The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
Cultural Organization

IAEA
International Atomic Energy Agency

2054-8

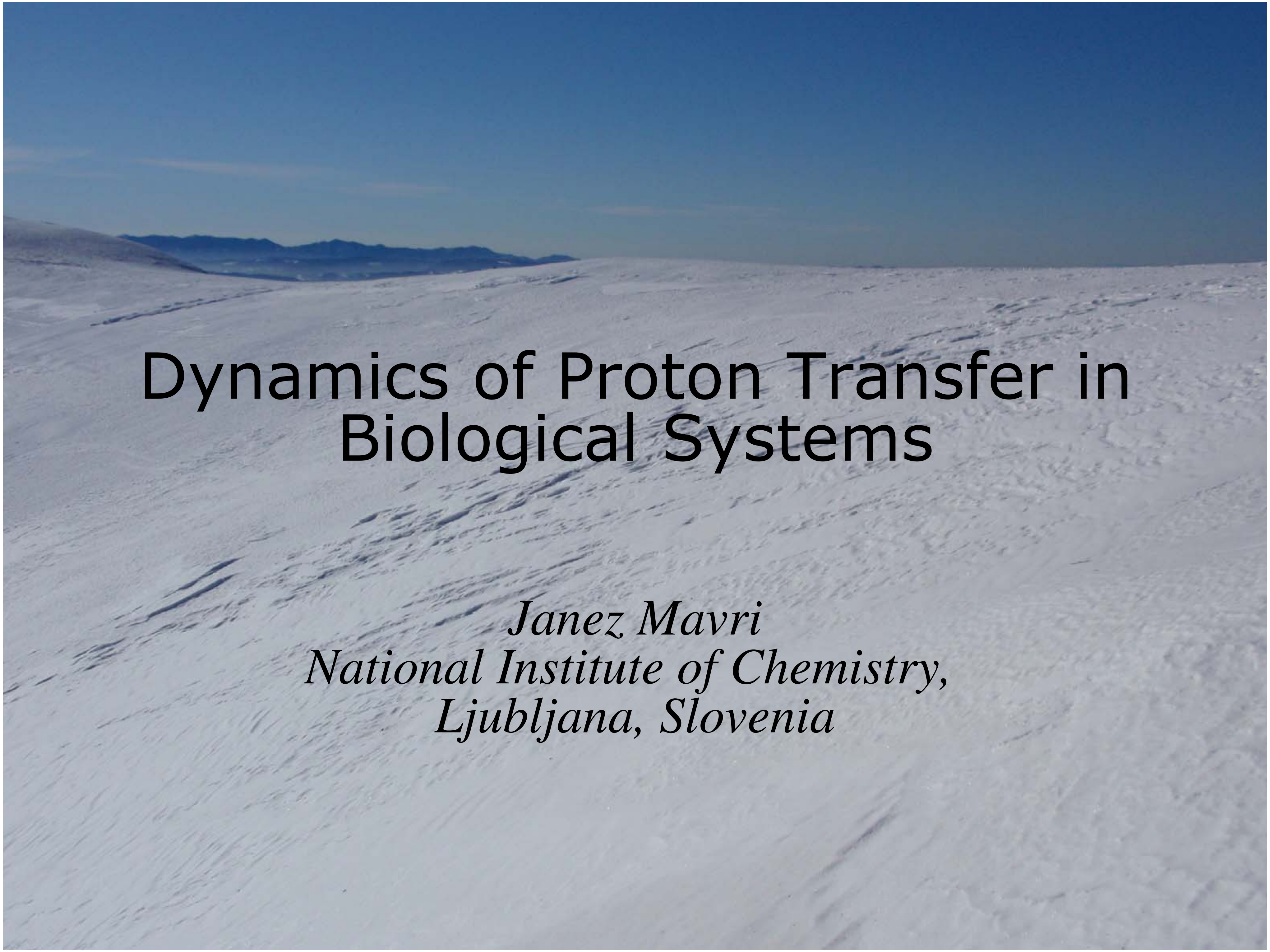
Structure and Dynamics of Hydrogen-Bonded Systems

26 - 27 October 2009

Dynamics of Proton Transfer in Biological Systems

Janez MAVRI

National Institute of Chemistry
Hajdrihova 19, POB 680
Ljubljana, SI-1001
SLOVENIA



Dynamics of Proton Transfer in Biological Systems

Janez Mavri
National Institute of Chemistry,
Ljubljana, Slovenia

Outline

Progress in pKa values calculation

Simulation of vibrational spectra of hydrogen bonded systems

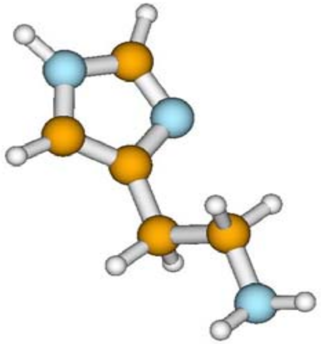
Catalytic step of lipoxygenase

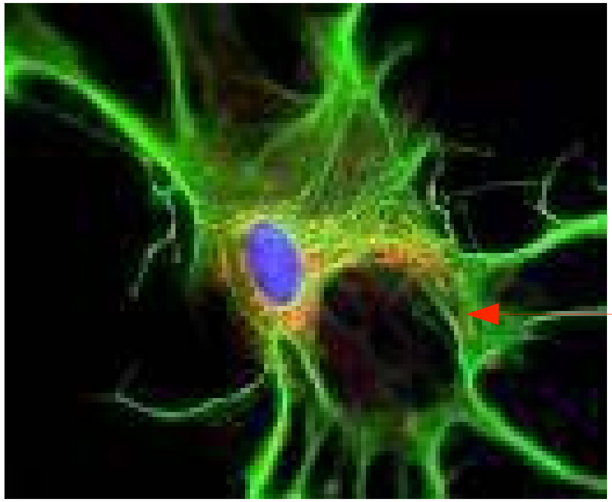
Why deuterated receptors do not work anymore

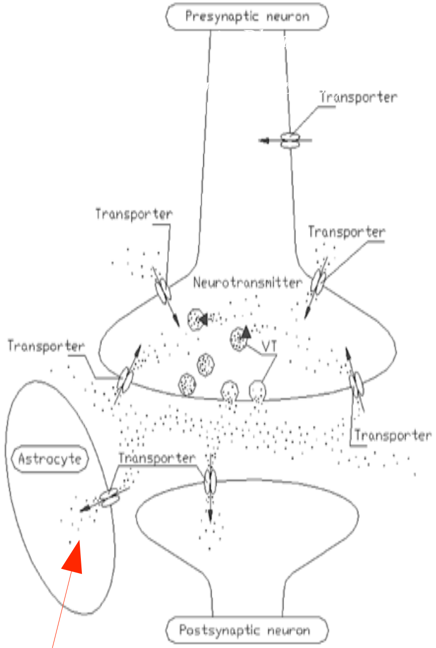
Conclusions

Histamine in Central Nervous System

Histamine is an inflamation mediator and neurotransmitter in the central nervous system.






Presynaptic neuron
Transporter
Transporter
Transporter
Neurotransmitter
Transporter
VT
Transporter
Astrocyte
Transporter
Postsynaptic neuron

astrocyte

Recent studies of histamine transport in rat brain tissue give evidence that there are two transport mechanisms involved :

- (electro)diffusion

-ATP driven sodium-dependent active transport

In neonatal rat astrocytes about ½ of the transport proceeds via diffusion and ½ is ATP driven.

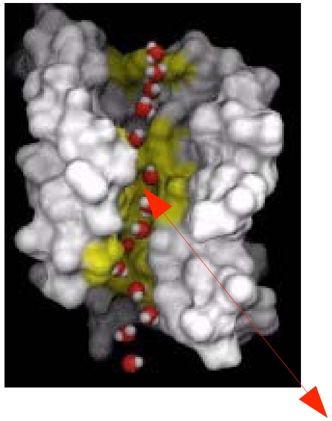
For adult astrocytes this ratio is different (1/3 passive, 2/3 active).

Structures of the transport protein(s) are not known, but a lot of effort is going in this direction.

K. Perdan-Pirkmajer, JM and M. Kržan,
resub. J. Mol. Model., 10/2009

Structures of the transport proteins are not yet known but as soon it will be known it will become a challenge to model histamine transport by microscopic simulation

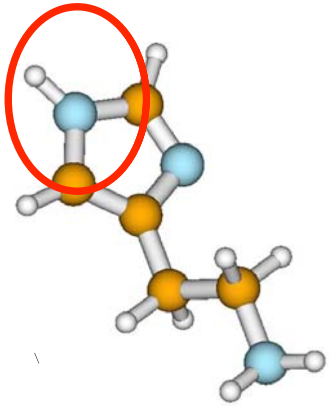
It should be clear that during the transport the protonation states are changed since the interior of the transport protein has different dielectric properties than extracellular solution and cytoplasm.



Dielectric properties and protonation states will be different at this two sites

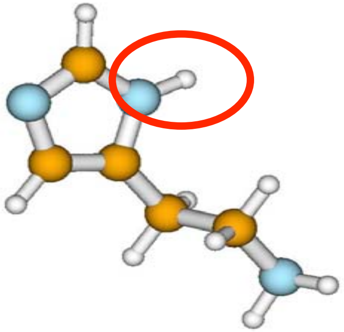
As a first step we calculate stabilities of protonation states in aqueous solution that are known experimentally.

Protonation States of Histamine



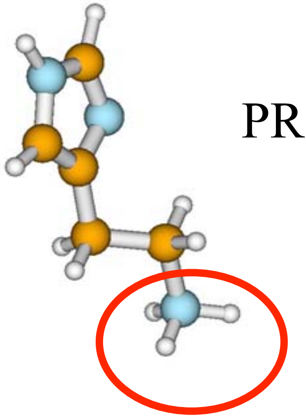
charge=0

Nα



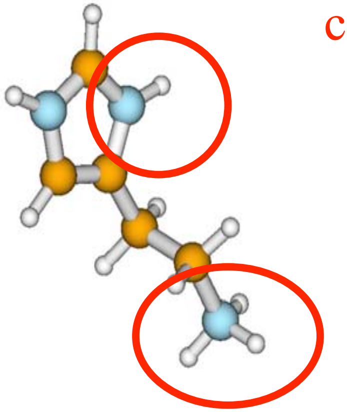
charge=0

Nπ



charge=1

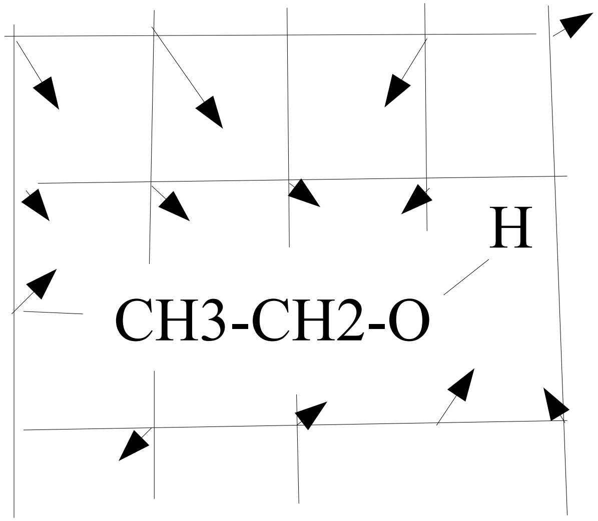
PROT1



charge=2

PROT2

Langevine Dipoles of Florian and Warshel



Arieh Warshel, University of Southern California

Jan Florian, Loyola University, Chicago

Method	Nα	PROT1	PROT2	H_3O^+	H_2O
HF/6-31G(d,p)	-10.58	-69.91	-193.86	-97.27	-8.44
HF/6-31++G(2d, 2p)	-9.97	-69.53	-194.13	-97.26	-6.94
B3LYP/6-31+G(d,p)	-9.79	-68.55	-192.88	-96.21	-8.04
B3LYP/6-31++G(2d,2p)	-9.11	-68.13	-193.03	-96.80	-6.39
MP2/6-31+G(d,p)	-10.55	-69.17	-193.18	-96.76	-8.37
MP2/6-31++G(2d,2p)	-9.84	-68.59	-193.27	-96.88	-6.58
Experimental value				-105	-6.4

Table 4: Free energies of hydration calculated by Langevin dipoles solvation model.

All values are in kcal/mol. Nα is the α tautomer neutral form of histamine. PROT1 is monoprotonated at amino nitrogen atom form and PROT 2 is a double protonated form.

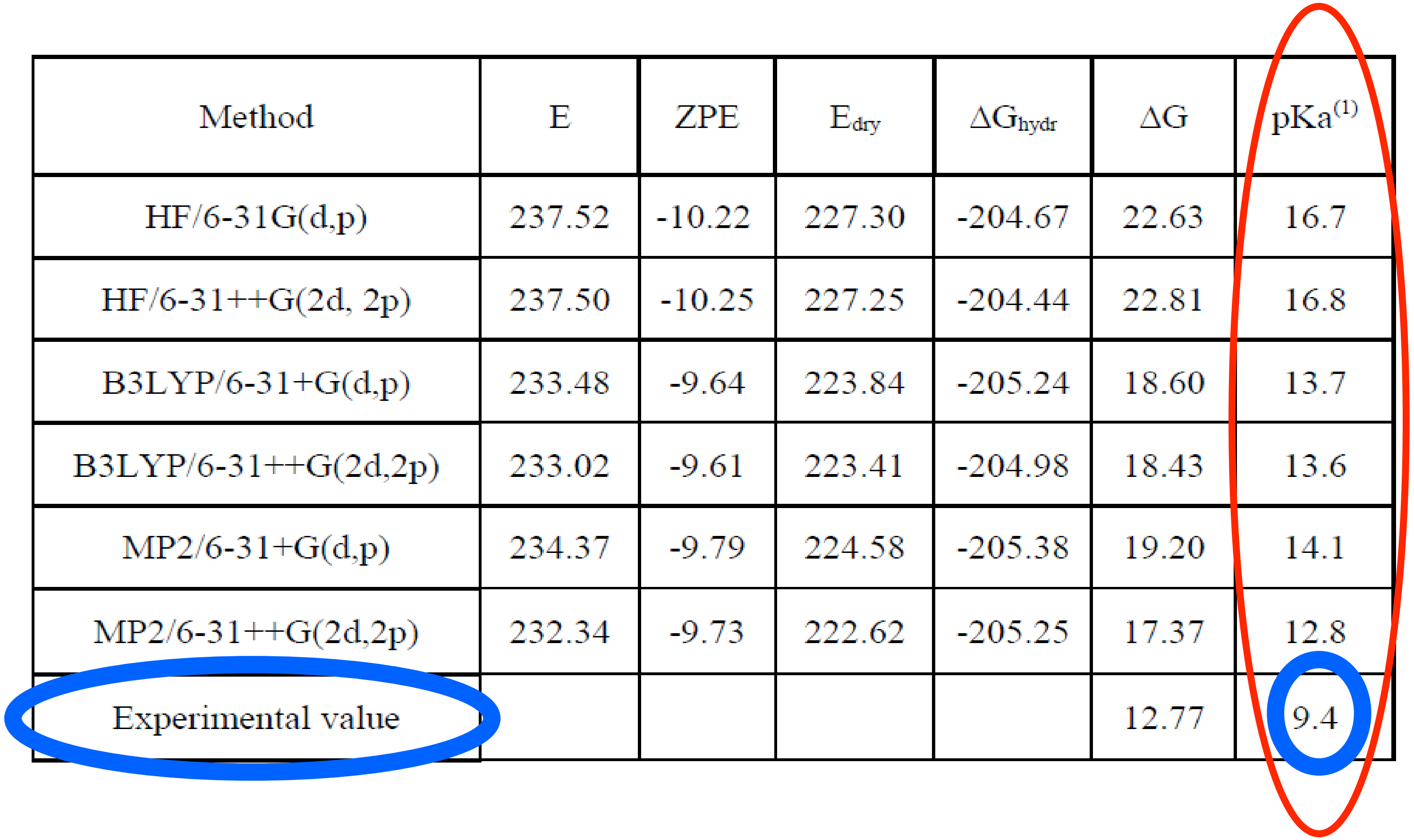
Experimental Free energies of Proton Hydration

$H^+(g) \longleftrightarrow H_3O^+(aq)$

Y. Marcus, Ion Solvation, Wiley, New York, 1985
-252.4 kcal/mol

C. Lim, D. Bashford, M. Karplus, *J. Phys.Chem.* 95 (1991) 5610
-259.2 kcal/mol
(This is a weighted average of values obtained from measurments of standard hydrogen potentials in the range [-253.5, -260.8] kcal/mol.)

M.D. Tissander, K. A. Cowen, W.Y. Feng, *J. Phys. Chem.* 102 (1998) 7787
-264 kcal/mol
(Most recent cluster-ion solvation data. This value we adopted in our work.)



Method	E	ZPE	E_{dry}	ΔG_{hydr}	ΔG	pKa(1)
HF/6-31G(d,p)	237.52	-10.22	227.30	-204.67	22.63	16.7
HF/6-31++G(2d, 2p)	237.50	-10.25	227.25	-204.44	22.81	16.8
B3LYP/6-31+G(d,p)	233.48	-9.64	223.84	-205.24	18.60	13.7
B3LYP/6-31++G(2d,2p)	233.02	-9.61	223.41	-204.98	18.43	13.6
MP2/6-31+G(d,p)	234.37	-9.79	224.58	-205.38	19.20	14.1
MP2/6-31++G(2d,2p)	232.34	-9.73	222.62	-205.25	17.37	12.8
Experimental value					12.77	9.4

The first pKa value for histamine

Method	E	ZPE	E_{dry}	ΔG_{hydr}	ΔG	pKa$^{(2)}$
HF/6-31G(d,p)	165.68	-9.07	156.61	-140.05	16.56	12.2
HF/6-31++G(2d, 2p)	165.57	-9.06	156.51	-139.40	17.11	12.6
B3LYP/6-31+G(d,p)	160.62	-8.61	152.01	-139.67	12.34	9.1
B3LYP/6-31++G(2d,2p)	160.76	-8.62	152.14	-139.11	13.03	9.6
MP2/6-31+G(d,p)	158.58	-8.65	149.93	-139.99	9.94	7.3
MP2/6-31++G(2d,2p)	156.97	-8.59	148.38	-139.32	9.06	6.7
Experimental value					7.88	5.8

The second pKa value

Langevin dipoles solvation model in conjunction with correlated wave function and flexible basis set is able to reproduce pKa values of histamine in aqueous solution if experimental value of proton hydration is considered

Hydration free energy of hydronium ion is not reproduced and experimental value must be considered. There is still space for reparametrization of the solvation model.

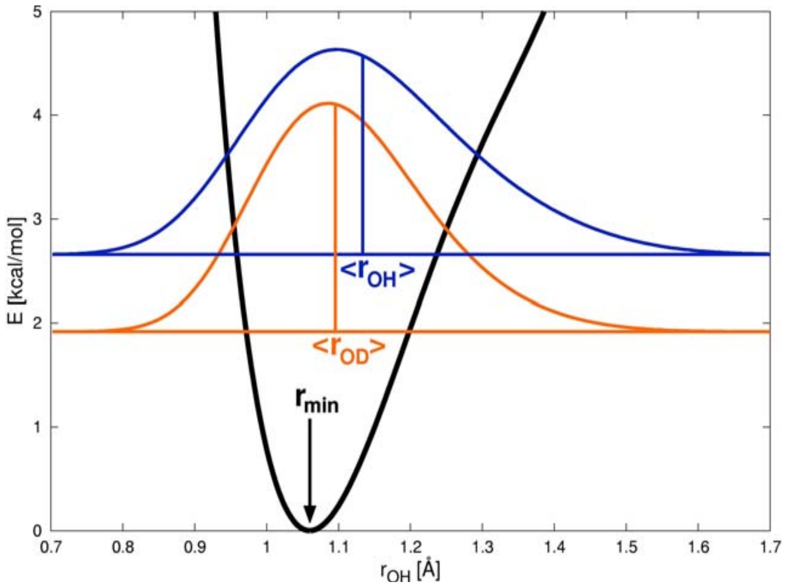
The experimental values of hydronium ion is associated with large uncertainty and the experimental uncertainty is about 10 kcal/mol.

QM/MM simulation of histamine transport with variable protonation states remains a challenge for future.

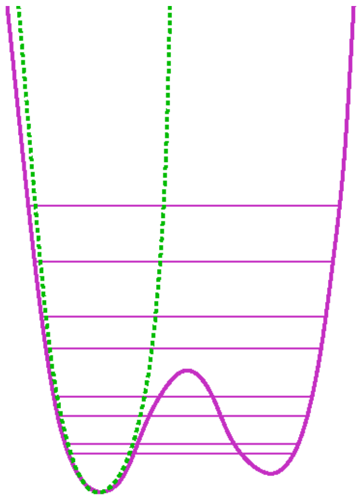
A closely related approach with solvent reaction field was used to calculate the pKa values of amino acids.
N. Peran and Z.Maksic, to be published.

Quantization of the Nuclear Motion

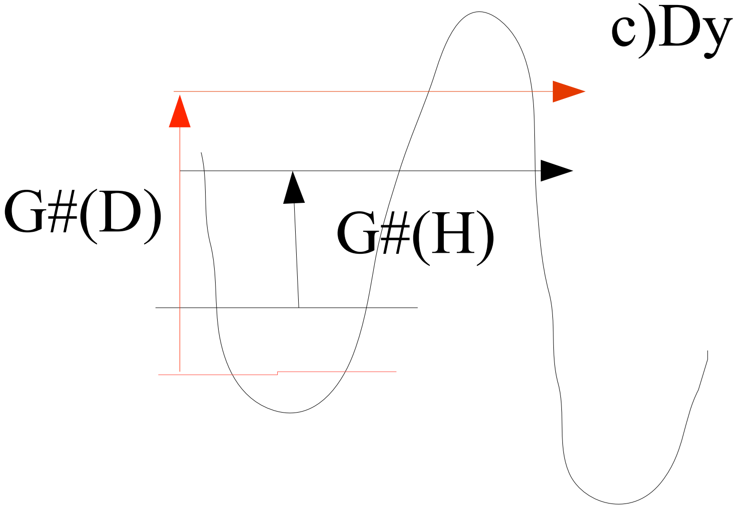
a) Expectation Values



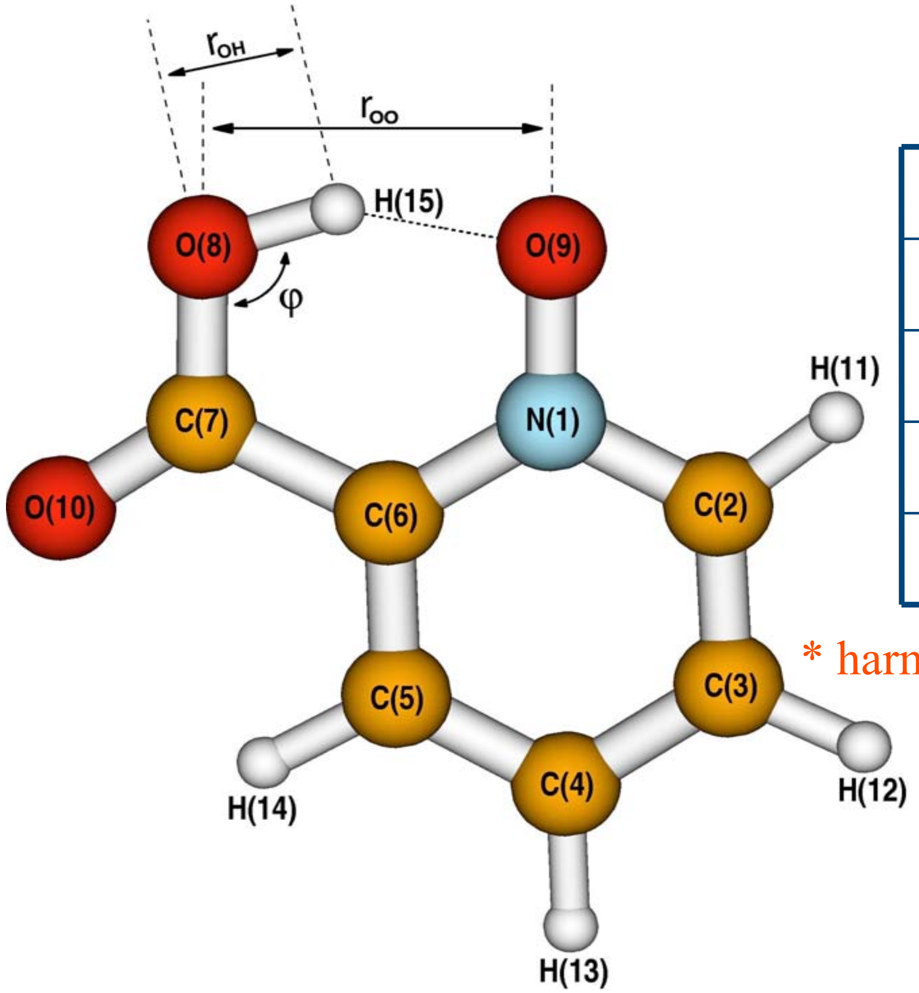
b)Vibrational Eigenvalues



c)Dynamics of Barrier Crossing



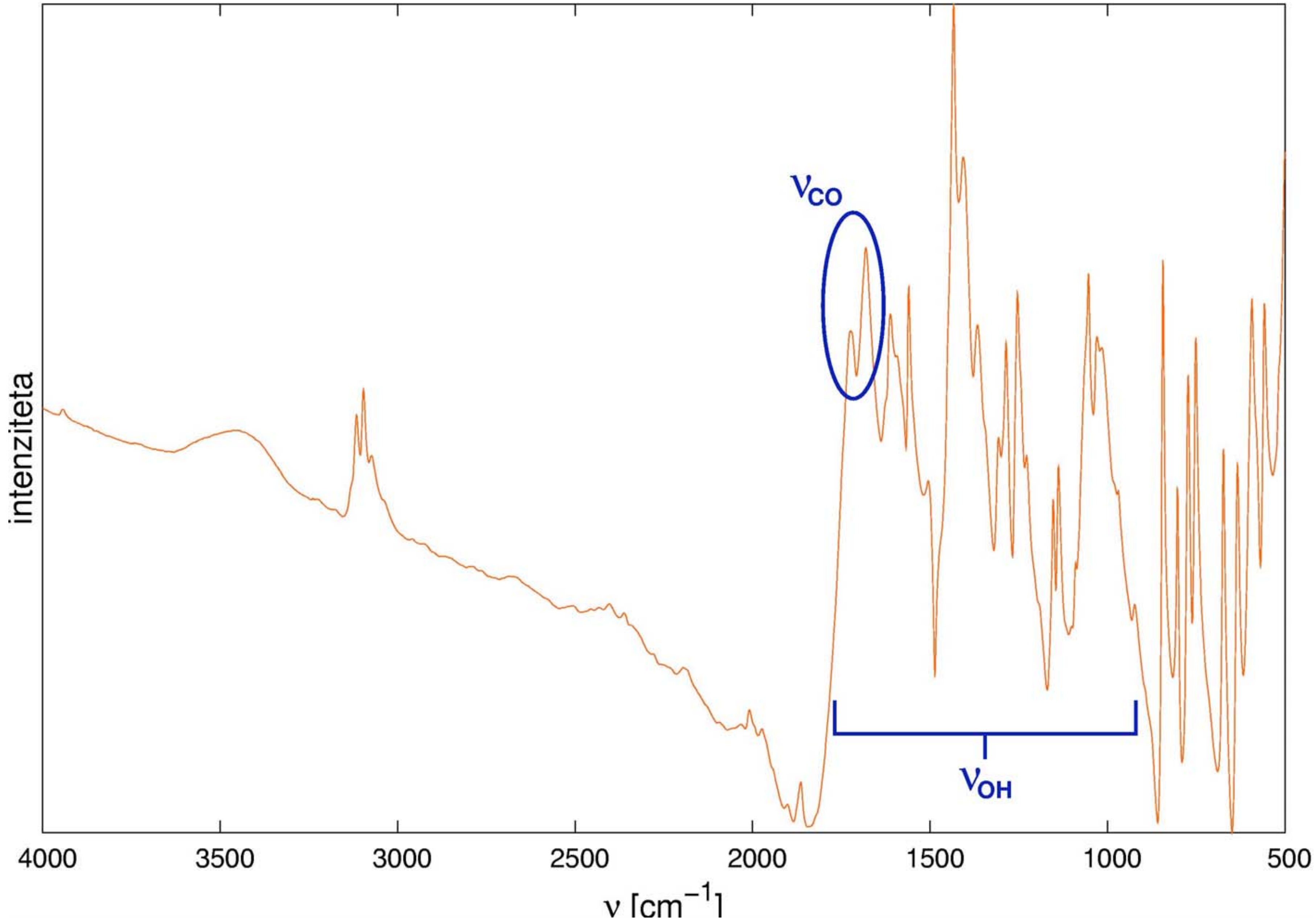
Picolinic Acid N-oxide (PANO)



	experiment	calculated*
r_{OO} [Å]	2.426	2.509
r_{OH} [Å]	1.091	1.011
ν_{OH} [cm^{-1}]	500-1800	2965
δ [ppm]	~18	15.3

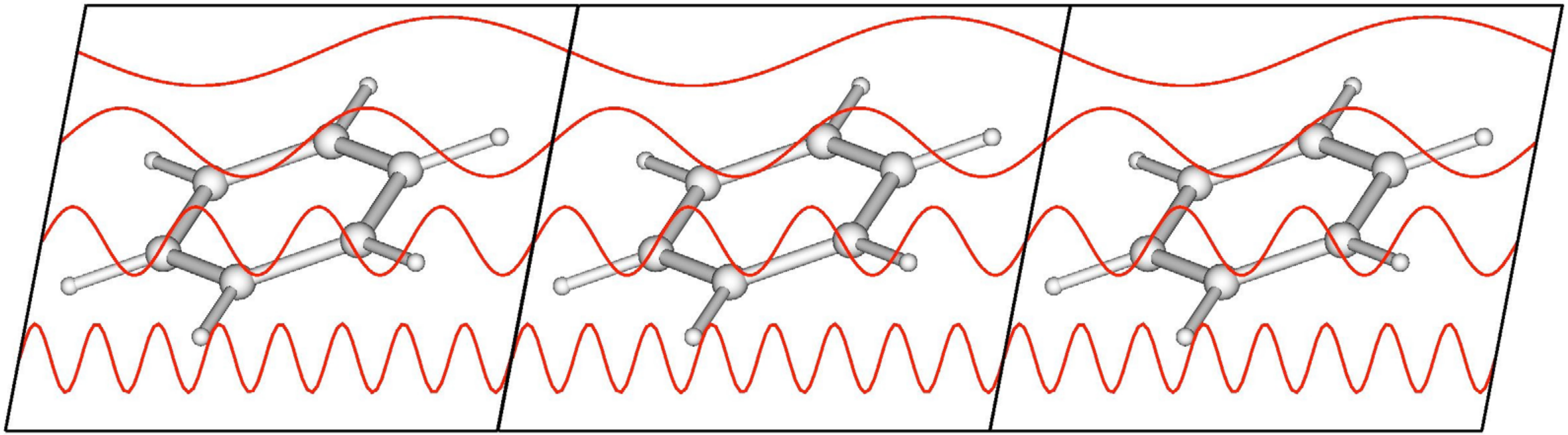
* harmonic approximation, in vacuo B3LYP/6-31+G(d,p)

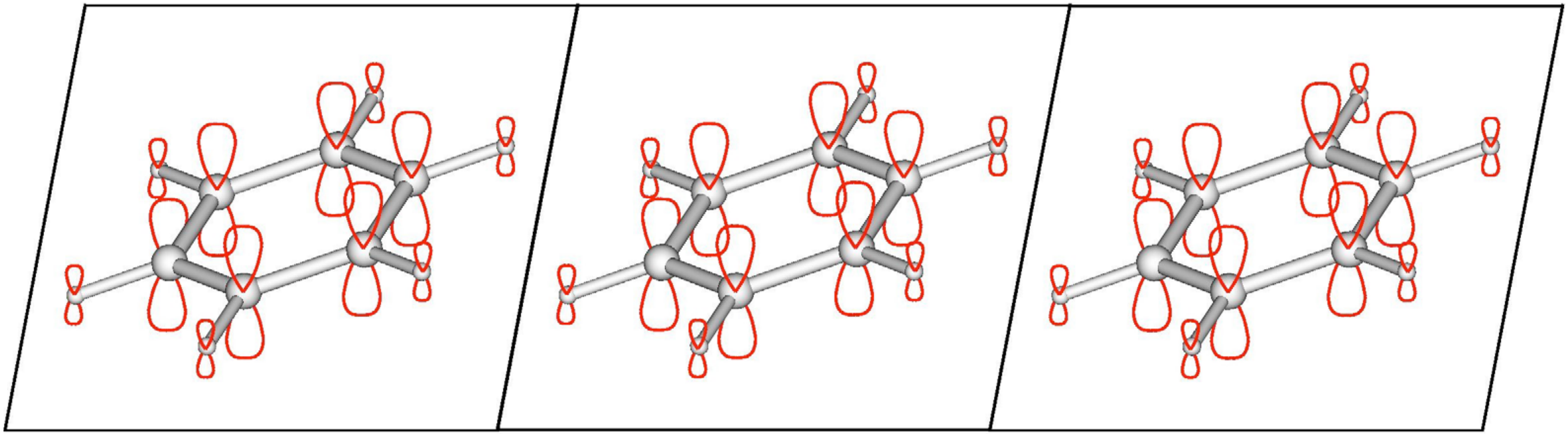
Experimental IR spectrum of PANO crystal



- We would like to simulate the OH stretching band of
- PANO by considering
-
- quantum chemical description
- first principle description of the environment
- thermal fluctuations

Plane–wave basis sets: intrinsically periodic





Periodized local atomic basis sets

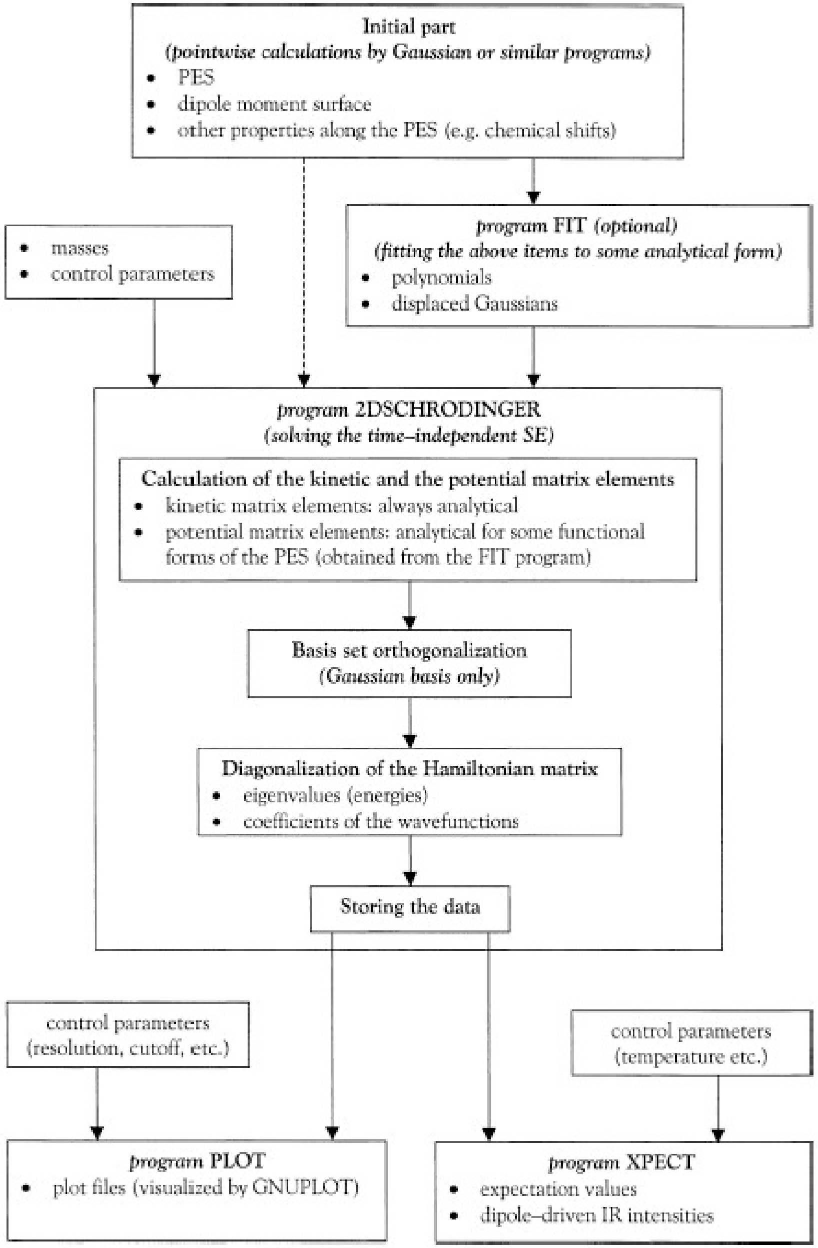
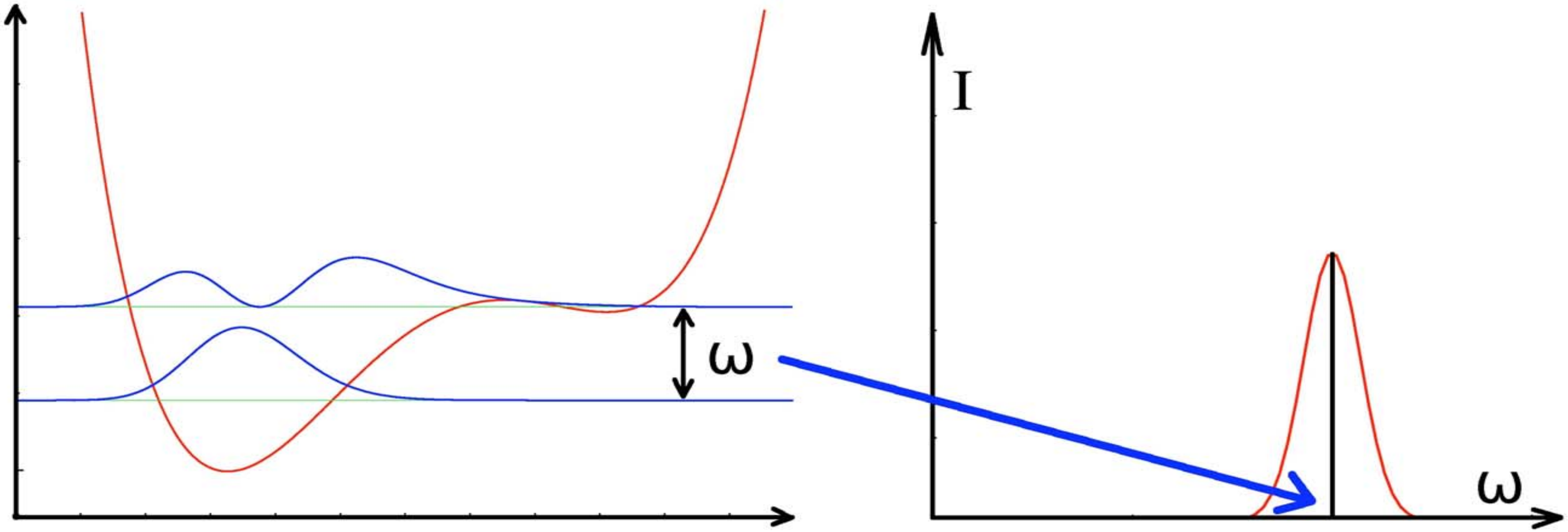
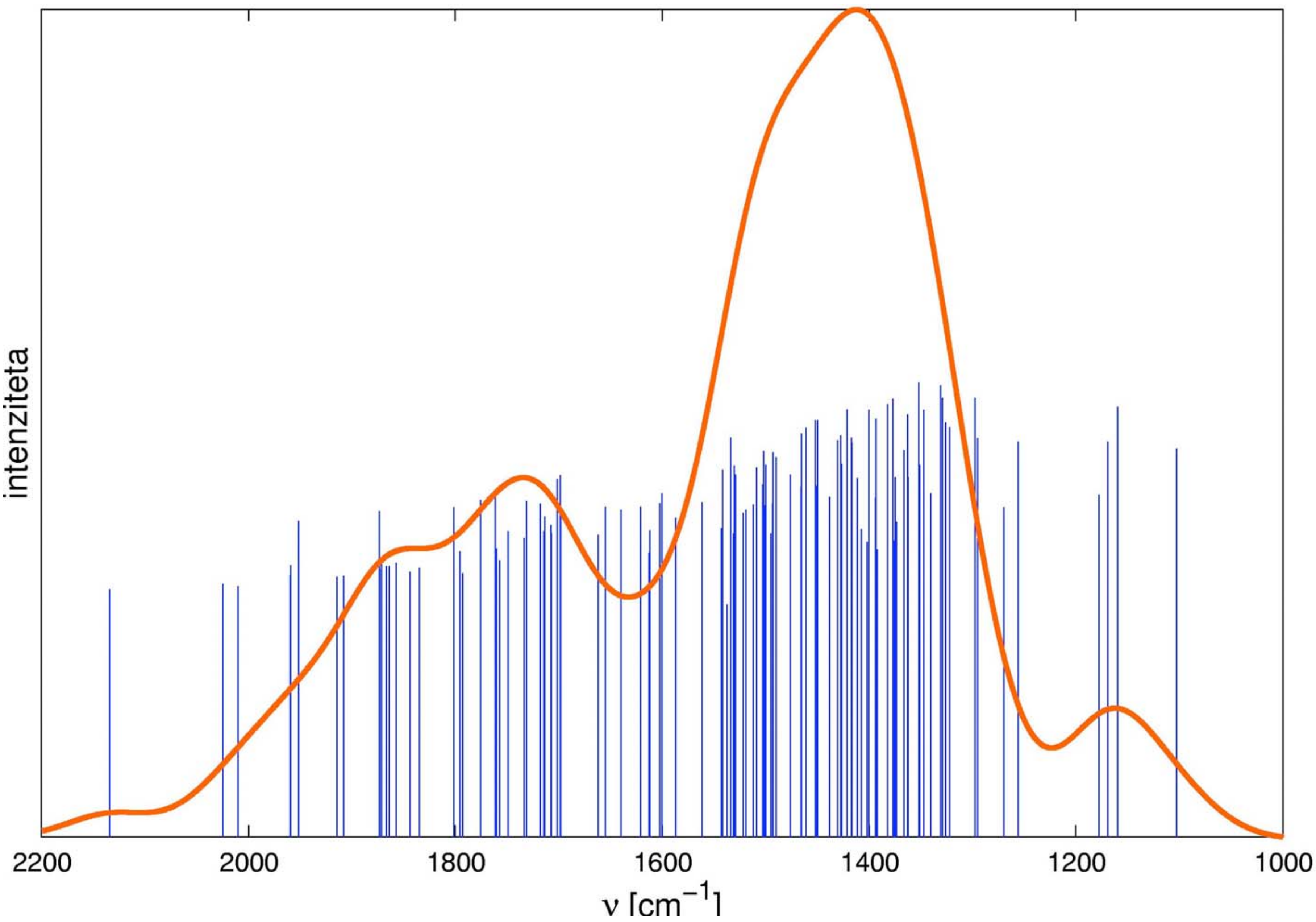
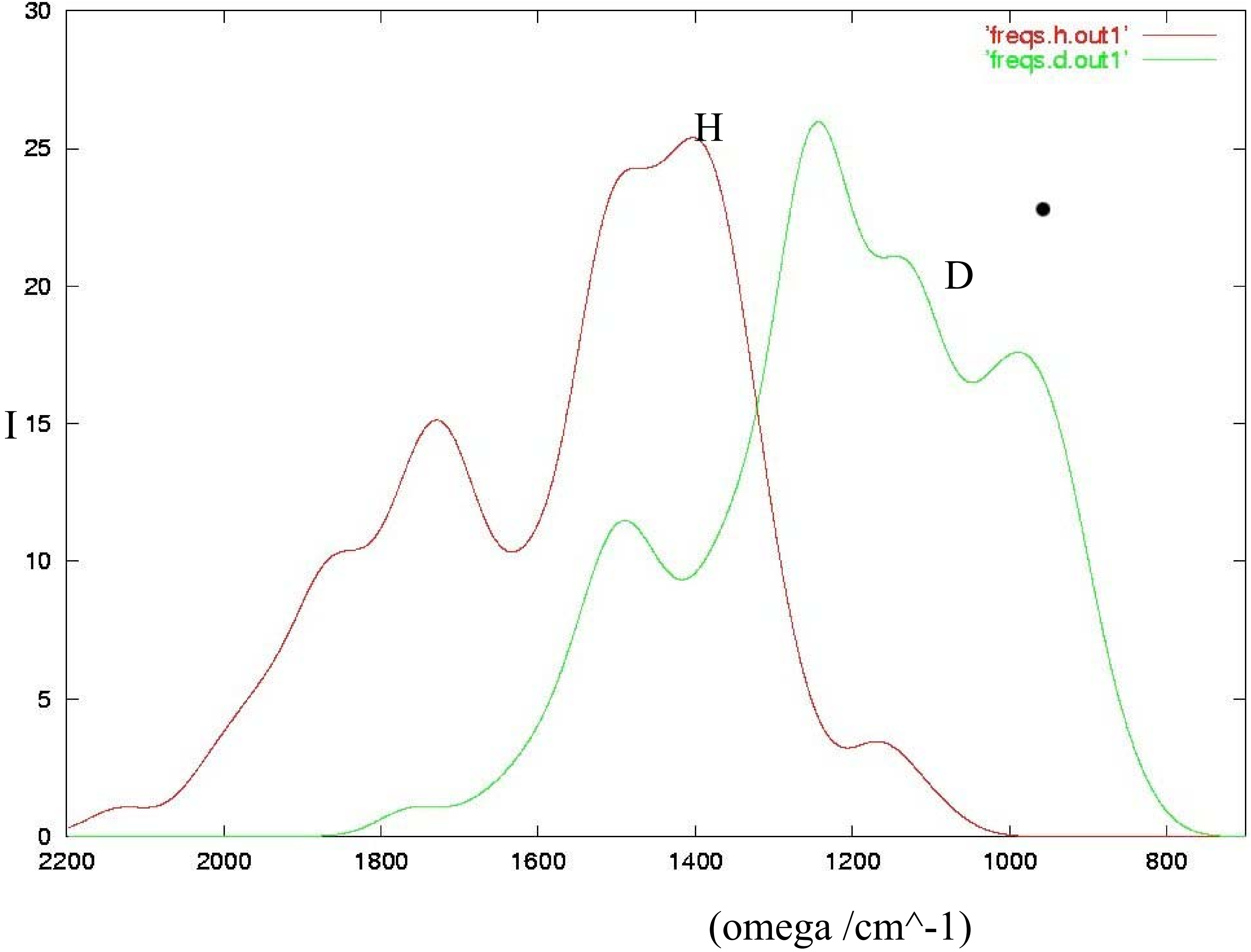


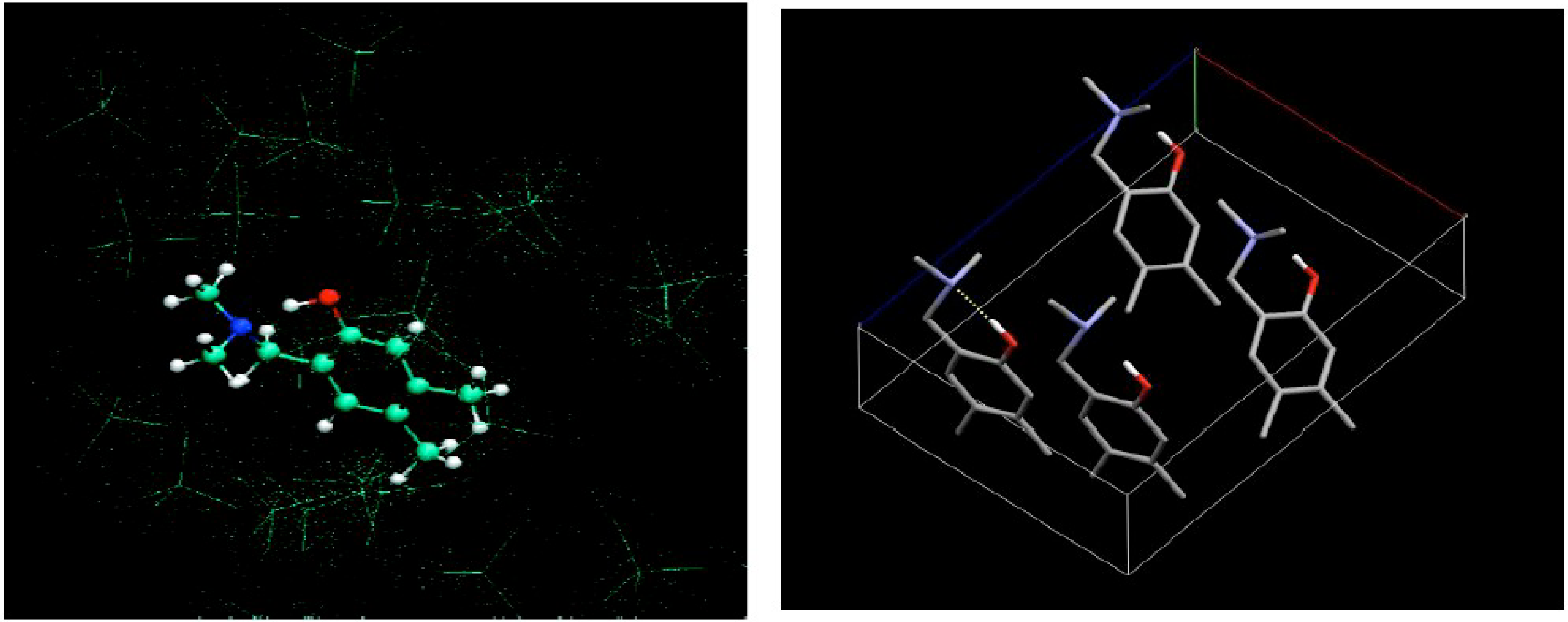
Fig. 1. Program package flowchart.

J.Stare, JM, Comput.Phys.Comm. 143 (2002) 222-240.


ω
I
ω


intenziteta
2200
2000
1800
1600
1400
1200
1000
ν [cm^{-1}]


'freqs.h.out1'
'freqs.d.out1'
H
D
I
30
25
20
15
10
5
0
2200
2000
1800
1600
1400
1200
1000
800
(omega /cm^-1)



Applied simulation boxes for Mannich base in carbontetrachloride solution and in the solid state

The snapshot methodology in conjunction with Car-Parrinello was applied to picolinic acid N-oxide

J. Stare, J. Panek, J. Eckert, JM, D. Hadži,
J. Phys. Chem A, 112 (2008) 1576-1586.

CPMD simulation of a Mannich base in solid, gas and solution
A. Jezierska, J. Panek, A. Koll, JM,
J. Chem. Phys., 126 (2007) 205101-205109.

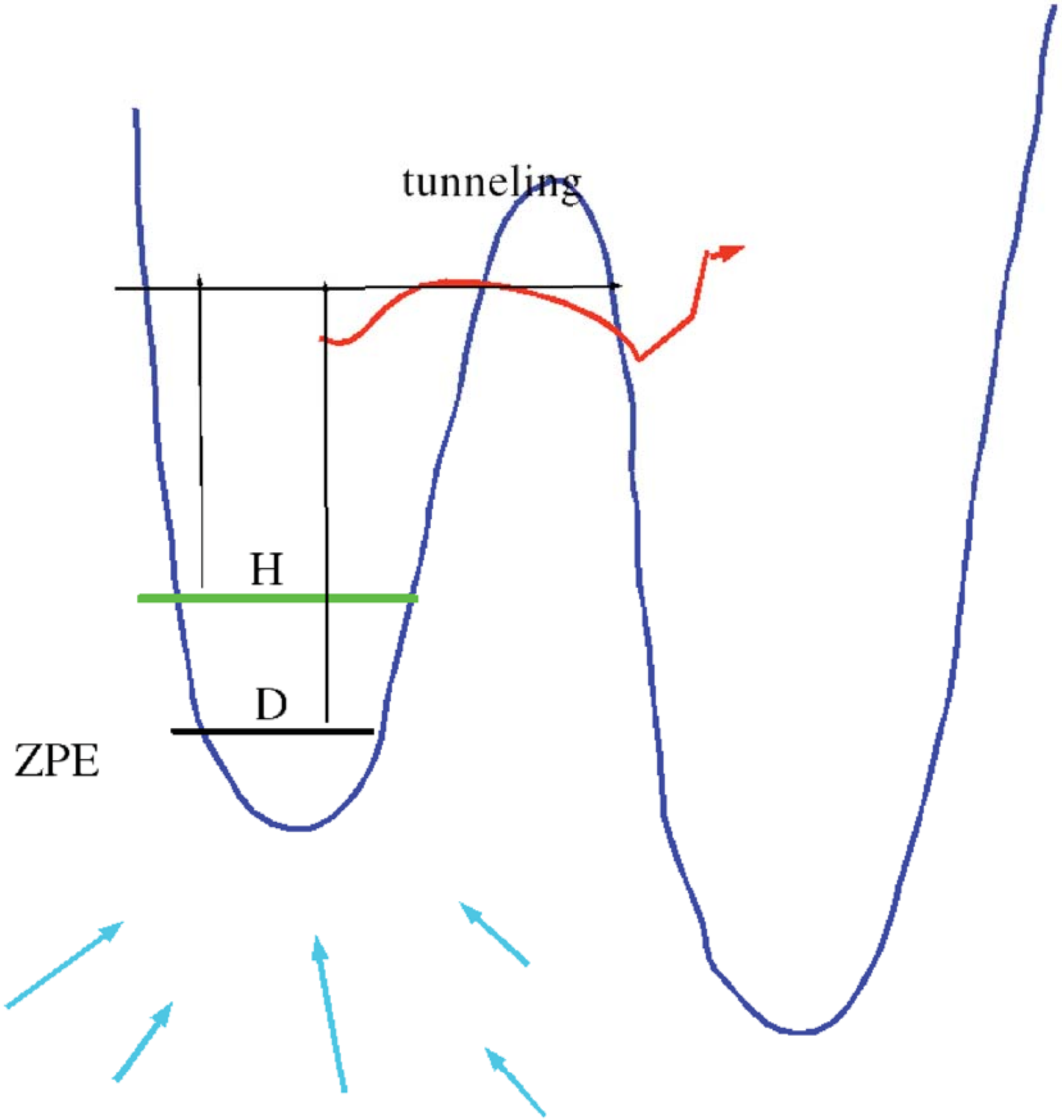
A. Jezierska, J. Panek, U. Borštnik, JM and D. Janežič,
J. Phys. Chem. A, 111 (2007) 5243-5248

Car–Parrinello MD in conjunction with quantization of the OH motion can be applied to calculate the OH stretching band of strong hydrogen bonded systems and potential of mean force for OH motion.

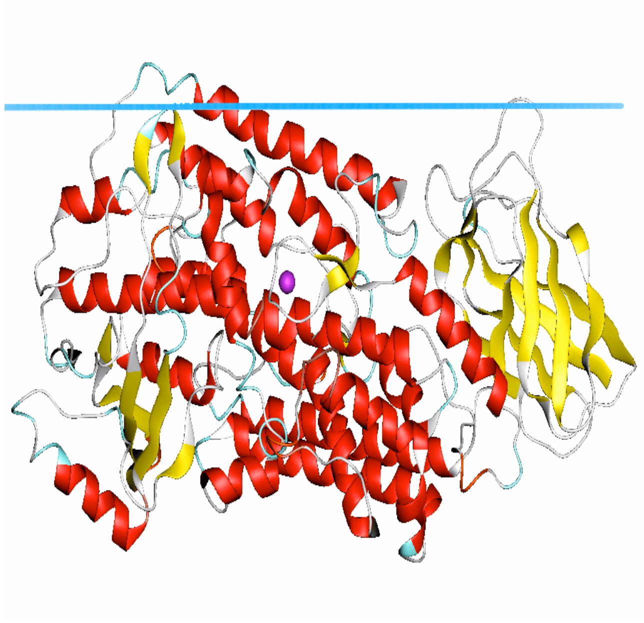
Application of the snapshot idea to the QM/MM calculation of the NH stretching band in Watson-Crick base pair
Y. Yan, G. M. Krishnan, O. Kühn, Chem. Phys. Lett., 464 (2008) 230

Tunneling in enzymatic reactions

H/D kinetic isotope effects for most of the enzymes are 3-8.


tunneling
H
D
ZPE

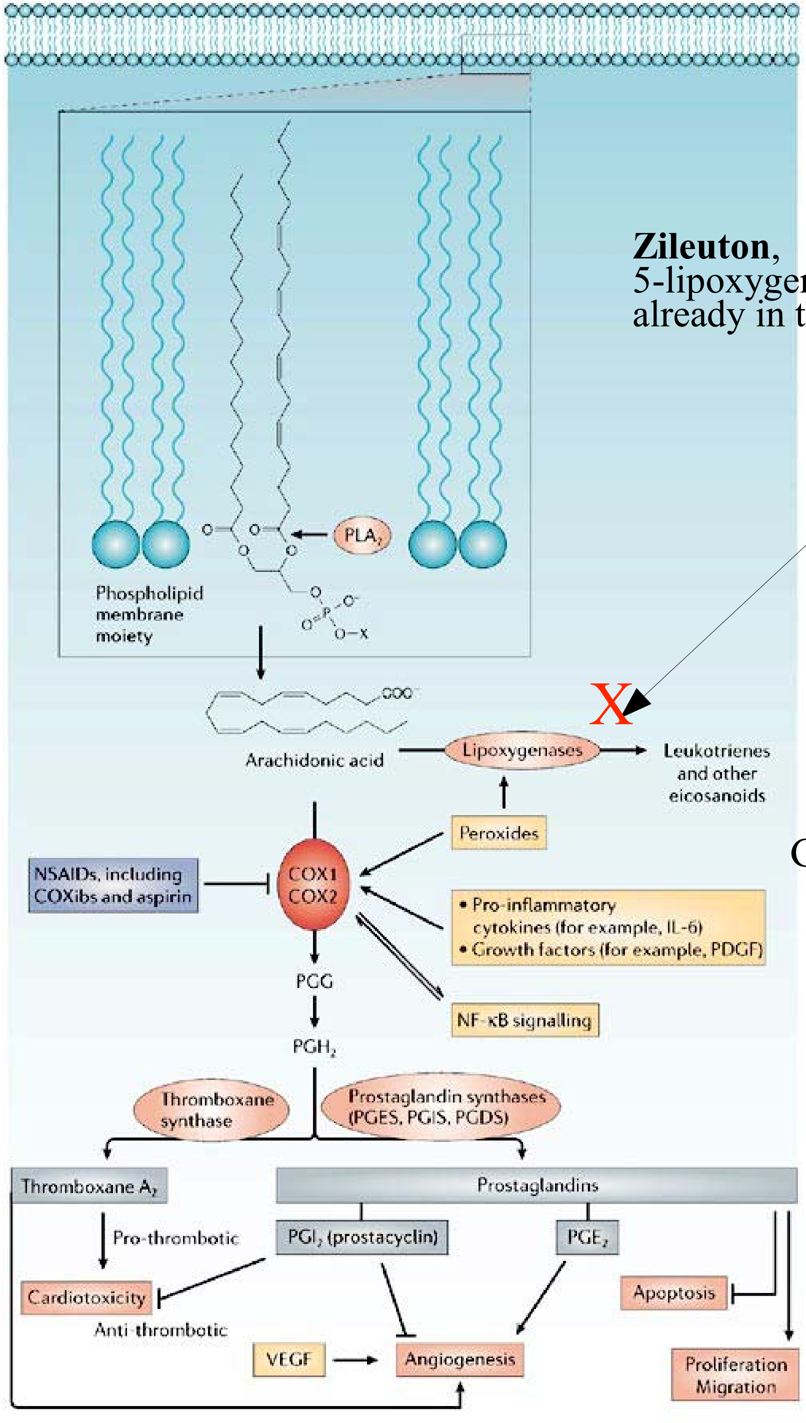
Lipoxygenase is an enzyme involved in oxydation of fatty acids



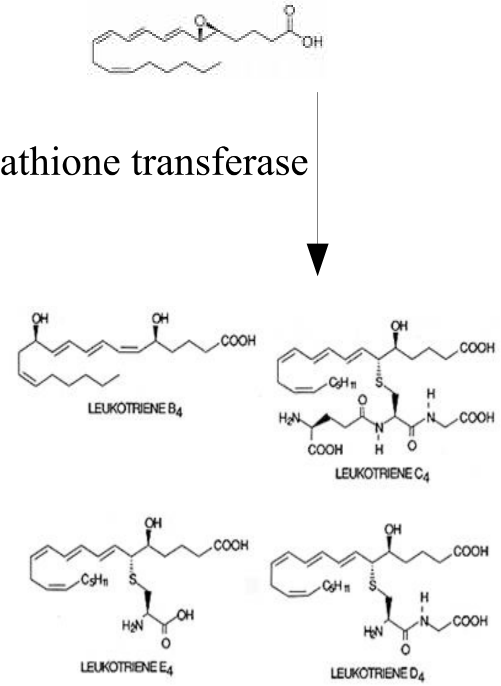
D.R.Tomchick et al. (2001).

Biochemistry, 40, 7509-7517.

898 residues, 1 nonheme iron atom
resolution 1.40 A

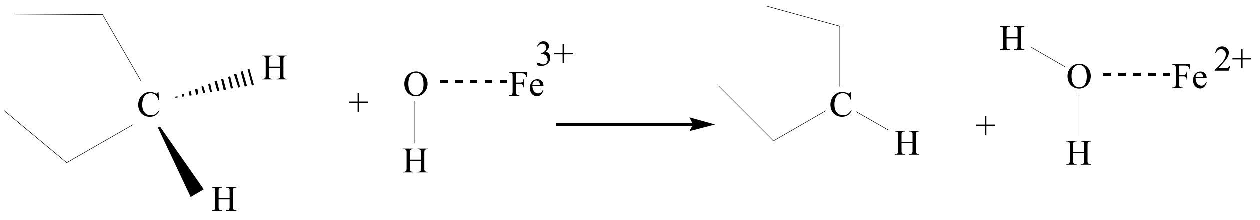


Zileuton,
5-lipoxygenase inhibitor for asthma treatment
already in the clinical use



Glutathione transferase

Chemical Reaction of Soybean Lipoxygenase-1



H/D Kinetic Isotope Effect=81

Most enzymatic reactions have H/D KIE 3-8

M.J Knapp, K.Rickert, J.P. Klinman, JACS, 2002, 124, 3865

Judith P. Klinman

We try to reproduce H/D kinetic isotope effect of SLO-1 by three different methods :

path integrals QCP

numerical propagation of hydrogen wavefunction

(vibronic formula)

MOLARIS all atom simulation

Spherical system

Region 1

Empirical Valence Bond (EVB) description of the reactive subsystem (bond Morse potentials, harmonic angle potentials, point charges ...)

Region 2

Protein and solvent atoms up to 18A radius, ENZYMIX force field

Region 3

Surface constrained all-atom solvent boundary and local reaction field model

Region 4

2 A shell of Langevin dipoles

The reaction is driven by changing the force field parameters from the reactant region to the product region by using thermodynamic perturbation (21 windows).

Nuclear quantum effects are calculated by Quantum Classical Path (QCP) version of path integration, 18 Trotter beads.

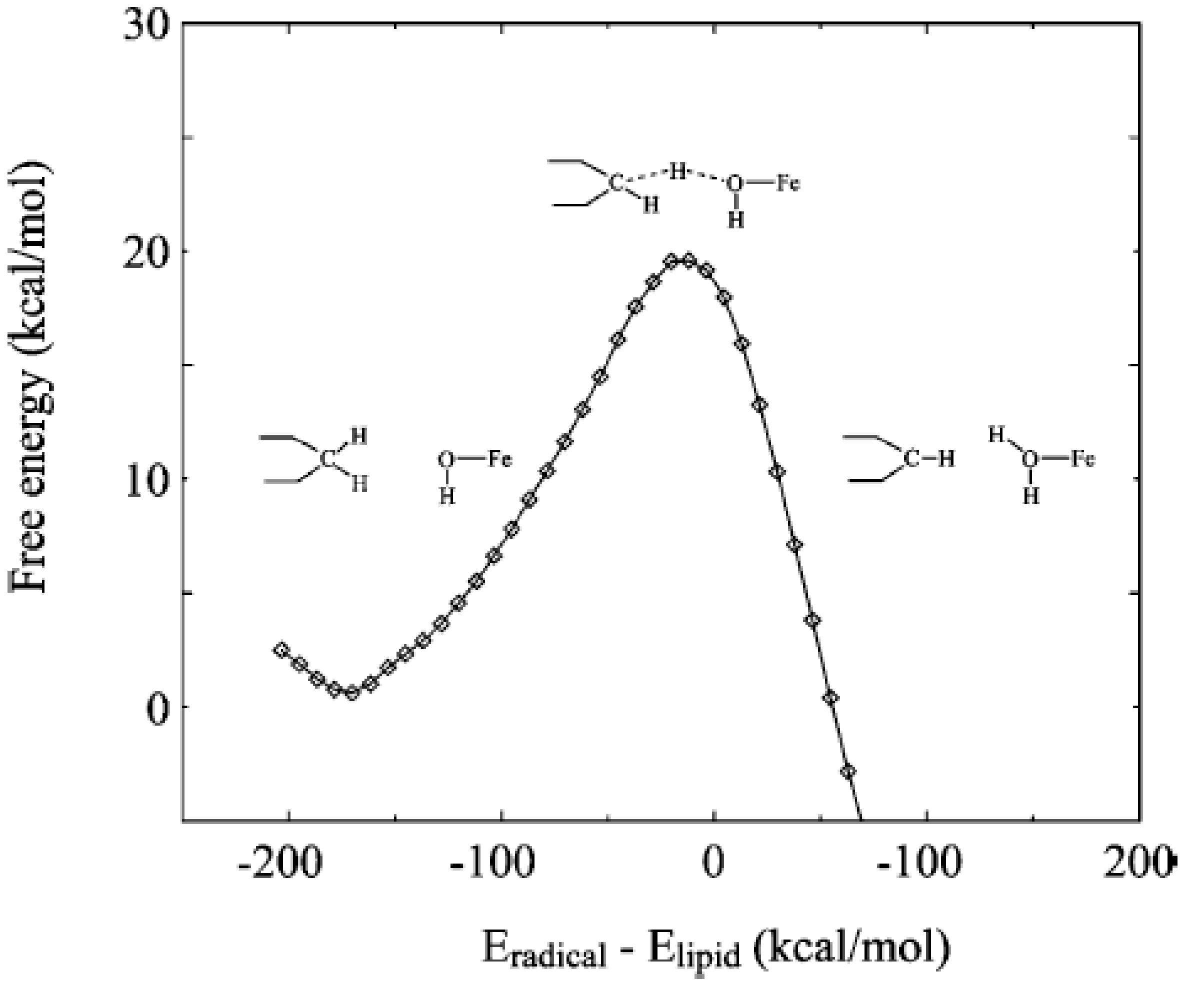
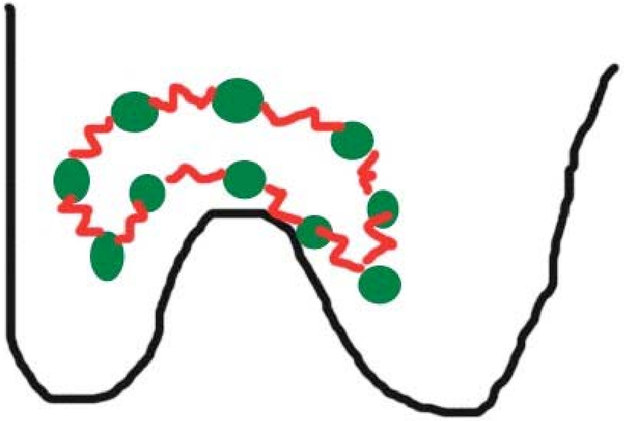


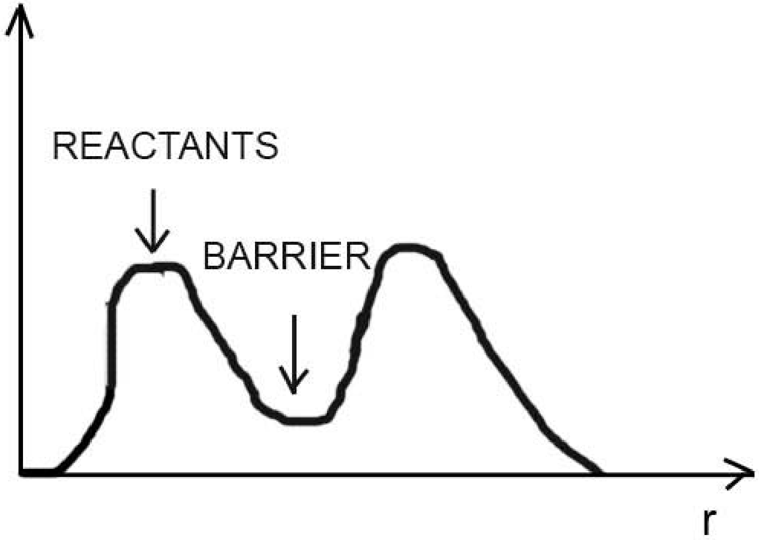
Figure 1. The classical free-energy barrier for transferring the hydrogen atom from the lipid substrate to form the radical intermediate.

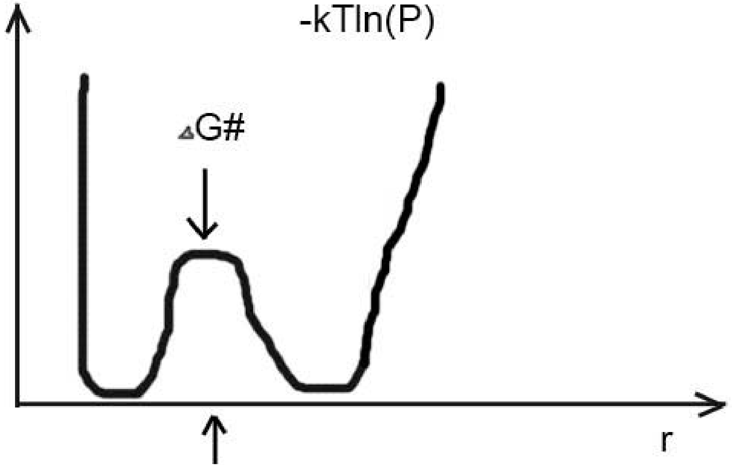
P= number of beads

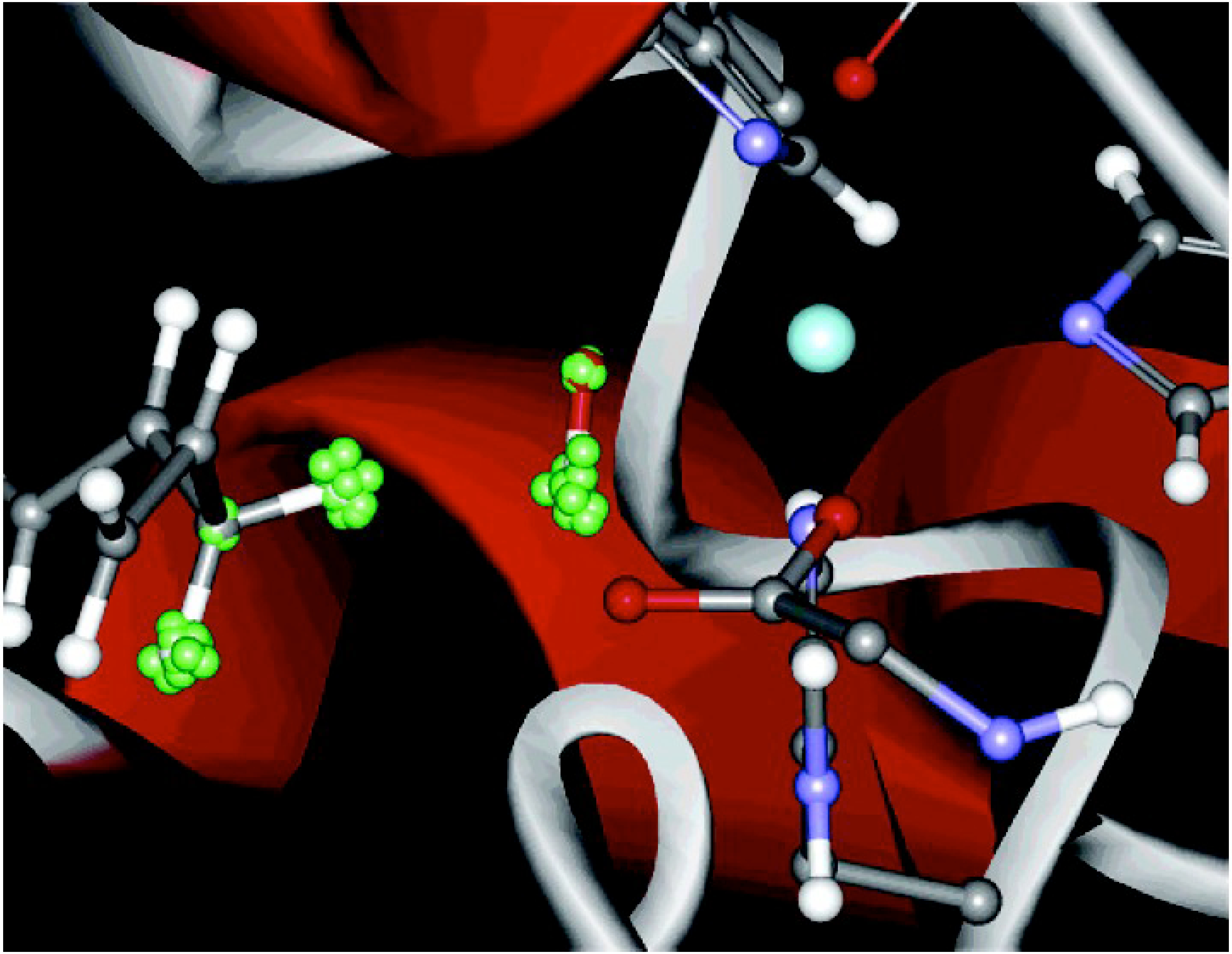
$$V_P = \frac{1}{2}\frac{P m_H k_B^2 T^2}{\hbar^2}\sum_{\alpha=1}^{P}\left(r^{(\alpha)} - r^{(\alpha+1)}\right)^2 +$$

$$+ \frac{1}{P}\sum_{\alpha=1}^{P}\sum_{i=1}^{class} V_{\alpha i}\,(\text{prot-class})$$









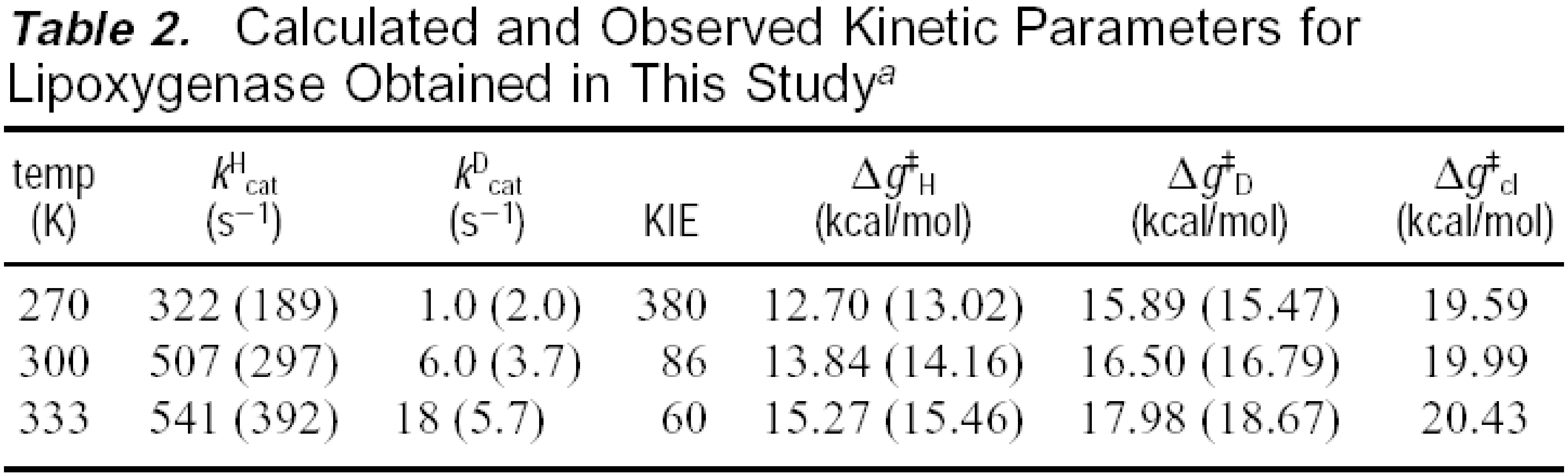


Table 2. Calculated and Observed Kinetic Parameters for Lipoxygenase Obtained in This Study[a]

temp (K)	k^{H}_{cat} (s^{-1})	k^{D}_{cat} (s^{-1})	KIE	$\Delta g^{\ddagger}_{H}$ (kcal/mol)	$\Delta g^{\ddagger}_{D}$ (kcal/mol)	$\Delta g^{\ddagger}_{cl}$ (kcal/mol)
270	322 (189)	1.0 (2.0)	380	12.70 (13.02)	15.89 (15.47)	19.59
300	507 (297)	6.0 (3.7)	86	13.84 (14.16)	16.50 (16.79)	19.99
333	541 (392)	18 (5.7)	60	15.27 (15.46)	17.98 (18.67)	20.43

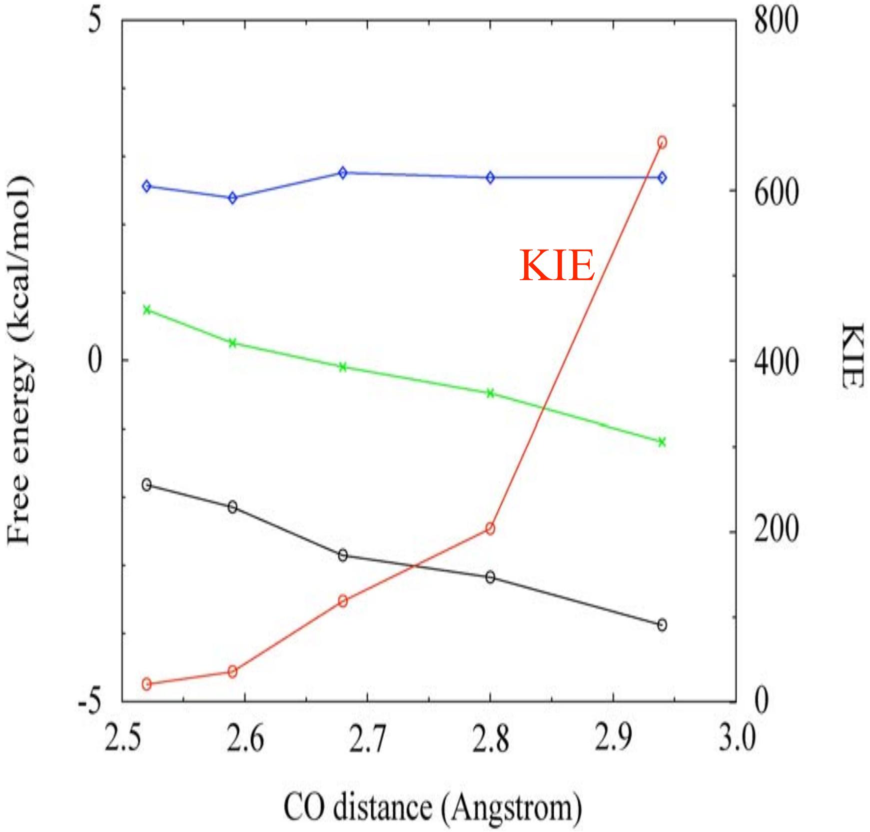
The QCP calculations were performed with full protein environment
Temperature dependence was reproduced for the H transfer
and less consistent results were obtained for the D transfer.

M.H.M. Olsson, P.E.M. Siegbahn, A.Warshel, JACS 2004,126,2820

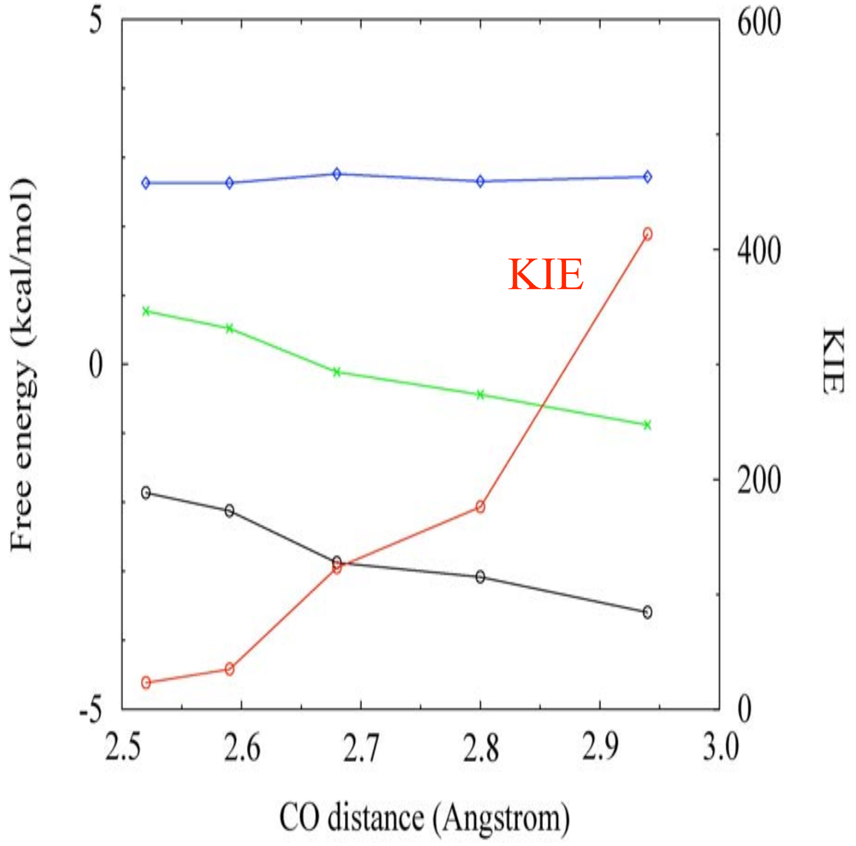
M.H.M. Olsson, JM and A. Warshel, Phil. Trans. Roy. Soc. B, 361 (2006) 1417-1432.

Path integral calculation of H/D KIE as a function of the CO distance

Microscopic description of the enzyme



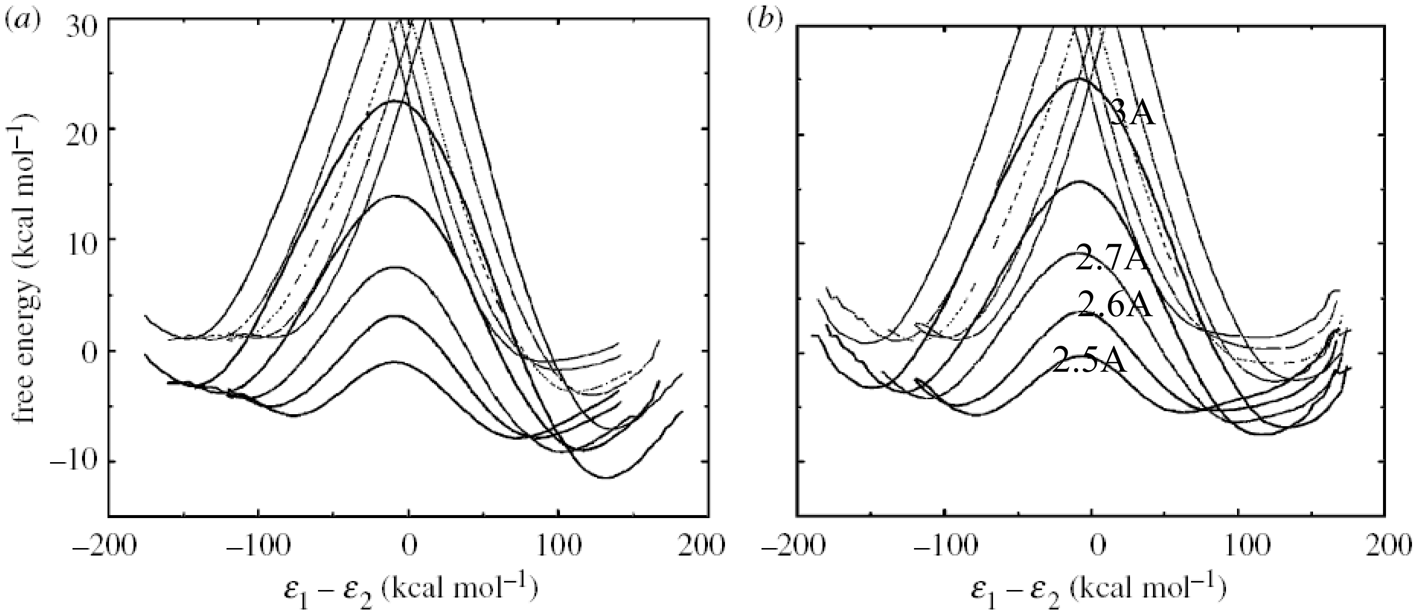
Aqueous solution



Protein

M.H.M. Olsson, JM, A.Warshel, Phil.Trans.Roy.Soc. B, 361 (2006) 1417-1432.

The classical barrier height (in terms of free energy) decreases with compressing the CO distance



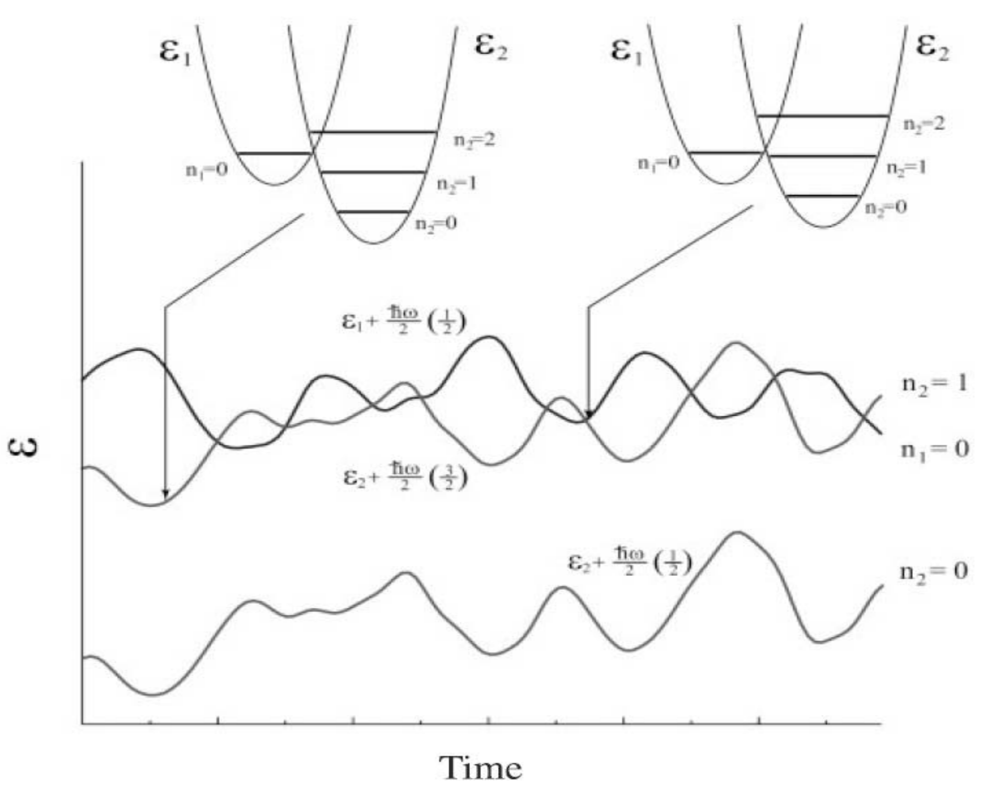
aqueous solution

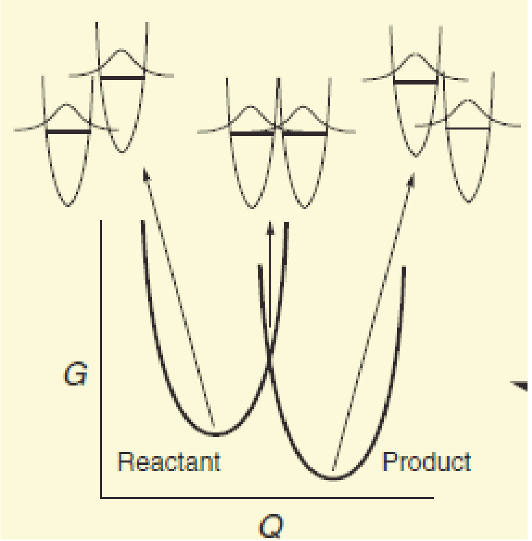
protein

Potential of mean force was calculated for reaction with fixed CO distances.

Tunneling is always associated with a barrier. If the barrier decreases the extent of tunneling decreases. By compressing the CO distance tunneling and KIE decrease.

Semiclassical vibronic treatment of proton transfer

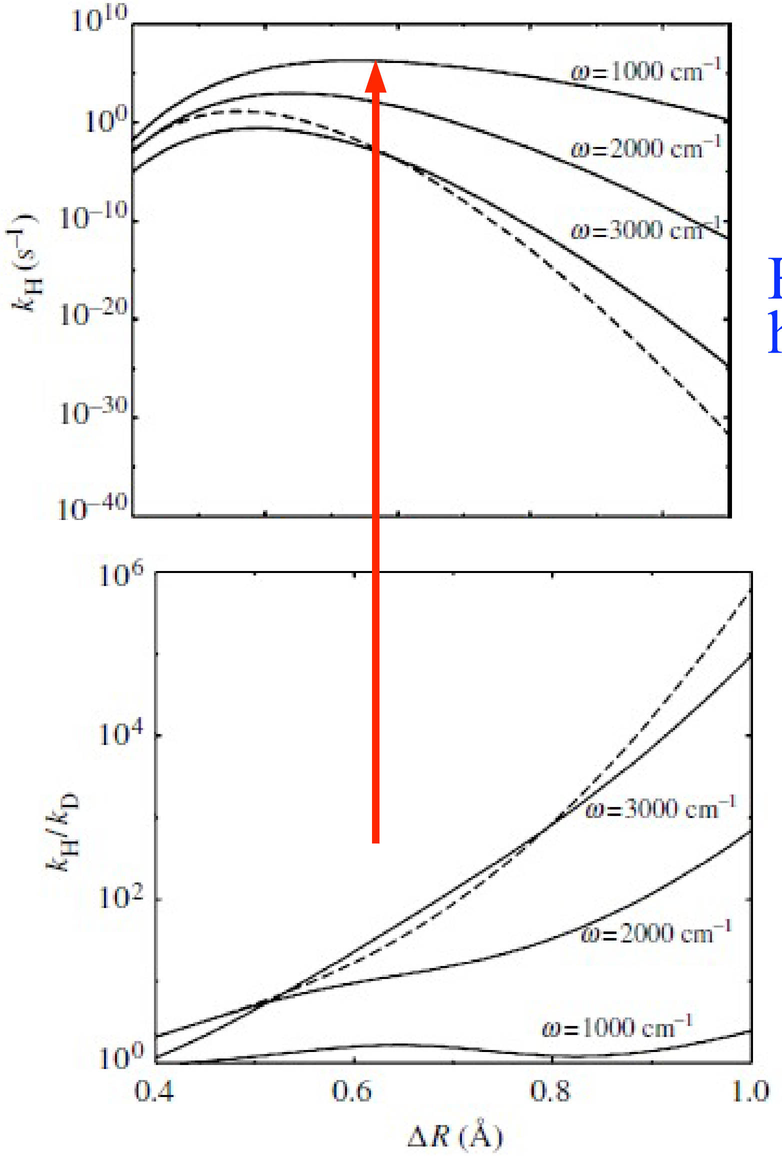




$$k_{\mathrm{tun}} = (\mathrm{Const.})\exp\left\{\frac{-(\Delta G^{o}+\lambda)^{2}}{4\,\lambda RT}\right\}\int_{r_1}^{r_0} e^{-m_{H}w_{H}r_{H}^{2}/2\hbar}\; e^{-E_x/k_bT}\,dx$$

Extension of Marcus's approach

Vibronic treatment of proton transfer



How the rate constant depends on the hydrogen transfer distance

How H/D kinetic isotope effect depends on the hydrogen transfer distance

Large distance are associated with large kinetic isotope values, but the rate constant there is vanishingly small. The main contribution to the rate constant and KIE comes from the narrow range labeled by red arrow.

Simulation of tunneling by direct propagation of hydrogen wave function

Place initial one-dimensional hydrogen wavefunction to the reactant well

Solve the time-dependent Schrodinger equation numerically by the propagator Kosloff and Kosloff and observe 'leaking' of the wavefunction to the product well

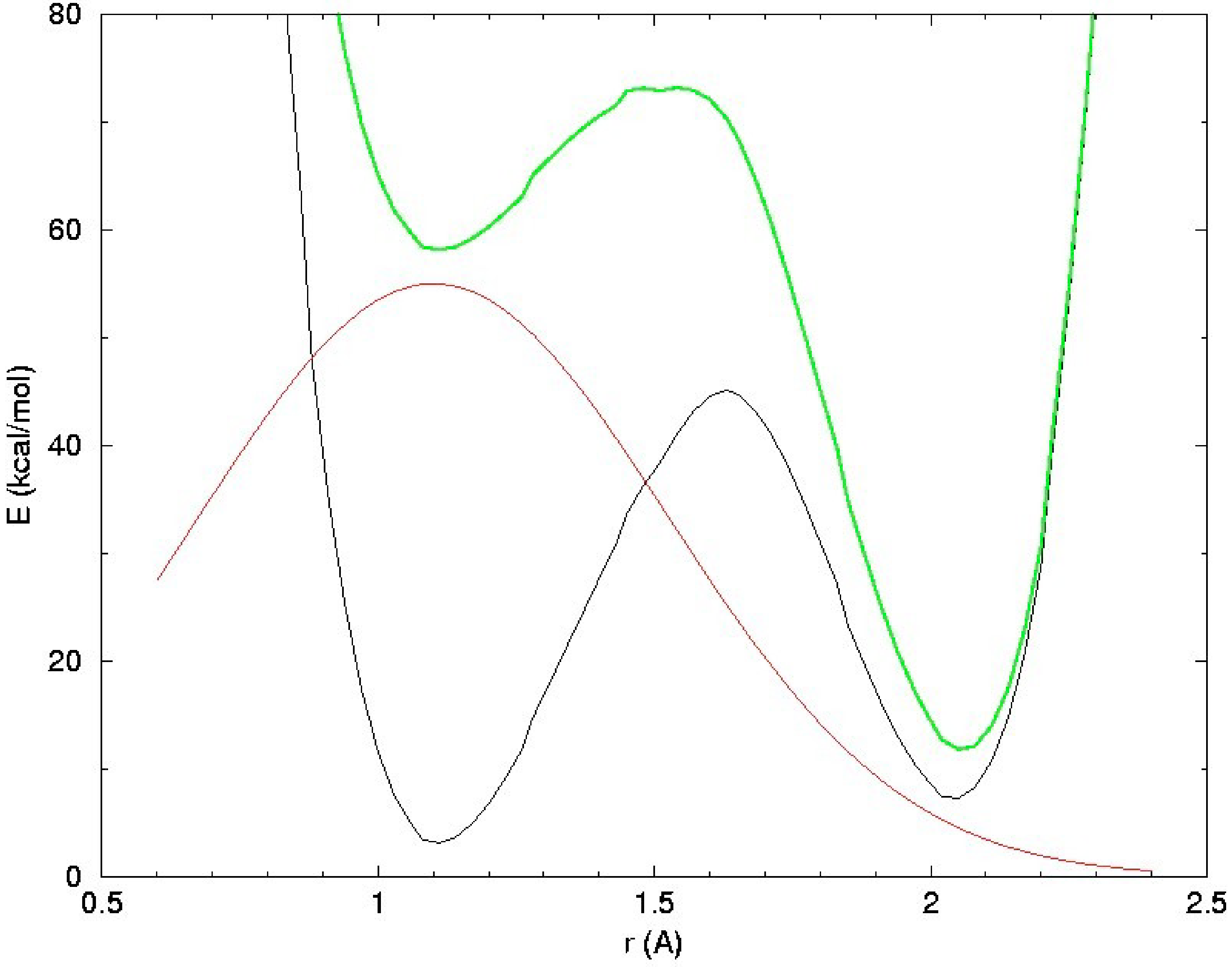
Repeat the exercise for several time origins, the slope of the population vs. time is rate constant

Repeat the procedure for D

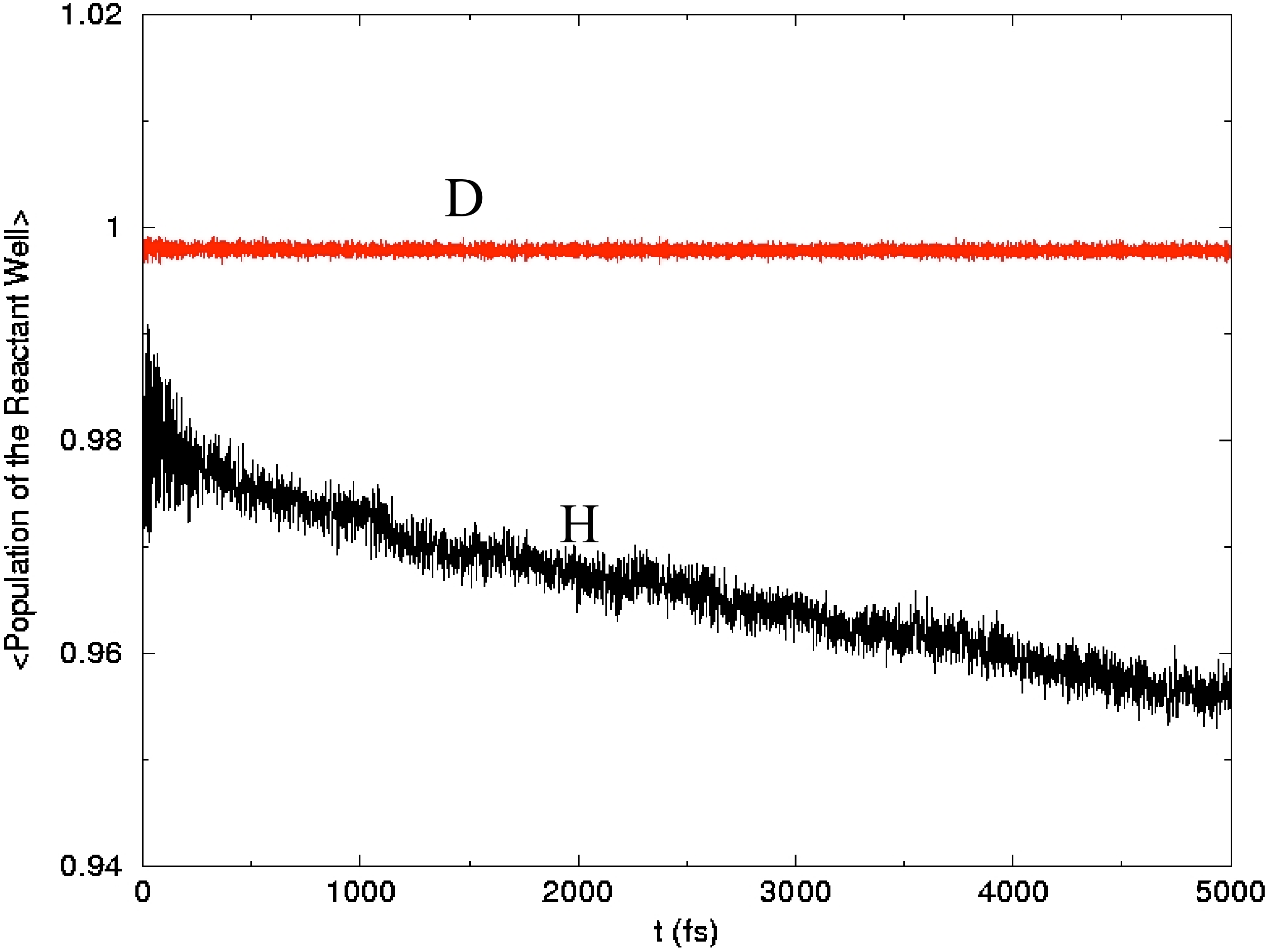
The ratio of both rate constants is H/D KIE.

A problem: one can simulate quantum-dynamically few picoseconds of real time.

Biased Sampling



Lipoxygenase
BIAS = 55*exp(-2.77*(1.1-x))**2)

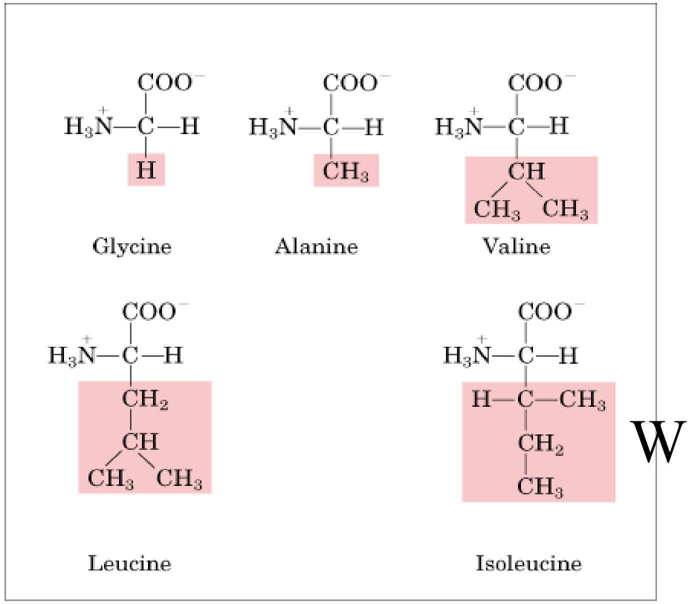


EFFECTS OF POINT MUTATIONS

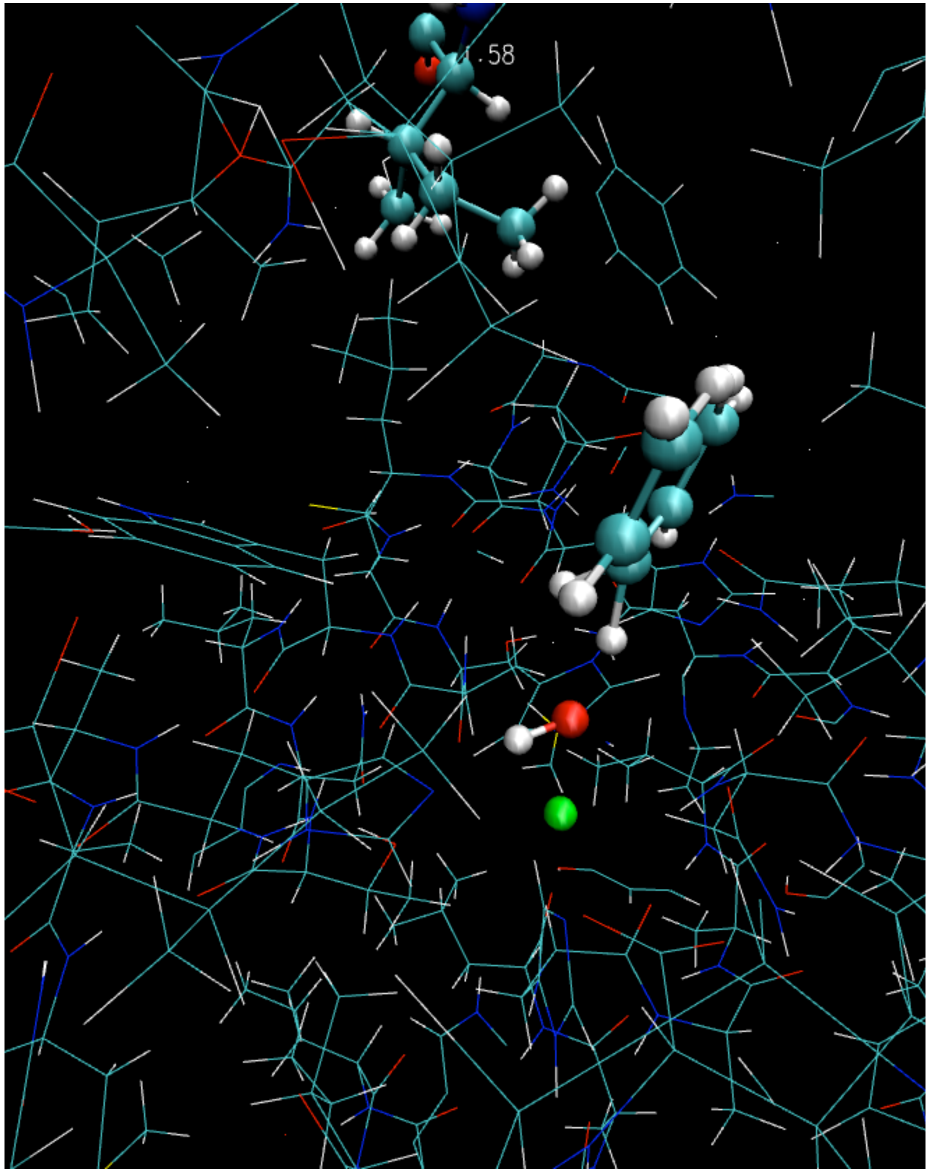
EXPERIMENTAL DATA

	k_rate(/sec)	H/D KIE
WT	297	81
I553G	59	180 *
I553V	99	84
I553L	322	117
I553A		103

* Path integral simulation in progress



WT



M.P. Meyer, D.R. Tomchick and J.P Klinman, PNAS **105** (2008) 1146-1151.

In lipoxygenase the CH motion is <u>decoupled</u> form the polar environment since by H transfer the charge distribution is not much changed. Additional proof: in aqueous solution the H/D KIE is basically the same as in protein. SLO-1 kinetics is controlled by intrinsic dynamics of CH cleveage reaction.

Path integration (QCP) is a practical method of choice to study isotope effects in enzyme active centers.

Calculation of KIE by numerical propagation of H/D wavefunction is less practical.
JM, H. Liu, M.H.M. Olsson and A. Warshel,
J. Phys. Chem. B, 112 (2008) 5950-5954.

Point mutations and temperature slightly change potential of mean force for the CO motion. Effective CH distance is changed giving rise to different barrier shapes and different extent of tunneling.

Tunneling is hydrogen transfer through the barrier.

If there is no hill it is difficult to drill the tunnel through the hill.

Bohinj railway tunnel at Podbrdo, Slovenia

We learned that for compressed donor-acceptor distances the barrier vanishes.

But still

A 21^{st} century revisionist's view at a turning point in enzymology

Zachary D Nagel & Judith P Klinman

NATURE CHEMICAL BIOLOGY VOLUME 5 NUMBER 8 AUGUST 2009 543

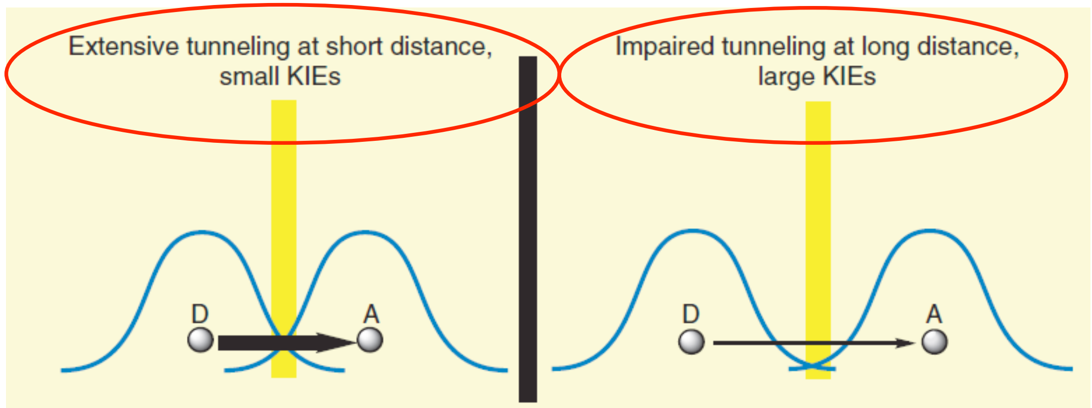


Figure 2 A basic representation of the tunneling phenomenon is illustrated. Hydrogen wave functions are shown as blue curves, and a thermal barrier is represented as a yellow block between the donor (D) and the acceptor (A). The thicker arrow on the left represents the higher probability of tunneling at short distance.

Kinetic isotope effect for most of the enzymes
is in the range 3-8.

Deuterated enzymes would work on average
5 times slower.

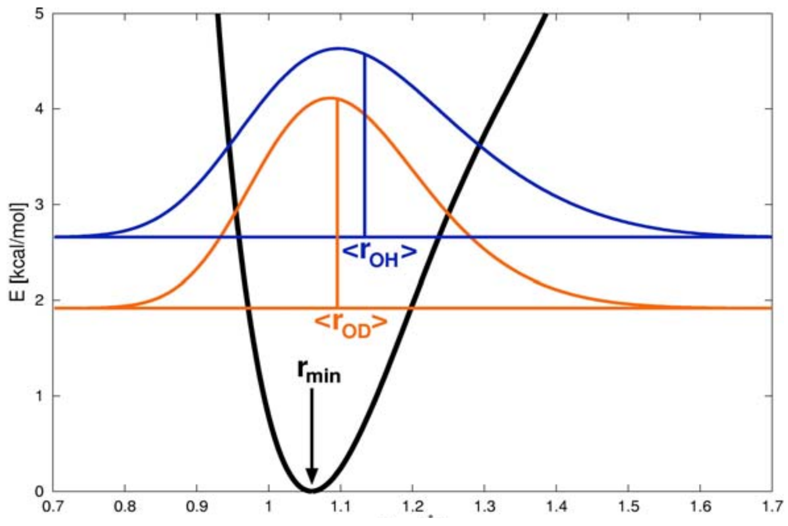
By drinking D_2O one would simply extend the time axis
for a factor of 5 giving rise to slow and long life
of 5*80 =400 years

E. coli lives and proliferates in D2O, while most of the eucaryotes do not.

Rodents can not survive D2O plasma concentration of more than 30%.

A possible explanation is that structure and stability of receptors has been changed upon deuteration.

Ubbelohde effect is a secondary isotope effect. By deuteration of a X-H..Y hydrogen bond the X..Y distance is increased. This applies to protein-protein, water-protein and water-water hydrogen bonds.



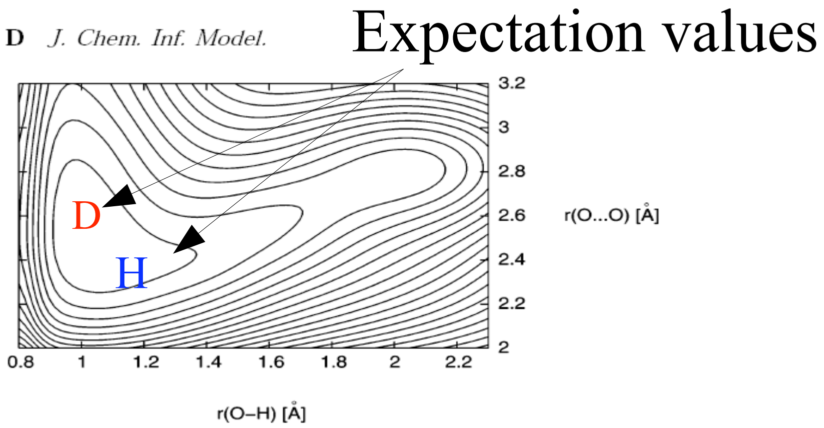
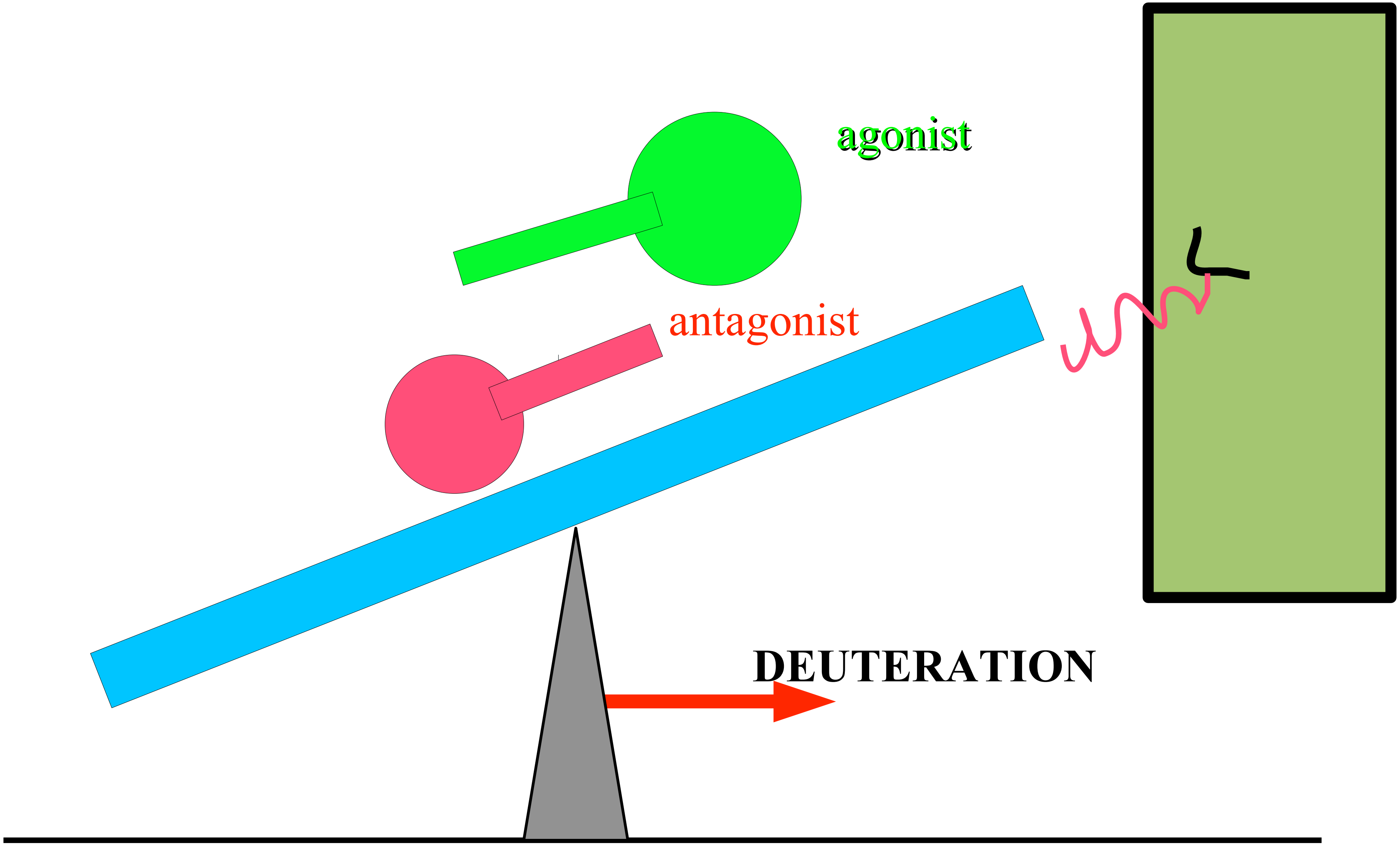
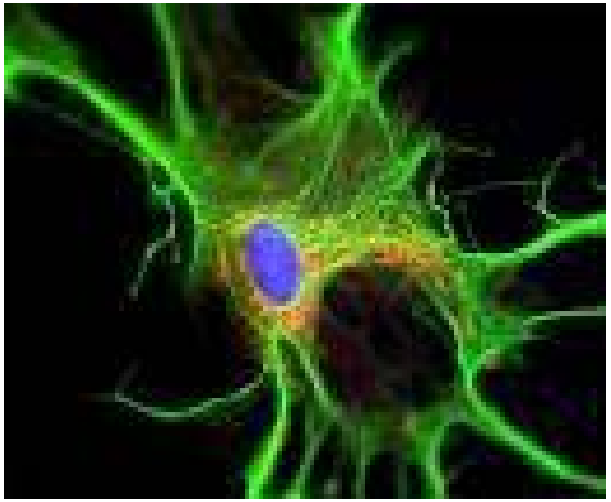


Figure 2. Contour plot of the potential energy surface of 4-methoxypicolinic acid *N*-oxide. The innermost contour represents the energy value of 5 kcal/mol; each successive outer contour represents a 5 kcal/mol increase in energy.


agonist
antagonist
DEUTERATION

Work on rat histamine H3 receptor deuteration is in progress



astrocytes

Proton (hydrogen) tunneling is important in the systems of biological interest

Contra: Arieh Warshel claims that there is about the same contribution of tunneling to the rate constant in the enzyme reaction than in the corresponding reaction in aqueous solution.

Pro: No rodents survive when 35% of water in the body is replaced by D2O.
Inelastic incoherent neutron scattering on living *E. coli* in H2O and D2O (average of all macromolecules) were interpreted by stronger hydrophobic effect in D2O.
(M. Jasnin *et al.* , Eur.Biophys.J. **37** (2008) 613-617.

Take home question

Enzymes catalyse reactions by praeorganized electrostatics. Electrostatics is a longe range interaction and this is a possible explanation why enzymes are so large.

If CH motion in the rate limiting step of lipoxygenase is basically decoupled from the polar macromolecular environment why evolution made lipoxygenase that large ?

- Jernej Stare

- Dusan Hadži, National Institute of Chemistry

- Arieh Warshel, University of Southern California

- Katja Perdan-Pirkmajer, Faculty of Medicine

- Mojca Kržan, Faculty of Medicine, University of Ljubljana

- Mats H.M. Olsson, University of Southern California
-
- Hanbin Liu, Lawrence Livermore National Laboratory
-
- Juergen Eckert, UCLA & UCSB
-
- Aleksander Koll, University of Wroclaw, Poland
-
- Anetka Jezierska, University of Wroclaw, Poland
-
- Jaroslaw Panek, University of Wroclaw
-
- Dušanka Janežič, National Institute of Chemistry
-
- Urban Borštnik, National Institute of Chemistry & University of Zurich, Switzerland
-
- Rok Borštnar, National Institute of Chemistry

$Ministry for Science and Higher Education of Slovenia,
grants P1-0012, J1-2014, P3-067