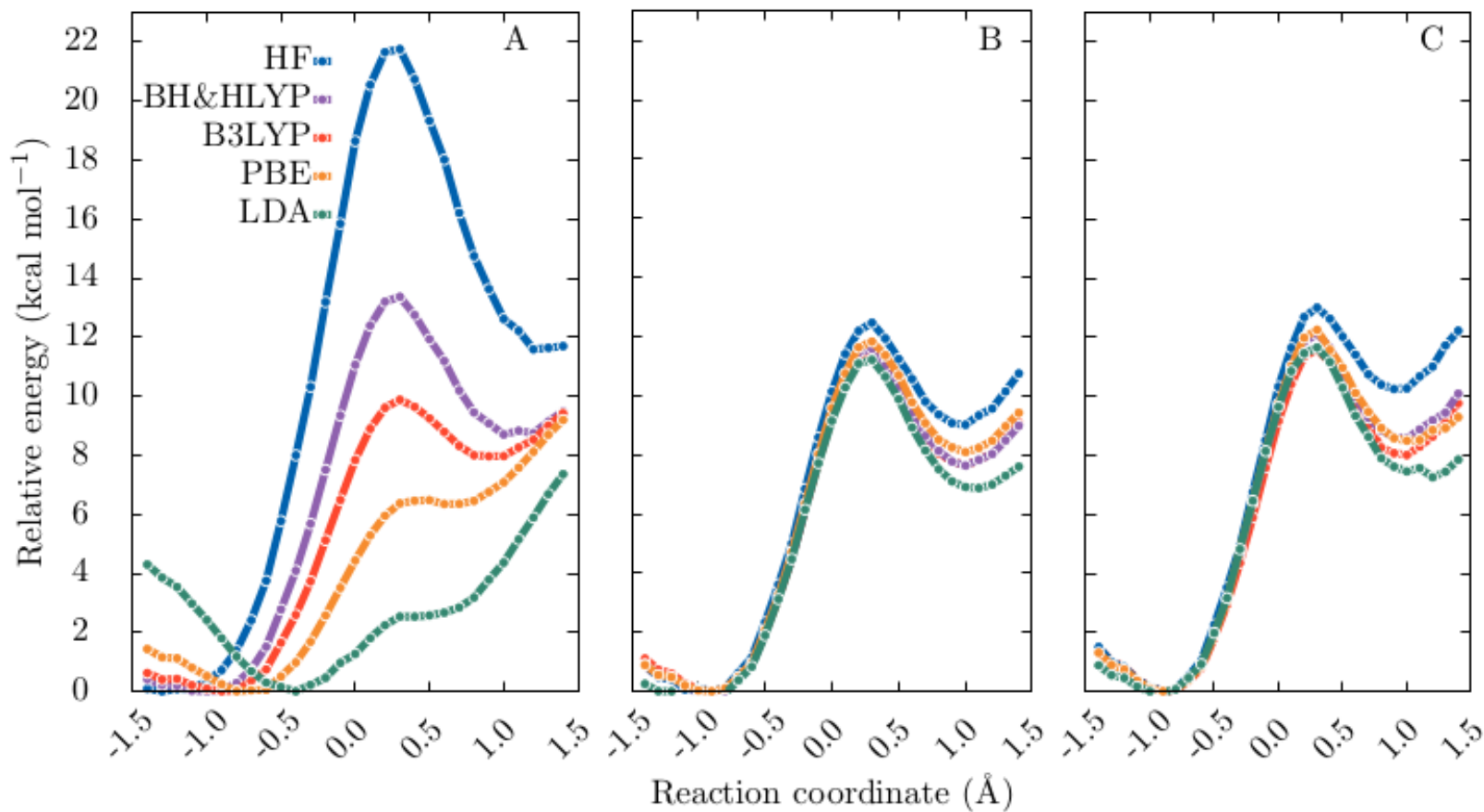
[Lever et al. JPC Lett. \(2014\)](#)

QM/MM with projector embedding: citrate synthase, CCSD(T):DFT/MM



- Citrate synthase forms citric acid via an enolate intermediate
- Proton abstraction from acetyl-CoA by Asp375
- Structures from B3LYP/6-31+G(d)/CHARMM27 QM/MM
- Projector-based embedding: Molpro

QM/MM with projector embedding: citrate synthase, CCSD(T):DFT/MM



Embedding removes DFT/HF variation of of ~20 kcal mol⁻¹ for the reaction barrier and ~6 kcal mol⁻¹ for the reaction energy

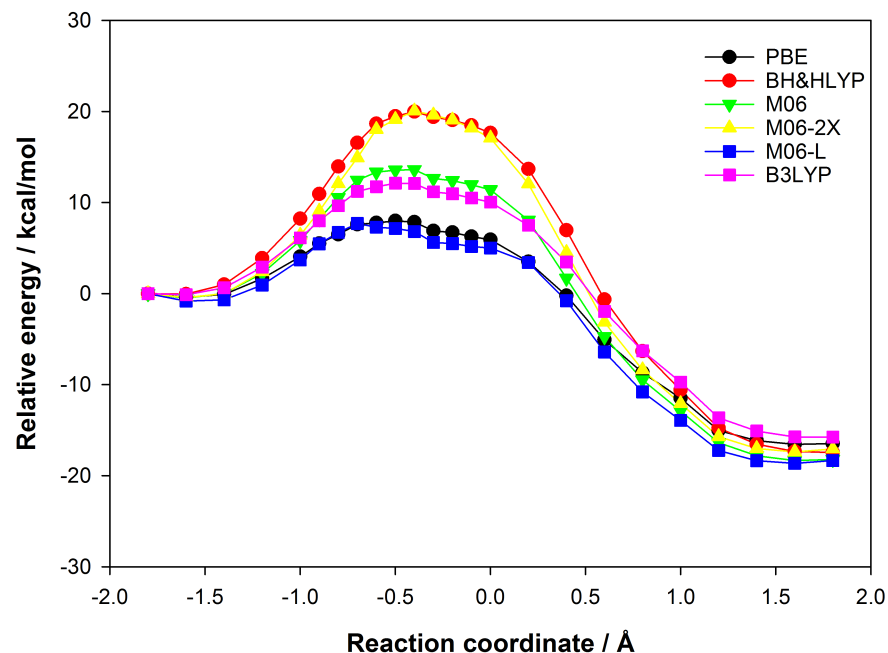
B: 0.0001 truncation threshold (62% of the environment)

Bennie *et al.*

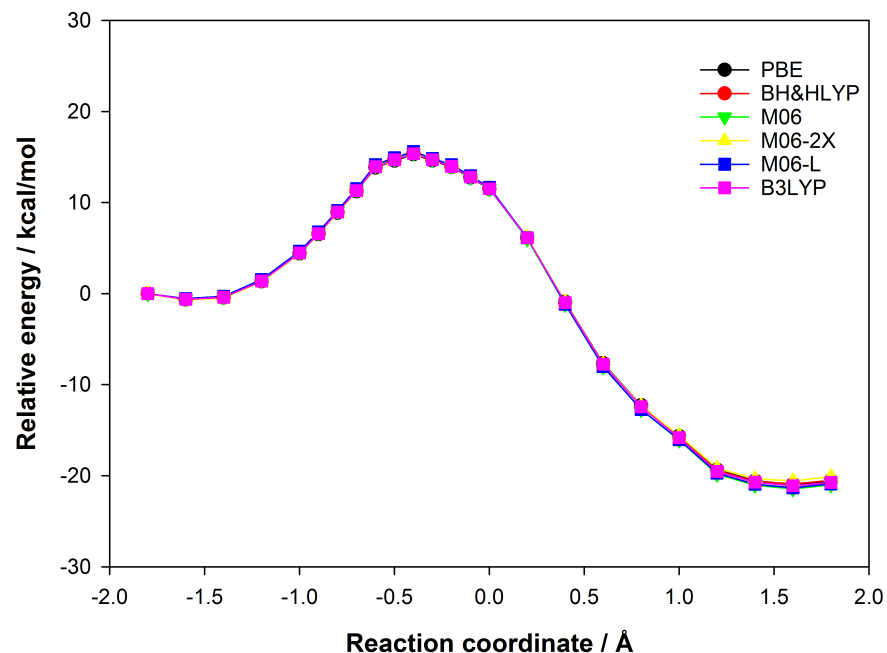
C: 0.001 truncation threshold (40% of the environment)

JCTC 2016

🔥 Testing QM/MM: chorismate mutase

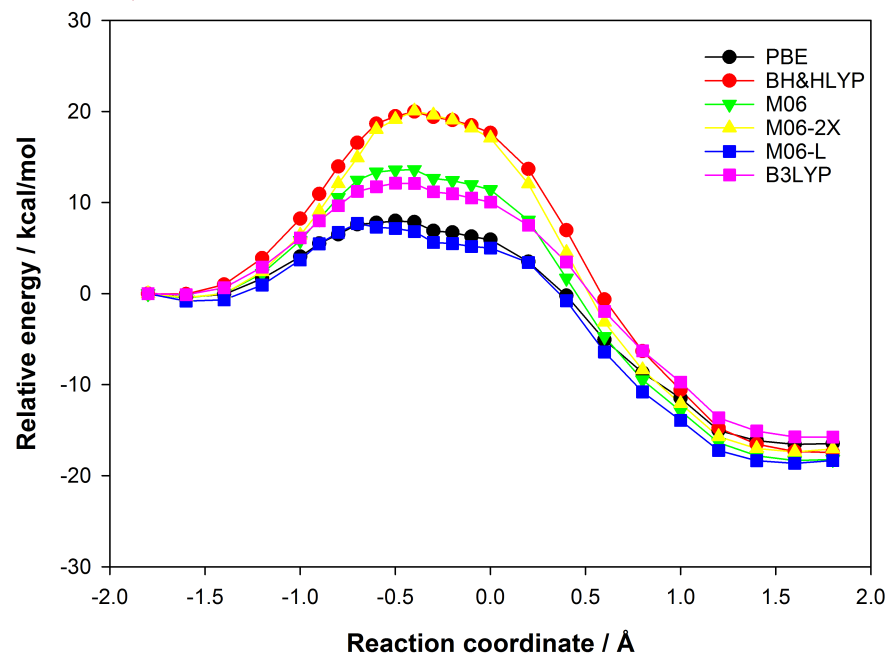


DFT/aug-cc-pVDZ//B3LYP/6-31G(d)/CHARMM27 QM/MM theory, using PBE, BH&HLYP, M06, M06-2X, M06-L and B3LYP

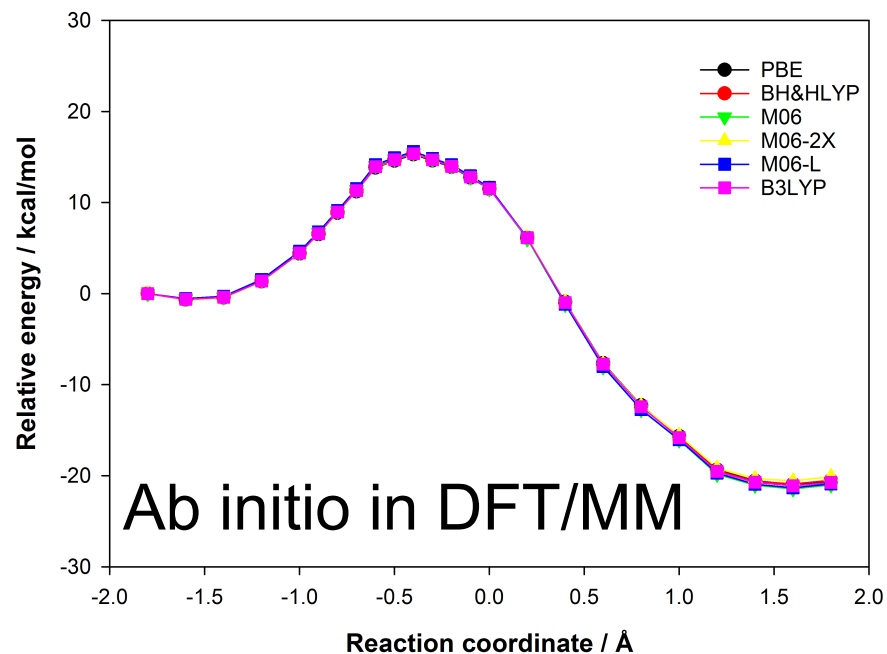


Projector-based embedding profiles
SCS-MP2-in-DFT/aug-cc-pVDZ//
B3LYP/6-31G(d)/CHARMM27 level
of QM/MM theory

🔥 Projector-based embedding eliminates density functional dependence in DFT QM/MM calculations: chorismate mutase



DFT/aug-cc-pVDZ//B3LYP/6-31G(d)/CHARMM27 QM/MM theory, using PBE, BH&HLYP, M06, M06-2X, M06-L and B3LYP



Ab initio projector-based embedding
SCS-MP2-in-DFT/aug-cc-pVDZ//
B3LYP/6-31G(d)/CHARMM27 level of
QM/MM theory

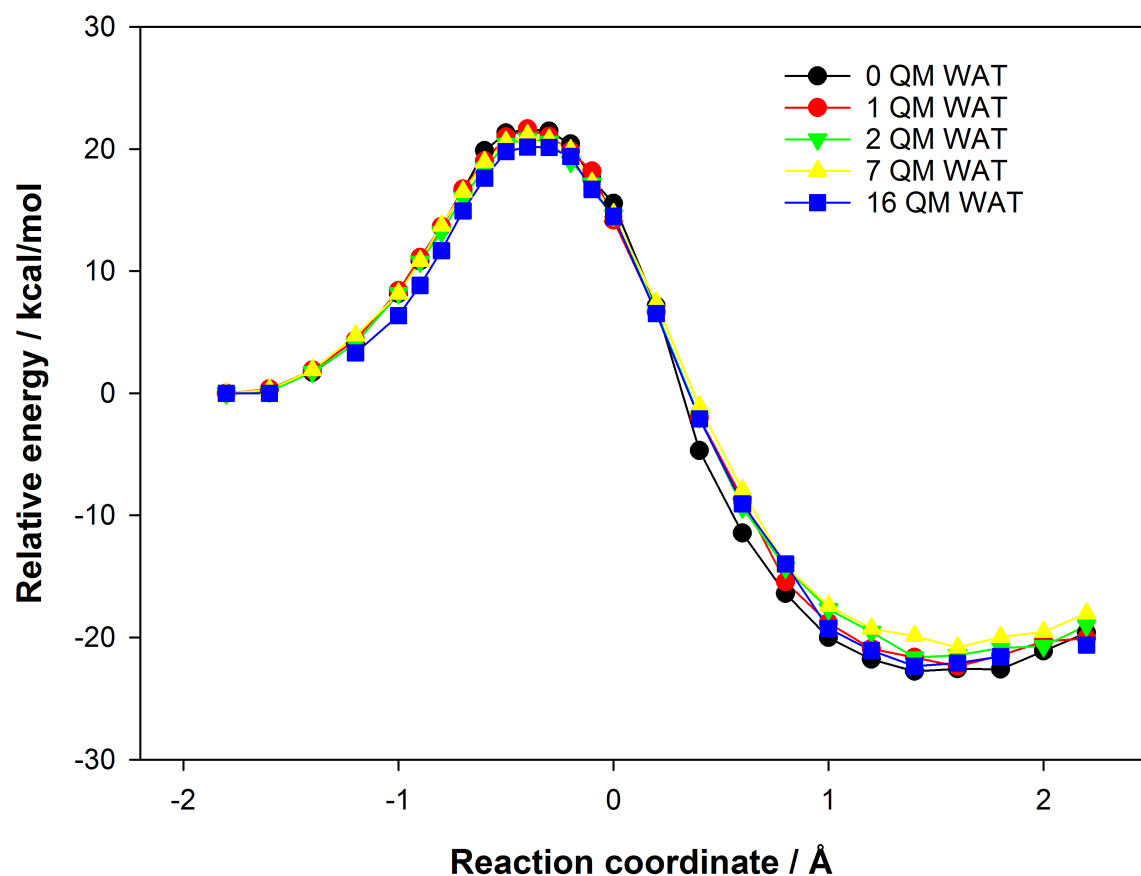
🔥 Testing QM/MM: chorismate-prephenate reaction in solution

- Potential energy barriers ($\Delta^\ddagger V$) and reaction energies for uncatalyzed reaction in water
- B3LYP/6-31G(d)/CHARMM27 QM/MM
- Varying no. of water molecules treated QM

No. of QM water molecules	$\Delta^\ddagger V /$ kcal/mol	$\Delta_r V /$ kcal/mol
0	19.6	-14.1
1	20.0	-13.9
2	19.5	-12.9
5	19.6	-12.7
7	19.0	-13.3
10	17.8	-14.4
11	17.4	-13.9
14	17.0	-14.4
16	17.7	-14.7

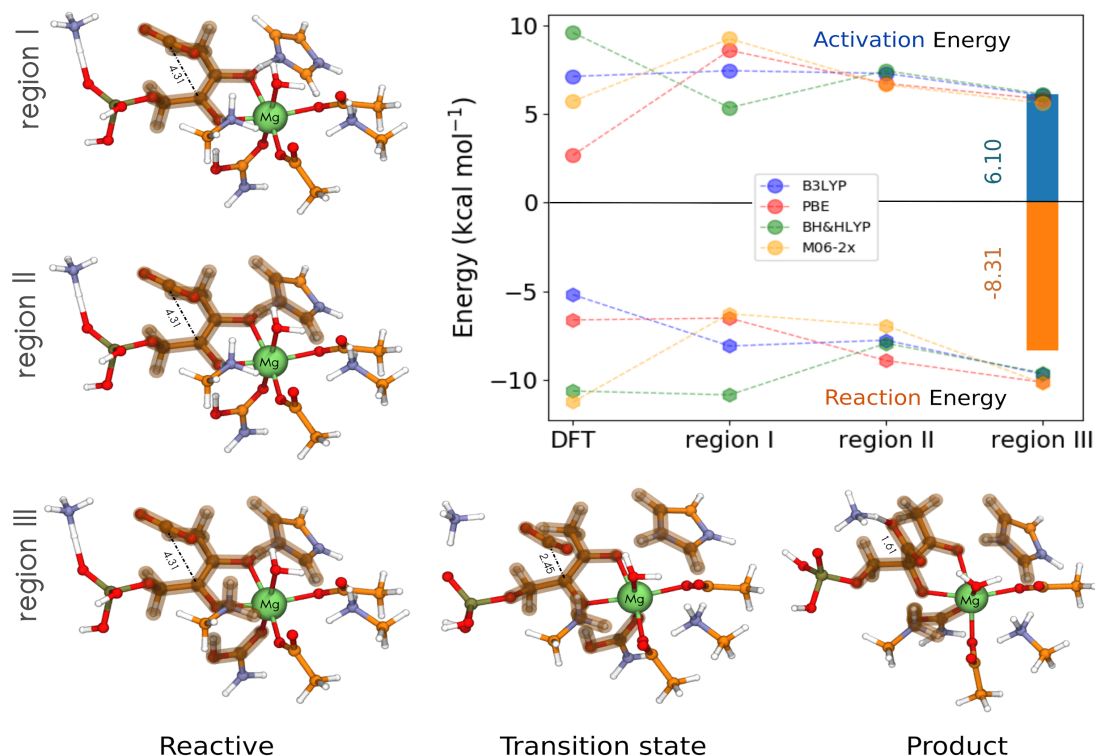
🔥 Testing QM/MM: chorismate-prephenate reaction in solution, projector-based embedding

Projector-based embedding SCS-MP2 in B3LYP, QM/MM with different numbers of DFT water molecules (cc-pVDZ basis)





Ribulose 1,5-bisphosphate carboxylase-oxygenase (RuBisCO)

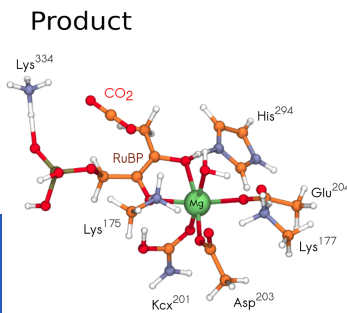


Projector-based embedding QM/MM removes dependence on DFT functional
MP2, SCS-MP2, CCSD, and CCSD(T)/aug-cc-pVDZ and cc-pVDZ

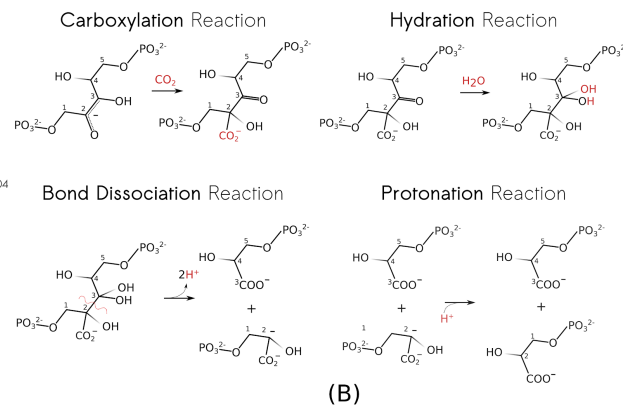
Douglas-Gallardo et al.
J. Comput. Chem. 2020



University of
BRISTOL

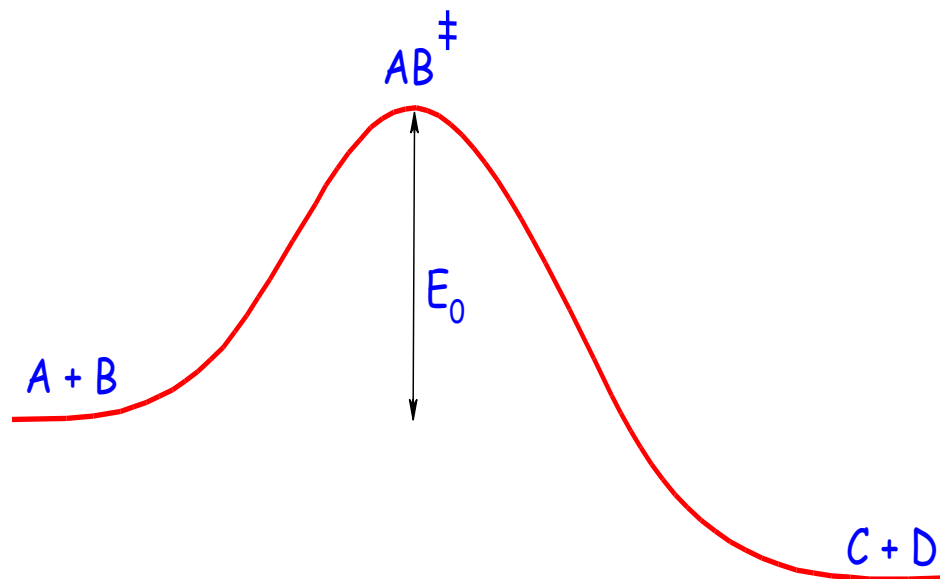


(A)



(B)

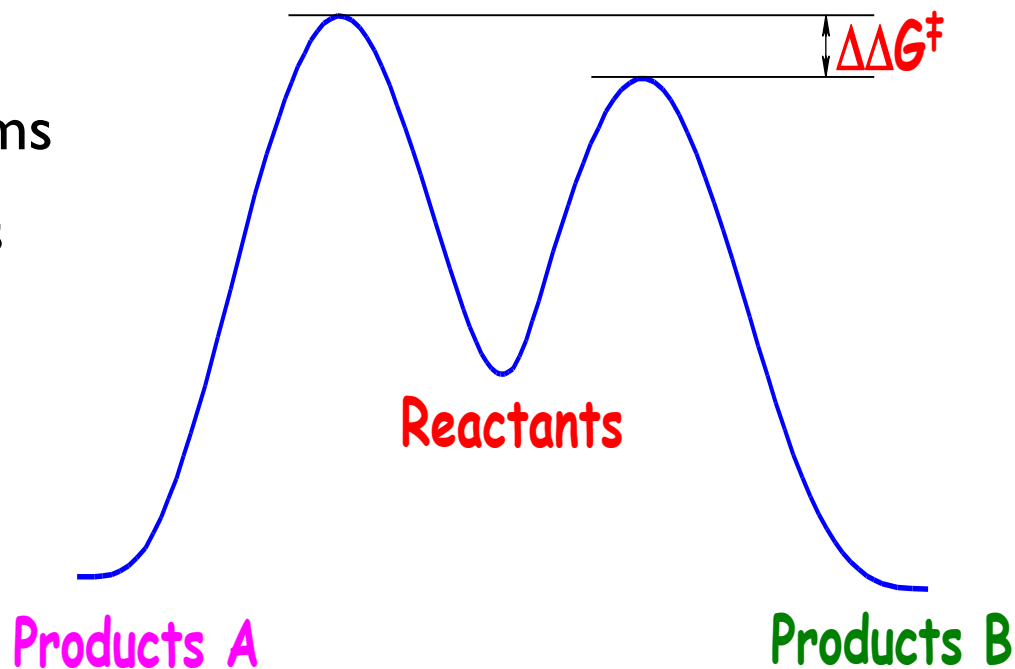
🔥 Predicting reactivity and selectivity



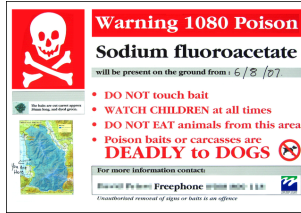
$$k = \frac{k_B T}{h} \times e^{-\Delta G^\ddagger / k_B T}$$

- Explore alternative mechanisms
- Rationalize substituent effects

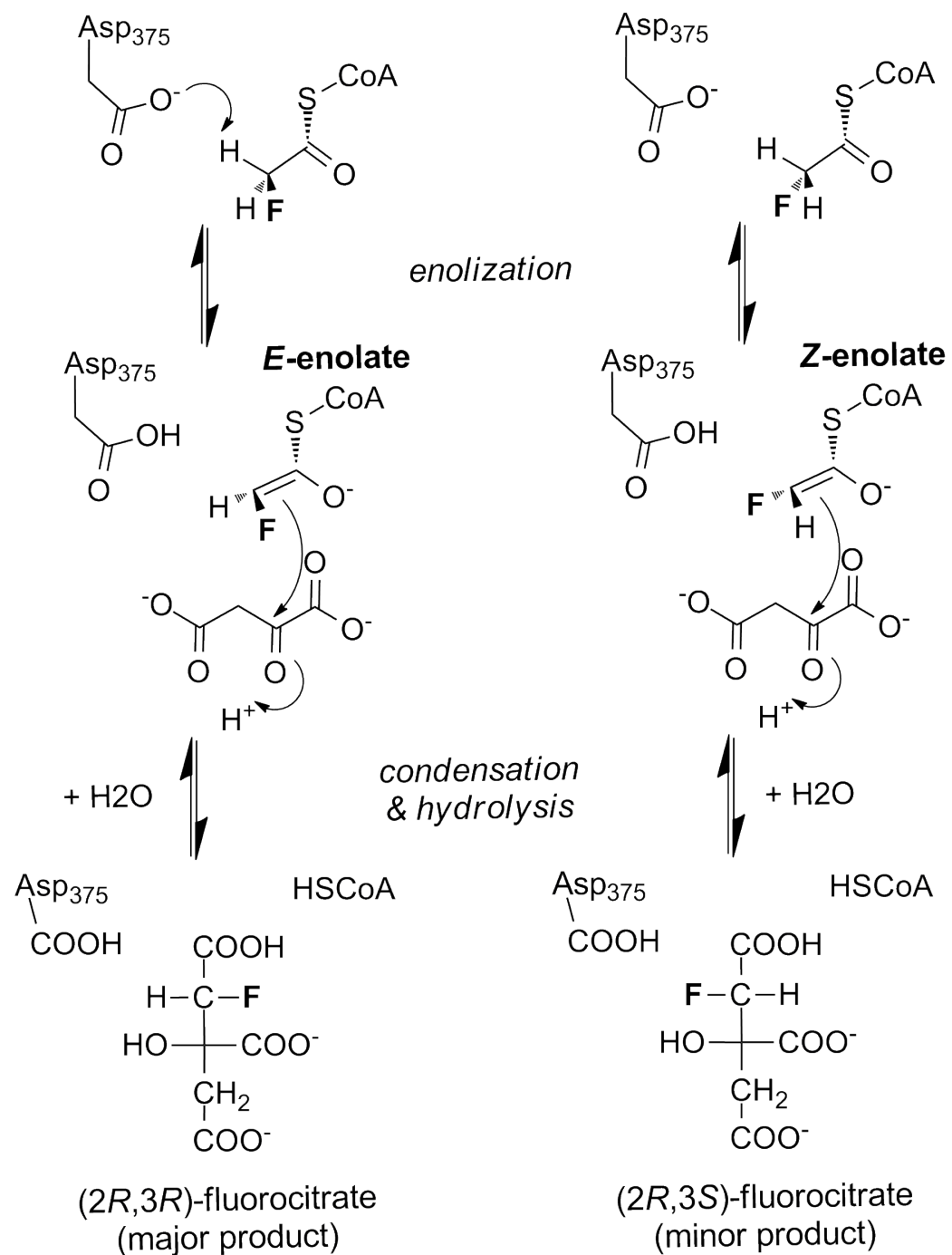
$$\text{Yield A} / \text{Yield B} = \exp(-\Delta\Delta G^\ddagger / RT)$$



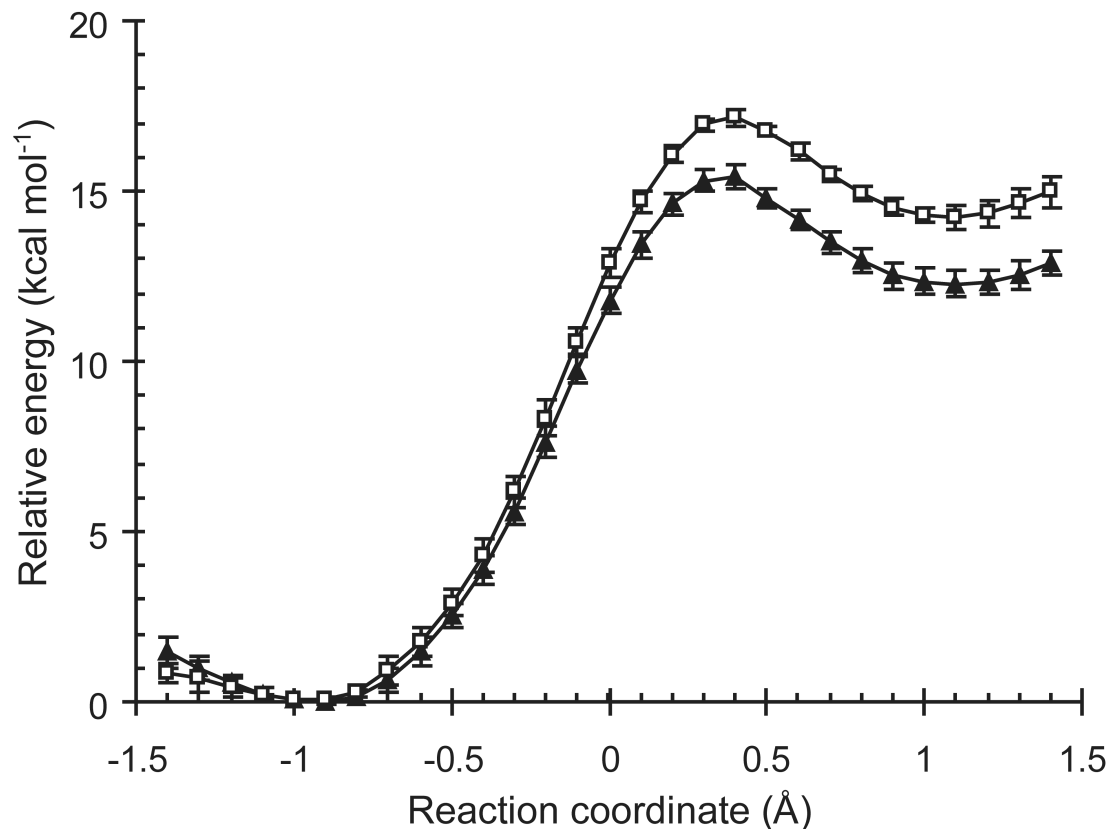
'Lethal synthesis' of fluorocitrate by citrate synthase



- (2*R*,3*R*)-fluorocitrate formed predominantly from fluoroacetyl-CoA
- This enantiomer inhibits aconitase
- Responsible for the lethal toxicity of fluoroacetate
- Minor product, (2*R*,3*S*)-fluorocitrate, is 2-3% i.e. $\Delta\Delta^\ddagger G = 2.1\text{-}2.3 \text{ kcal mol}^{-1}$

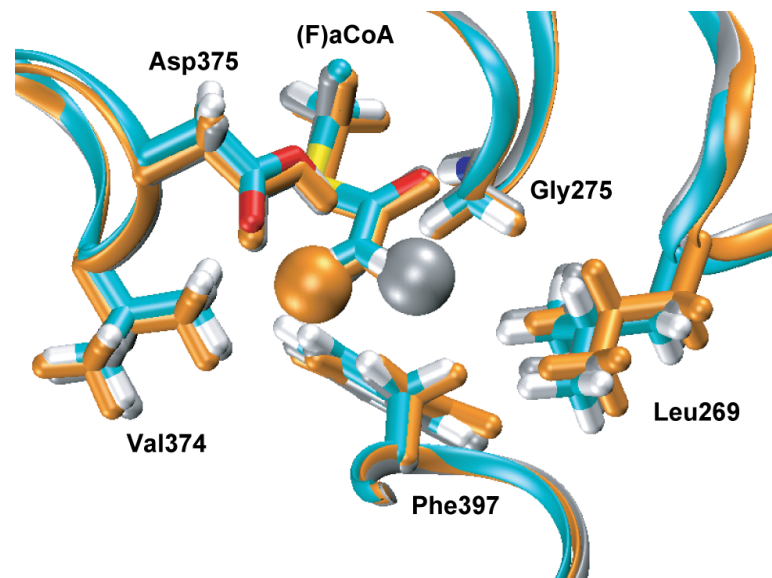


QM/MM calculations reproduce observed stereospecificity of 'lethal synthesis'



SCS-MP2/AVDZ//B3LYP/6-31+G(d)/CHARMM27
energy profiles for formation of *E*- (▲) and *Z*- (□)
enolates from fluoroacetyl-CoA in CS. Average profiles
from 5 different enzyme-substrate conformations

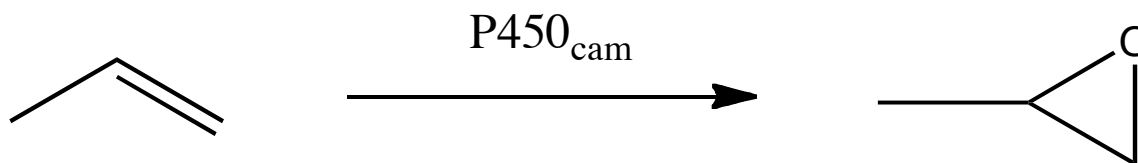
$$\begin{array}{cc} \Delta\Delta E_{act} & \Delta\Delta E_{react} \\ 1.8 \pm 0.3 & 2.1 \pm 0.4 \end{array}$$



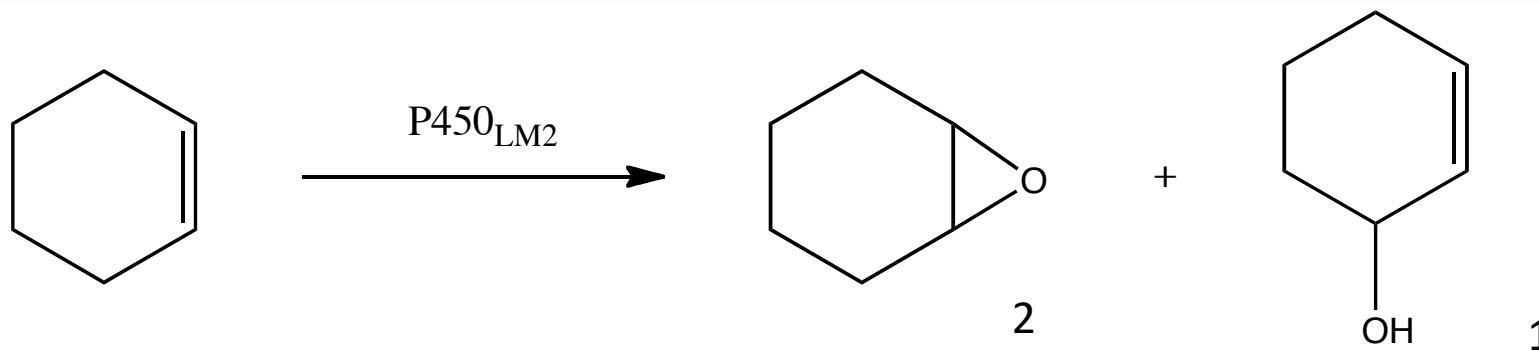
Van der Kamp *et al.* *Angewandte Chemie* (2011); Zhang *et al.* *Royal Soc. Open Science* (2018)

Alkene oxidation in bacterial P450s

Propene – epoxidation only



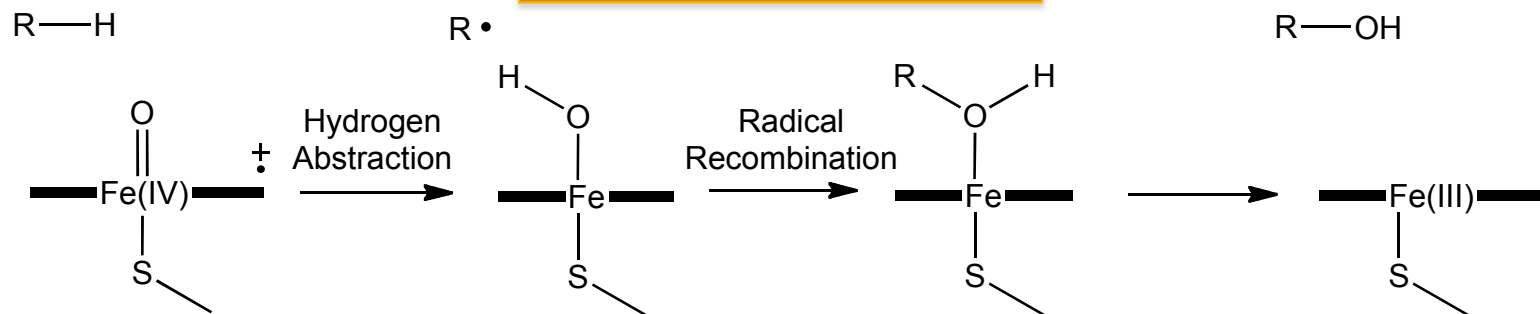
Cyclohexene – epoxidation and hydroxylation observed



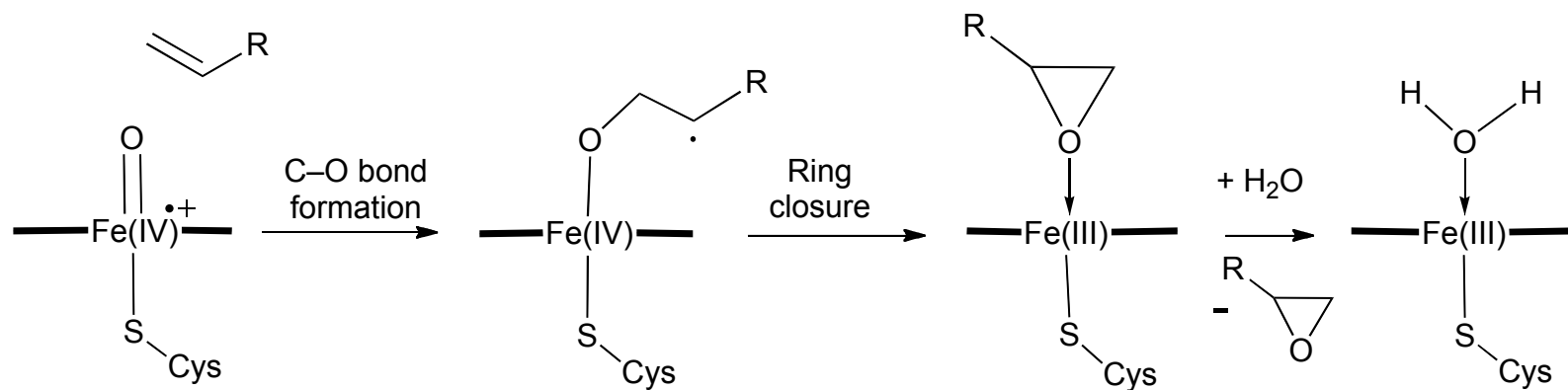
Prediction of Enzyme Selectivity by

Mechanisms

Hydroxylation



Epoxidation



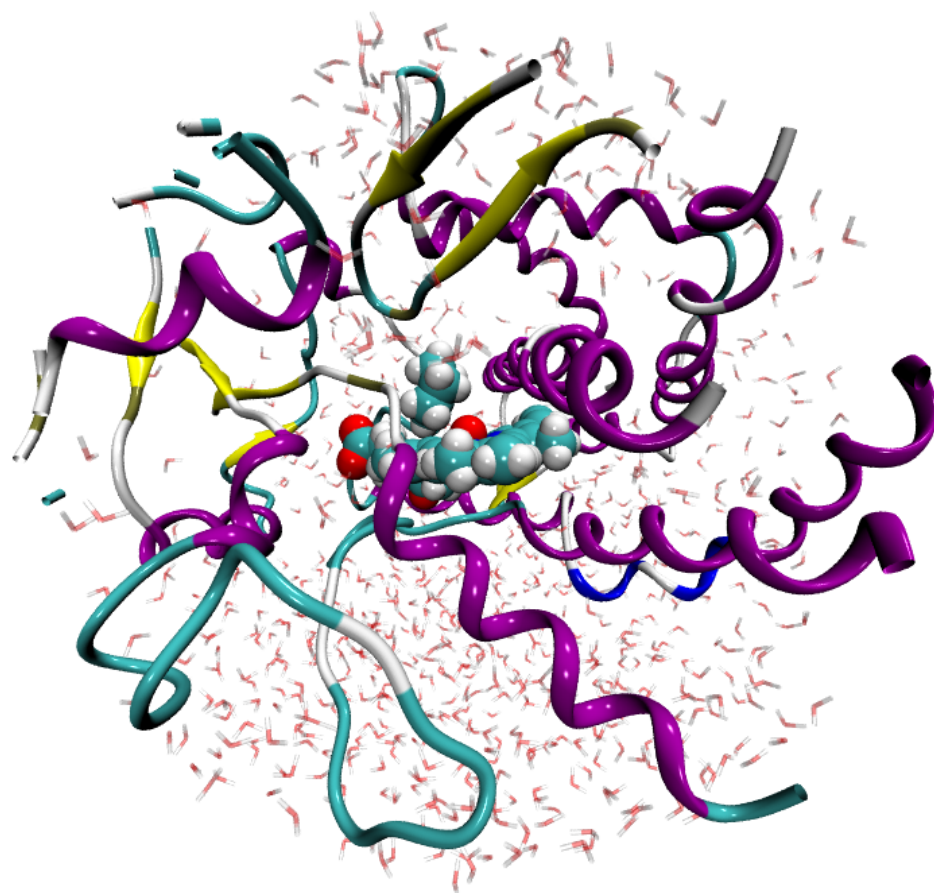
Alkene oxidation in bacterial P450s

Relating to experiments: what should we expect to find?

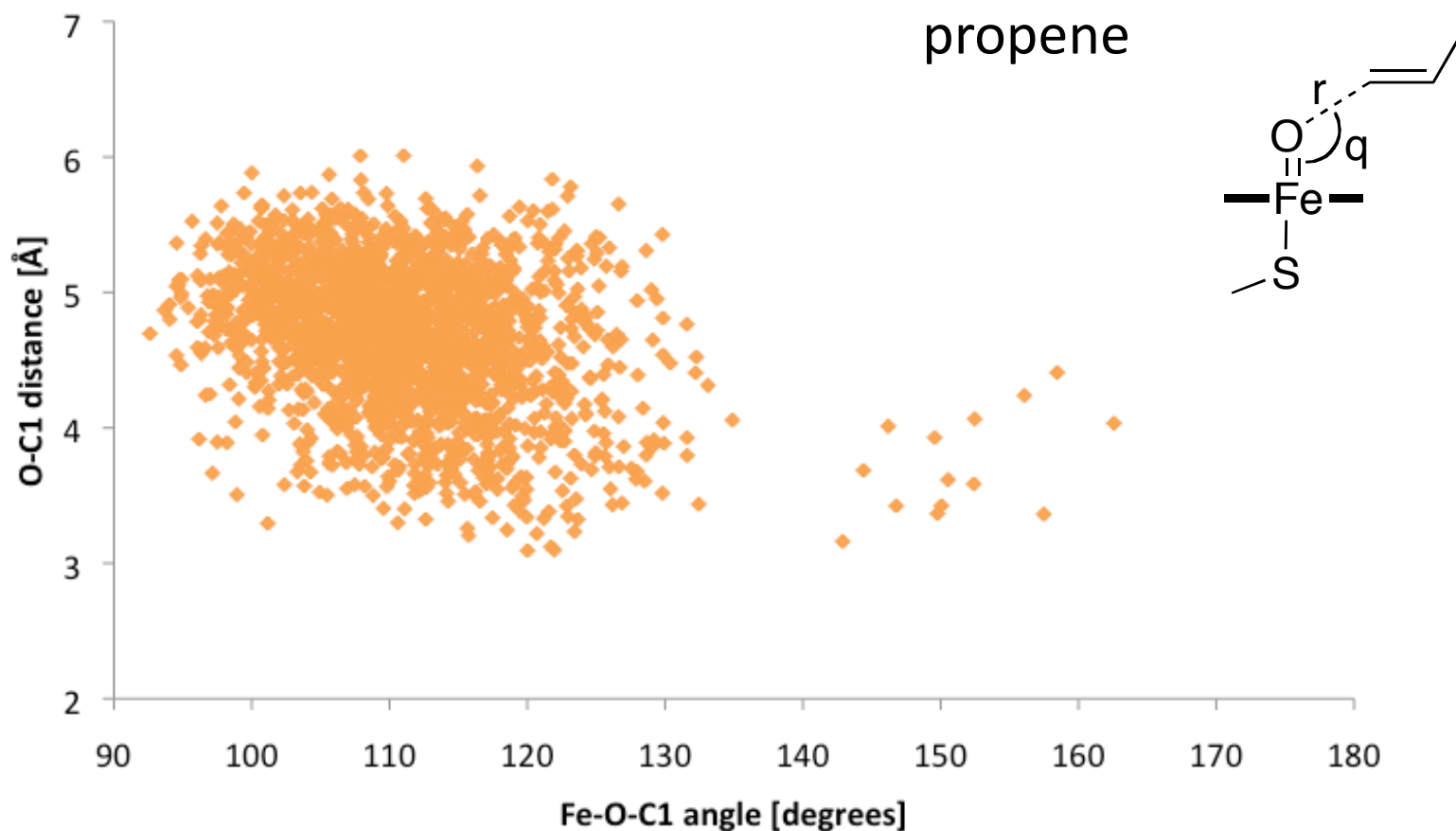
- Lower barrier for epoxidation of propene
- Approximately equal barriers for hydroxylation and epoxidation of cyclohexene

P450_{cam} Alkene oxidation: QM/MM calculations

- QM region:
 - Compound I + substrate
 - B3LYP/6-31G** and LACVP (Jaguar) for QM/MM optimization
 - B3LYP and BP86 single point QM/MM calculations using the LACV3P basis set for iron, with 6-311G* and 6-311G**
 - Focus on quartet state
- MM region:
 - 25Å radius sphere of protein + water
 - CHARMM27 (Tinker)
- QM/MM optimization
 - QoMMMa interface
 - QM region polarized by MM atoms

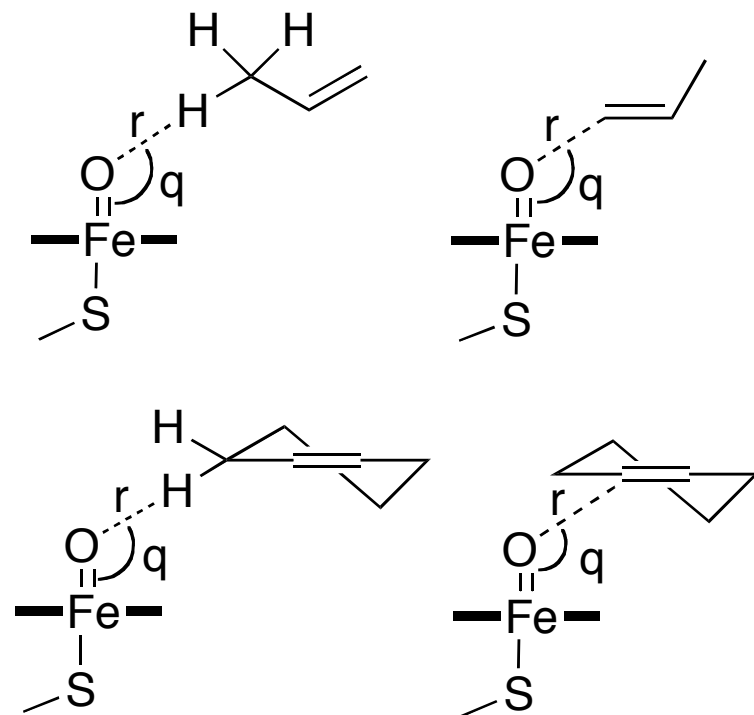
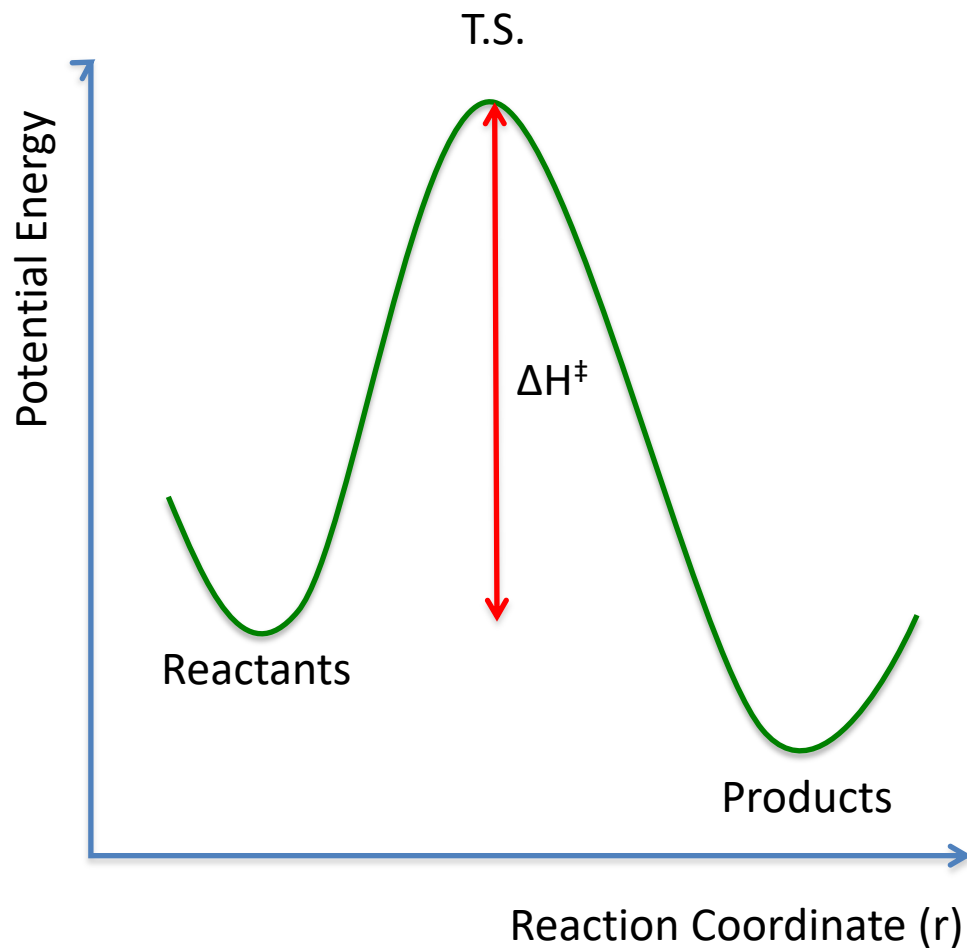


Molecular dynamics simulations



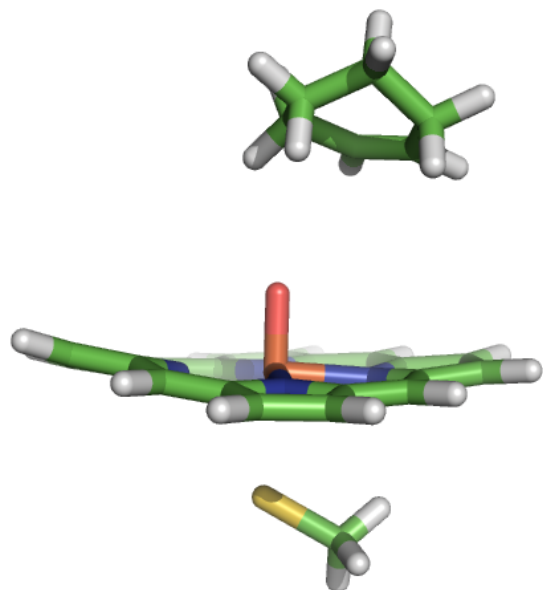
Free tumbling of substrate in active site found for both substrates

Reaction modelling

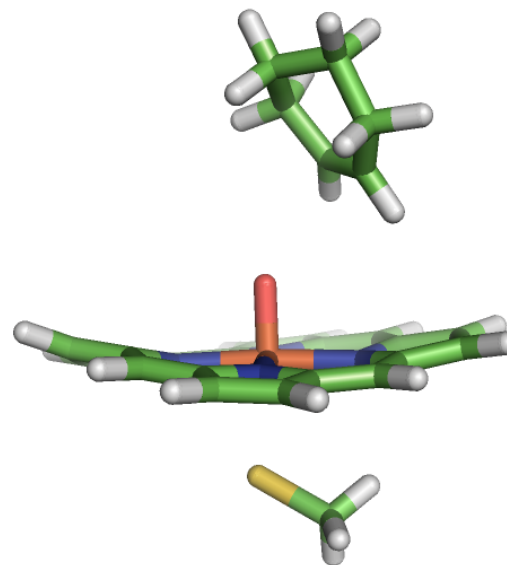


$$r < 4.0\text{\AA}, 110 < q < 140^\circ$$

P450 alkene epoxidation versus hydroxylation: need for conformational sampling



$$\Delta H^\ddagger_E = 12.5 \text{ kcal/mol}$$



$$\Delta H^\ddagger_E = 20.2 \text{ kcal/mol}$$

- Substrates move and tumble freely in the active site
- Large variation in energy barrier depending on substrate orientation

Predicting selectivity for hydroxylation vs. epoxidation: need for conformational sampling

$\Delta\Delta H^\ddagger = \Delta H_E^\ddagger - \Delta H_H^\ddagger$	Cyclohexene	Propene
Experiment (est.)	−0.5	−2.3
Cohen et al. ¹	+7.6 ^a	+1.4 ^a
This work ²	+1.5 ^b	+0.3 ^b

^a LACVP (Fe), 6-31G (all other atoms)

^b LACV3P (Fe), 6-311G* (all other atoms)

Energies in kcal/mol

Barriers for this work are Boltzmann-weighted averages from a minimum of 6 snapshots

1. Cohen *et al.*, *J. Am. Chem. Soc.*, 2006, **128**, 11028
2. Lonsdale *et al.*, *J. Phys. Chem. B* 2010, 114, 1156

Dispersion correction improves agreement with experiment for P450 alkene oxidation

- B3LYP-D calculated $\Delta\Delta E^\ddagger$ values are within 3 kcal/mol of the experimental values for both substrates.

$\Delta\Delta E^\ddagger = \Delta E_E^\ddagger - \Delta E_H^\ddagger$	cyclohexene	propene
experiment	-0.5	-2.3 (upper bound estimate)
B3LYP/BS2//B3LYP	+3.1	+1.8
B3LYP-D/BS2//B3LYP-D	-0.4	+0.4

Alkene oxidation in bacterial P450s

Improvement over previous calculations for cyclohexene

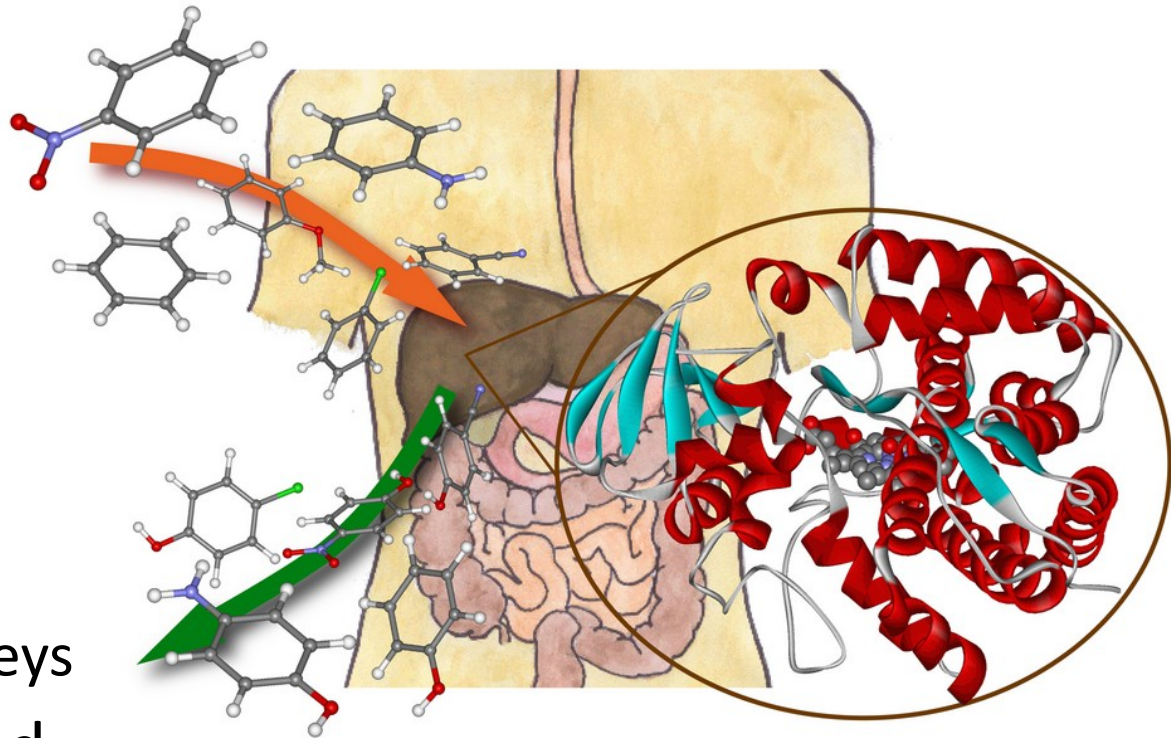
- Better agreement with experiment (to within error of DFT/QMMM method (“~3 kcal/mol?”)?)
- B3LYP consistently underestimates hydroxylation barriers, compared to epoxidation; need dispersion

Large variation in barriers – dependence on geometry

- This is despite pre-screening starting geometries
- Conformational sampling must be included by using multiple starting geometries – the more the better

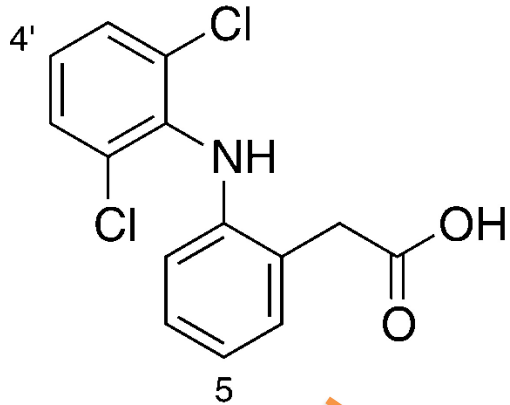
Drug metabolism by cytochrome P450 enzymes

- A small number of P450 isoforms metabolise most drugs
- Catalyse a variety of reactions
 - E.g. hydroxylation, epoxidation, N/O-dealkylation
- Increases solubility
 - aids removal by kidneys
- Toxic products formed with some drugs
 - e.g. paracetamol

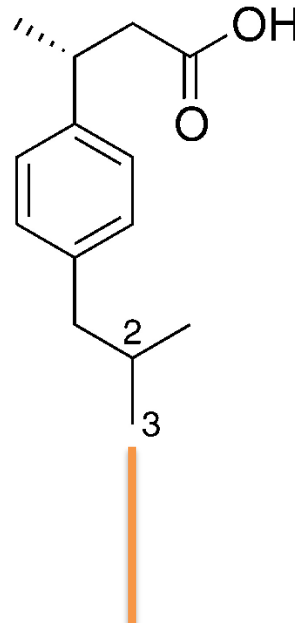


Modelling drug metabolism in 2C9

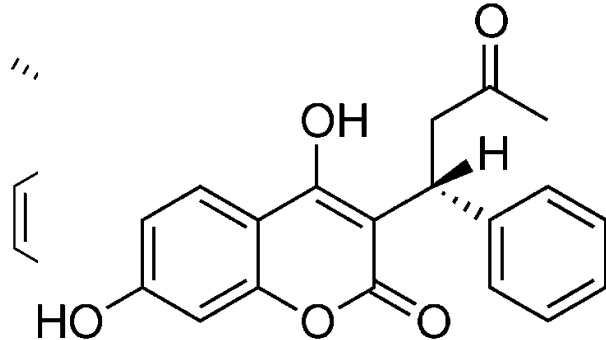
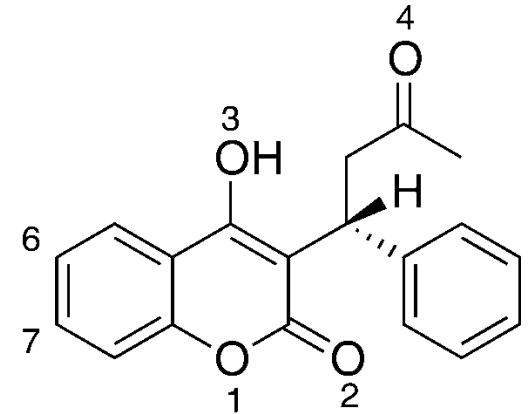
diclofenac



ibuprofen

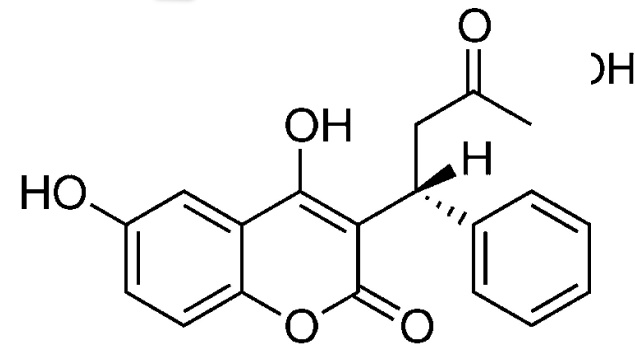


warfarin



major product

major products



minor product

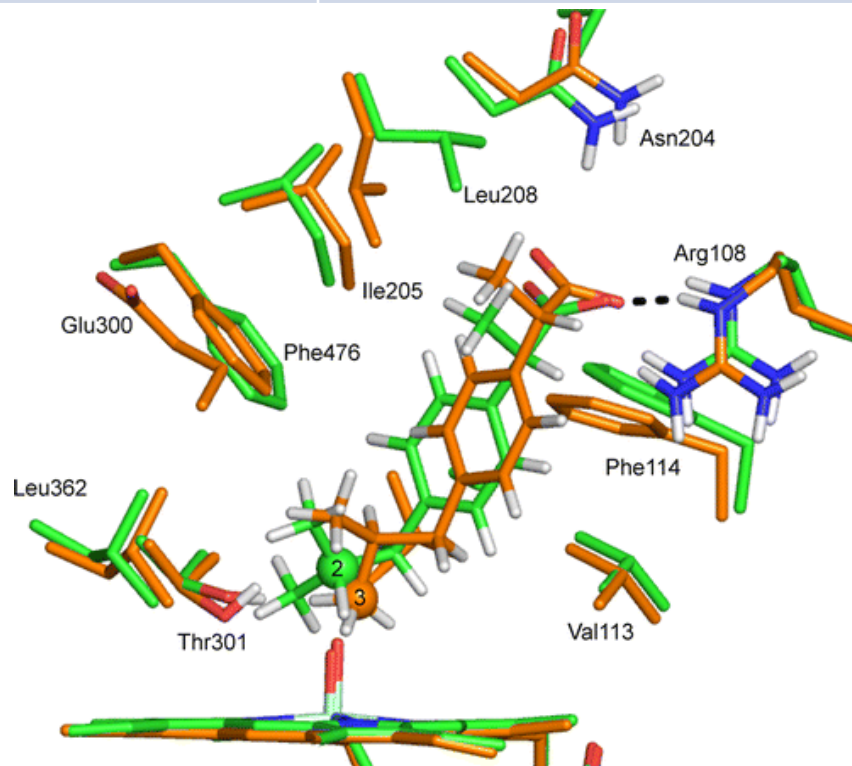
minor product

in 4

Ibuprofen

[kcal/mol]	C3	C2
ΔE^\ddagger (Boltzmann average)	19.2	26.5

- Correct preference for C3 hydrogen abstraction

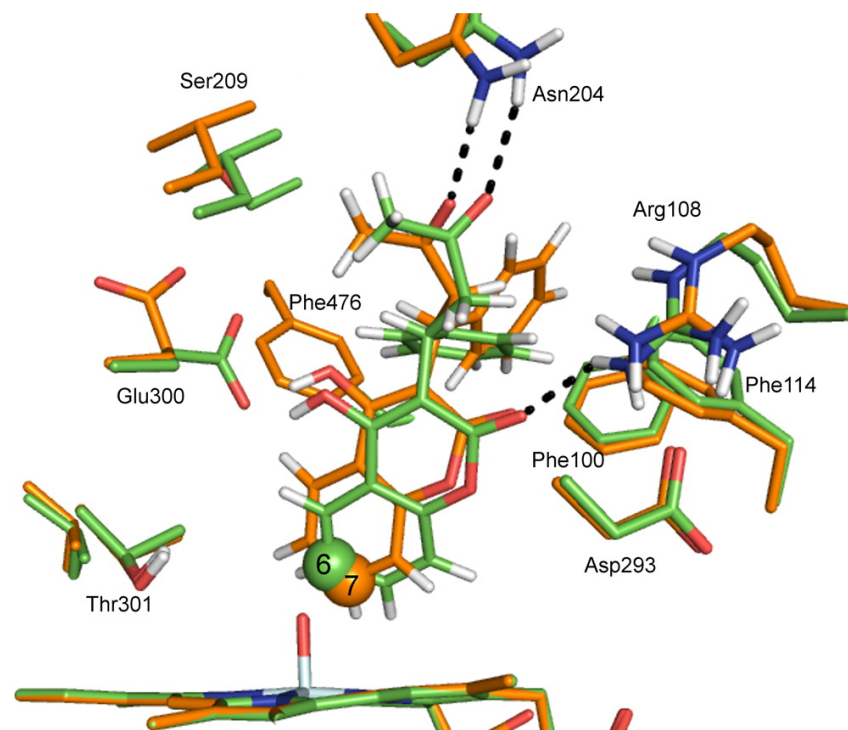


green = C2 transition state
orange = C3 transition state

Warfarin

[kcal/mol]	C7	C6
ΔE^\ddagger (Boltzmann average)	15.1	22.4

- Correct preference for C7 C-O bond formation

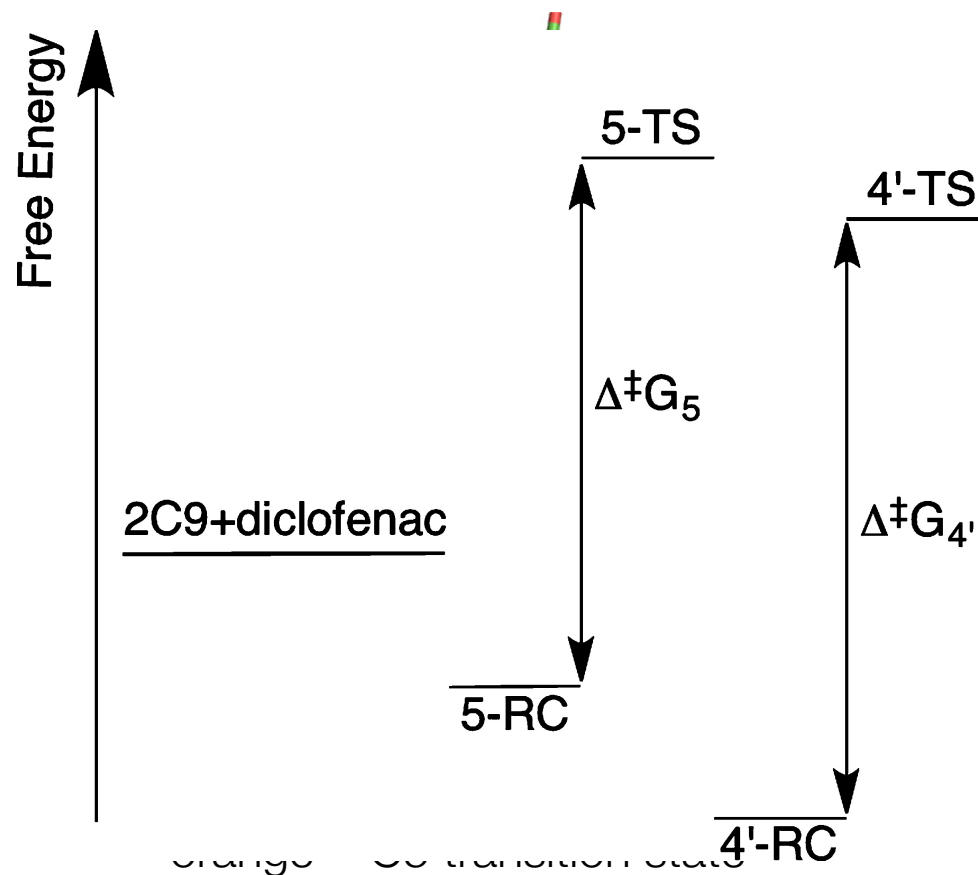


green = C6 transition state
orange = C7 transition state

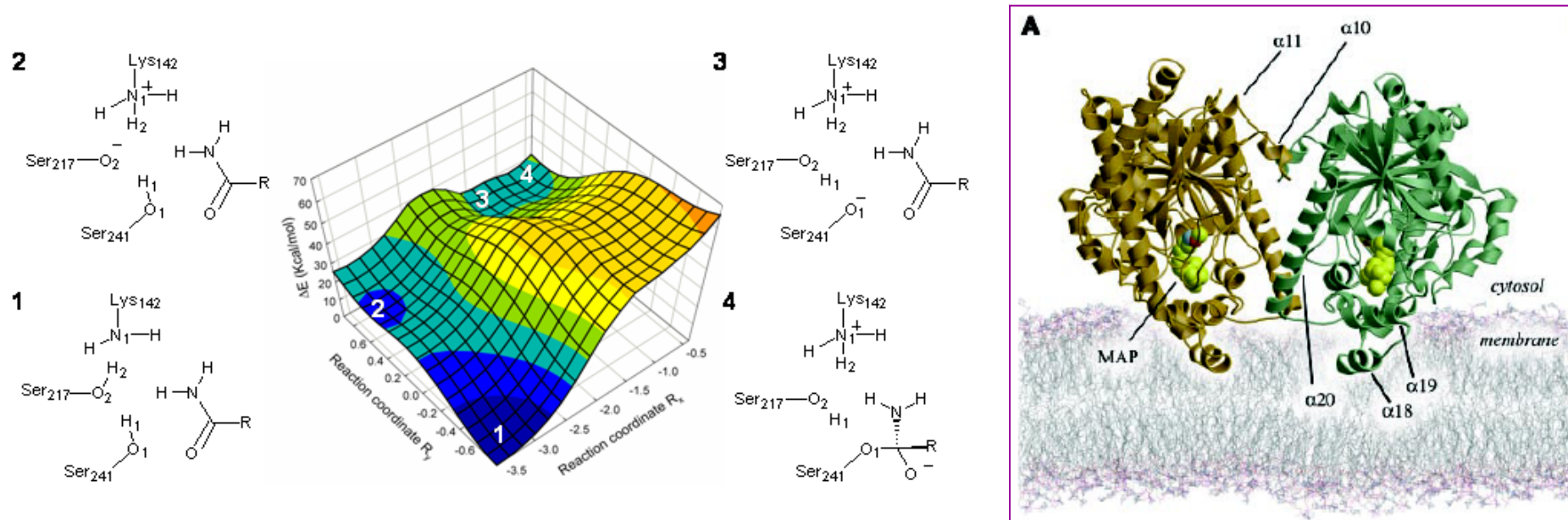
Diclofenac

[kcal/mol]	C4'	C5
ΔE^\ddagger (Boltzmann average)	17.8	12.1

- Incorrect preference for C5 C-O bond formation
- Different binding orientations required



Conformational effects in catalysis: Fatty Acid Amide Hydrolase (FAAH)



Inactivates neuromodulatory fatty acid amides:

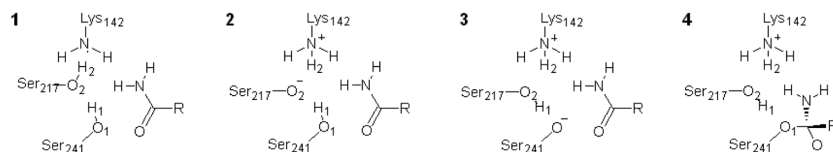
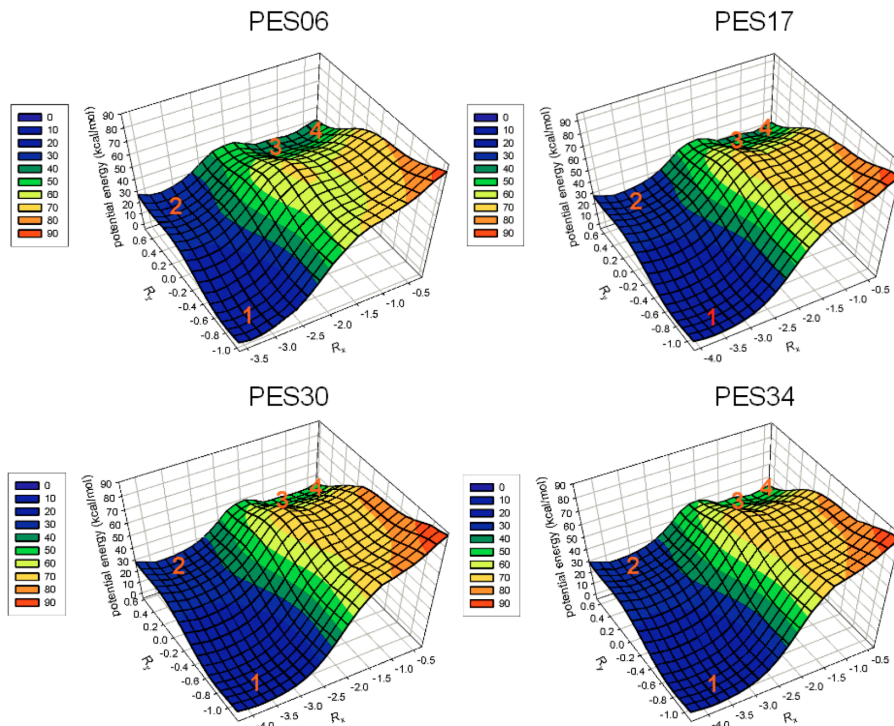
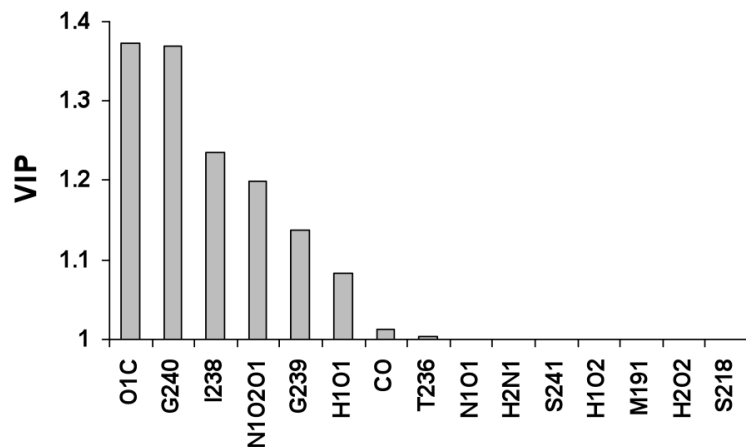
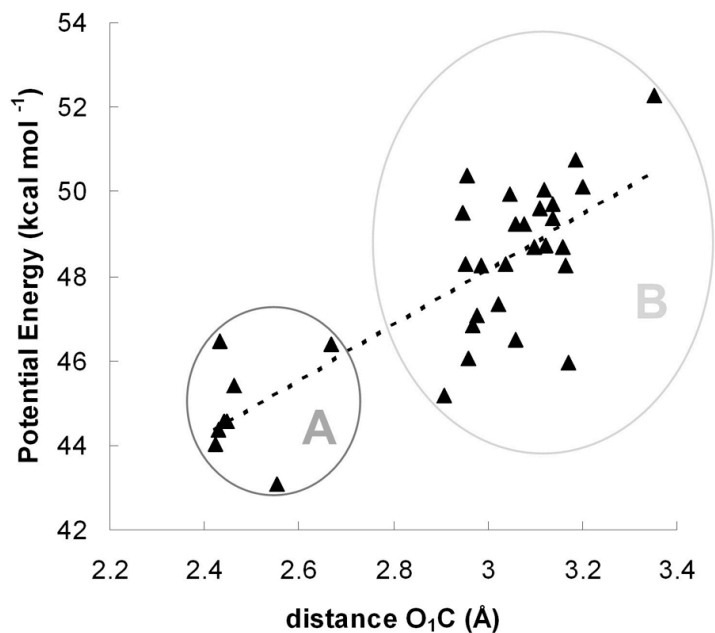
- Anandamide, endogenous cannabinoid involved in the regulation of pain and anxiety
- Oleamide ('sleep-inducing substance')
- Unusual Ser-Ser-Lys catalytic triad

Fatty acid amide hydrolase: reactive and unreactive conformations

Snapshot	O ₁ -C (Å)	H ₁ -O ₂ (Å)	N ₁ -H ₂ (Å)	N ₁ -O ₁ (Å)	PM3/MM kcal/mol	B3LYP/MM kcal/mol
1	2.679	1.816	1.752	4.668	43	28
2	2.705	1.807	1.791	4.660	49	32
3	2.613	1.816	1.757	4.510	53	30
4	2.795	1.758	1.762	4.097	36	18

PM3/CHARMM22: CHARMM; B3LYP/CHARMM22: Jaguar/Tinker

🔥 Analysis of QM/MM profiles in FAAH identifies 'families' of reactive conformations

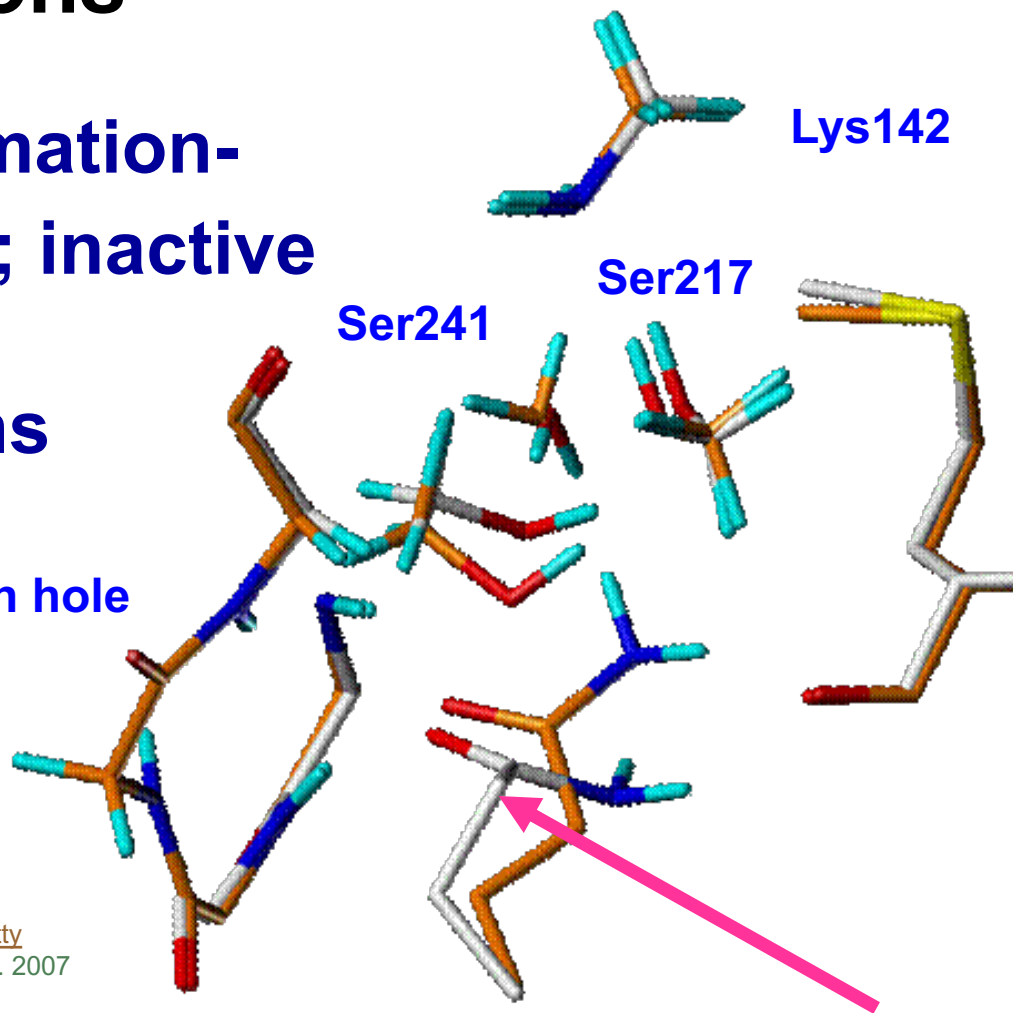


- PCA, PLS and MLR analysis
- Lodola *et al* JCTC 2010

FAAH: reactive and unreactive conformations

Active conformation-
white carbons; inactive
conformation-
orange carbons

Oxanyan hole



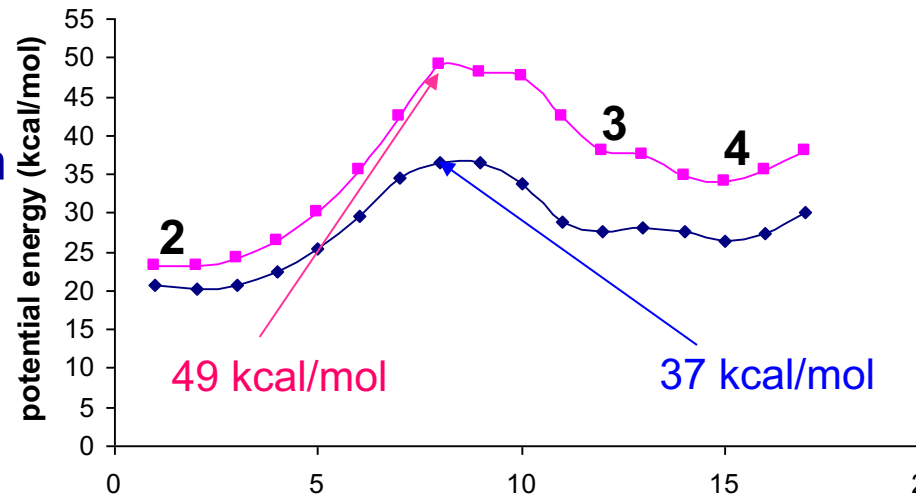
Substrate dihedral angle
changes from 180° to -90° .

Conformational effects in enzyme catalysis:
reaction via a high energy conformation in fatty
acid amide hydrolase Lodola et al. *Biophys J.* 2007
Structural Fluctuations in Enzyme-Catalyzed
Reactions: Determinants of Reactivity in Fatty Acid
Amide Hydrolase from Multivariate Statistical
Analysis of Quantum Mechanics/Molecular
Mechanics Paths Lodola et al. *J Chem Theory*
Comput. 2010
Computational enzymology Lodola & Mulholland
Methods Mol Biol. 2013

Better transition state stabilization (by Lys142) in reactive conformation

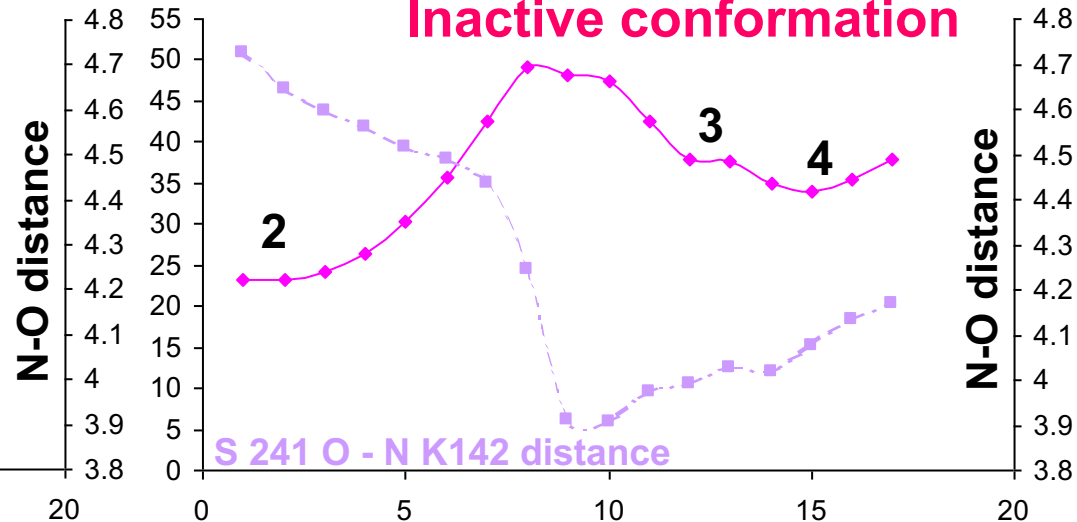
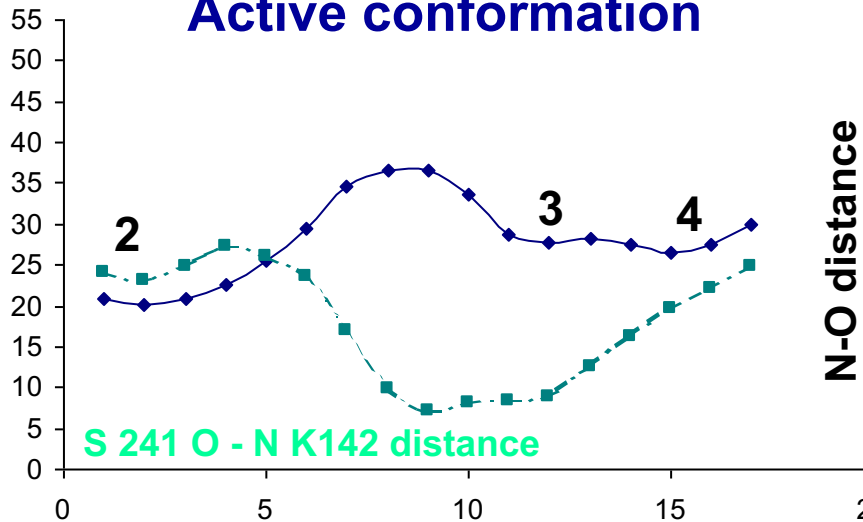
Active conformation

Inactive conformation



Active conformation

Inactive conformation



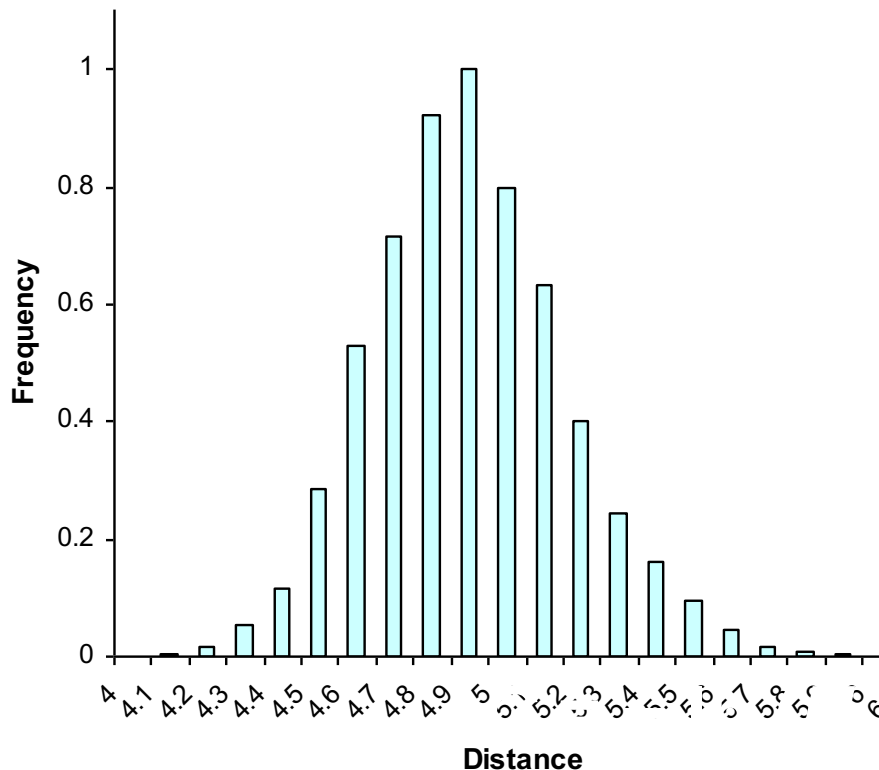
Restraining the N-O distance to 4.1 Å

reduces the barrier from 49 to 42 kcal/mol - part (not all) of the effect

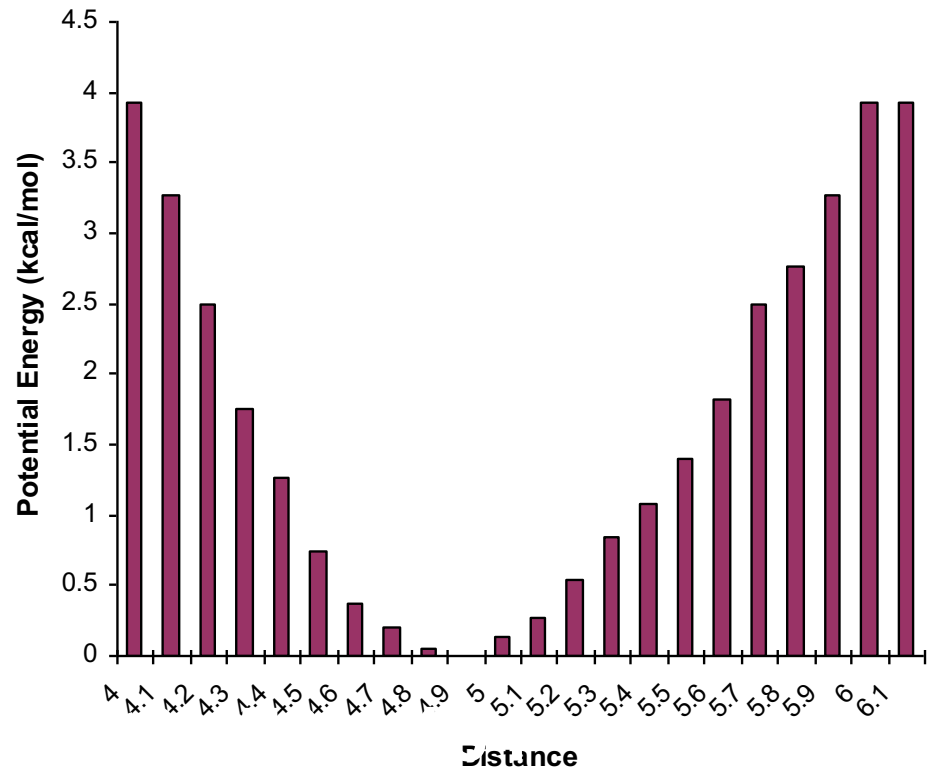
FAAH: reactive conformation ~ 3 kcal/mol above ground state

- cooperative fluctuations at active site
- important for selectivity?

Lys142 N1 Ser241 O1 Distance Distribution



Conformers Energy Distributions

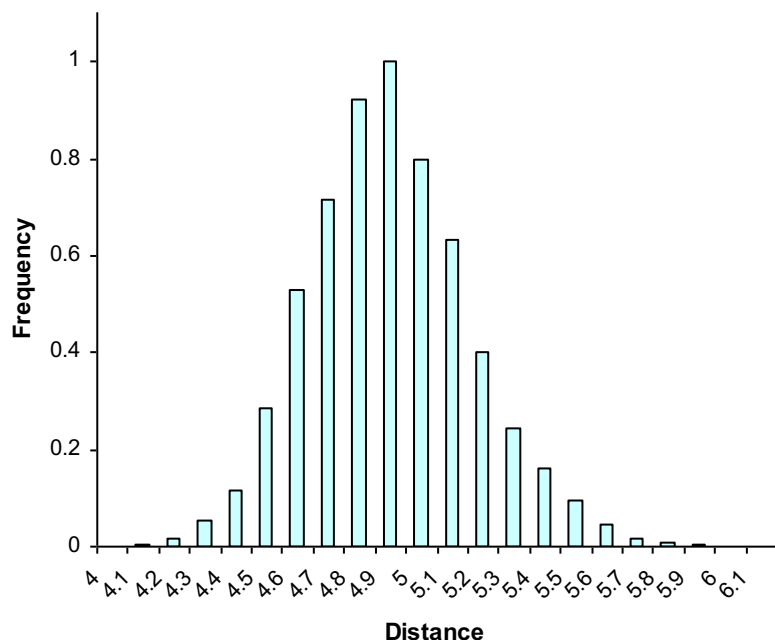


1 ns stochastic boundary MD; (umbrella sampling gives similar result)

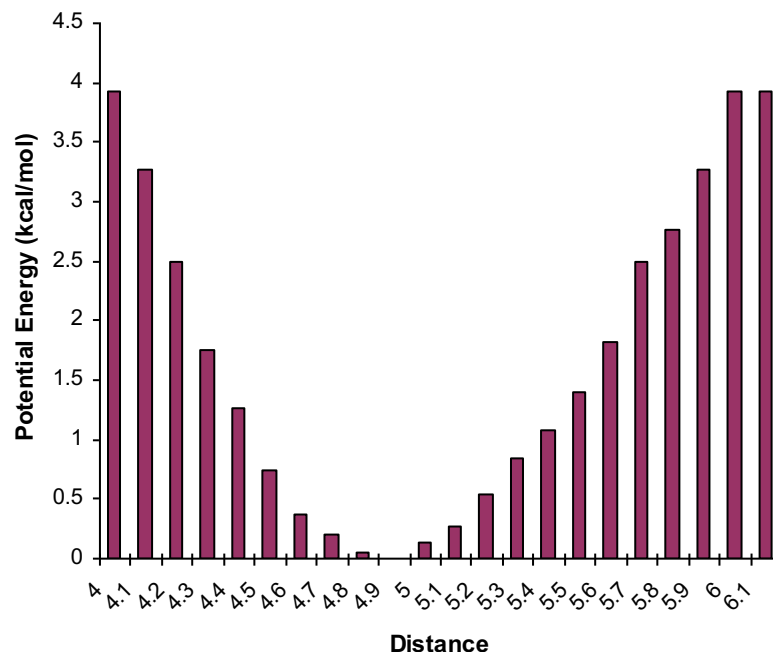
FAAH: Conformer populations

Populations from MD

Lys142 N1 Ser241 O1 Distance Distribution



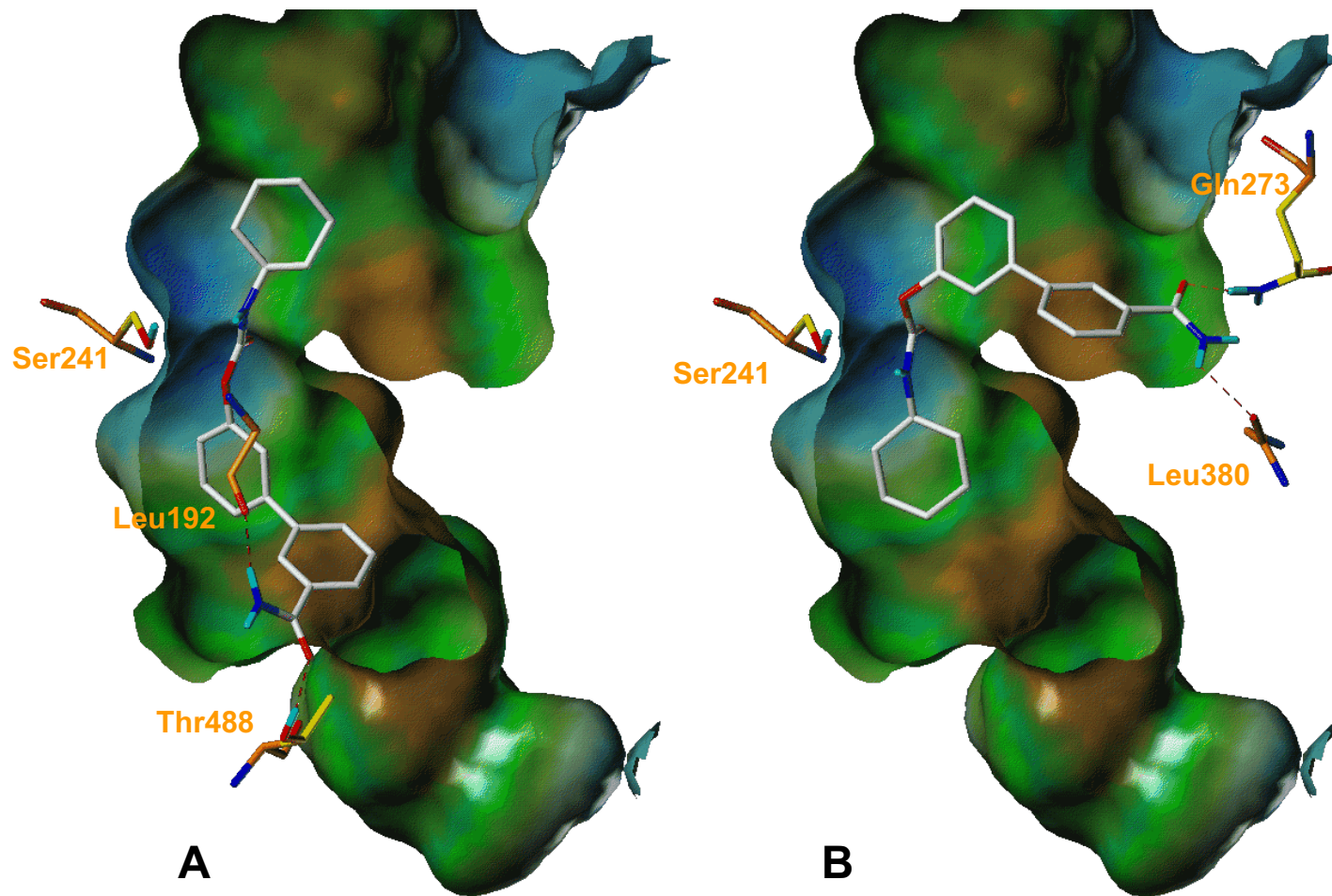
Conformers Energy Distributions



$$\Delta G = -\ln(N1/N0) * 2.479 / 4.184$$

Reactive conformation is ~3 kcal/mol higher
(umbrella sampling gives similar result)

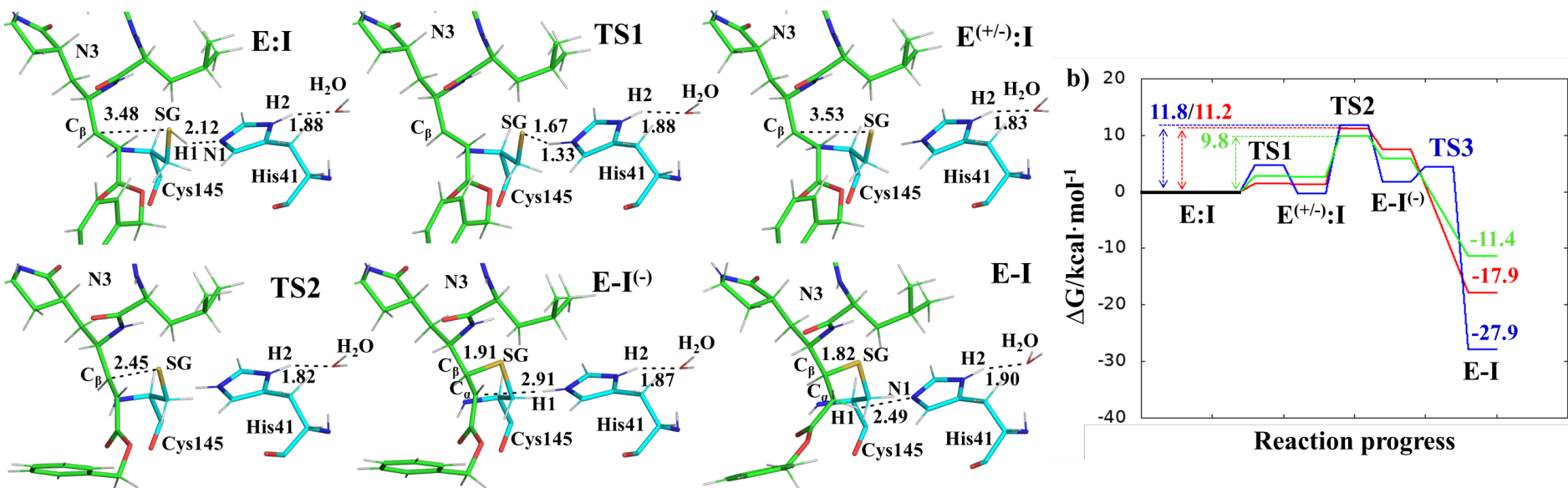
Fatty acid amide hydrolase: QM/MM identifies covalent inhibitor binding mode



QM/MM prediction (Lodola *et al Chem Commun* 2008) validated by subsequent crystal structure (Mileni *et al. J. Mol. Biol.* 2010)

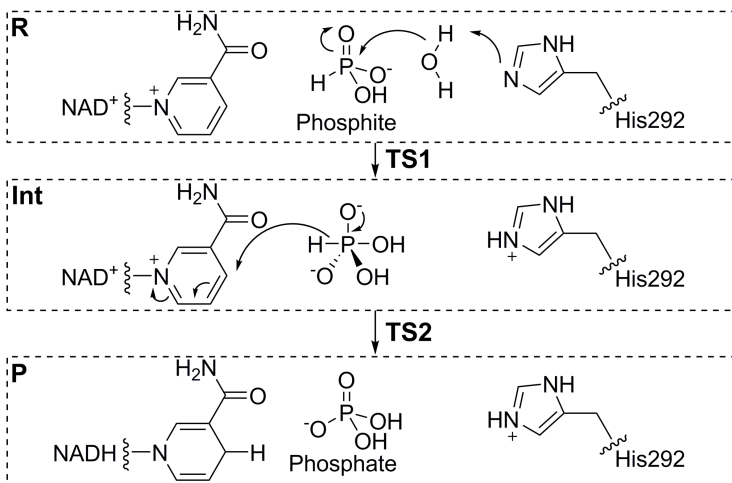
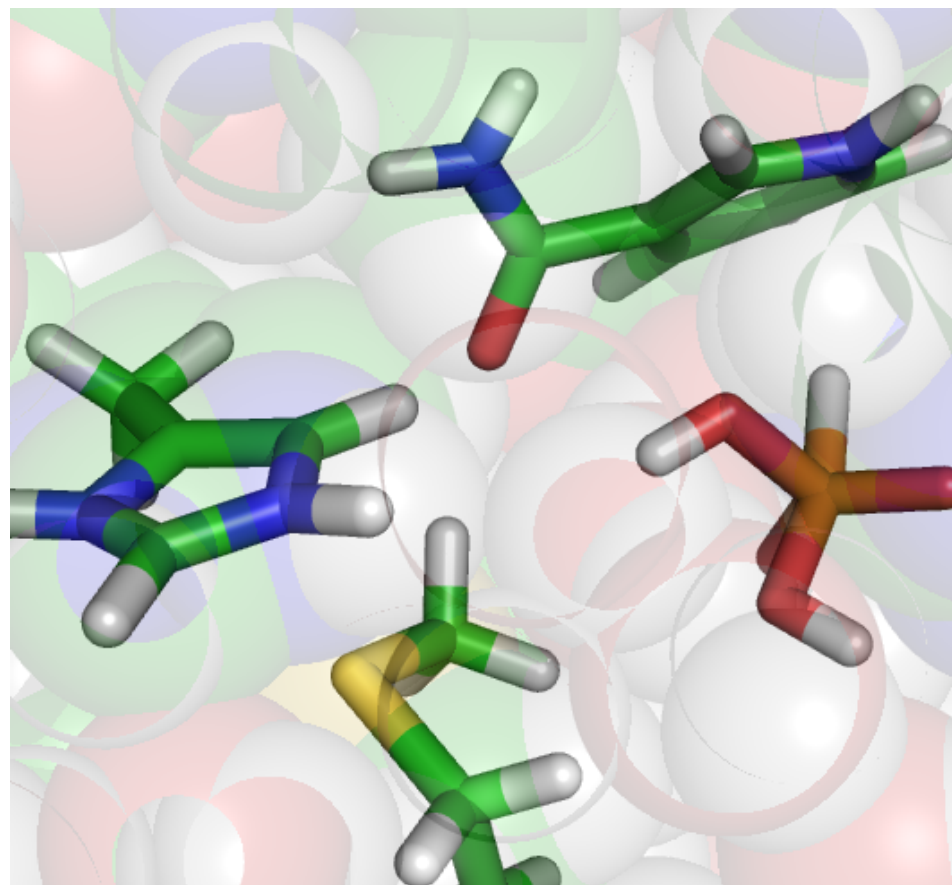
🔥 QM/MM modelling suggests inhibitors of the SARS-CoV-2 main protease

- Main protease is a potential drug target for COVID-19
- QM/MM simulations identify mechanism of reaction of N3 covalent inhibitor, and suggest chemical modifications to tune reversibility of inhibition



Transition state stabilization by methionine: phosphite dehydrogenase

- Methionine stabilizes TS for hydride transfer by interaction with histidine
- Potentially wide importance (since found in PDB and CSD)



TS from AM1/CHARMM27 QM/MM MD

Experiments: John Hung, Wilfred van der Donk, UIUC,
Ranaghan *et al.*, *Chemical Science* (2014)

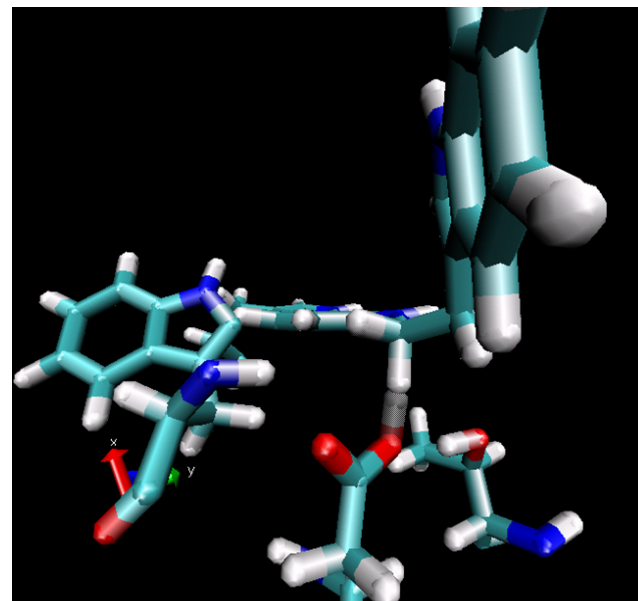
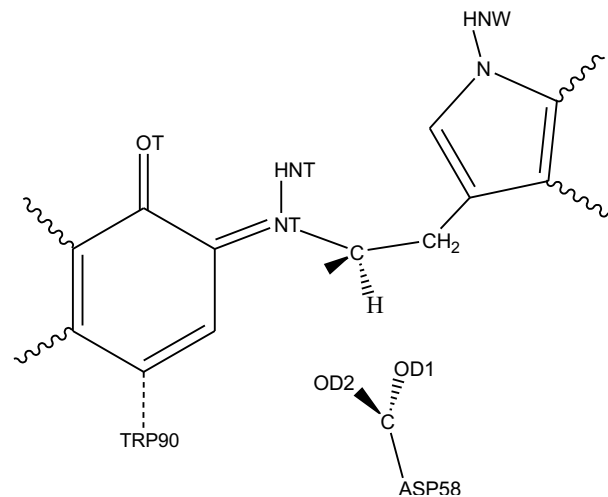
A novel role for methionine in catalysis

- Methionine not previously thought to be involved in enzyme catalysis
- QM/MM simulations of phosphite dehydrogenase identified interaction
- Tested by experiments: natural and non-natural amino acid mutants (e.g. Nle, SeMet)
- Met53 mutation affects k_{cat} , not K_{M}
- Methionine stabilizes transition state

Ranaghan *et al*, *Chemical Science* (2014)

Quantum tunnelling in enzyme reactions: aromatic amine dehydrogenase

- Experimental KIE = 55
- Calculated KIE ($k_{\text{H}}/k_{\text{D}}$) = 30-58
- Expt. $\Delta^\ddagger G = 12.7$ kcal/mol
- Calculated $\Delta^\ddagger G = 9.4$ -11.3 kcal/mol (VTST/SCT QM/MM PM3(corrected)/CHARMM using CHARMMRATE)
- Tunnelling reduces barrier by ~ 3 kcal/mol



Antibiotic resistance

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24 January 2013 Last updated at 13:18 GMT



Antibiotic 'apocalypse' warning

By James Gallagher

Health and science reporter, BBC News

The rise in drug resistant infections is comparable to the threat of global warming, according to the chief medical officer for England.

Prof Dame Sally Davies said bacteria were becoming resistant to current drugs and there were few antibiotics to replace them.

She told a committee of MPs that going for a



Drug resistance is a problem in tuberculosis

theguardian

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News Society Antibiotics

Antibiotic-resistant diseases pose 'apocalyptic' threat, top expert says

Chief medical officer Dame Sally Davies tells MPs issue should be added to national risk register of civil emergencies

Ian Sample, science correspondent

The Guardian, Wednesday 23 January 2013 19.41 GMT

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Antibiotics Resistance A Growing Health Threat

Some antibiotics are losing their effectiveness at a rate that is both alarming and irreversible, says Chief Medical Officer.

2:50pm UK, Friday 16 November 2012

Carbapenem resistant enterobacteria: resistance to 'last resort' antibiotics

BMJ

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Healthcare-associated Infections (HAIs)

Healthcare-associated Infections

HAIs: The Burden

Monitoring HAIs

Types of Infections

Diseases and Organisms

Acinetobacter

Burkholderia cepacia

Clostridium difficile

Clostridium Sordellii

► Carbapenem-resistant Enterobacteriaceae (CRE)

Tracking CRE

Patients

Patient FAQ

General Information

[Healthcare-associated Infections](#) > [Diseases and Organisms](#)

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Carbapenem-resistant Enterobacteriaceae (CRE)

CRE, which stands for carbapenem-resistant Enterobacteriaceae, are a family of germs that are difficult to treat because they have high levels of resistance to antibiotics. *Klebsiella* species and *Escherichia coli* (*E. coli*) are examples of Enterobacteriaceae, a normal part of the human gut bacteria, that can become carbapenem-resistant. Types of CRE are sometimes known as KPC (*Klebsiella pneumoniae* carbapenemase) and NDM (New Delhi Metallo-beta-lactamase). KPC and NDM are enzymes that break down carbapenems and make them ineffective.

Healthy people usually do not get CRE infections. In healthcare settings, CRE infections most commonly occur among patients who are receiving treatment for other conditions. Patients whose care requires devices like ventilators (breathing machines), urinary (bladder) catheters, or intravenous (vein) catheters, and patients who are taking long courses of certain antibiotics are most at risk for CRE infections.

Some CRE bacteria have become resistant to most available antibiotics. Infections with these germs are very difficult to treat, and can be deadly—one report cites they can contribute to death in up to 50% of patients who become infected.



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NEWS

Resistance of enterobacteria to carbapenem antibiotics is a global crisis

BMJ 2012; 344 doi: <http://dx.doi.org/10.1136/bmj.e1646> (Published 6 March 2012)

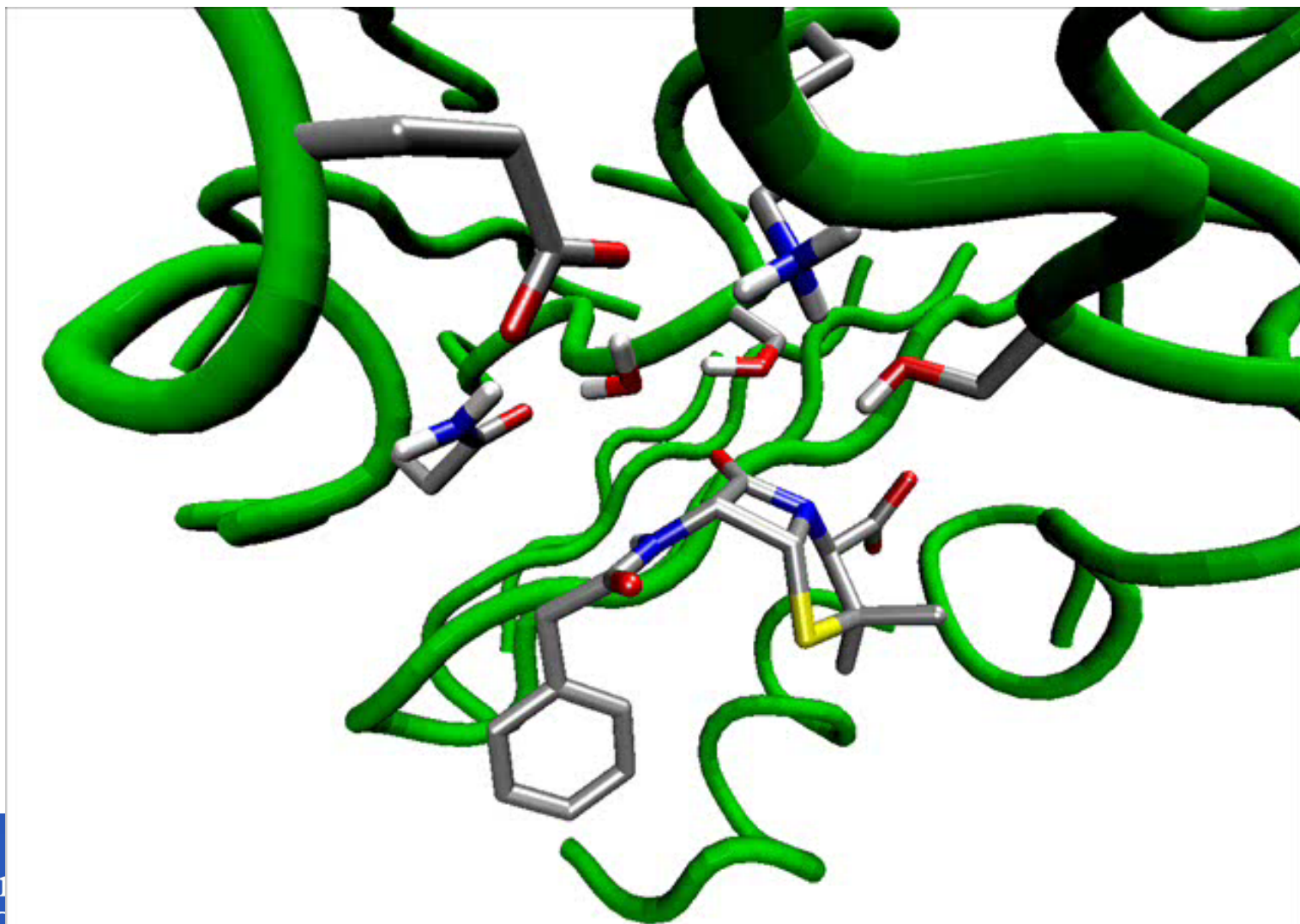


QM/MM modelling of antibiotic breakdown in *E. coli* TEM1 β -lactamase

Class A
 β -lactamase

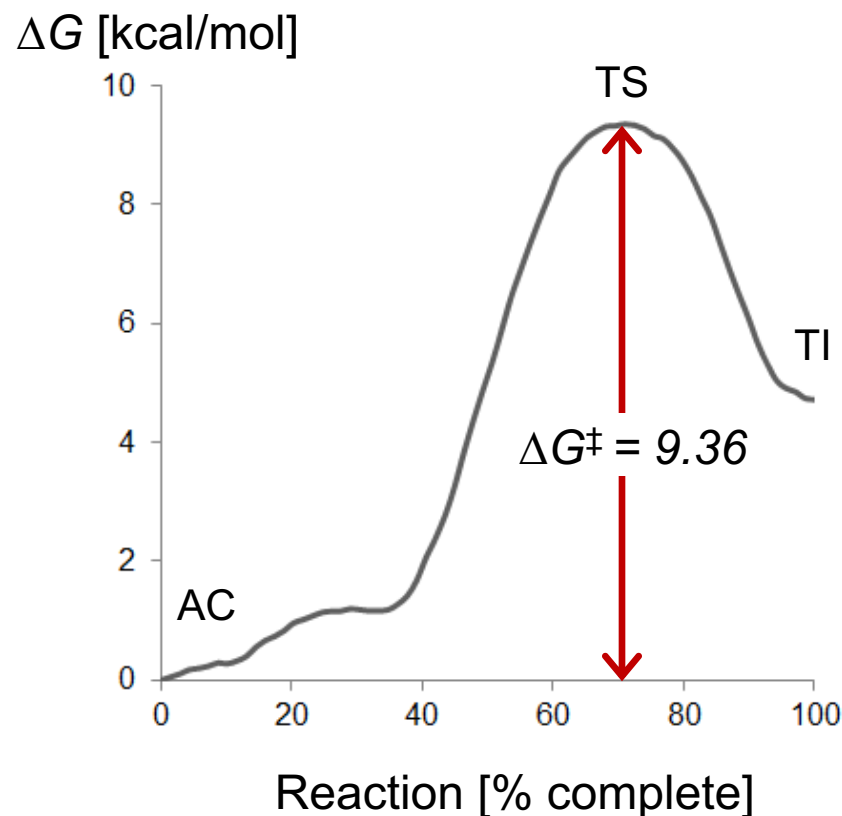
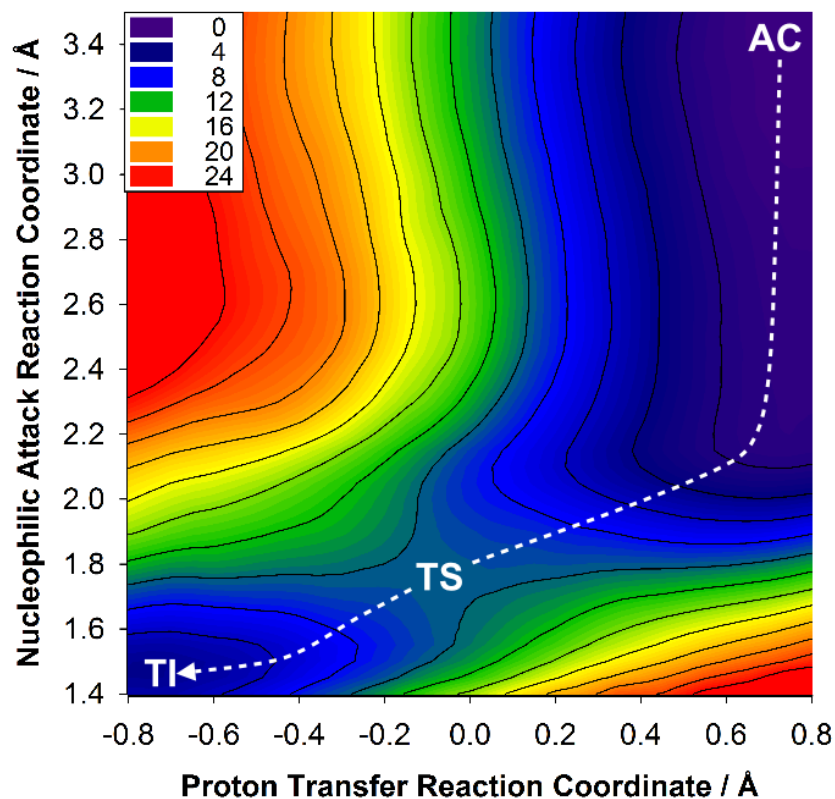
Hermann et al.
JACS 2003,
JACS 2005,
OBC 2006,
JPCA 2009

<https://www.youtube.com/watch?v=FRsjMm4eop4>



🔥 QM/MM 'assay' for carbapenem breakdown?

- Follow processes in rate-limiting step with QM/MM MD¹



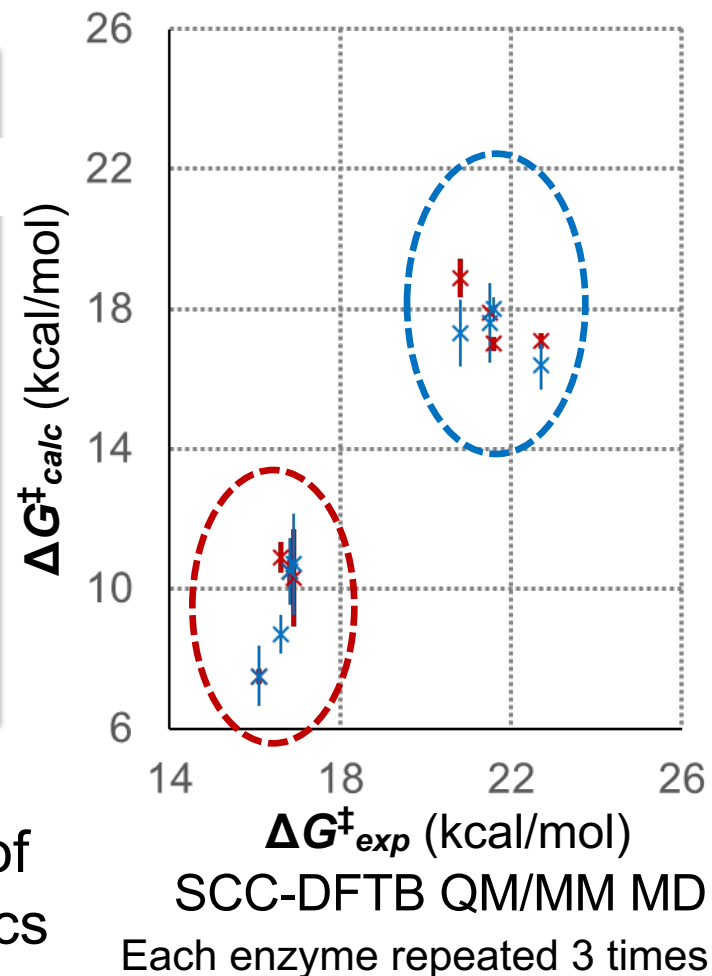
¹ EI Chudyk, MAL Limb, C Jones, J Spencer, MW van der Kamp, AJ Mulholland (2014) *Chem Comm* 50: 14736

🔥 A 'computational assay' for carbapenemases: QM/MM MD simulations of deacylation

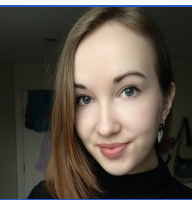
enzyme	k_{cat} (s ⁻¹)	$\Delta G^{\ddagger}_{\text{calc}}$ (kcal/mol)		
		Full		
BlaC	1.7×10^{-3}	17.9 (0.1)	17.6 (2.3)	21.8 (1.8)
CTX-M	4.2×10^{-3}	18.9 (1.1)	17.3 (1.9)	20.3 (3.5)
SHV-1	1.3×10^{-3}	17.0 (0.4)	18.0 (0.7)	20.8 (1.0)
TEM-1	2.3×10^{-3}	17.1 (0.4)	16.4 (1.4)	20.6 (1.3)
KPC-2	3.6	10.5 (0.9)	10.5 (1.9)	11.1 (2.3)
NMC-A	12.0	7.5 (0.4)	7.5 (1.7)	10.5 (1.3)
SFC-1	6.5	10.9 (0.9)	8.7 (1.1)	11.5 (0.8)
SME-1	3.2	10.3 (2.8)	10.7 (2.9)	12.9 (0.5)

Time for 1 $\Delta G^{\ddagger}_{\text{calc}}$ ~20 days 38 hrs <4 hrs

- QM/MM assays may help predict effects of mutations and in design of future antibiotics

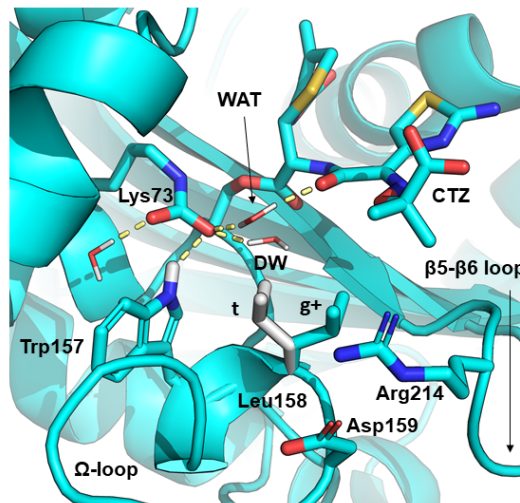


[Hirvonen, Spencer, van der Kamp et al.
J. Chem. Inf. Model. 2019](#)

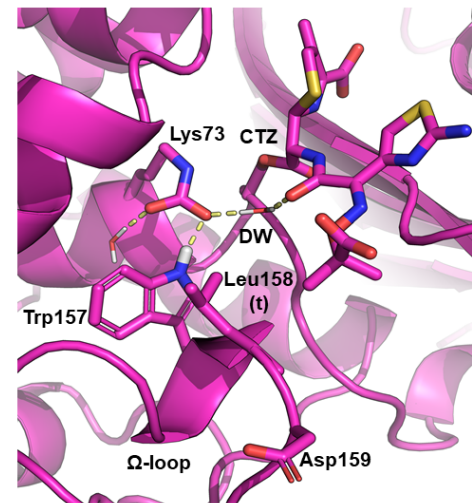


🔥 QM/MM modelling reveals origins of differences in antibiotic breakdown enzymes

- Class D β -lactamases
- QM/MM MD simulations reproduce differences in barriers between enzymes
- Small differences in active site hydration determine activity against cephalosporins

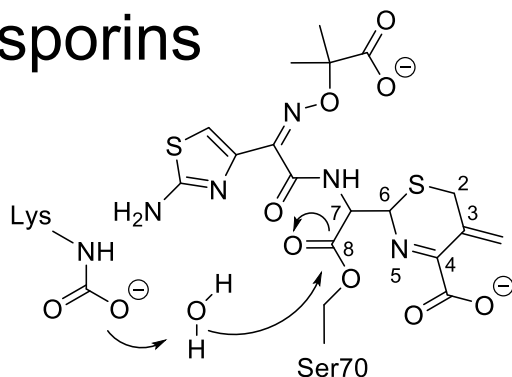
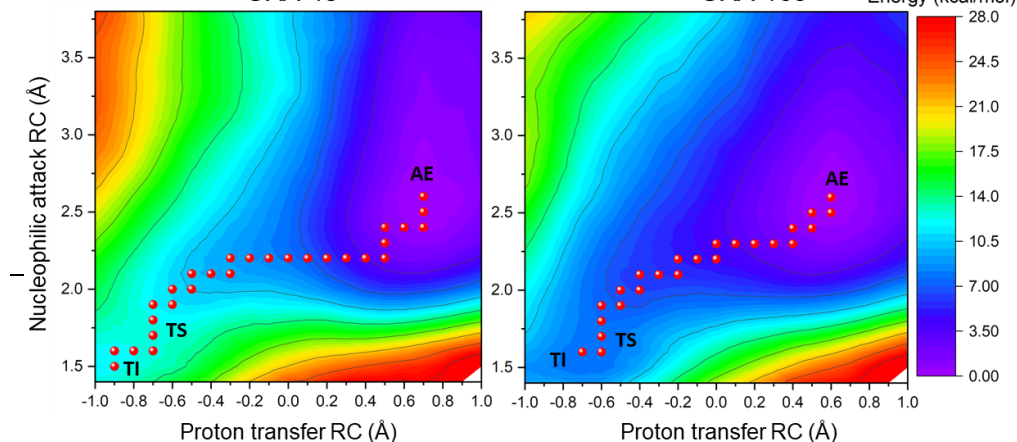


OXA-48



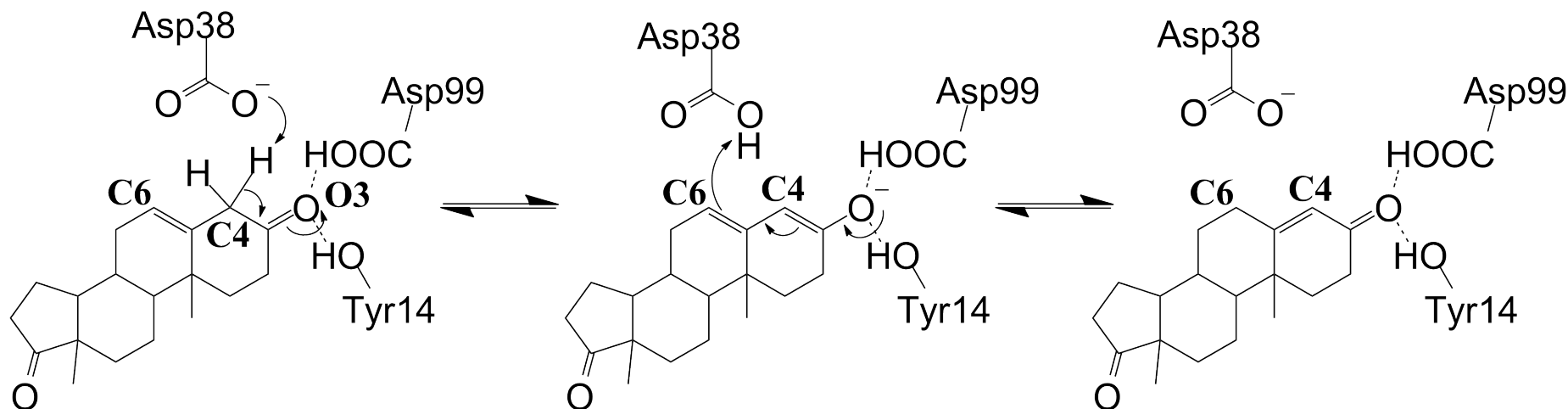
OXA-163

Energy (kcal/mol)



🔥 Ketosteroid isomerase (KSI)

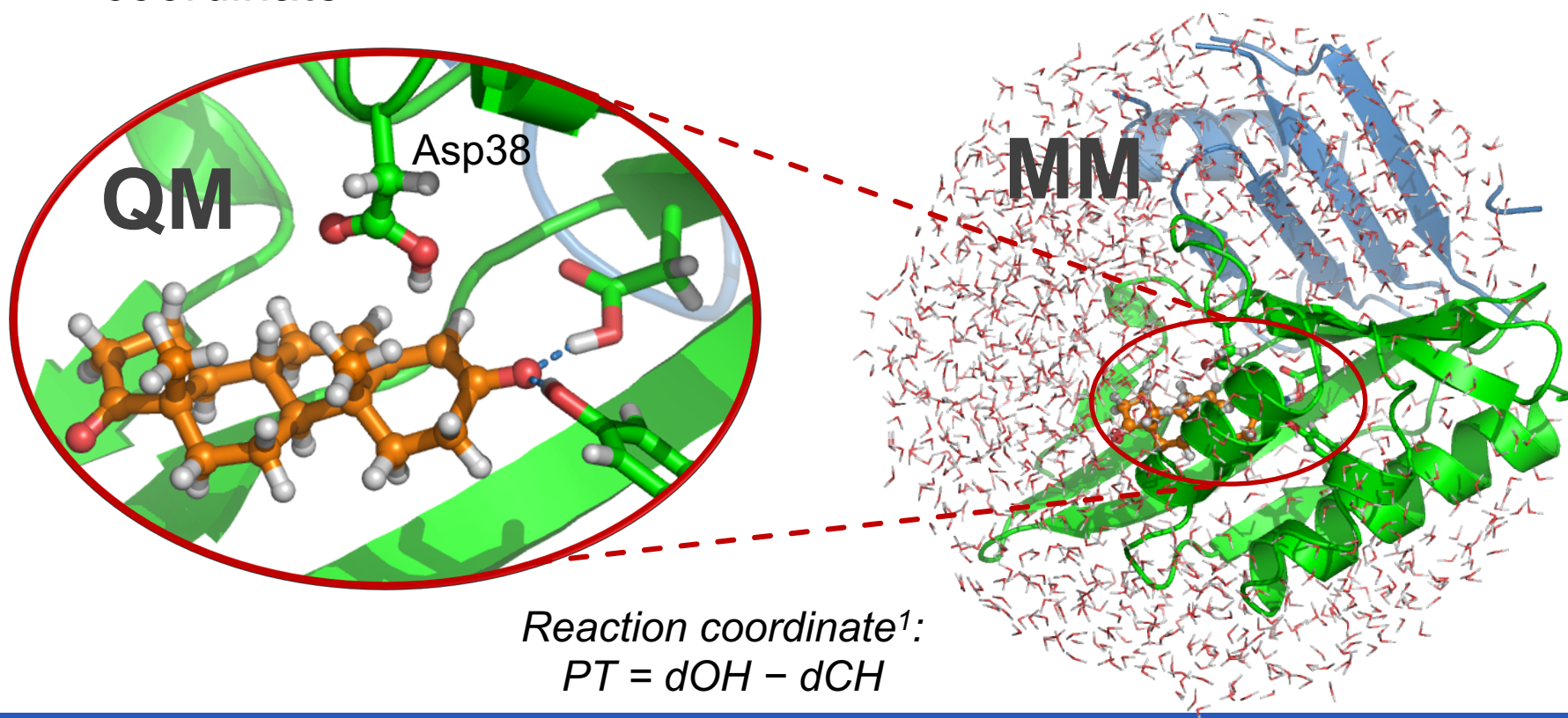
- *Highly efficient catalyst*: reaction rate near diffusion limit
- Catalyses isomerization of 3-oxo- Δ^5 -steroids to 3-oxo- Δ^4 -steroids



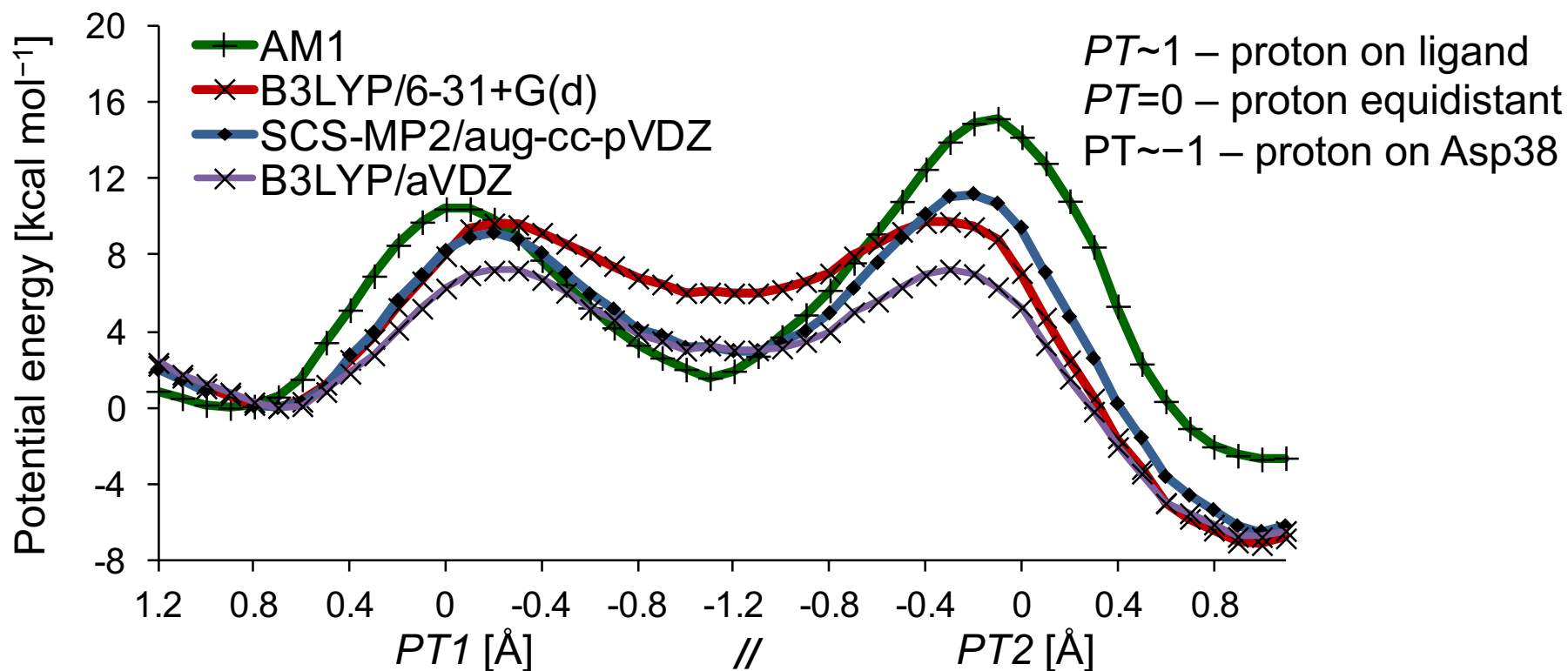
- Popular model system for enzyme catalysis
 - EVB modelling indicates catalysis is mainly *electrostatic*¹
 - MM MD studies suggest intermediate *more desolvated*²

🔥 KSI: QM/MM reaction modelling

- Run (short) AM1/CHARMM27 MD with intermediate state
- Calculate potential barriers for proton transfers along reaction coordinate

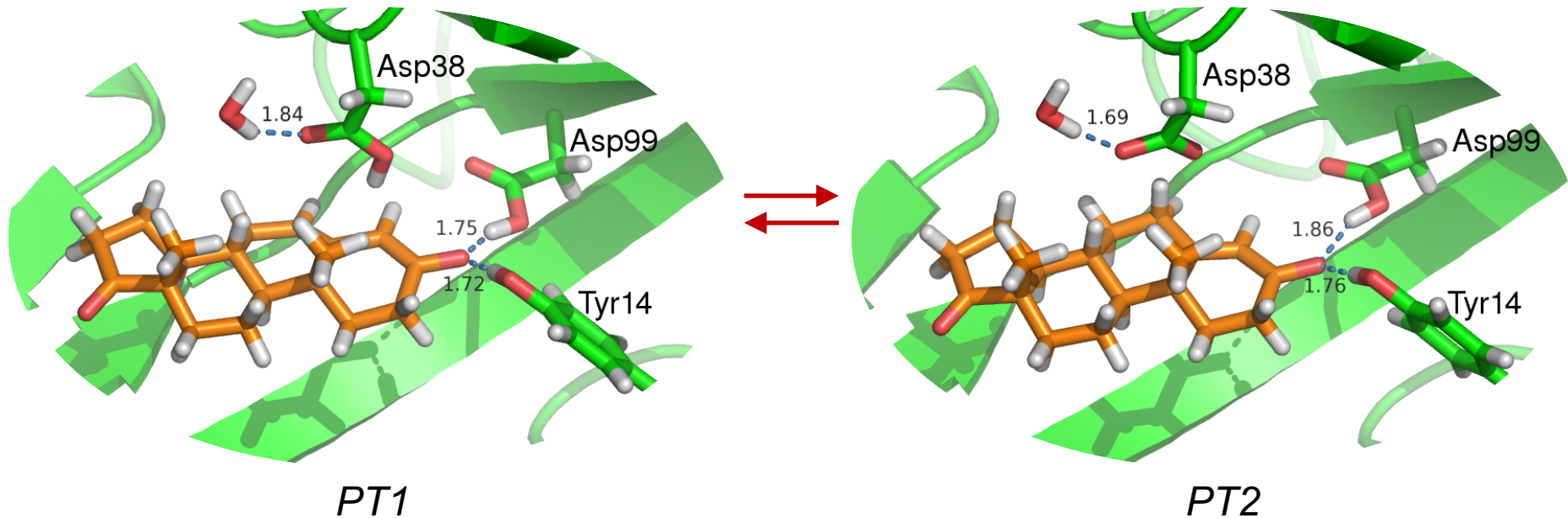


🔥 KSI: QM/MM potential energy profiles for reaction



- Barriers in line with experiment (diffusion-limited reaction)
- For first step, AM1 is similar to SCS-MP2/aug-cc-pVDZ

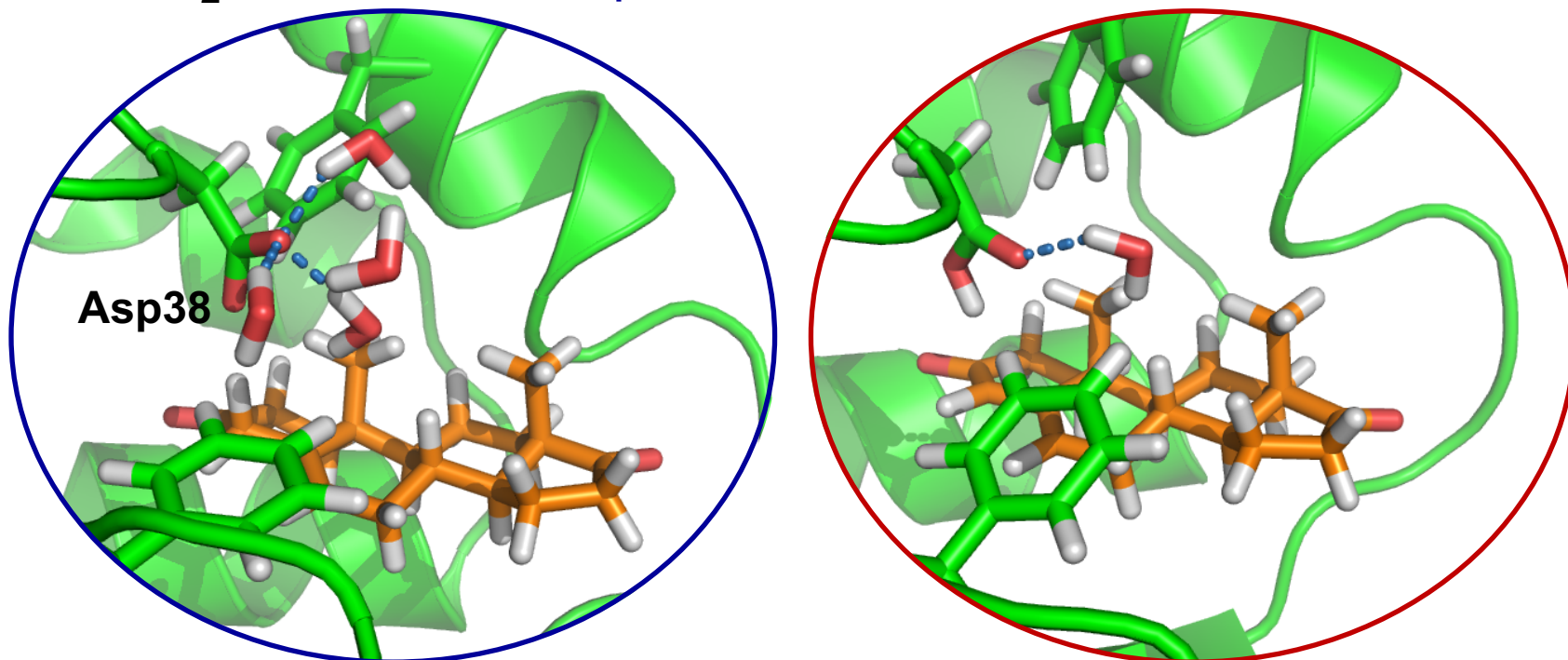
🔥 KSI: structures during reaction



- Change required between PT1 and PT2 is small and sampled in QM/MM MD (AM1/CHARMM)

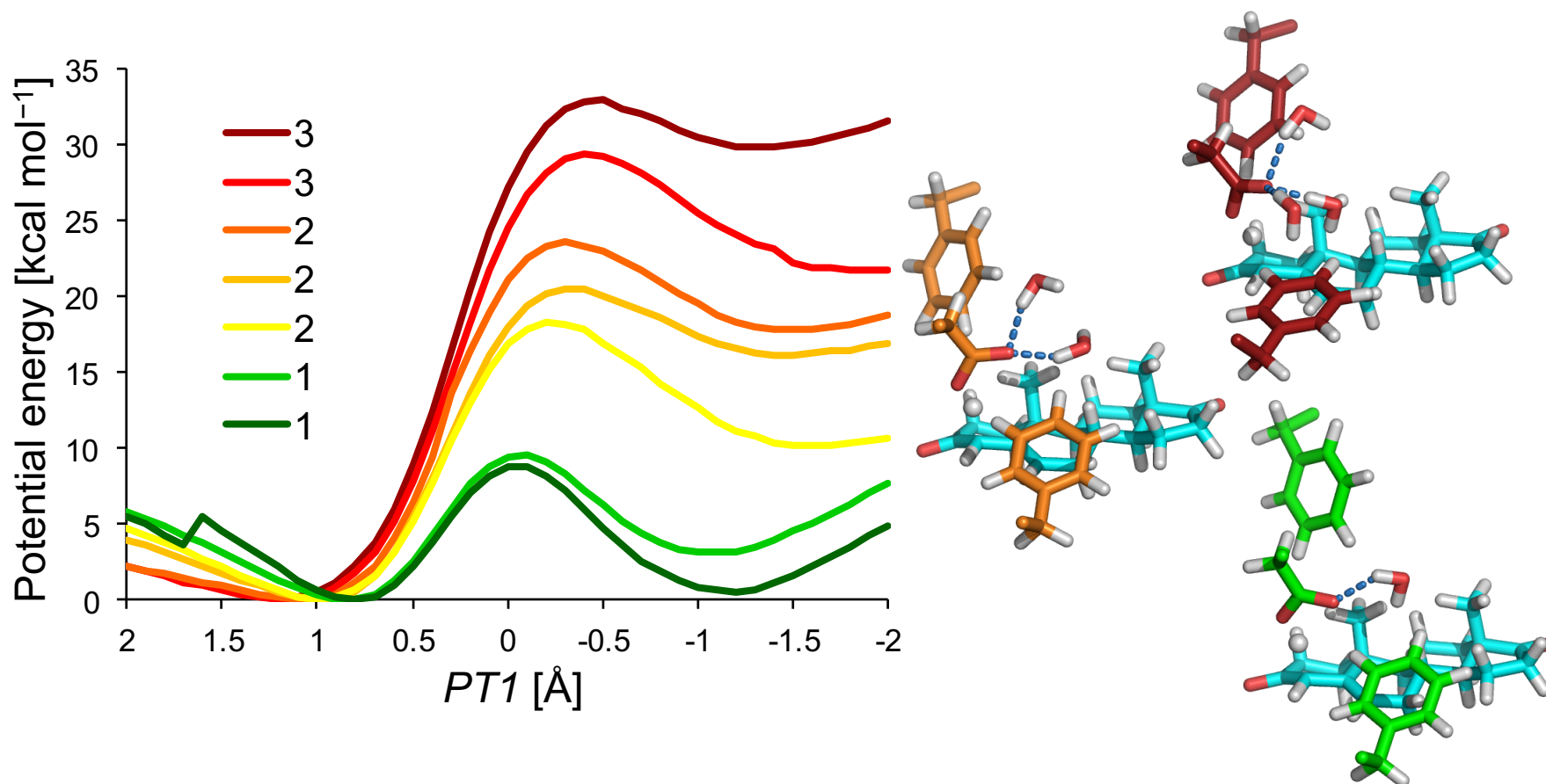
🔥 KSI: (de)solvation of Asp38

- AM1 QM/MM MD: 1 H₂O around Asp38 in **intermediate** state, up to 4xH₂O in **reactant or product** state



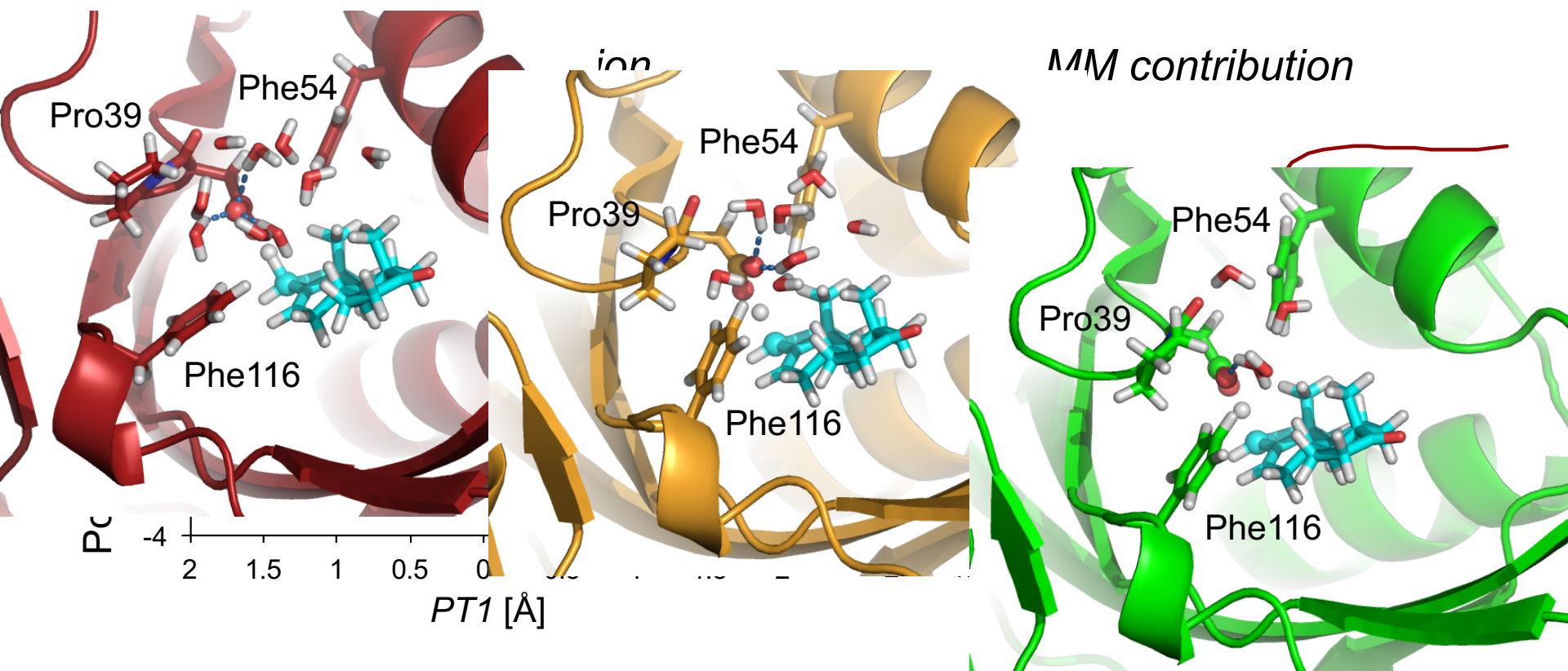
- Strong dependence of free energy on starting structure in QM/MM umbrella sampling MD: not converged

🔥 KSI: Asp38 desolvation is crucial for catalysis



- The more hydrated Asp38 becomes, the higher the barrier

🔥 MM region responsible for barrier change

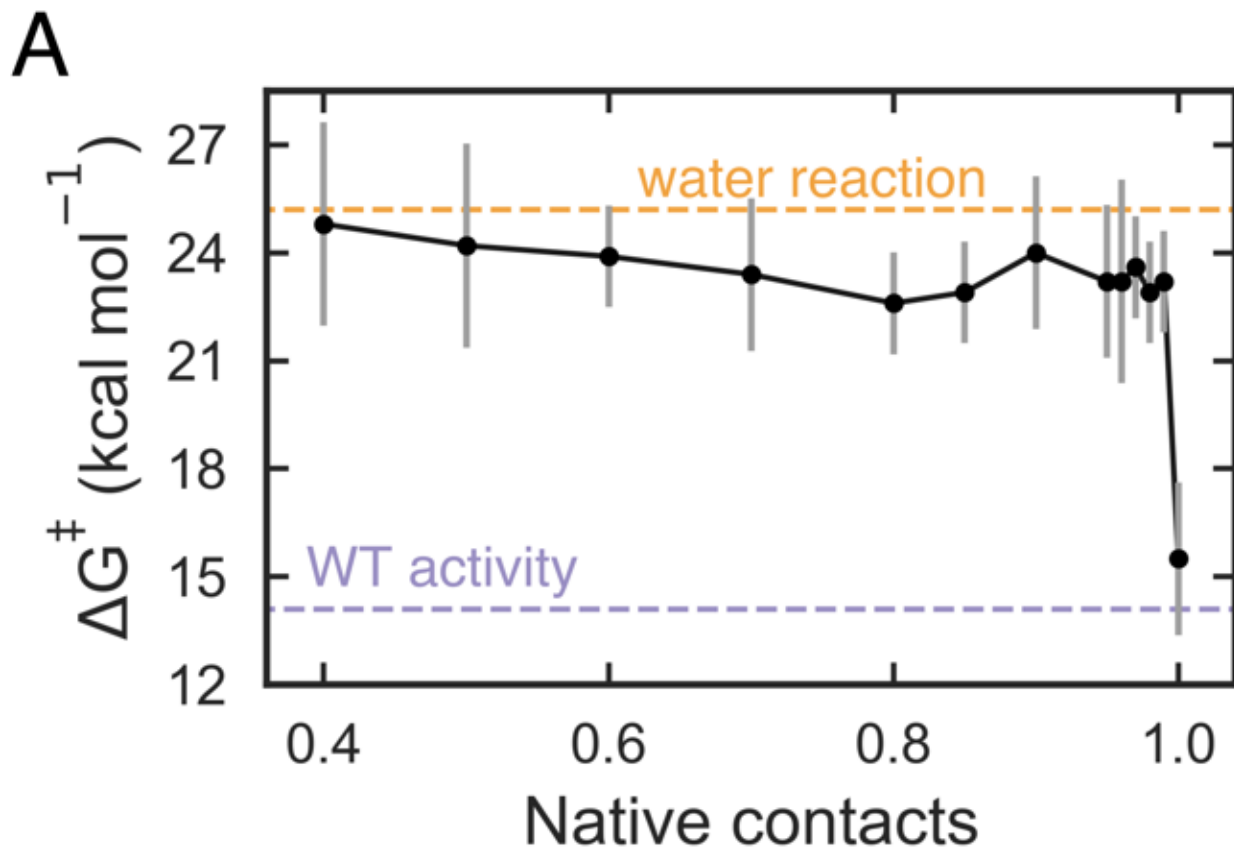


- QM barrier (gasphase) and TS structure similar throughout
- Electrostatic interactions with MM region affect barrier
- Water is 'squeezed' out so that efficient reaction can occur



🔥 Conformational changes and catalysis: loop motion in TIM

- Effective catalysis requires full closure of loop 6 ($Q > 0.99$)
- Desolvation of Glu165 raises pK_a



EVB simulations on structures from BE-metaD



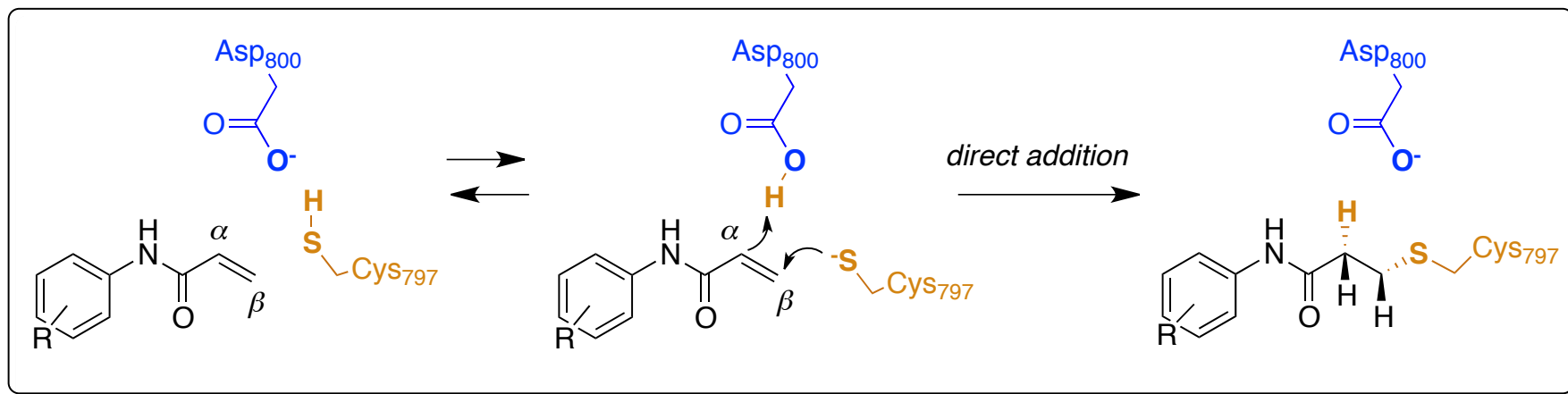
Multiscale analysis of drug resistance: Osimertinib and mutant epidermal growth factor receptor (EGFR)

- Osimertinib: AstraZeneca treatment for non-small cell lung cancer, approved 2017
- Third generation EGFR tyrosine kinase receptor
- Covalent inhibitor: alkylates Cys797
- Targets T790M mutant EGFR
- Most patients respond well, but drug resistance appears in around a year
- L718Q resistant mutant; origins of resistance not clear
- QM/MM umbrella sampling of reactivity; WaterSwap binding affinity predictions; MM metadynamics



EGFR/osimertinib: impact of L718Q mutation on reactivity

- Mechanism of EGFR Cys797 alkylation modelled by QM/MM
 - Cys797 modelled as thiolate ($\text{pK}_{\text{a,exp}} = 5.5$)
 - Asp800 involved in the protonation of acrylamide C α
 - Direct addition taken as likely mechanism
- Reaction modelled for two complexes
 - EGFR T790M (osimertinib sensitive)
 - EGFR T790M/L718Q (osimertinib resistant)

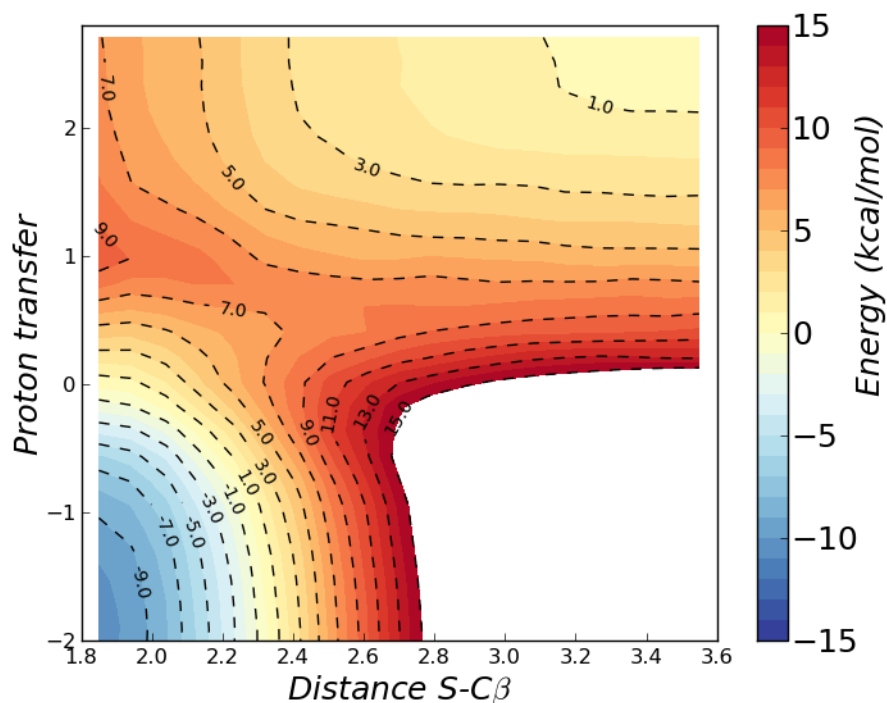


SCC-DFTB/AMBER99SB QM/MM umbrella sampling

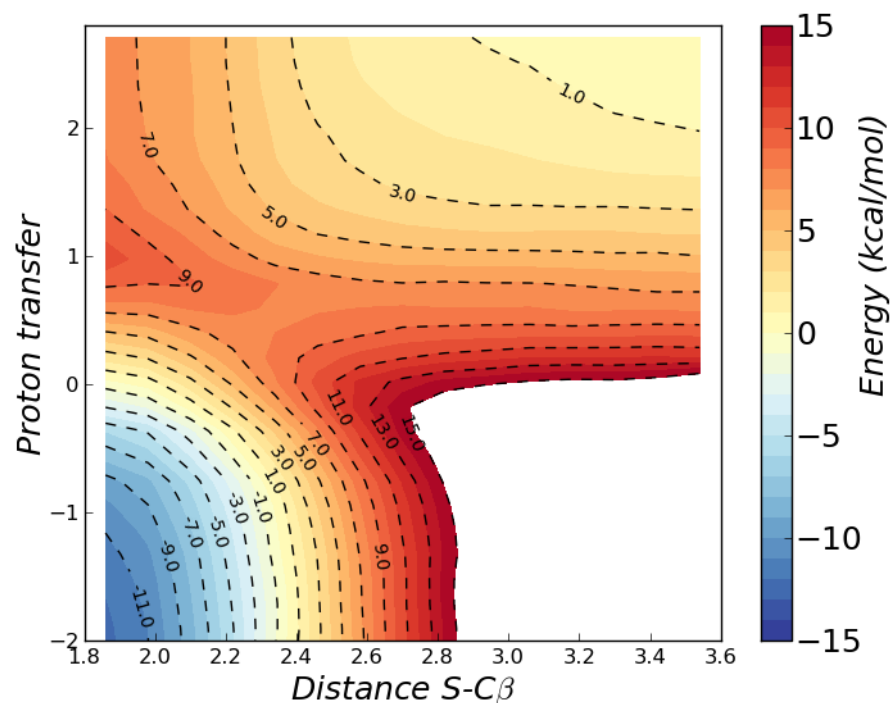
QM/MM free energy surface for EGFR Cys797 alkylation by Osimertinib

- L718Q mutation does not affect
 - Activation barrier ($\Delta A^* = 9$ kcal/mol) for sensitive and resistant EGFR mutant
 - Reaction energy ($\Delta A = -10$ kcal/mol) for sensitive and resistant EGFR mutant

T790M



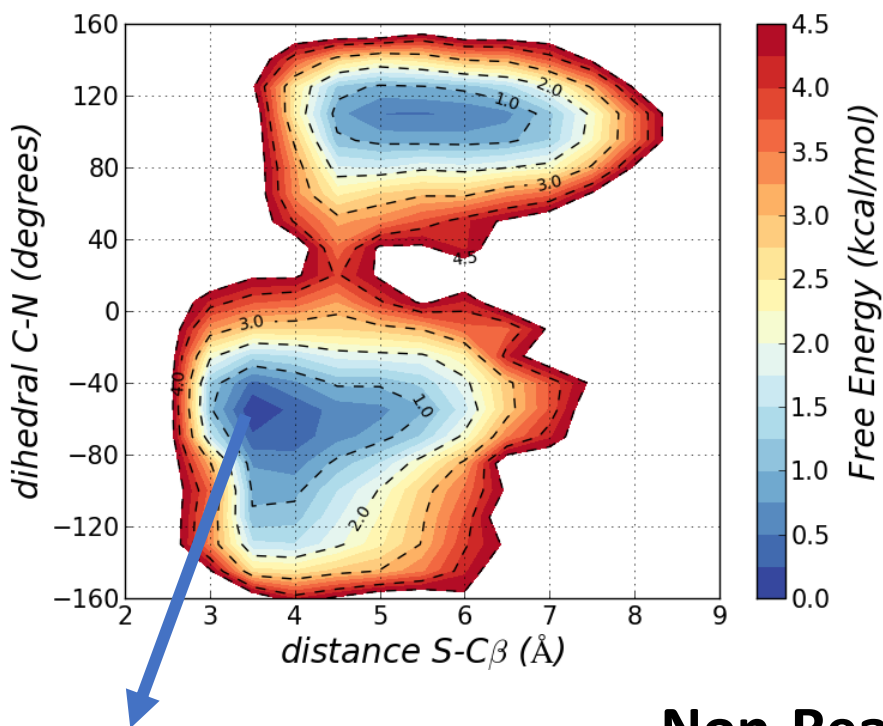
T790M/L718Q



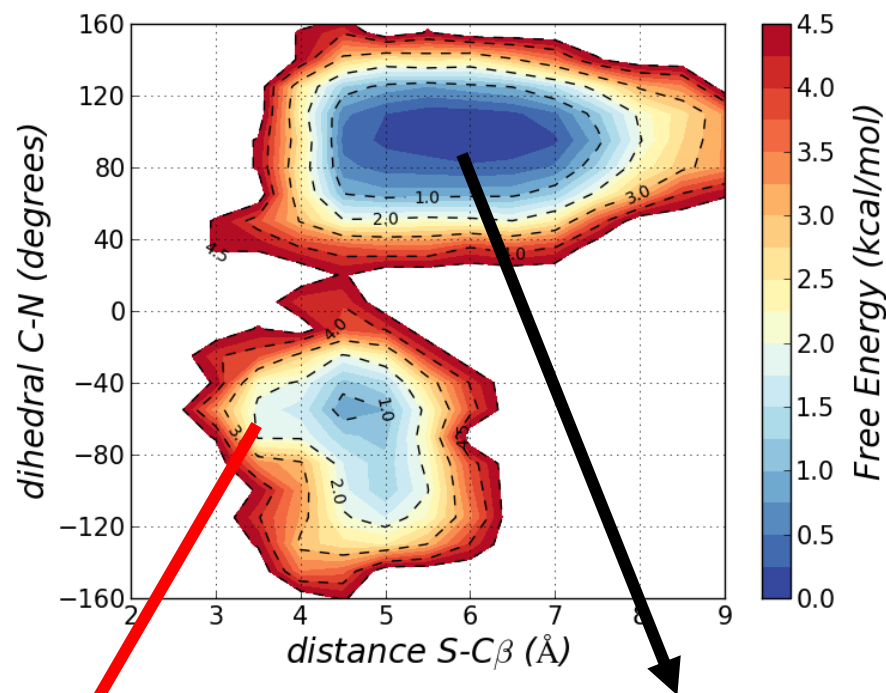
SCC-DFTB/AMBER99SB QM/MM with umbrella sampling

Drug resistance in EGFR: Reactive vs non-reactive conformations from molecular dynamics

T790M



T790M/L718Q

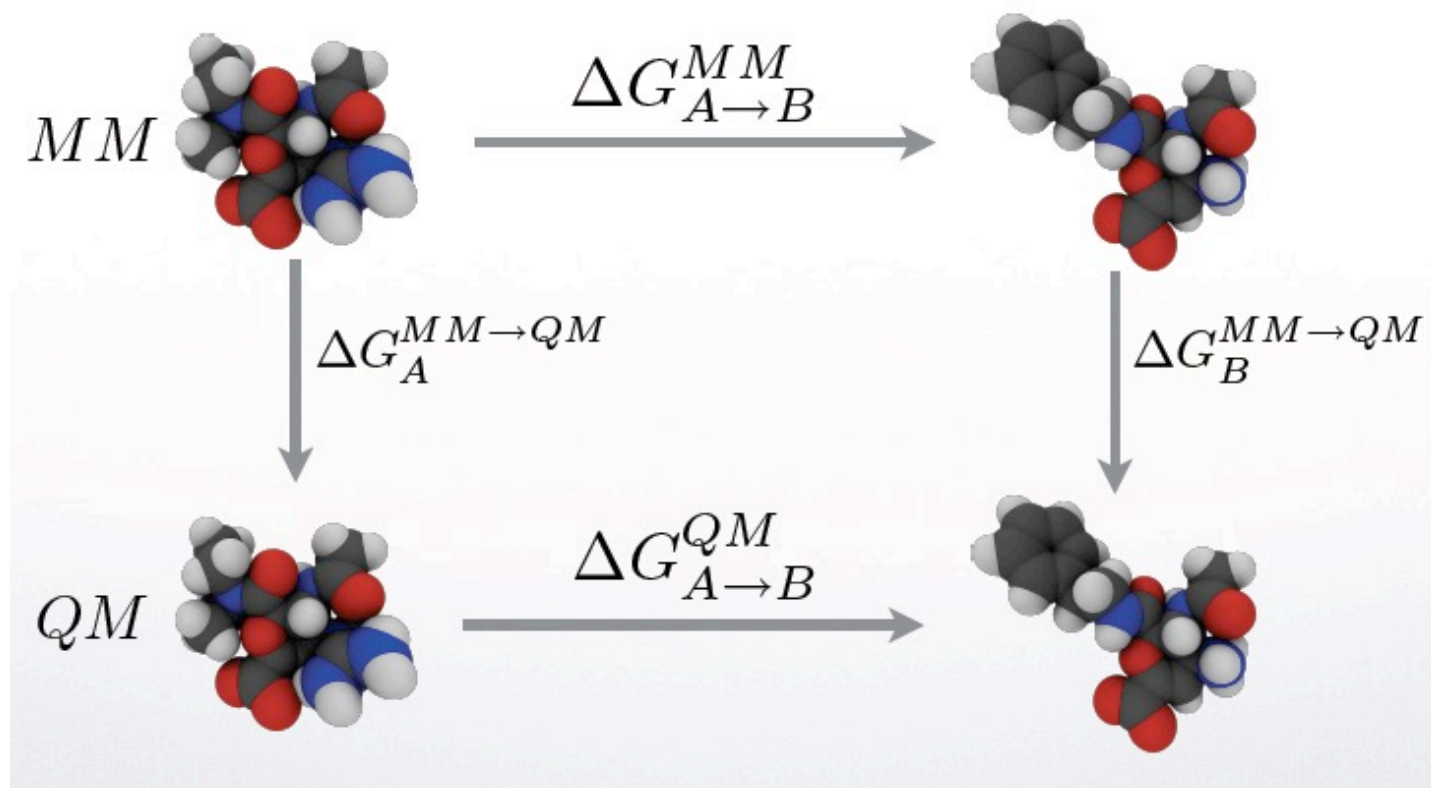


**Non-Reactive conformation is global minimum
Resistant to Osimertinib**

**Reactive conformation is no longer a
minimum for T790M/L718Q mutant**

**Reactive conformation is
the global minimum
Sensitive to Osimertinib**

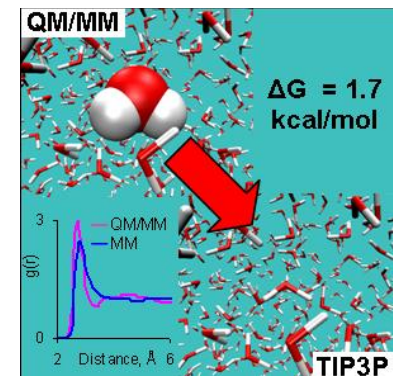
QM/MM methods for predicting protein-ligand binding affinities



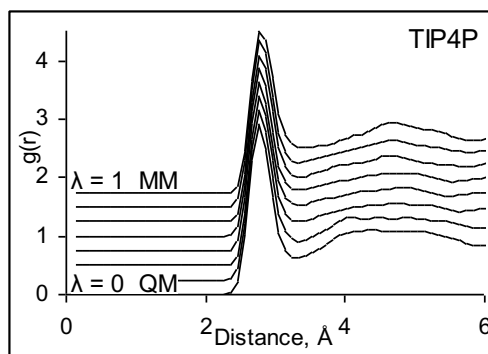
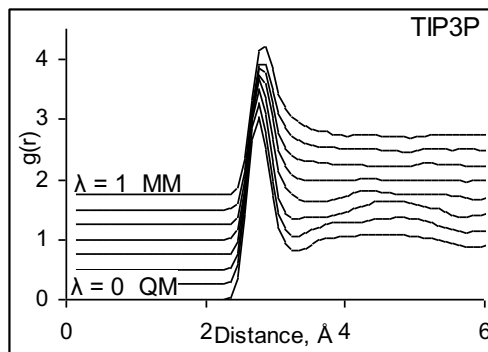
**Predict relative binding affinities through
QM/MM free energy simulations**

🔥 Free energy calculations to test QM/MM interactions

- Software: SIRE with Molpro
- Free energy Monte Carlo simulations (60 M steps) using *ab initio* QM (MP2/AVDZ) in 2 days
- Free energy changes from replica exchange thermodynamic integration (**RETI**) over a λ coordinate
- Calculate ΔG for QM to MM perturbation
- QuantoMM app: <https://siremol.org/pages/apps/quantomm.html>



QM/MM testing of water models



Perturbation	QM Method	ΔG
QM \rightarrow TIP3P	BLYP	0.5 (\pm 0.3)
	HF	3.3 (\pm 0.5)
	MP2	1.7 (\pm 0.4)
QM \rightarrow TIPS3P	BLYP	0.5 (\pm 0.3)
	HF	2.7 (\pm 0.4)
	MP2	1.5 (\pm 0.5)
QM \rightarrow TIP4P	BLYP	0.0 (\pm 0.3)
	HF	2.4 (\pm 0.2)
	MP2	1.2 (\pm 0.2)
QM \rightarrow TIP5P	BLYP	10.4 (\pm 1.9)
	HF	12.7 (\pm 1.8)
	MP2	11.8 (\pm 1.9)

• TIP5P not suitable for QM/MM; TIP4P better than TIP3P

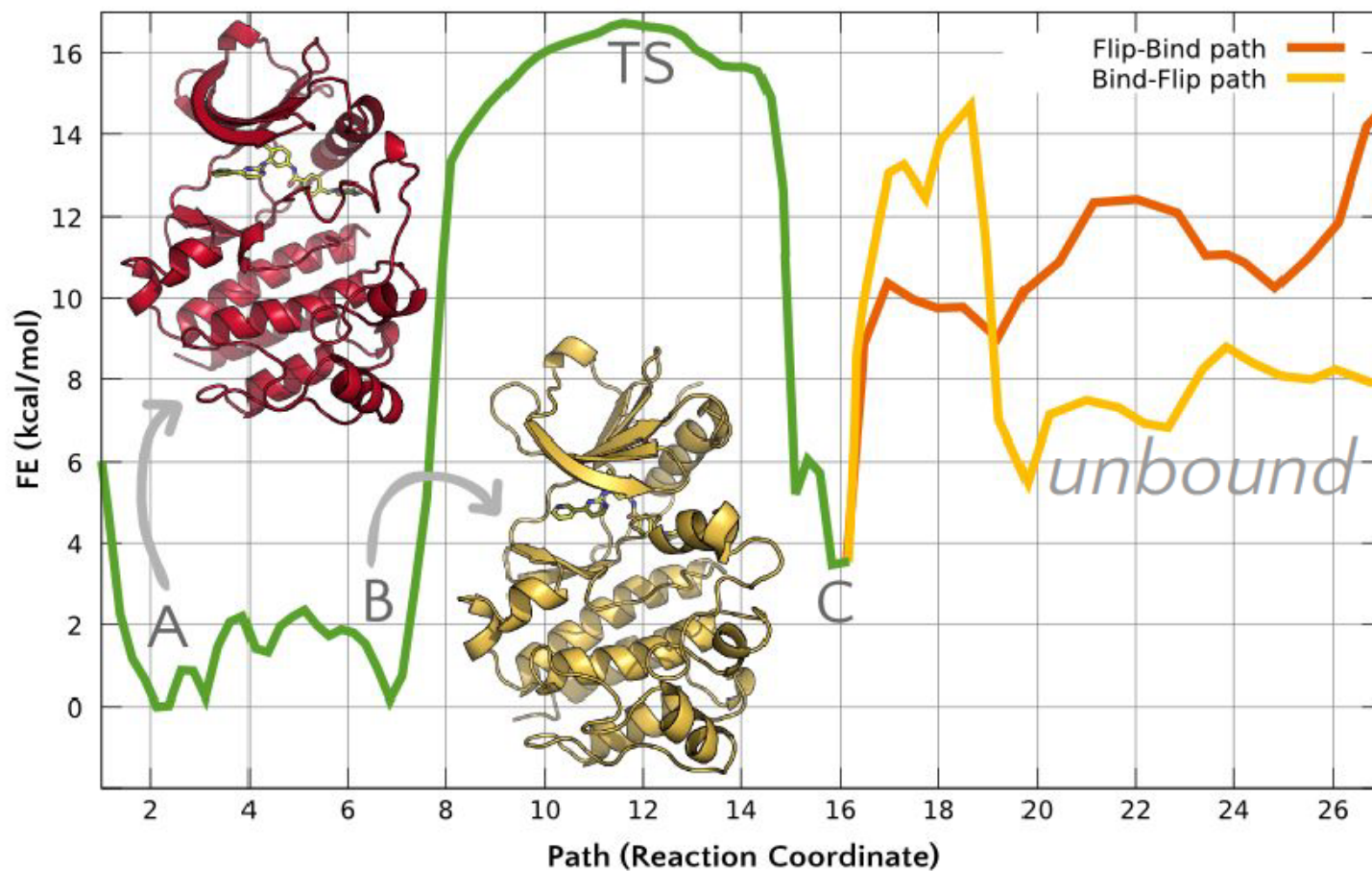
🔥 QM/MM calculations of binding affinity show importance of polarization

- QM to TIP4P perturbation for bound water molecules
- Flu Neuraminidase
- Compare to ΔG (BLYP \rightarrow TIP4P) in bulk water of 0.0 (± 0.3 kcal.mol⁻¹)

Shaw et al. *JPC Lett* (2014)

PDB structure	Water	$\Delta G_{\text{pert HF}}$	$\Delta G_{\text{pert BLYP}}$
	Wat A		
1f8c		2.0 ± 0.2	1.1 ± 0.2
1mwe		0.9 ± 0.2	0.5 ± 0.2
2qwj		0.7 ± 0.2	0.6 ± 0.2
2qwk		1.0 ± 0.2	0.7 ± 0.2
	Wat B		
1mwe		2.0 ± 0.2	0.7 ± 0.2
2qwj		1.8 ± 0.2	1.2 ± 0.2
2qwk		2.7 ± 0.2	1.5 ± 0.1
1lf7		3.7 ± 0.2	2.9 ± 0.2

Binding kinetics: Imatinib binding to c-Src

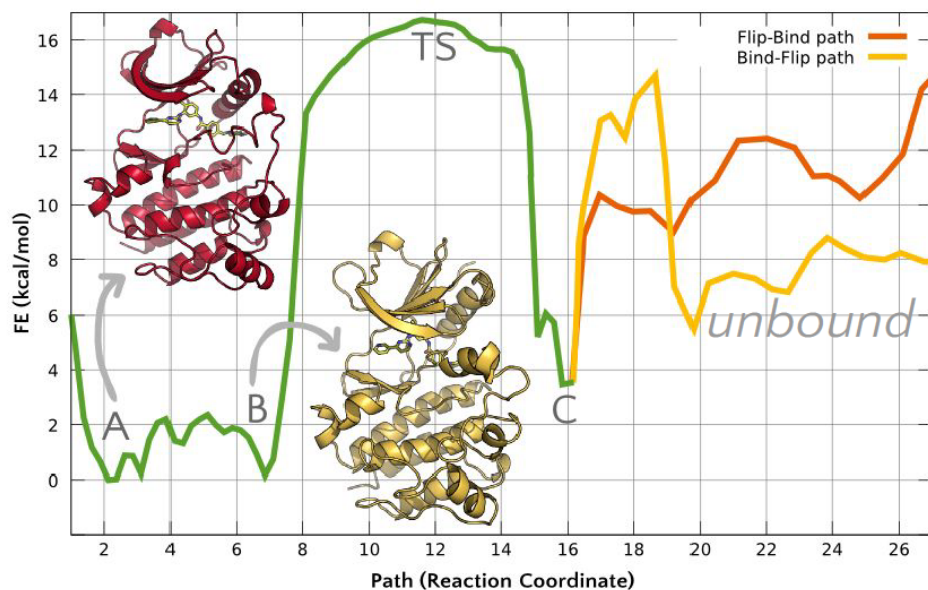


- Free energy profile for binding from well-tempered ensemble parallel tempering metadynamics using optimal path collective variable, atomistic MM MD

Imatinib/c-Src:

QM/MM for drug (un)binding

- Ligand is treated QM: BLYP/VDZ
- Replica Exchange Thermodynamic Integration (RETI) Metropolis-Hastings Monte Carlo using Sire (www.siremol.org); 8 λ values



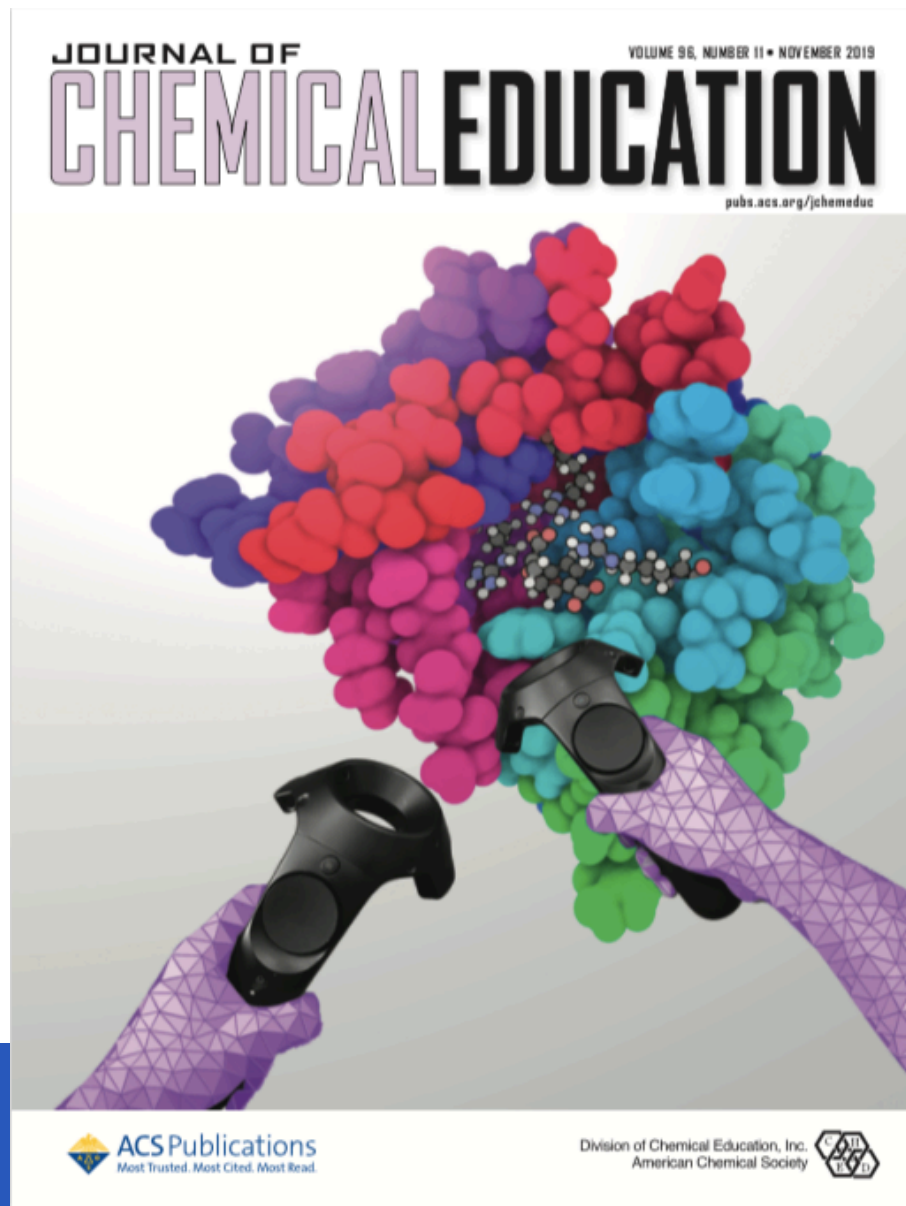
Minimum	QM/MM→MM correction (kcal/mol)
A	2.3
C	2.4
TS	3.9
Unbound	4.7

🔥 Interactive MD in VR for teaching

iMD-VR is effective and intuitive to teach and learn:

- Structure and dynamics
- Chemical reactivity
- Reaction discovery
- Catalysis
- Materials
- Drug docking

Immersive, multiuser environment for demonstration



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BRISTOL

Bennie et al.
J. Chem. Ed. 2019

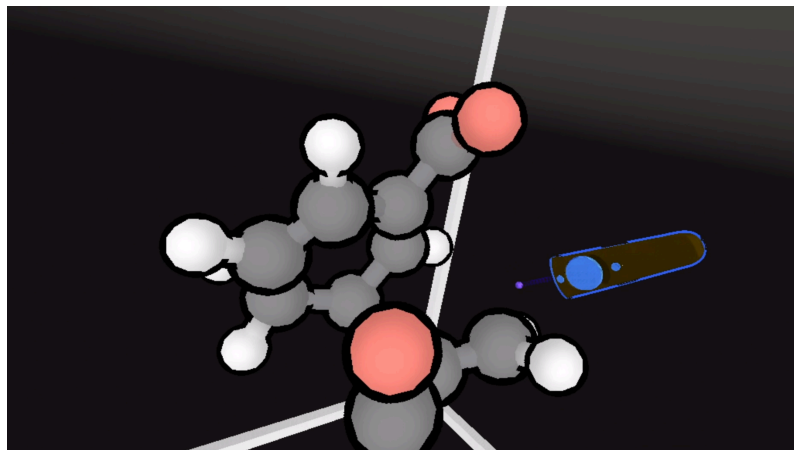
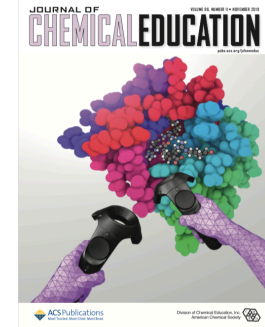
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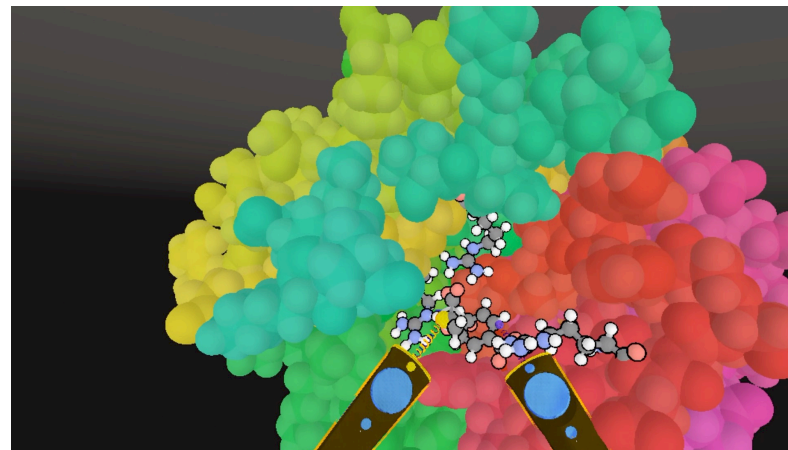




Teaching enzyme catalysis using interactive molecular dynamics in virtual reality



Claisen rearrangement via interactive DFT



Substrate docking in chorismate mutase via interactive MD

- Students found VR more engaging
- VR improved their perceived educational outcomes

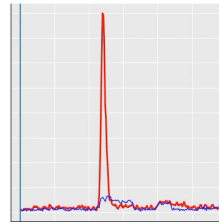
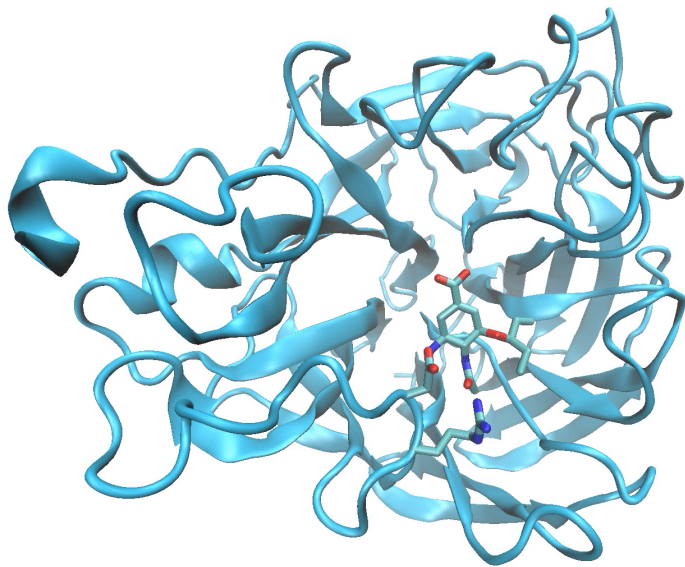


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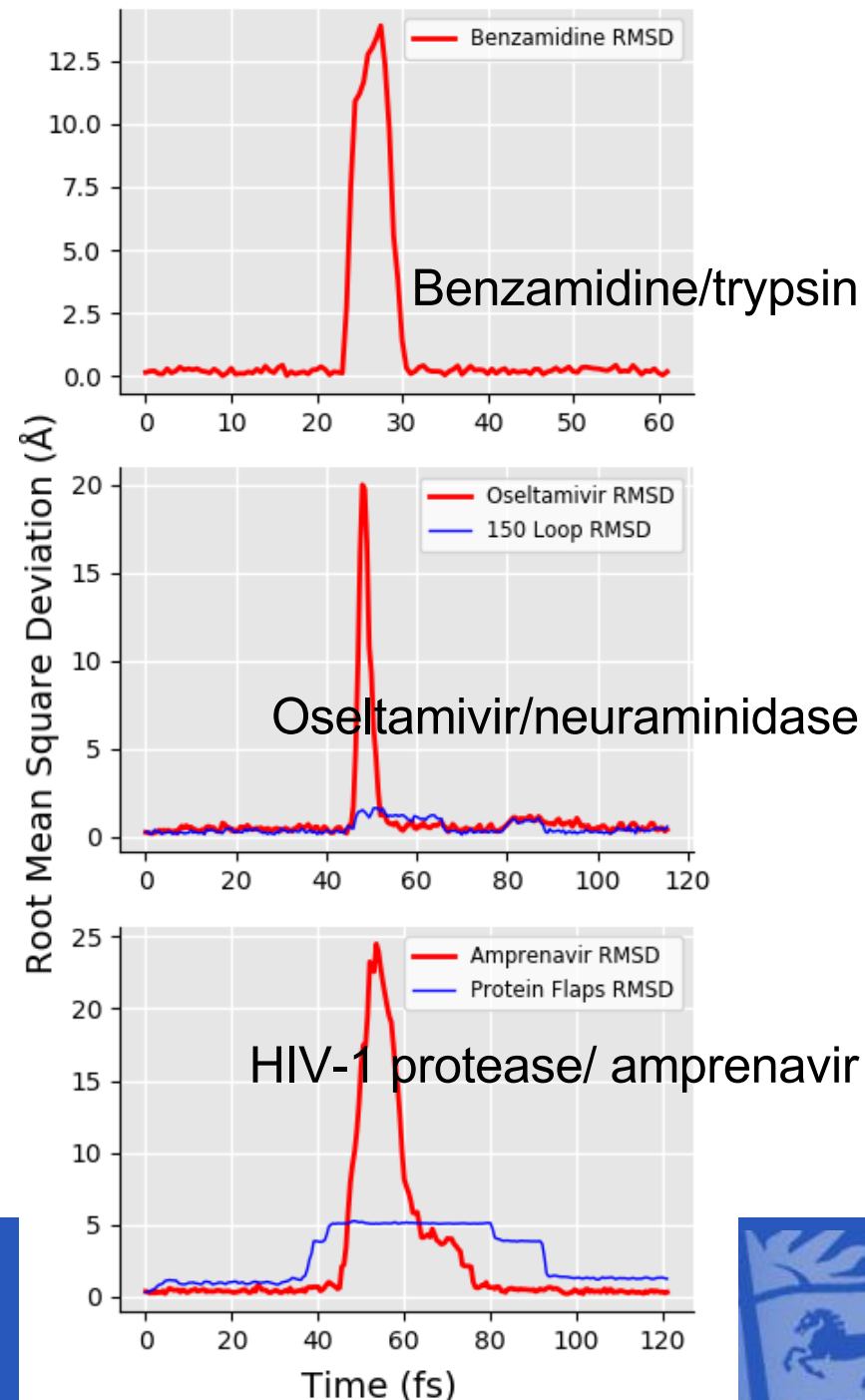
[Bennie et al. J. Chem. Ed. 2019](#)

<https://vimeo.com/320188113>

🔥 Interactive docking with iMD-VR correctly predicts enzyme-ligand complexes



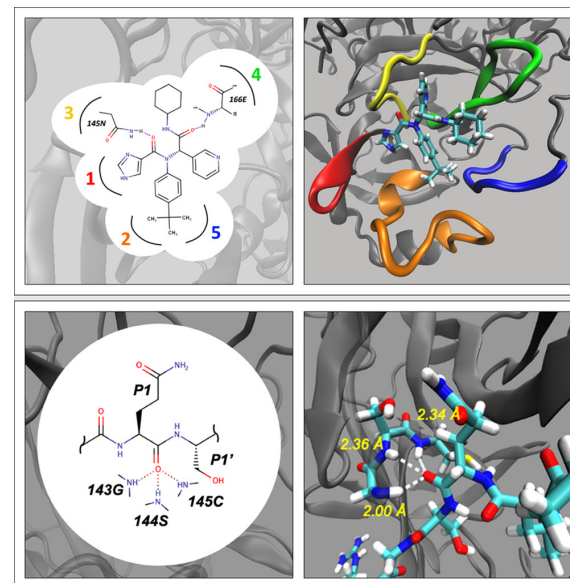
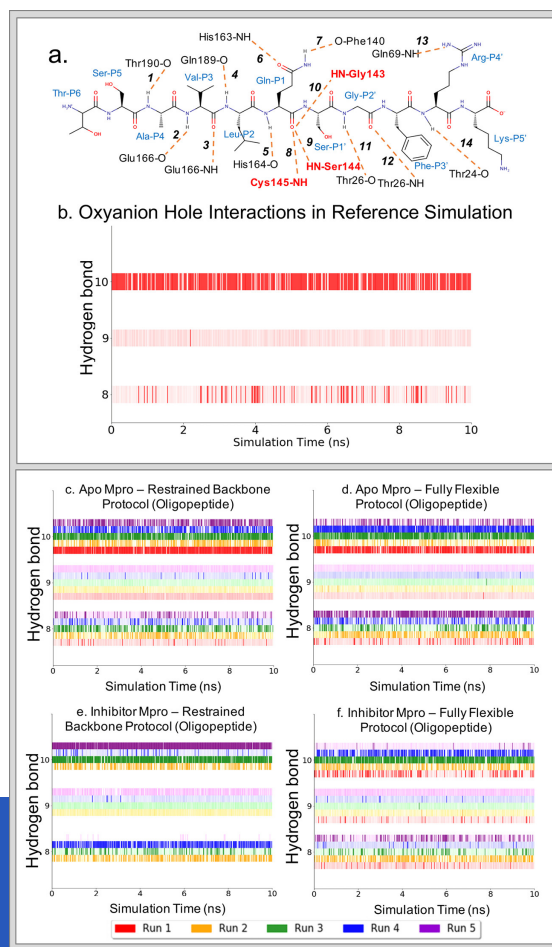
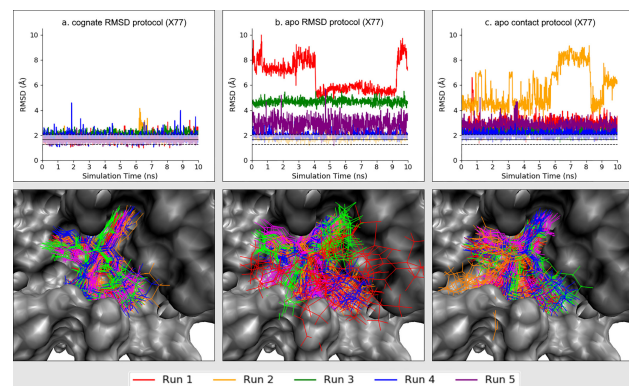
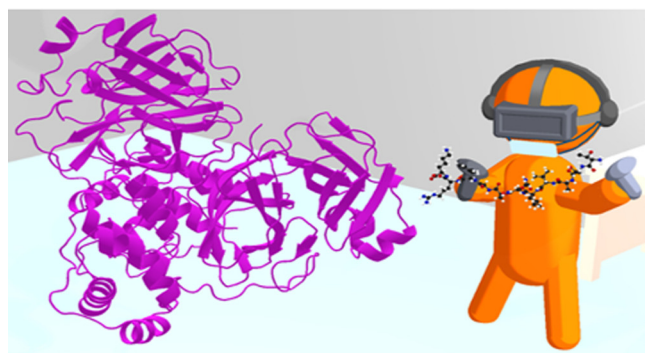
Oseltamivir/neuraminidase



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[Deeks et al.](#)
[PLoS One 2020](#)

🔥 Interactive Molecular Dynamics in Virtual Reality Is an Effective Tool for Flexible Substrate and Inhibitor Docking to the SARS-CoV-2 Main Protease





Interactive simulation in VR O'Connor et al. J. Chem. Phys. (2019)

Video Index	URL	Description	Force Engine
Video 1	vimeo.com/244670465	Multi-person demo showing: ⁸ (a) C ₆₀ being passed back and forth; (a) CH ₄ transit through a nanotube; (b) helicene changing from right to left-handed twist; (c) 17-ALA peptide being tied in a knot	(a) MM3 ⁴⁰ (b) MM3 ⁴⁰ (c) MM3 ⁴⁰ (d) OpenMM ⁴¹
Video 2	vimeo.com/305459472	Illustrating the iMD-VR selection interface with Cyclophilin A	OpenMM ⁴¹
Video 3	vimeo.com/315239519	Narupa secondary structure visualization demo of neuraminidase (PDB 3TI6)	OpenMM ⁴¹
Video 4	vimeo.com/315218999	Reactive & non-reactive OH + CH ₄ scattering	DFTB+ ⁴²
Video 5	vimeo.com/311438872	Exploring reactive PESs for CN + isobutane for NN fitting using interactive ab initio quantum chemistry ¹³	SCINE ⁴³
Video 6	vimeo.com/312963823	On-the-fly reaction discovery for OH + propyne using interactive ab initio quantum chemistry	SCINE ⁴³
Video 7	vimeo.com/306778545	Reversible Loop Dynamics in Cyclophilin A	OpenMM ⁴¹
Video 8	vimeo.com/274862765	Using the Narupa-OpenMM plugin to dock benzamidine with trypsin	OpenMM ⁴¹
Video 9	vimeo.com/296300796	Using the Narupa-OpenMM plugin to dock oseltamivir with neuraminidase	OpenMM ⁴¹
Video 10	vimeo.com/312957045	Guiding 2-methyl-hexane through a ZSM-5 zeolite using the Narupa-PLUMED interface	PLUMED/DL_POLY ^{44, 45}
Video 11	vimeo.com/312994336	Real-time sonification of a biomolecule's potential energy illustrated by tying a knot in 17-ALA peptide	OpenMM ⁴¹
Video 12	vimeo.com/305823646	Use of our custom Extentile VR gloves to tie a knot in 17-ALA peptide ⁴⁶	OpenMM ⁴¹



CCP-BioSim: the UK Collaborative Computational Project on Biomolecular Simulation



- BioSimSpace: interoperability of different simulation packages (biosimspace.org – tutorials etc)
- [4th Conference on Multiscale Modelling](#) Manchester
Mar 29th-31st 2021 (with CCP5)

www.ccpbiosim.ac.uk

CCPBioSim



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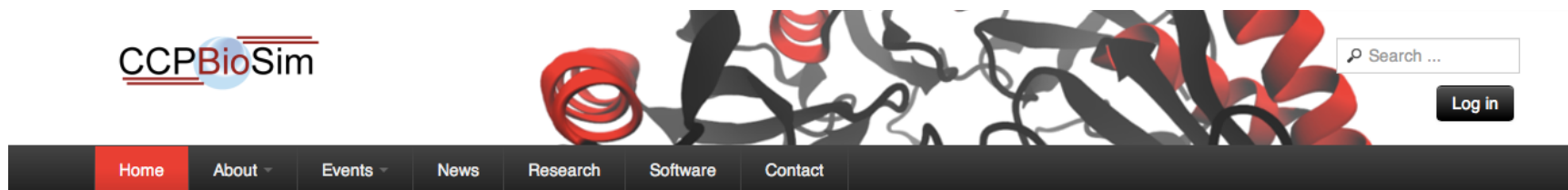
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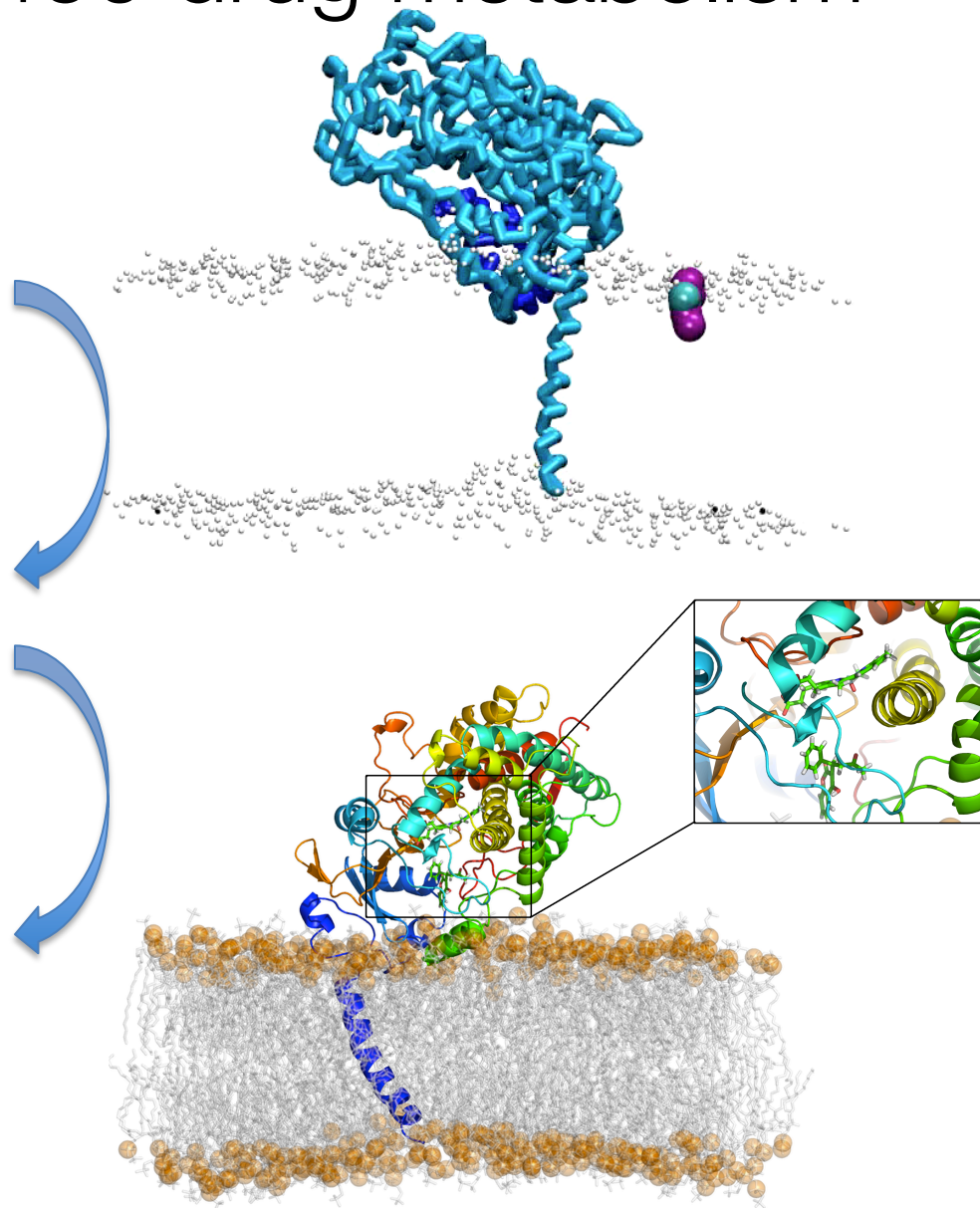
- Training workshops; www.ccpbiosim.ac.uk
- Software development: making new methods accessible



Multiscale modelling of CYP450 drug metabolism

- **Coarse-grained MD**
 - Build model of protein in the membrane
 - Study orientation of protein in membrane
- **Atomistic MD simulations**
 - Relaxation
 - Explore conformations of protein and binding modes of drugs
- **QM/MM**
 - Model reactions of drugs in P450s (e.g. Lonsdale *et al.* *JACS* 2013; PLoS *Comp. Biol.* 2014)

Amaro and Mulholland, *Nature Reviews Chemistry* 2018



Acknowledgements

bp



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