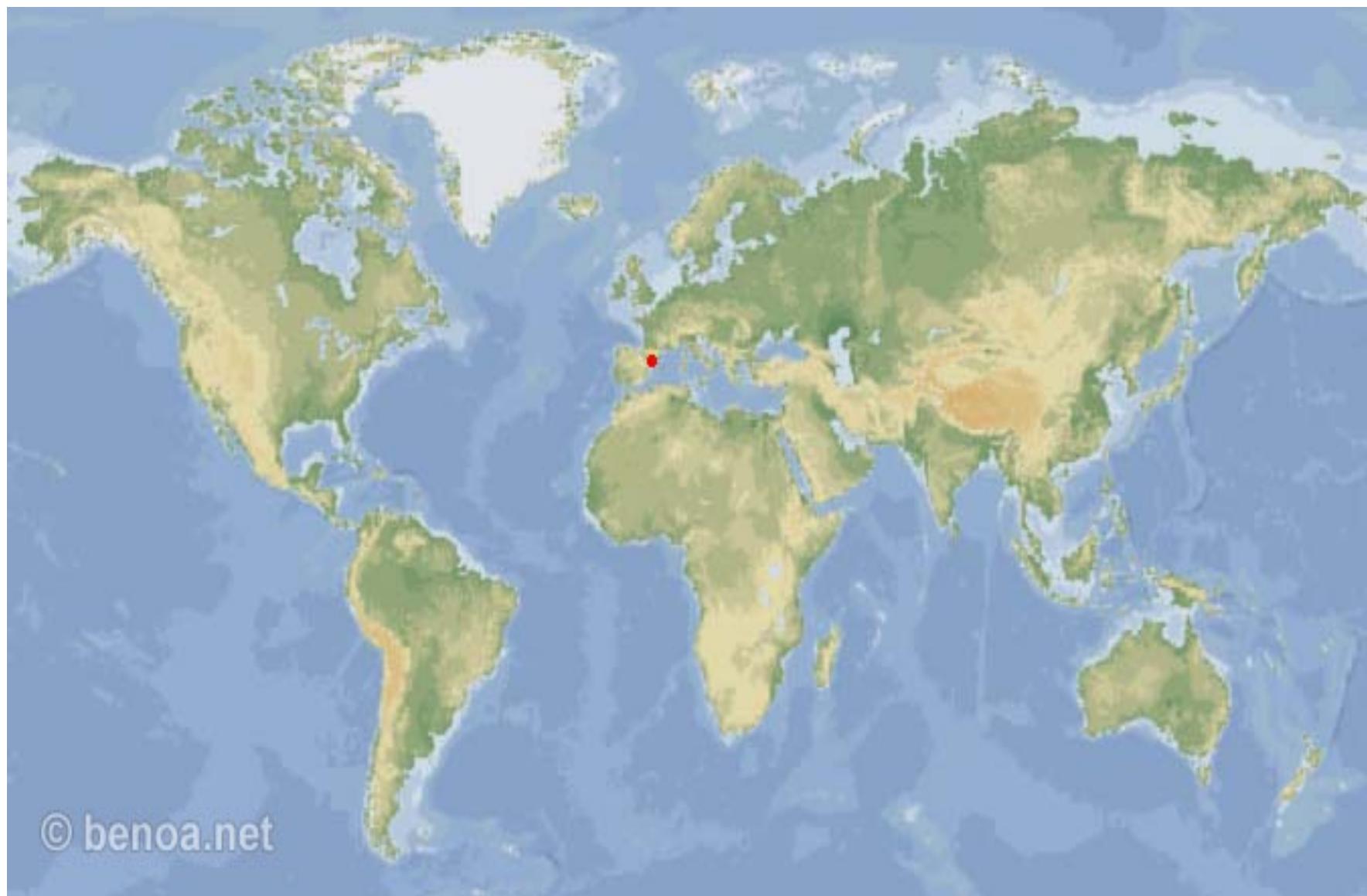


Reaction Mechanism of Mandelate Racemase in a QM/MM model: reaction path and catalysis

Xavier Prat-Resina
January 28th, 2005 AD





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Mandelate Racemase

Optimization of TS

PMF: Rc

PMF: mechanism

Overview

Mandelate Racemase: Structure and Reaction Mechanism

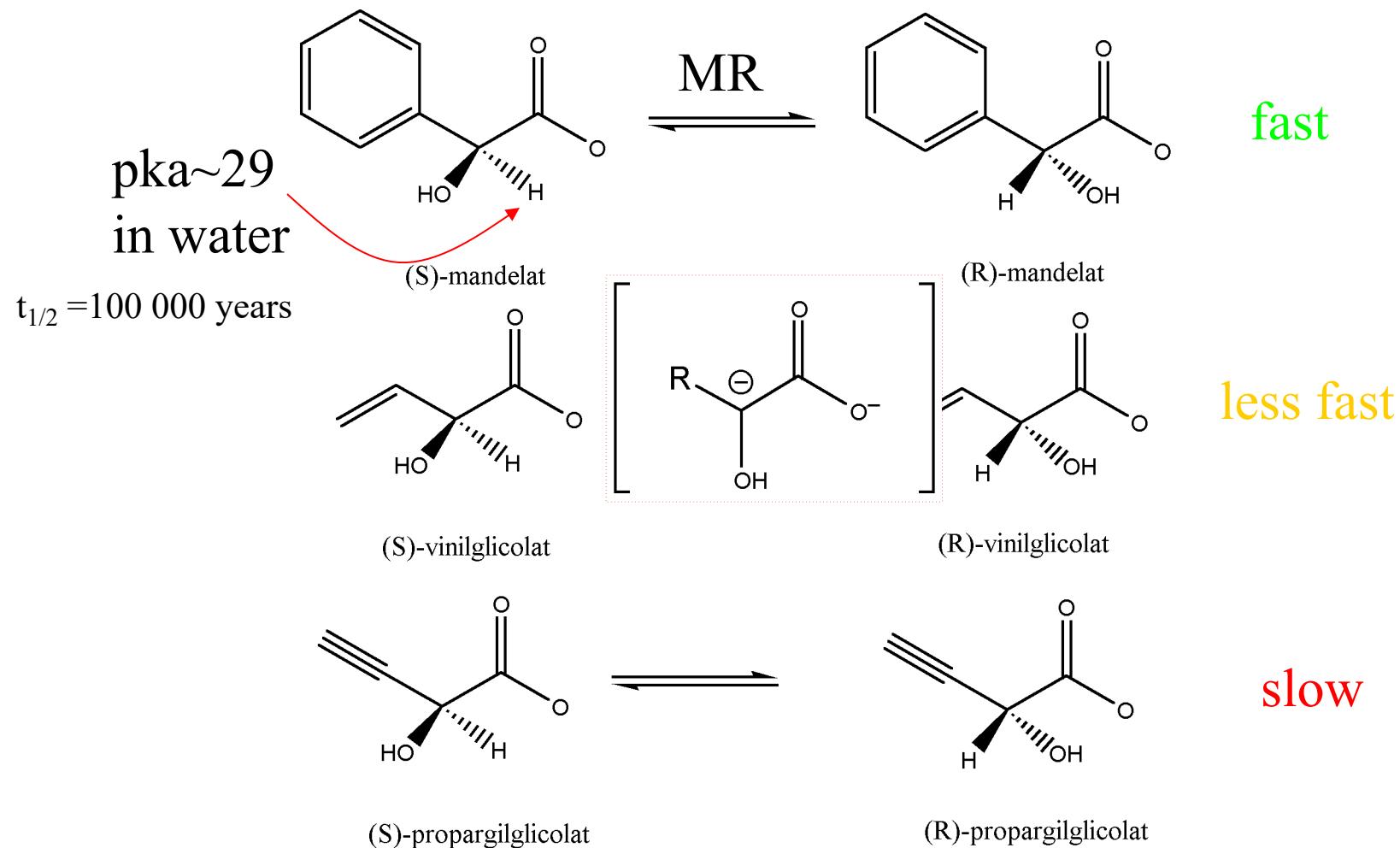
Optimization of Saddle Points in big systems

Potentials of Mean Force: Reaction coordinate

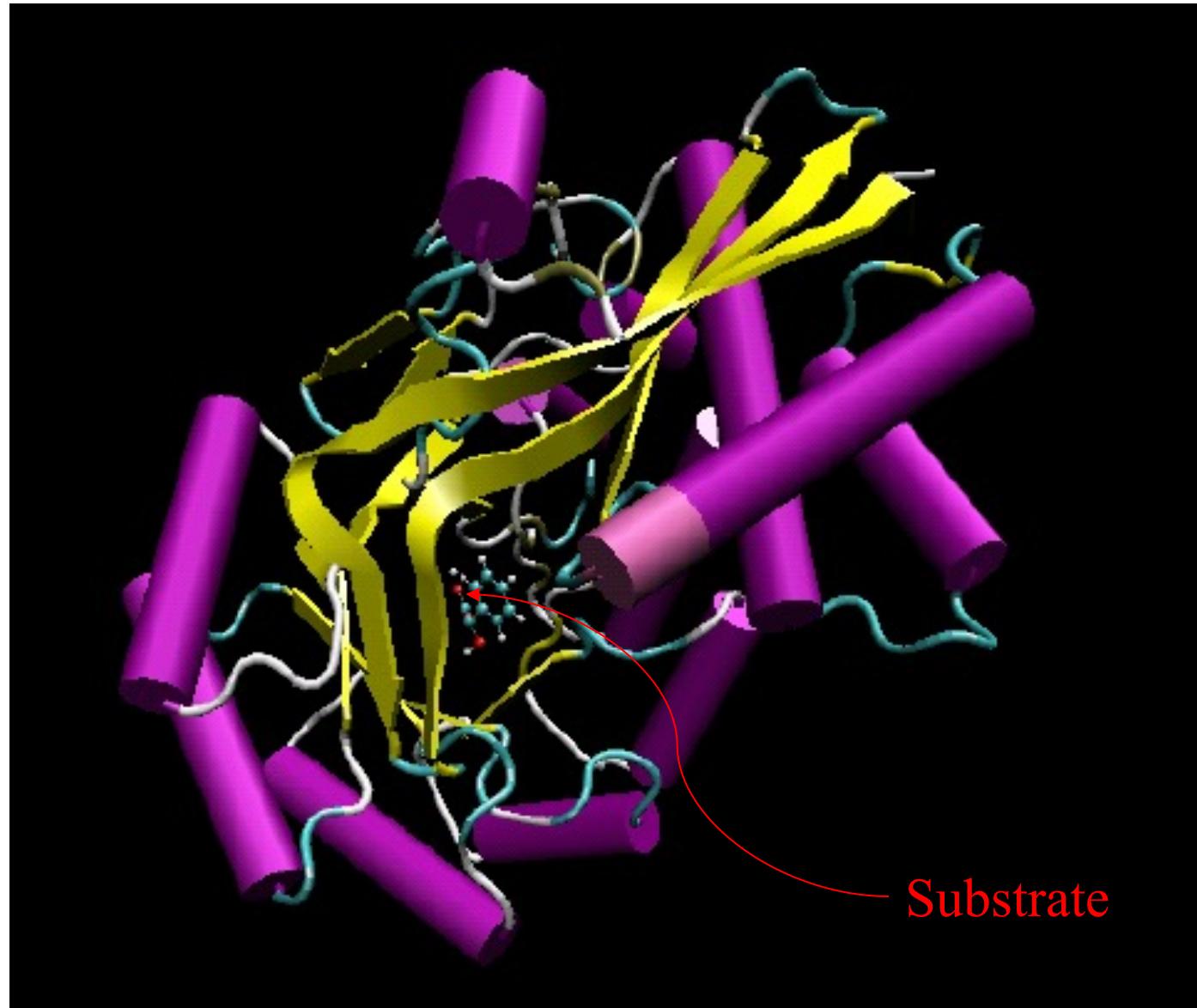
Potentials of Mean Force: mechanism and catalysis

Conclusions/General Discussion

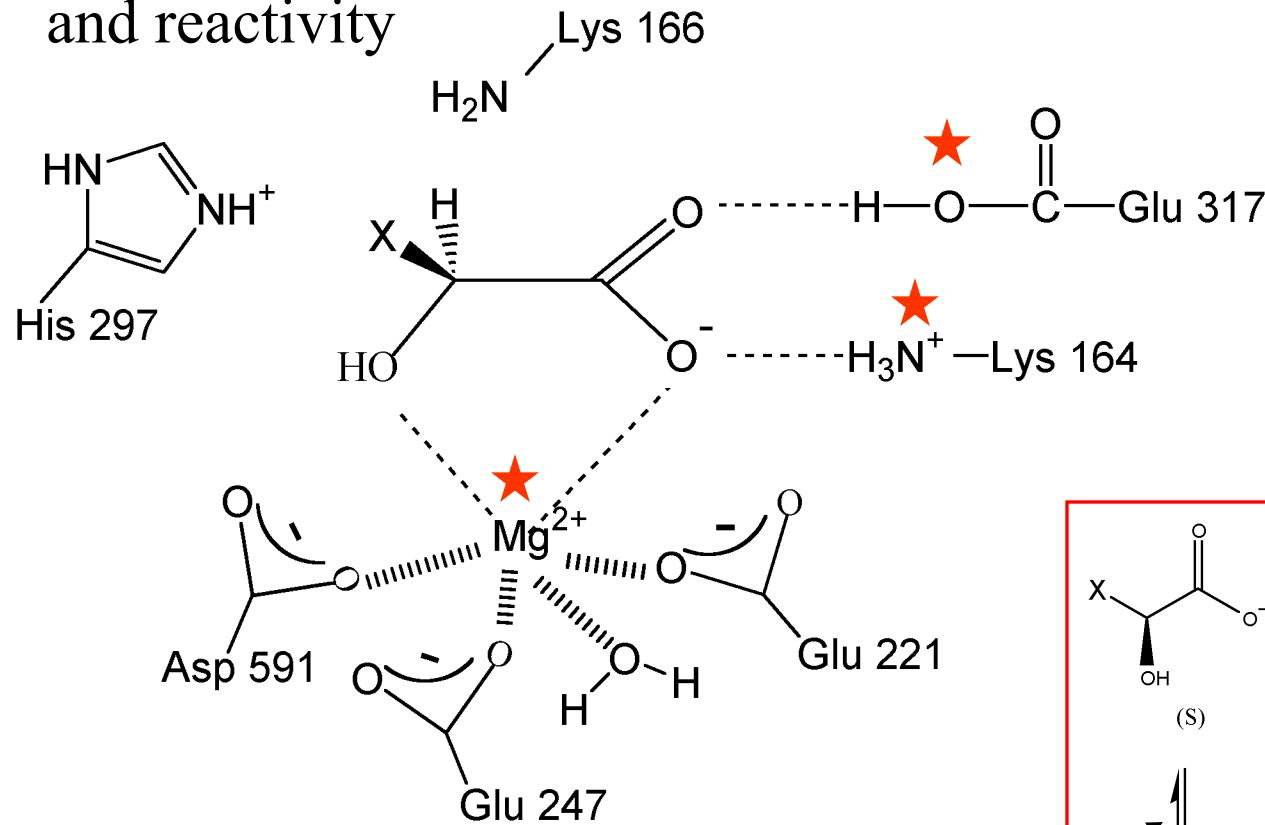
MR: the reaction



MR:
structure

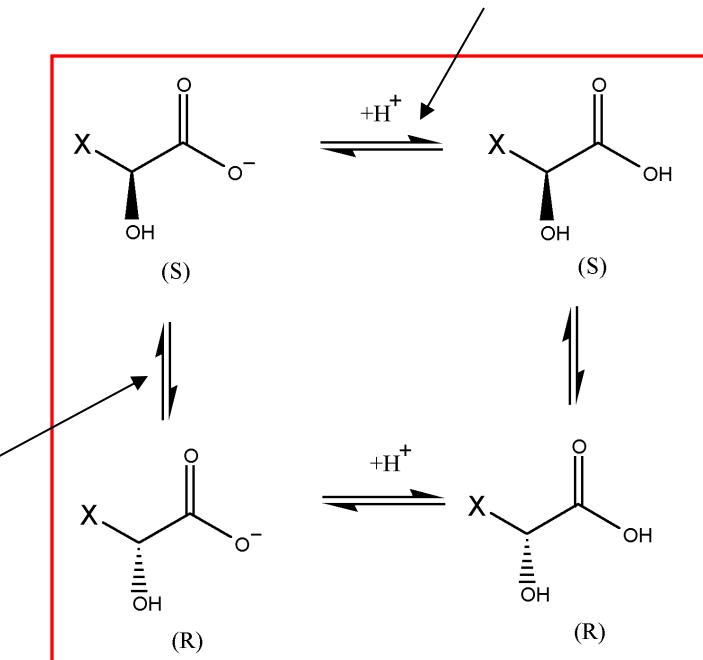


MR: Active site and reactivity

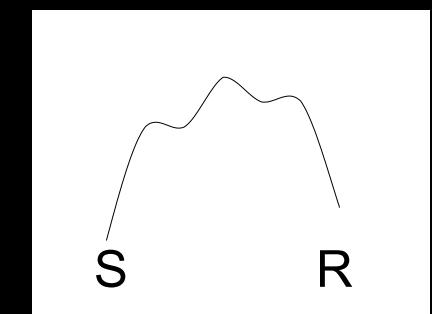
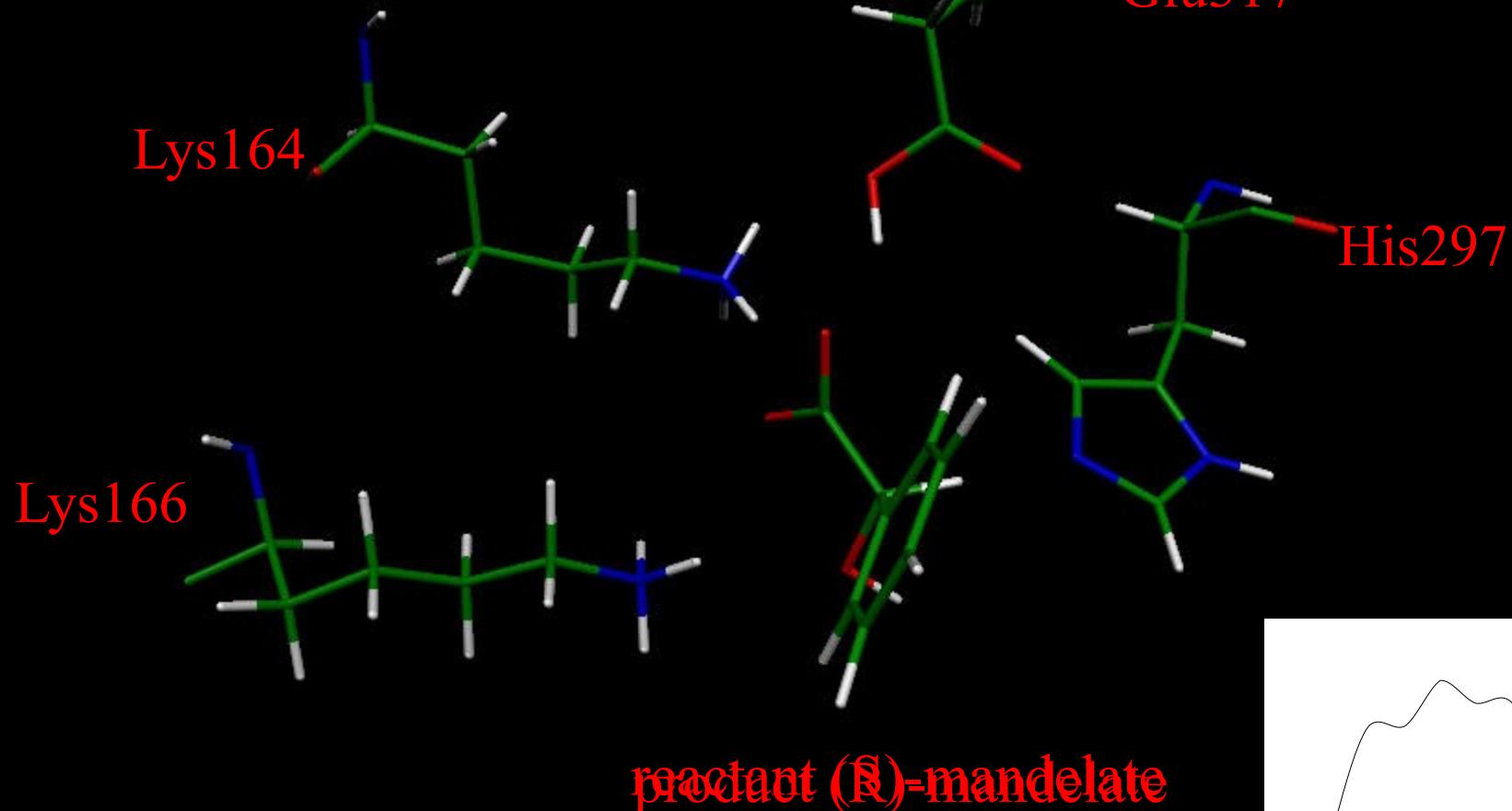


★ Elements stabilizing the anionic intermediate

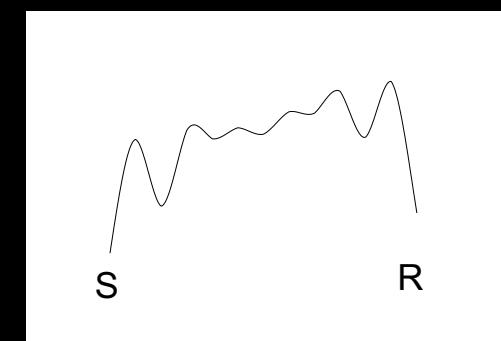
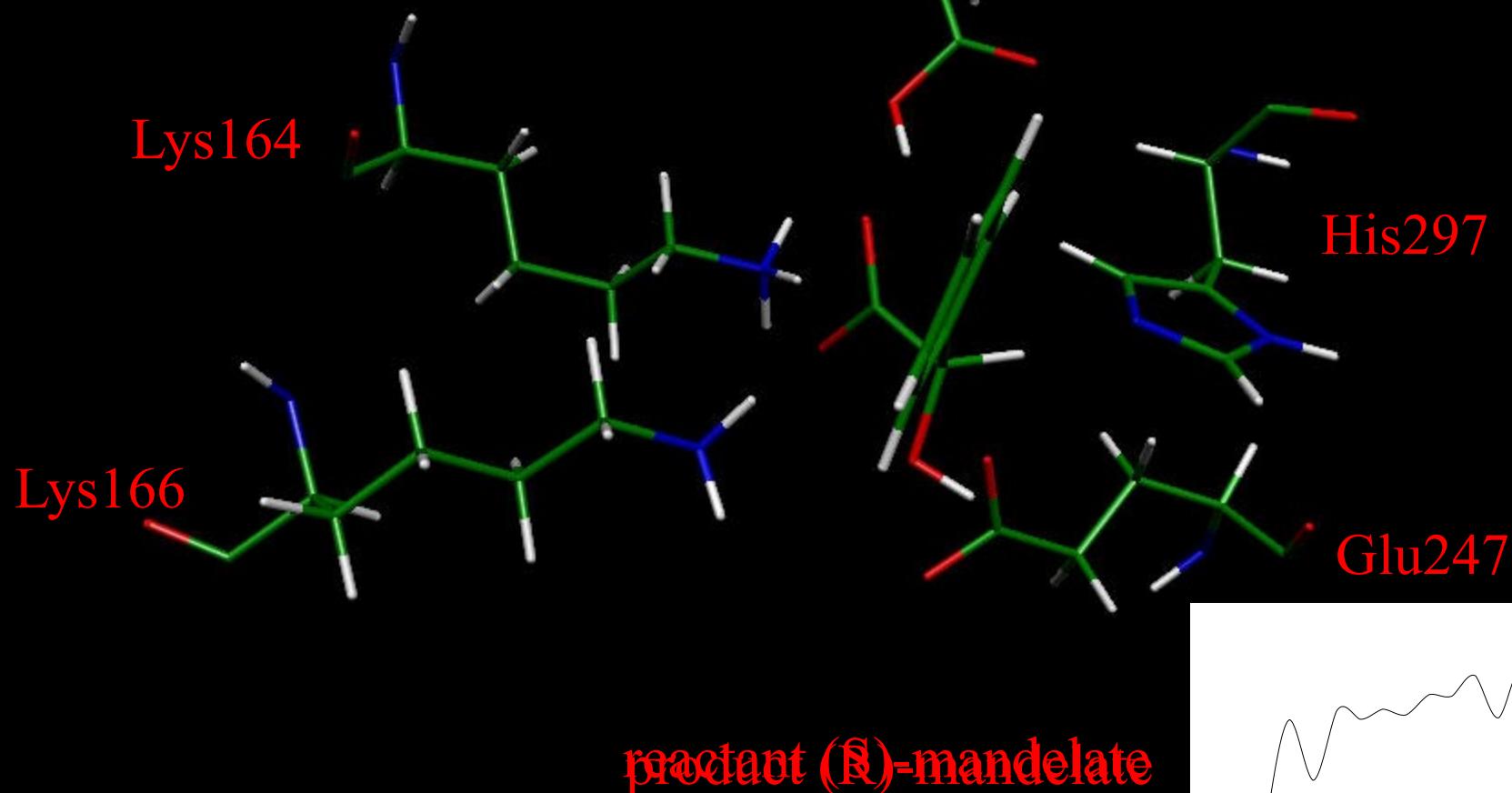
concerted mechanism



MR: Adiabatic mapping
concerted mechanism

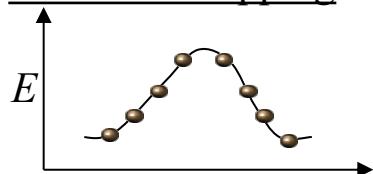


MR: Adiabatic mapping
stepwise mechanisms



Optimization of TS: possible options

Adiabatic mapping

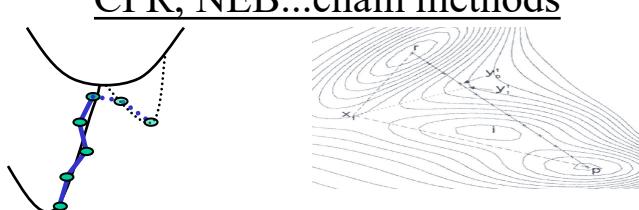


Easy to perform



Not always so
intuitive. Hysteresis
TS may not exist

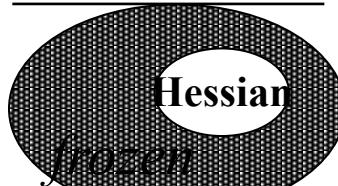
CPR, NEB...chain methods



Direct location
without Hessian
manipulation

There is no
transition vector
yet. Convergence
problems?

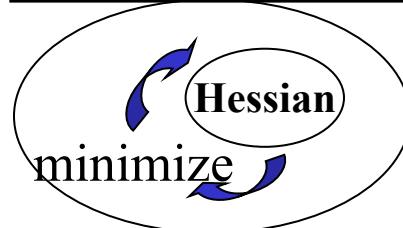
Hessian for a core



2nd order direct
location

The environment is
never relaxed

Iterative core/environment

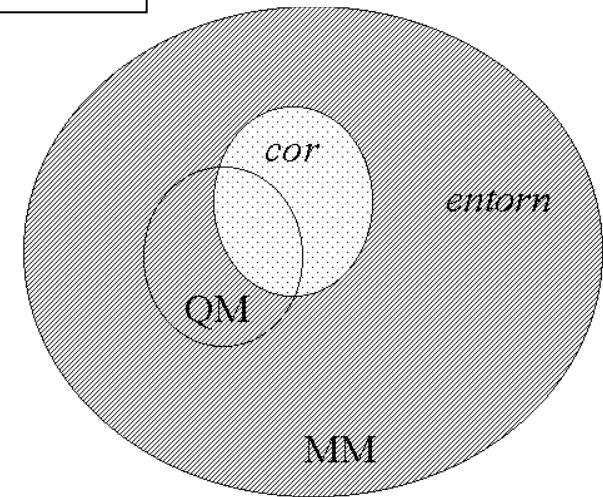
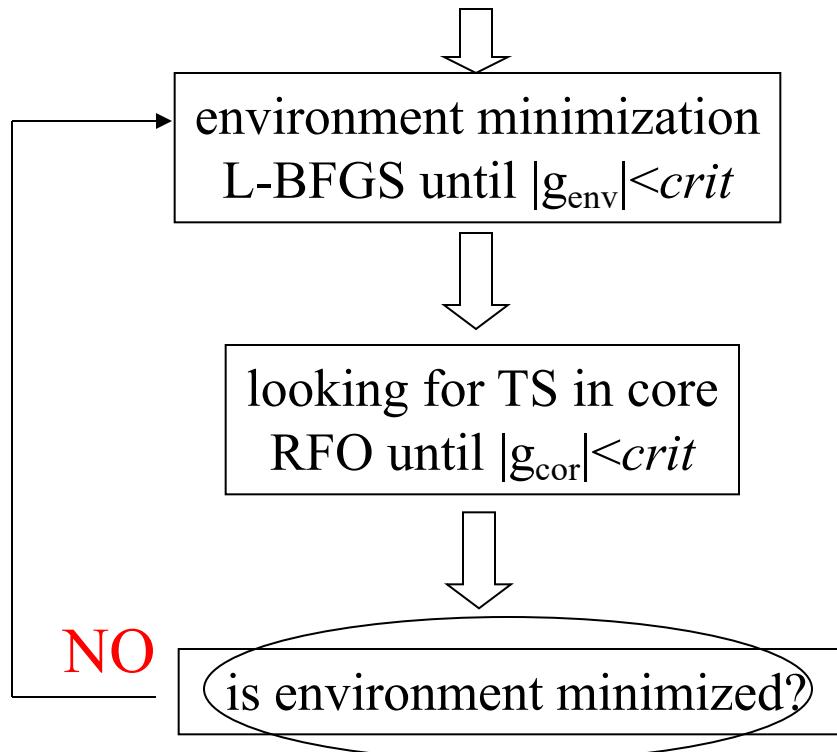


2nd order direct
location. Permits the
relaxation of the
environment

If core is not big
there will be
coupling between
the two zones

TS search: algorithm

Initial geometry with a core and an environment



Suggestions:

An adequate core size must be selected.

When minimizing,
the QM wavefunction can be
kept frozen (1SCF)

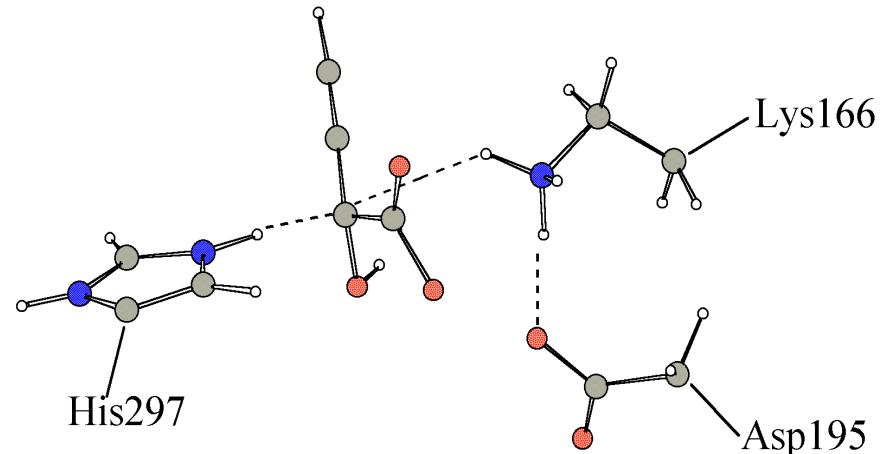
TS search: comparison refined TS with adiabatic mapping

mandelate substrate				propargyl-glyc. substrate		
Structure	Stepwise I	Stepwise II	Concerted	Stepwise I	Stepwise II	Concerted
S	0.00	0.00	0.00	0.00	0.00	0.00
TS1	17.77 (18.24)	17.69 (17.78)		15.90 (19.66)	11.83 (11.24)	
TS2	19.52 (19.65)	14.77 (14.46)		19.88 (19.92)	15.33 (15.32)	
TS3	20.04 (20.06)	14.55 (14.95)		22.18 (22.20)	16.96 (16.99)	
TS4	22.54 (22.56)		20.19 (19.50)	30.12 (22.20)	28.11 (20.68)	22.05 (21.98)
TS5	25.15 (25.75)	23.57 (23.83)		23.16 (22.60)	19.23 (20.08)	
TS6	27.22 (27.28)	28.14 (28.18)		23.89 (24.35)	26.45 (24.59)	
R	6.74	6.74	4.63	3.34	3.34	3.34

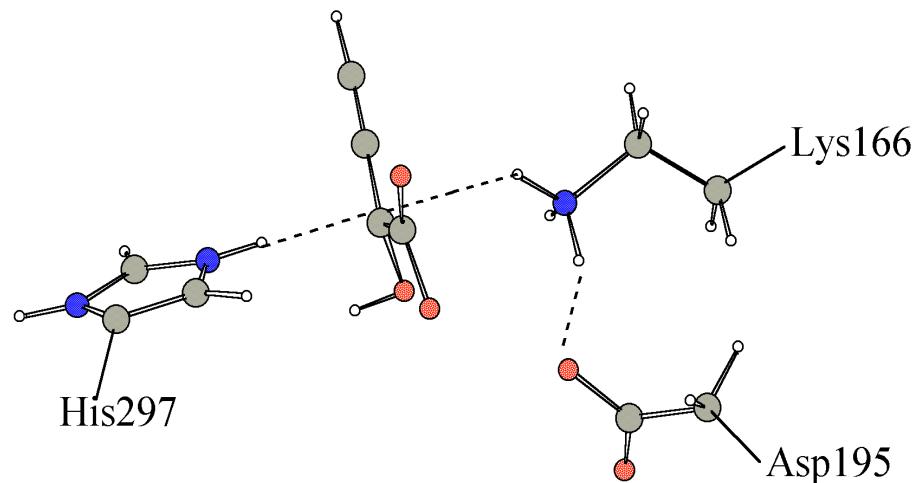
Energy in kcal/mol.

In brackets the value corresponding to the refined structure

TS search: comparison refined TS with adiabatic mapping



Adiabatic
mapping

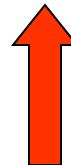


TS search

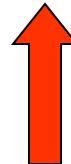
Energetic and structural differences exist

PMF: computational details

Free energy calculation: umbrella sampling



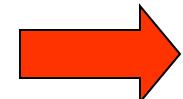
Molecular Dynamics: SBMD



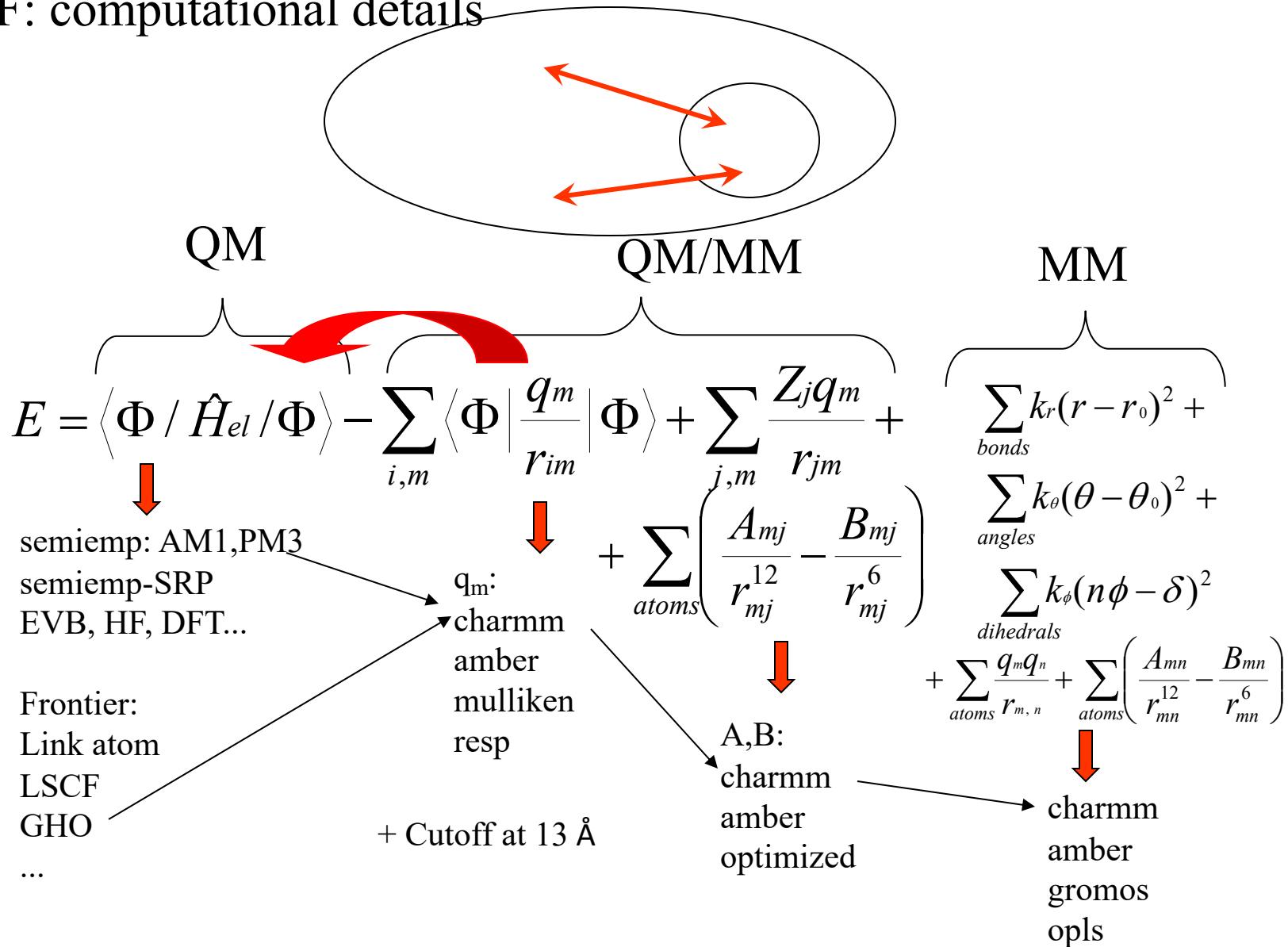
Potential Energy Surface: QM/MM



Structure: PDB



PMF: computational details



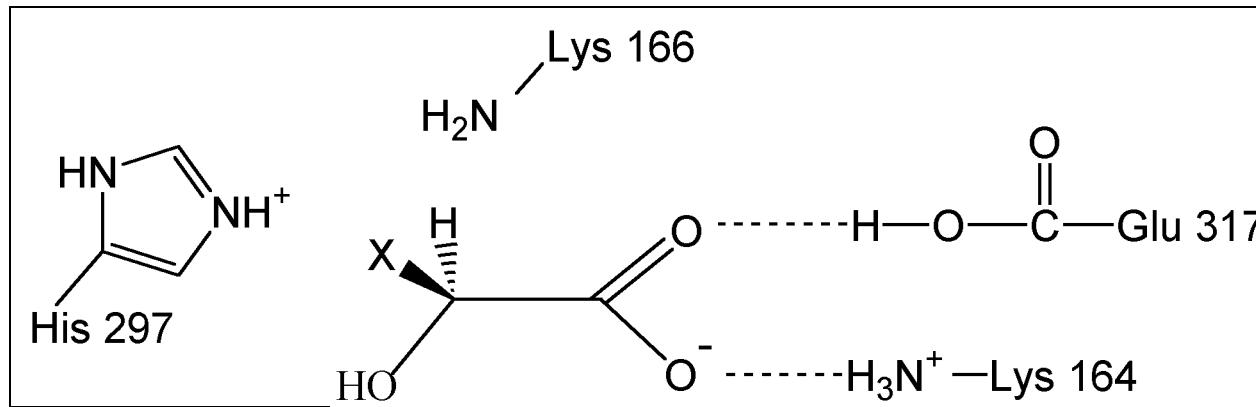
PMF: computational details

Are non-bonded interactions univoquely reproduced in QM/MM?

AMBER ARG		
N	N	-0.34790
H	H	0.27470
CA	CT	-0.26370
HA	H1	0.15600
CB	CT	-0.00070
HB2	HC	0.03270
HB3	HC	0.03270
CG	CT	0.03900
HG2	HC	0.02850
HG3	HC	0.02850
CD	CT	0.04860
HD2	H1	0.06870
HD3	H1	0.06870
NE	N2	-0.52950
HE	H	0.34560
CZ	CA	0.80760
NH1	N2	-0.86270
HH11	H	0.44780
HH12	H	0.44780
NH2	N2	-0.86270
HH21	H	0.44780
HH22	H	0.44780
C	C	0.73410
O	O	-0.58940

CHARMM ARG		
N	NH1	-0.47
HN	H	0.31
CA	CT1	0.07
HA	HB	0.09
CB	CT2	-0.18
HB1	HA	0.09
HB2	HA	0.09
CG	CT2	-0.18
HG1	HA	0.09
HG2	HA	0.09
CD	CT2	0.20
HD1	HA	0.09
HD2	HA	0.09
NE	NC2	-0.70
HE	HC	0.44
CZ	C	0.64
NH1	NC2	-0.80
HH11	HC	0.46
HH12	HC	0.46
NH2	NC2	-0.80
HH21	HC	0.46
HH22	HC	0.46
C	C	0.51
O	O	-0.51

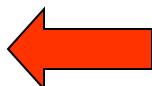
PMF: computational details



X: mandelate

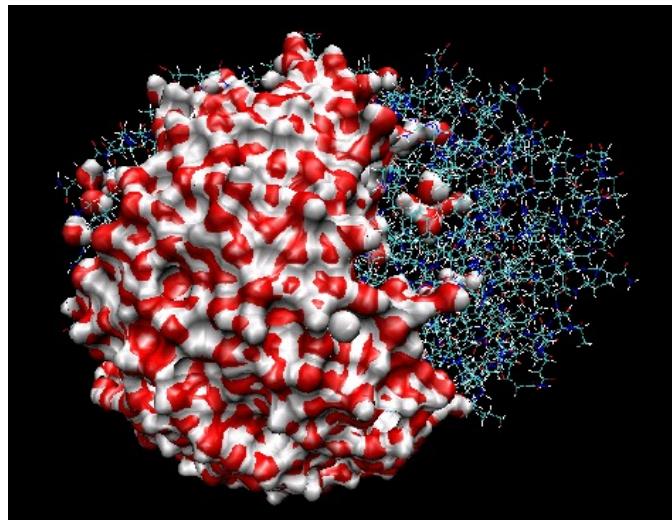
63 atoms PM3-GHO / 8208 CHARMM

Study of the concerted mechanism



PMF: computational details

Stochastic Boundary MD with a sphere of waters 24 Å

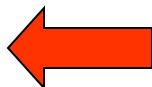


0-20:Newton MD zone

20-24: Langevin MD zone
harmonic restraint+friction + stochastic

$$m_i \ddot{q}_i(t) = F_i - m_i \Omega_i^2 [q_i(t) - q_i^{ref}] - m_i \beta_i \dot{q}_i(t) + f_i(t)$$

24-...: fixed zone



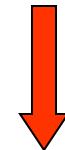
PMF: computational details

Scanning Rc in
increments of
0.2 Å windows

Every window:
15ps eq / 50ps sampling

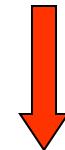
$$V_{\text{tot}} = V_{\text{QM/MM}} + k(Rc - Rc_0)^2 + Px(Rc)$$

Molecular Dynamics



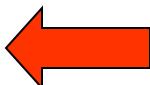
$$\langle \rho(Rc_0) \rangle = \frac{\int \delta(Rc(r) - Rc_0) \exp[-V_{\text{tot}}(r)/K_B T] dr}{\int \exp[-V_{\text{tot}}(r)/K_B T] dr}$$

*Statistical treatment
(WHAM)*

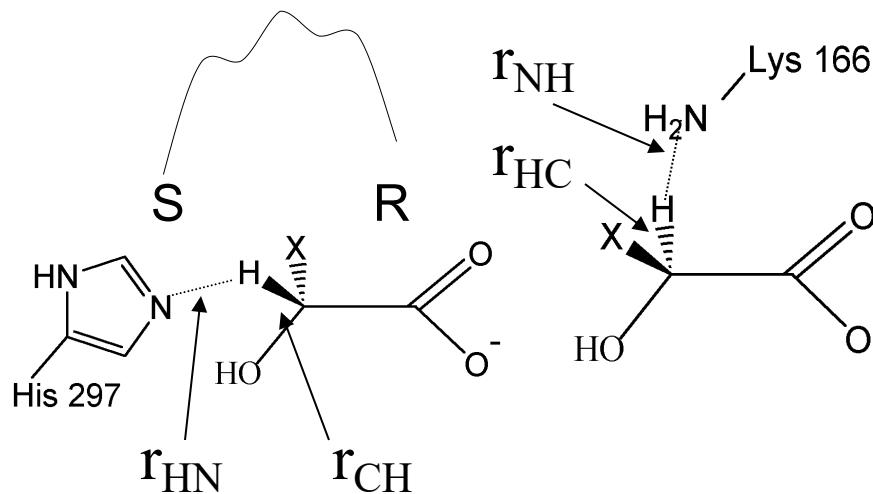


$$PMF = f(Rc) !!!$$

$$W(R_c) = -RT \ln \rho(R_c) + C$$



PMF: Selection of a Rc

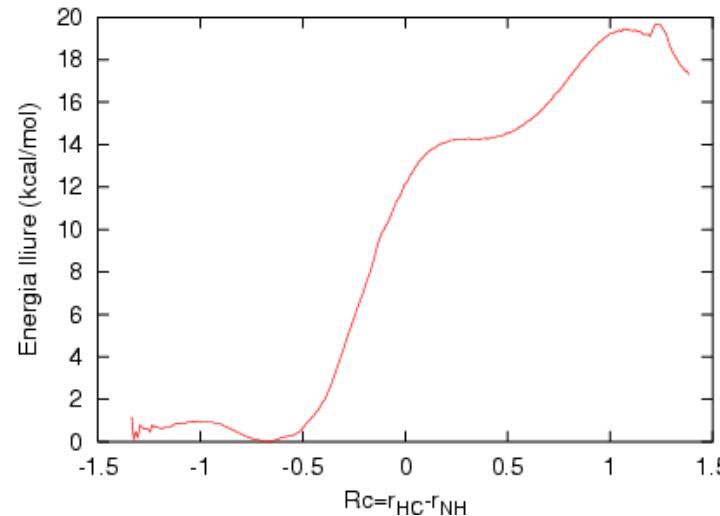
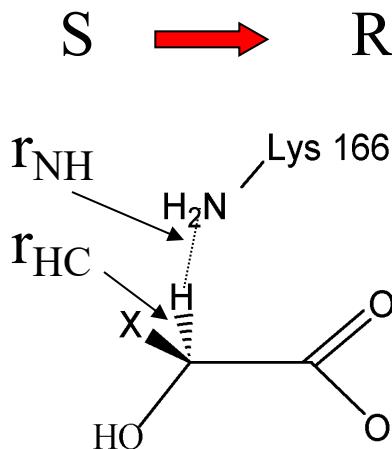


Data from TSsearch

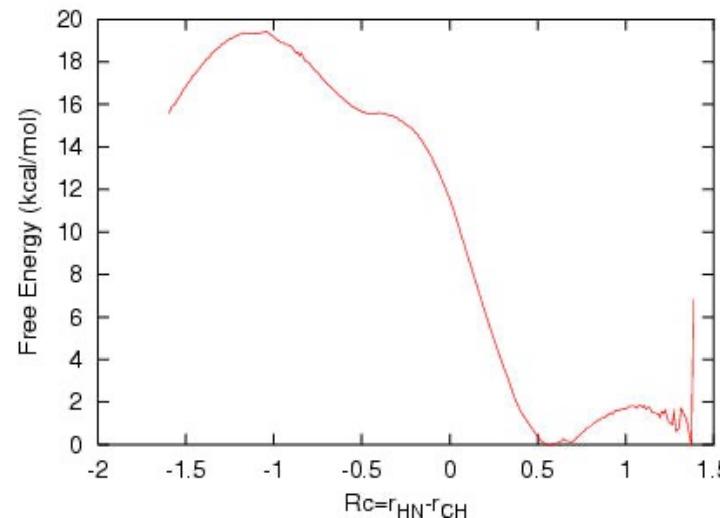
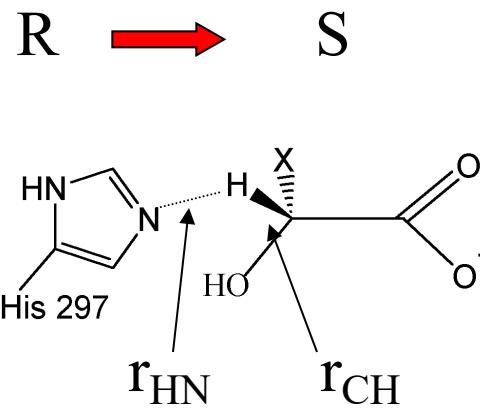
	r_{NH}	r_{HC}	r_{CH}	r_{HN}
S	1.804	1.158	2.927	0.995
Sts	1.194	1.522	2.865	0.996
Is	1.151	1.570	2.845	0.995
TS	1.011	2.258	2.093	1.013
Ir	1.055	2.612	1.607	1.117
Rts	1.004	2.736	1.509	1.200
R	1.004	2.868	1.165	1.772

R_c	$r_{HC}-r_{NH}$	$r_{HN}-r_{CH}$	$r_{HC}-r_{CH}$	$r_{HC}-r_{NH}$ $+r_{HN}-r_{CH}$	$r_{NC}-r_{CN}$	Improper Θ	ΔE
S	-0.646	-1.932	-1.769	-2.578	-0.043	0.421	0.0
Sts	-0.328	-1.869	-1.343	-1.541	-0.220	0.412	13.81
Is	0.419	-1.850	-1.275	-1.431	-0.201	0.398	13.79
TS	1.247	-1.080	0.165	0.167	0.992	-0.058	19.47
Ir	1.607	-0.490	1.005	1.117	1.828	-0.306	16.46
Rts	1.732	-0.309	1.227	1.423	2.057	-0.312	16.75
R	1.864	-0.607	1.703	2.471	2.543	-0.350	4.61

PMF: two bond distances Rc

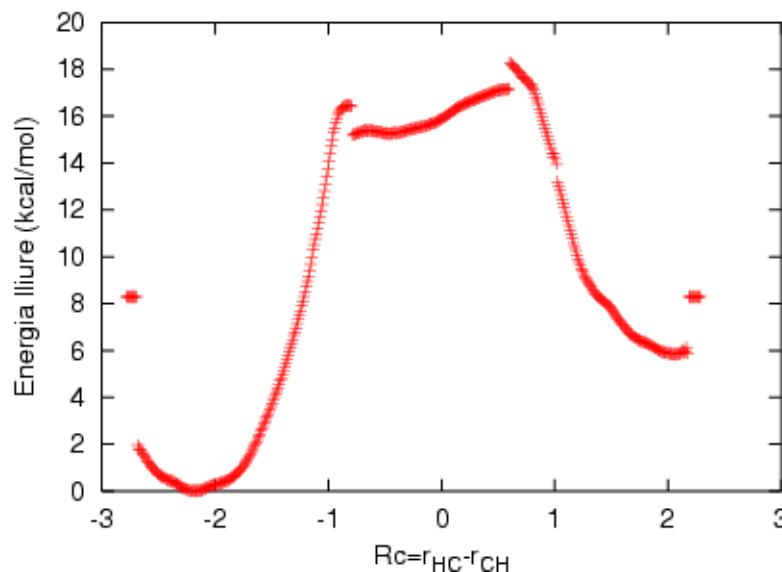
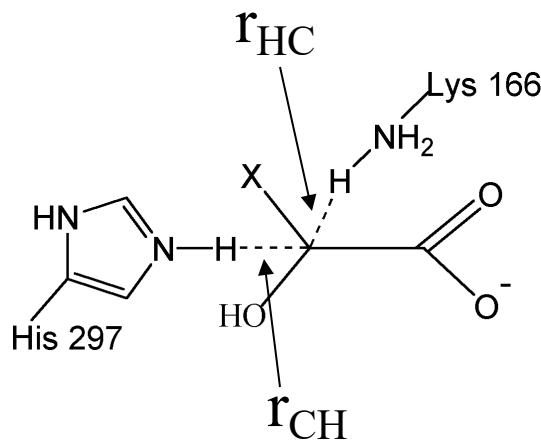


$$\mathbf{Rc} = \mathbf{r}_{\text{HC}} - \mathbf{r}_{\text{NH}}$$



$$\mathbf{Rc} = \mathbf{r}_{\text{CH}} - \mathbf{r}_{\text{HN}}$$

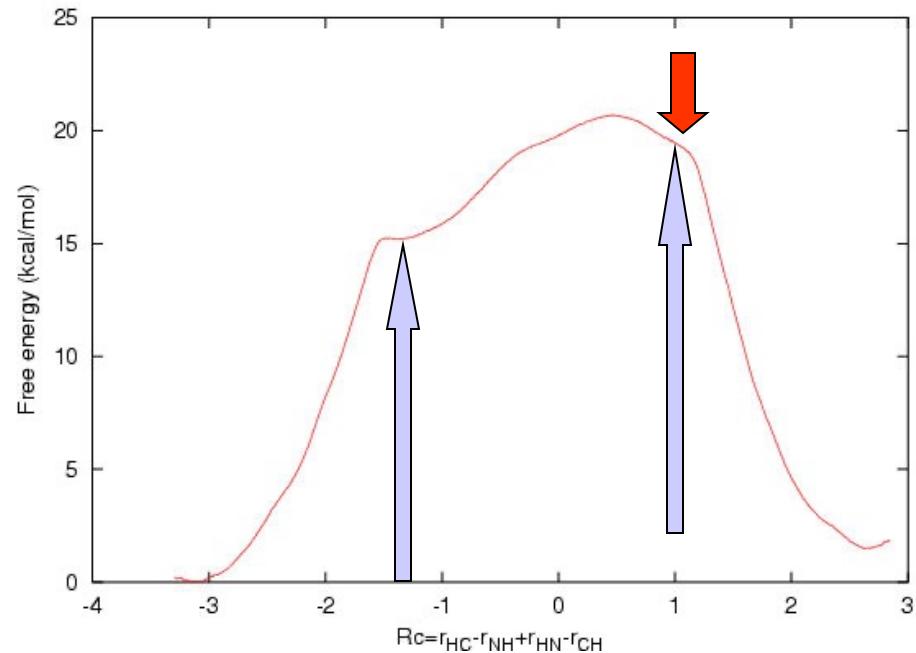
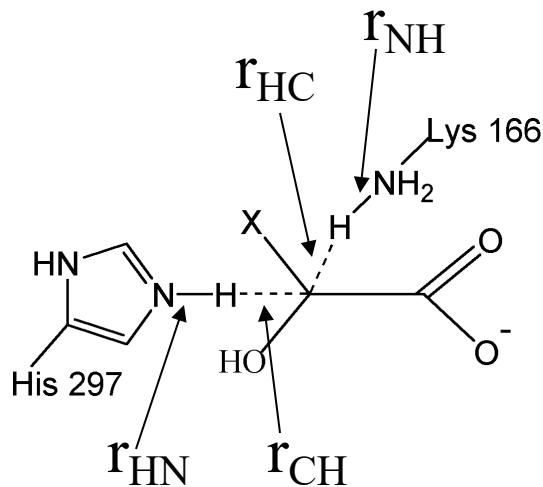
PMF: two bond distances Rc



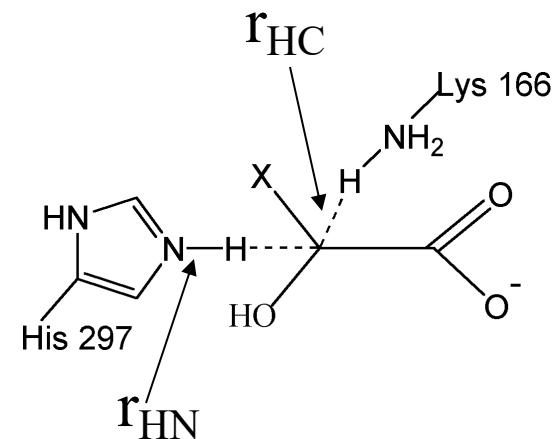
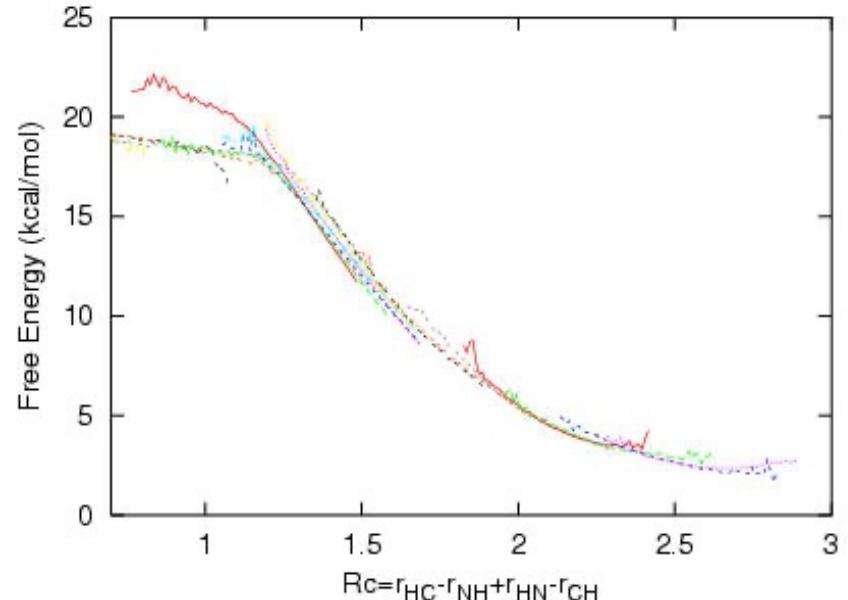
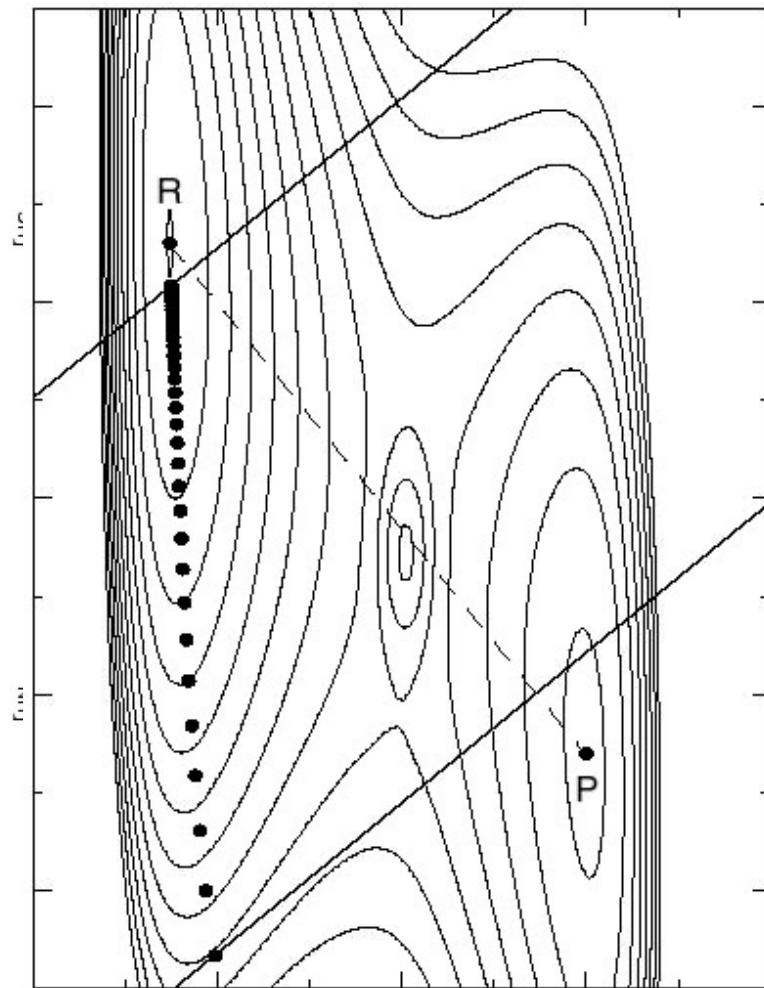
$$Rc = r_{HC} - r_{CH}$$

PMF: four bond distances Rc

$$Rc = r_{HC} - r_{NH} + r_{CH} - r_{HN} = R_4$$



PMF: some failures on R_4



PMF: switching functions with R_4

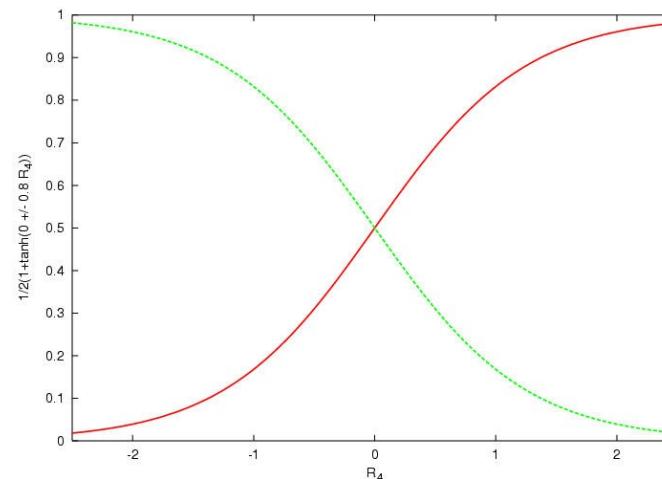
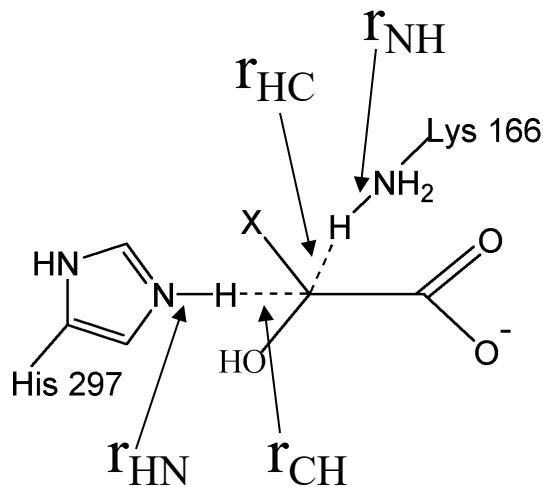
$$Rc = f_S(r_{HC} - r_{NH} + K) + f_R(r_{HN} - r_{CH} + K')$$

$$f_S(R_4) = \frac{1}{2}(1 + \tanh(c - p \cdot R_4))$$

$$f_R(R_4) = \frac{1}{2}(1 + \tanh(c + p \cdot R_4))$$

$K = r_{HN} - r_{CH}$ at S structure

$K' = r_{HC} - r_{NH}$ at R structure



PMF: mechanism. Distance analysis

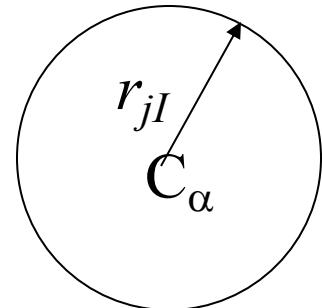
Pair of Interaction	Reactant	MinS	TS	MinR	Product
Interactions stabilizing reactants					
His297NH-Glu247OE1	2.48	2.83	2.88	2.93	3.15
His297NFH-Glu270OD1	1.75	1.72	1.85	1.84	2.28
Interactions stabilizing TS					
Lys166H-Asp195OD2	3.15	2.77	2.67	2.75	2.91
OE1-Ser139OH	1.92	1.90	1.79	1.79	2.08
OE1-Glu317H	1.78	1.72	1.72	1.72	1.76
OE2-Lys164NH	1.90	1.79	1.78	1.80	1.79
OE2-Lys164N	2.83	2.71	2.67	2.69	2.69
OE2-Mg	1.86	1.84	1.83	1.83	1.85
OG-Mg	2.17	2.16	2.10	2.12	2.14
Interactions stabilizing products					
Lys166H-Asn197OD1	3.74	2.06	2.27	2.76	2.38
The OH rotation					
OH-Glu247OE2	3.24	1.97	1.88	1.84	1.77
OG-Asn197HD22	3.01	2.02	2.28	2.27	2.25

Significative stabilization of TS with respect to reactants

PMF: mechanism. Electrostatic energy perturbation analysis

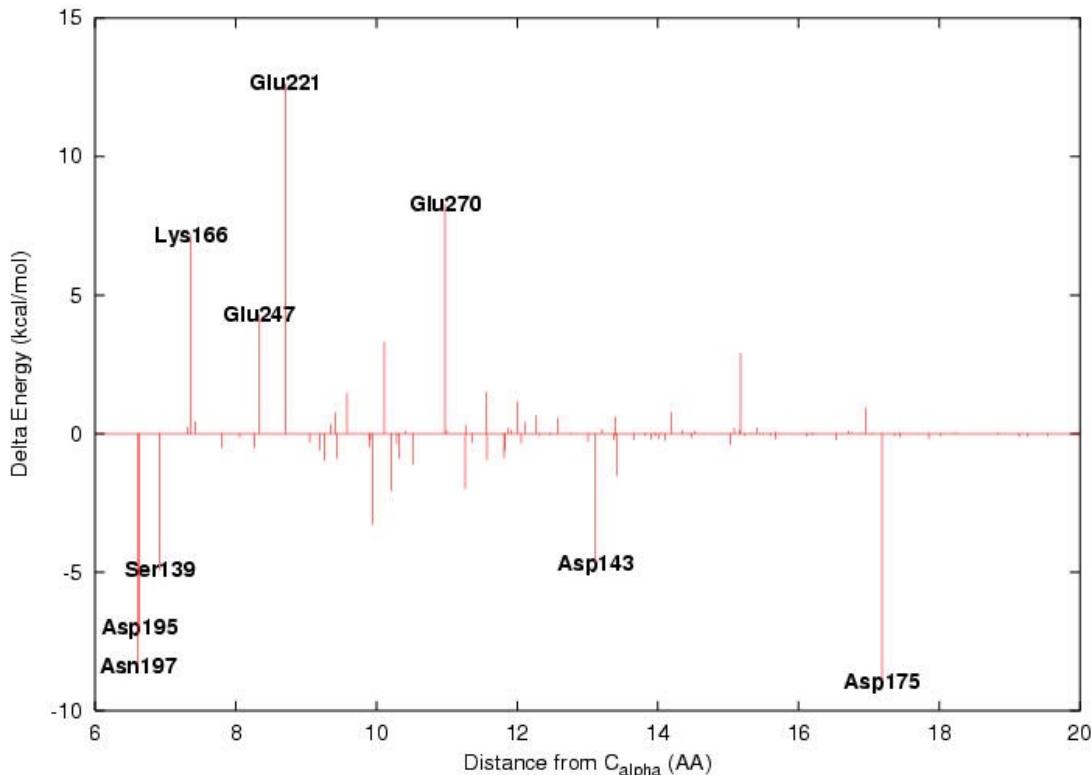
$$E_I = \left\langle \Phi / \hat{H}_{el} / \Phi \right\rangle - \sum_{i,m=I} \left\langle \Phi \left| \frac{q_m}{r_{im}} \right| \Phi \right\rangle + \sum_{j,m=I} \frac{Z_j q_m}{r_{jm}}$$

$$\Delta E_I = (E_I - E_{I-1})_{TS} - (E_I - E_{I-1})_{react}$$



TS

- ↑ destabilization
- ↓ stabilization

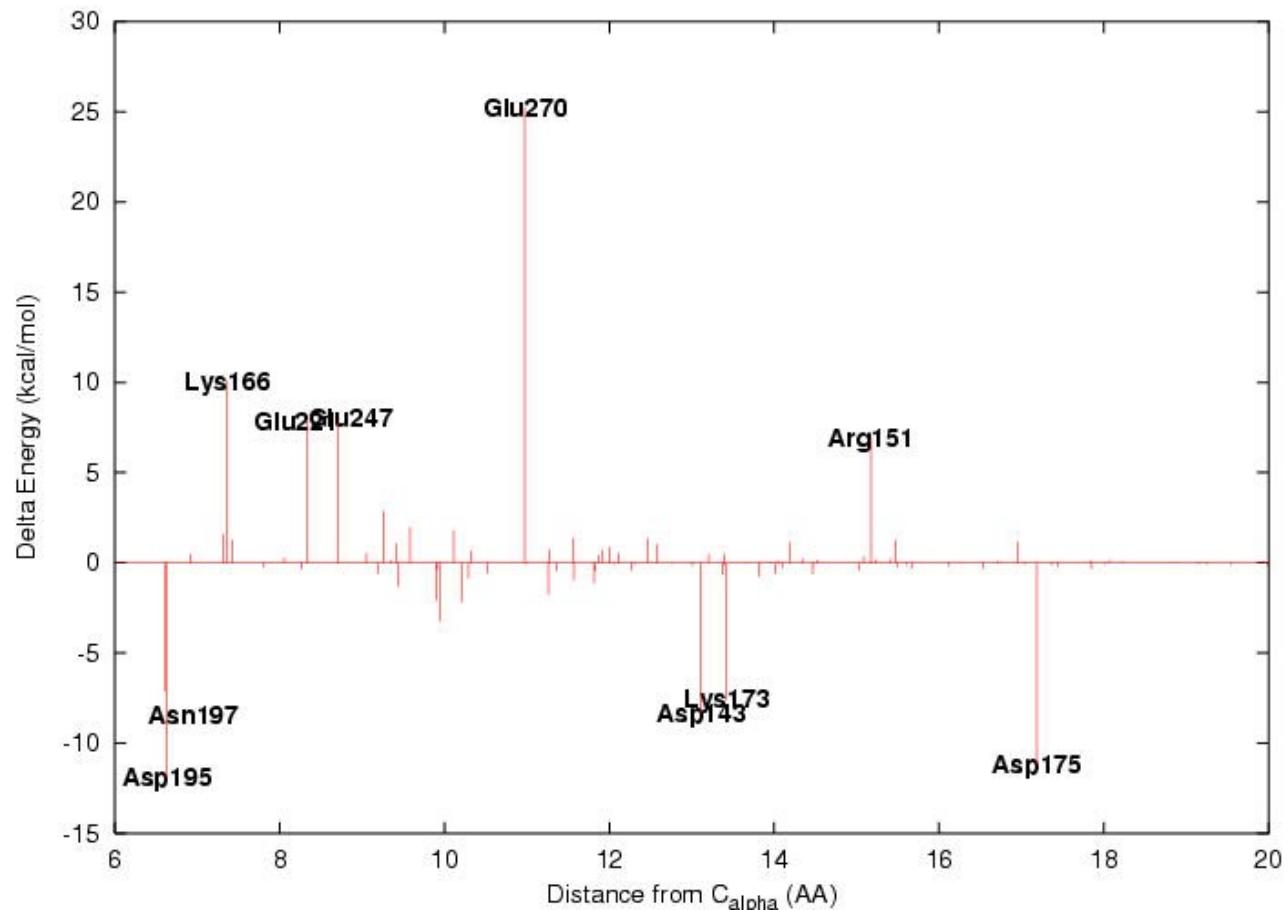


PMF: mechanism. Electrostatic energy perturbation analysis

$$\Delta E_I = (E_I - E_{I-1})_{prod} - (E_I - E_{I-1})_{react}$$

Product

- ↑ destabilization
- ↓ stabilization



PMF: mechanism. Comparison with uncatalyzed reaction

Usually found in the literature to know the origin of the catalysis

Feature Article

J. Phys. Chem. B, Vol. 105, No. 33, 2001 7905

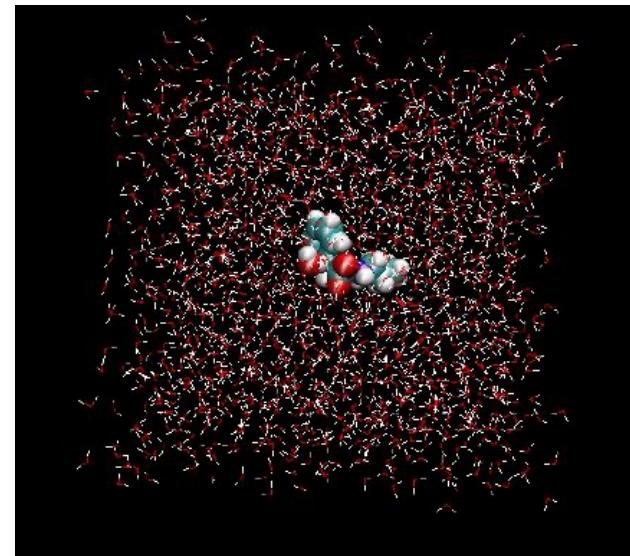
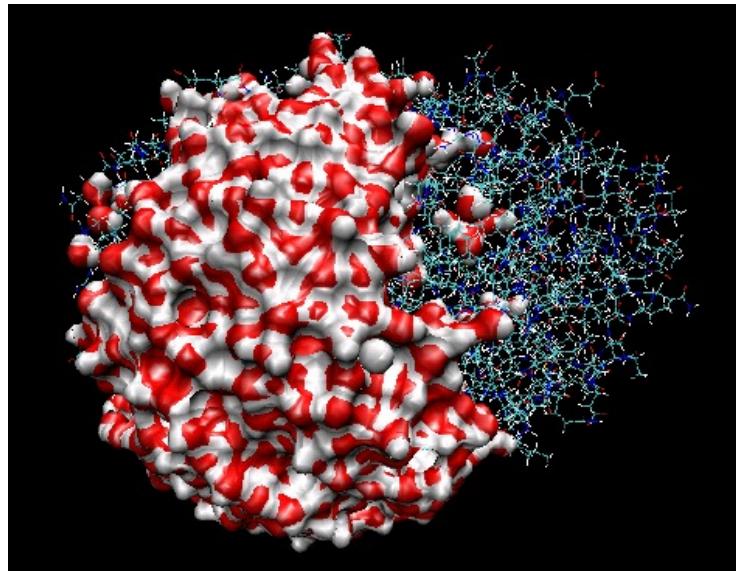
TABLE 1: Summary of Proposals for the Origin of Enzyme Catalysis

mechanisms	ts stabilization	consistent with mutations	supported by simulations	inconsistencies	quantitative analysis
desolvation ^{144,160–162}	–	–	–	a	75, 163
strain ^{1,144}	–	–	–	b	2, 16
entropy ^{40,144}	–	?	?	c	2, 151, 164
orbital steering ¹⁶⁵	–	?	–	d	2
dynamics ^{4,5,96,99}	–	?	–	e	2, 29
LBHB ^{166, 167}	+	+	–	f	168
electrostatics ^{16,77}	+	+	+		2, 76, 75
tunneling ^{4,5,122}	+	+	–	h	108, 128
diffusion ¹⁶⁹	+	+		h	58

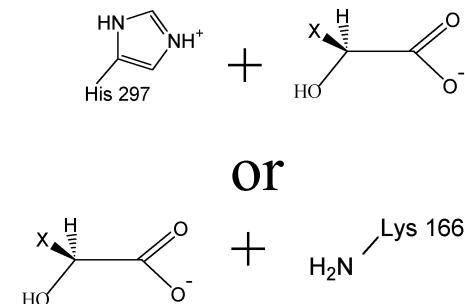
^a Groups that are supposed to be ionized are not ionized in the assumed nonpolar environment, and the desolvation energy is not taken into account.^{2,75} ^b Enzymes are too flexible.¹⁴⁴ ^c Original support from model compounds was found to be questionable^{2,164} and irrelevant,^{2,151} while simulation studies show that the actual catalytic effect is small.¹⁵¹ ^d The bending force constants at the TS are much smaller than the value needed for the proposed effect.² ^e Computer simulations produce very similar dynamical effects in enzymes and the corresponding solution reactions (this work, and refs 2 and 52). ^f Shown to be anticatalytic due to loss of solvation energy.¹⁶⁸ ^g Simulation studies produce a very small catalytic effect.¹²⁸

^h Observed diffusion effects are negligible, contributing around 1 kcal/mol to the reduction of Δg^\ddagger in cases where the overall reduction is larger than 10 kcal/mol.⁵⁸

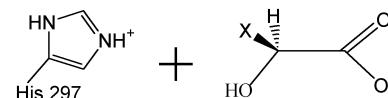
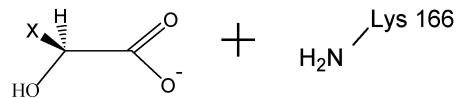
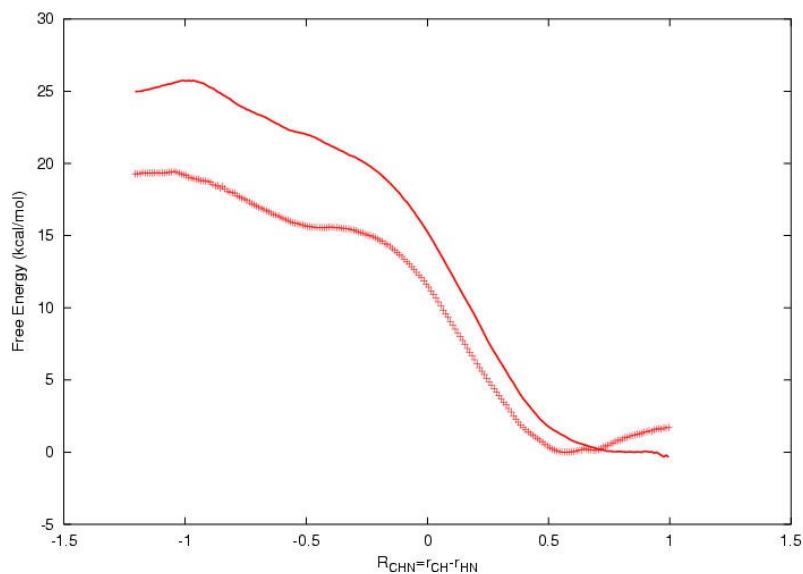
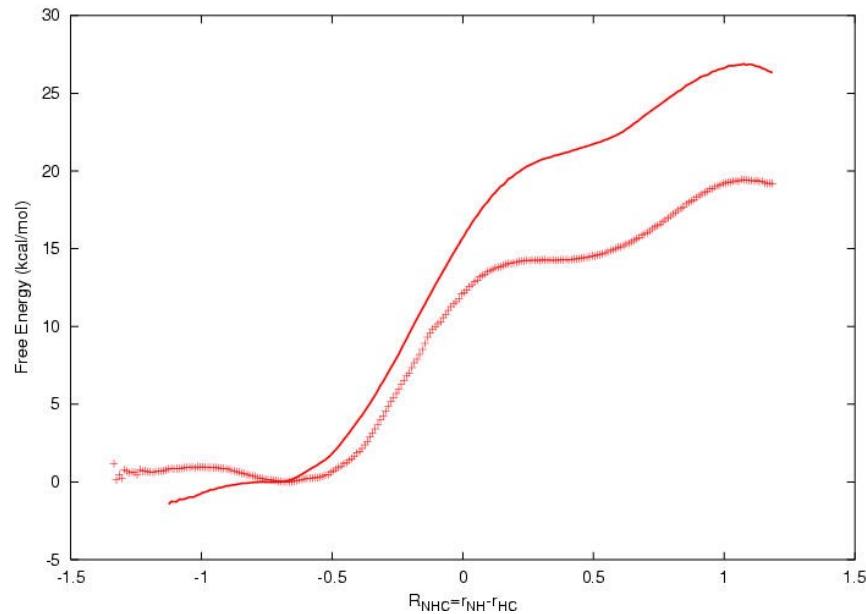
PMF: mechanism. Comparison with uncatalyzed reaction



Mandelate Racemase
+
substrate



PMF: mechanism. Comparison with uncatalyzed reaction



PMF: mechanism. Comparison with uncatalyzed reaction

Mulliken Charge(q) and Bond Order(BO) along
the reaction coordinate as descriptors of the chemical reaction

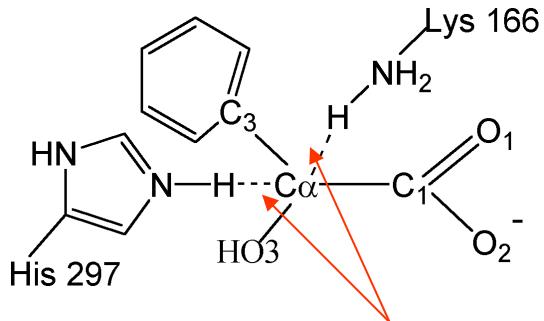
$$q_A = Z_A - \sum_{\mu \in A} (PS)_{\mu}$$

$$BO_{A-B} = \sum_{\mu \in A} \sum_{\nu \in B} (PS)_{\mu\nu} (PS)_{\nu\mu}$$

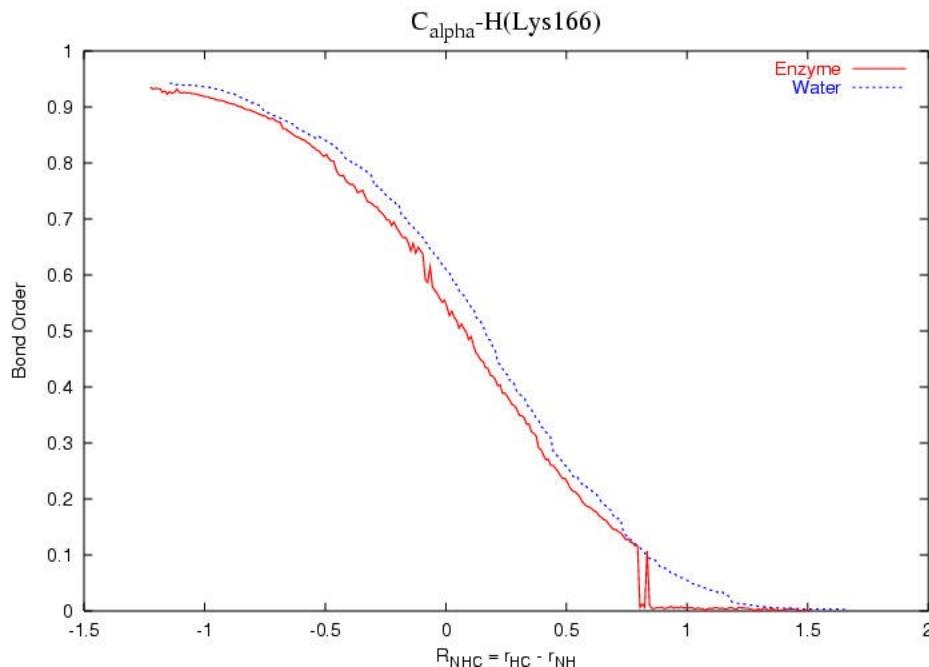
semiempirical $\sum_{\mu \in A} \sum_{\nu \in B} P^2_{\mu\nu}$

PMF: mechanism. Comparison with uncatalyzed reaction

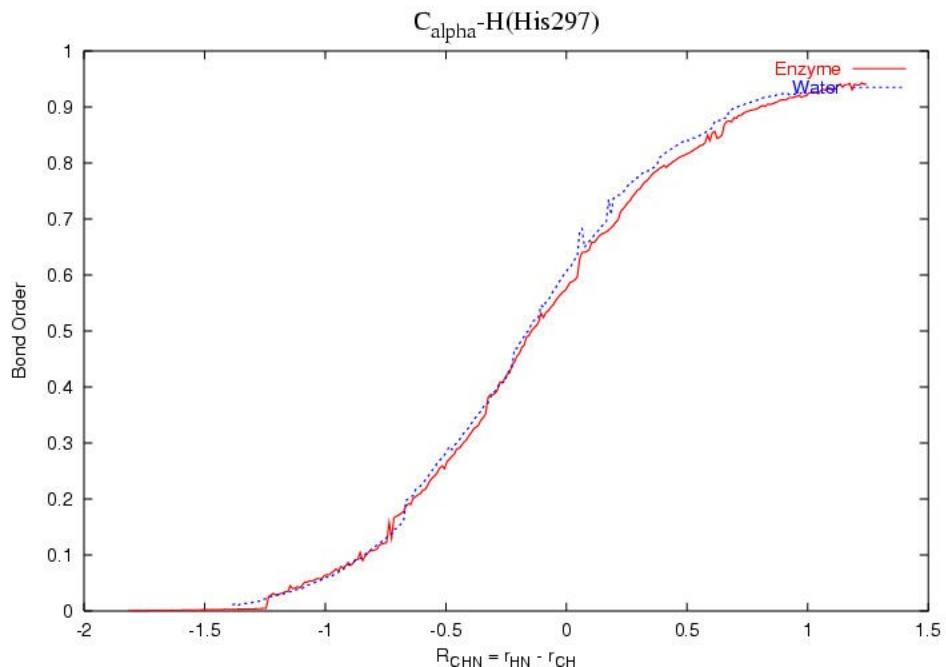
Bond Orders



substrate + Lys166

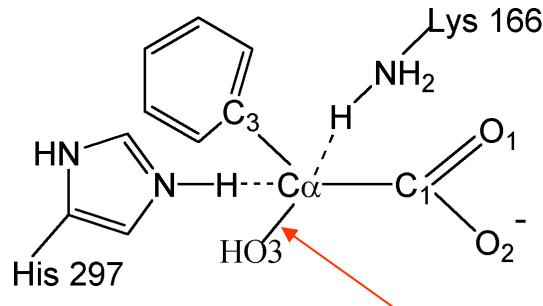


substrate + His297

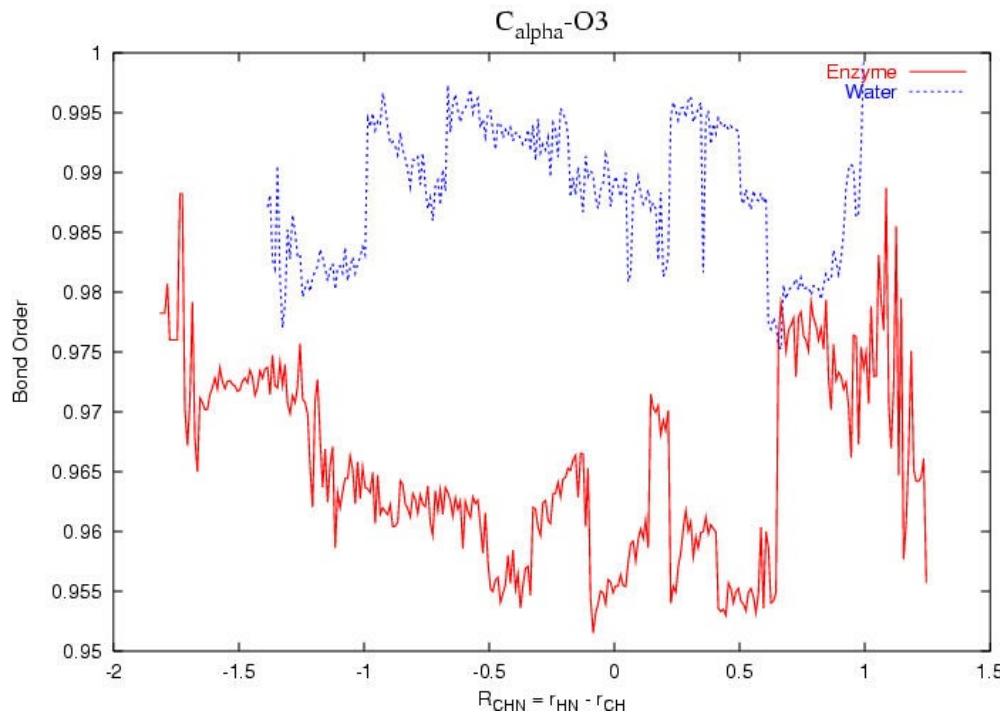


PMF: mechanism. Comparison with uncatalyzed reaction

Bond Orders

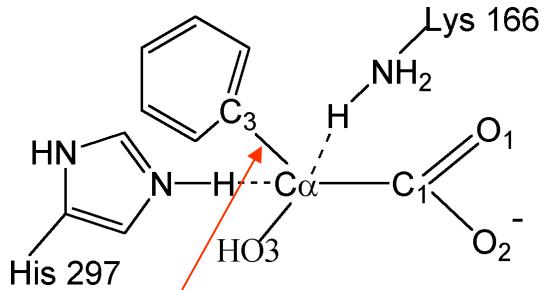


not correlated
values



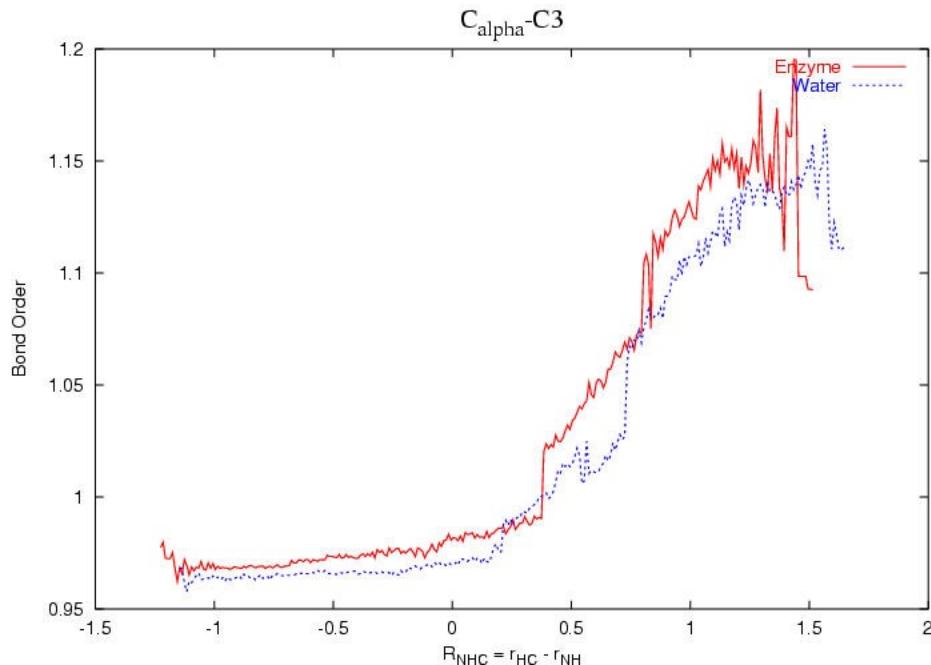
PMF: mechanism. Comparison with uncatalyzed reaction

Bond Orders

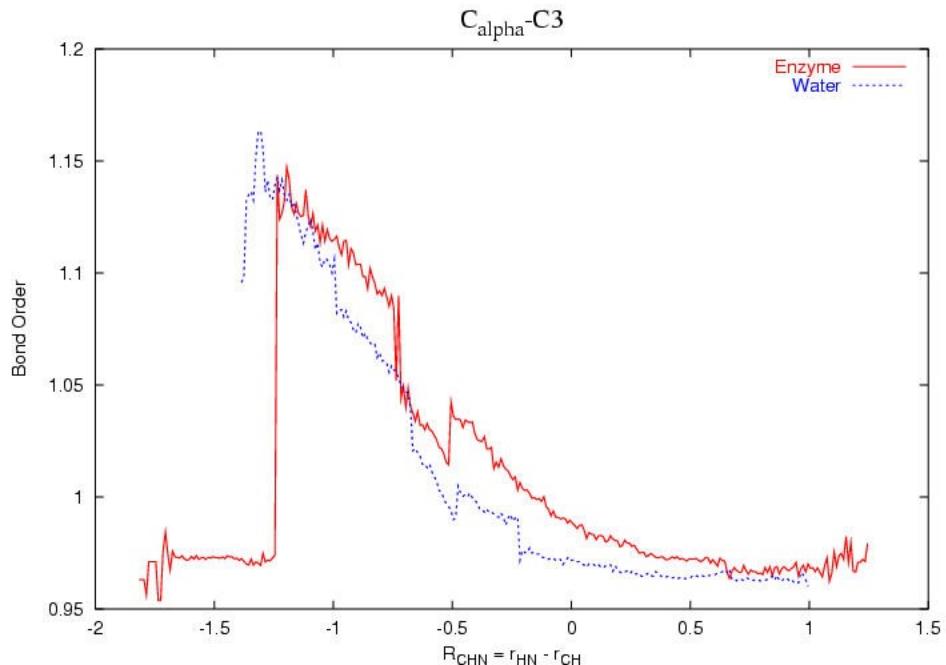


The enzyme delocalizes the charge in the phenyl ring

substrate + Lys166

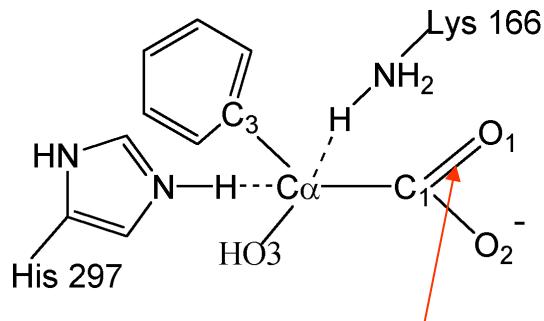


substrate + His297



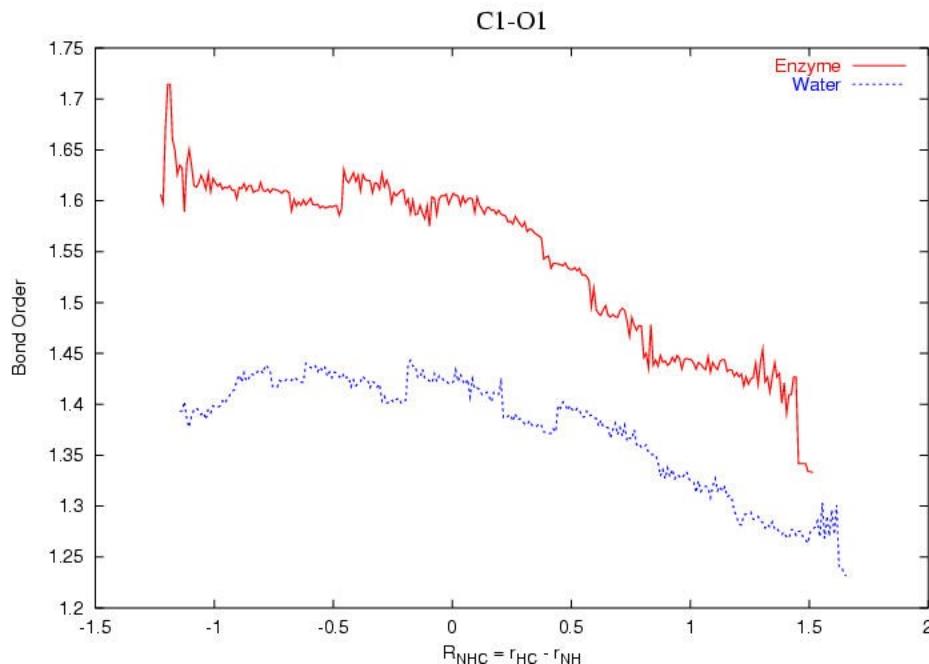
PMF: mechanism. Comparison with uncatalyzed reaction

Bond Orders

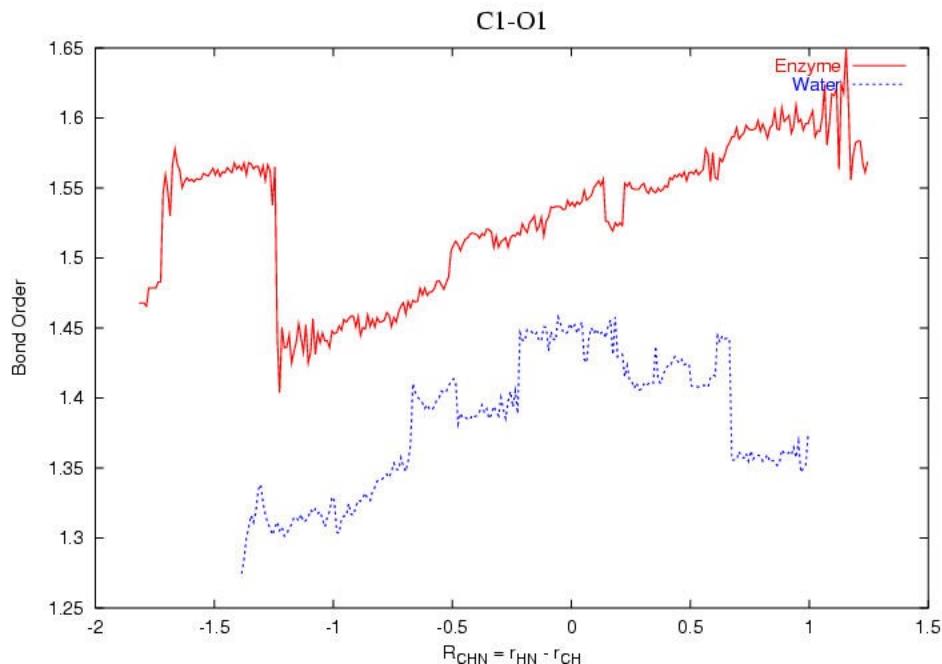


The symmetry of the carboxylic group is different but not correlated to the reaction: **Mg role**

substrate + Lys166

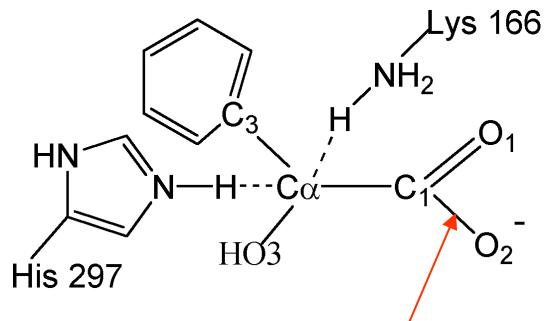


substrate + His297



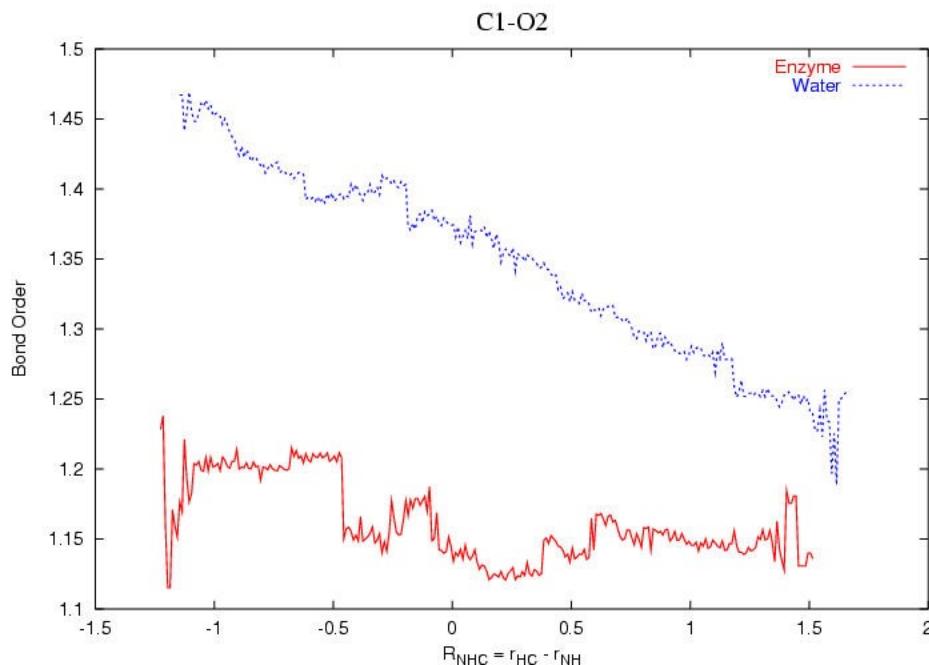
PMF: mechanism. Comparison with uncatalyzed reaction

Bond Orders

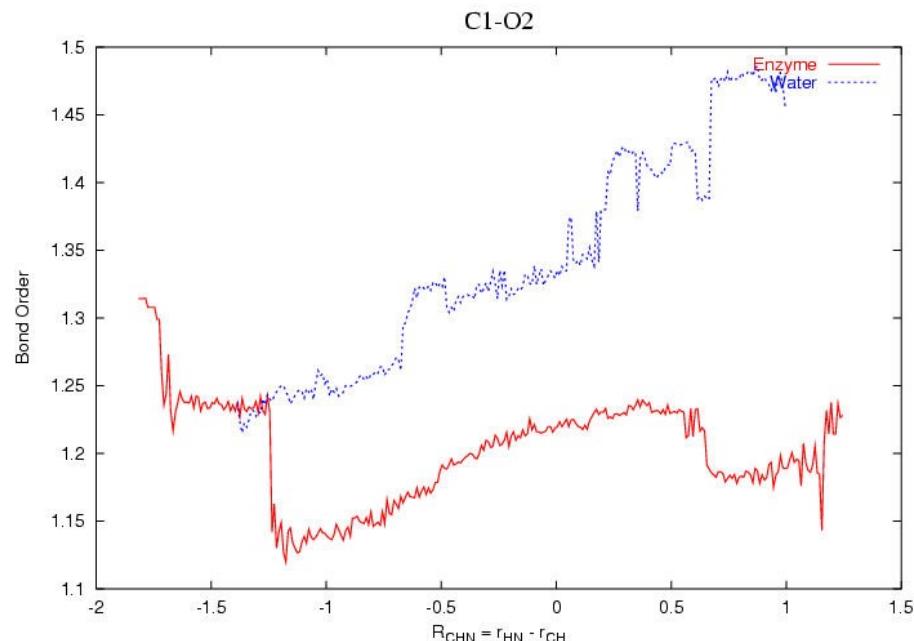


The symmetry of the carboxylic group is different but not correlated to the reaction: **Mg role**

substrate + Lys166

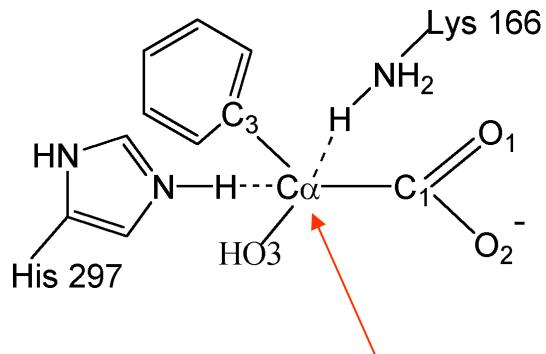


substrate + His297



PMF: mechanism. Comparison with uncatalyzed reaction

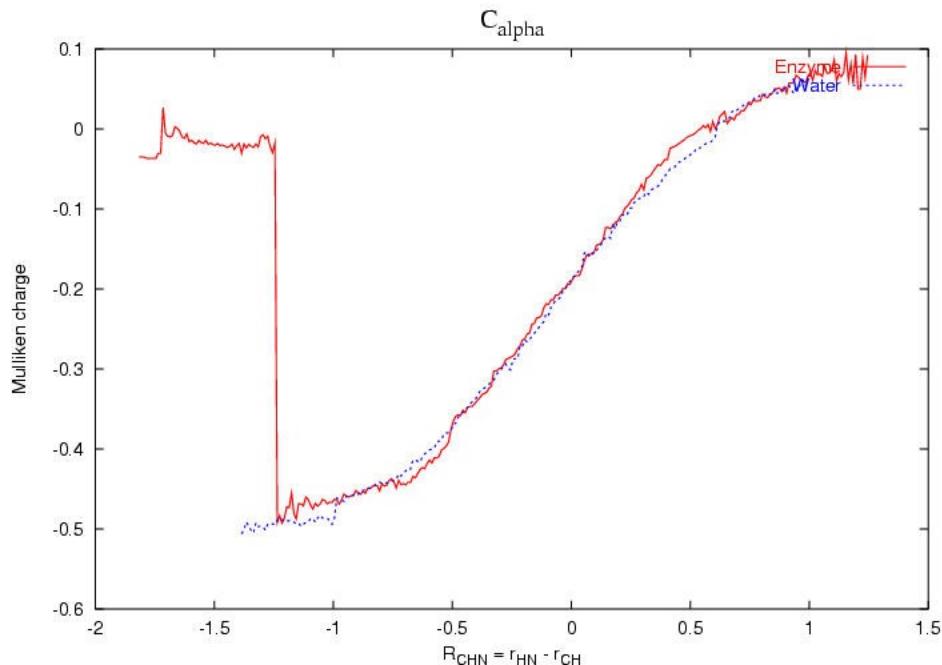
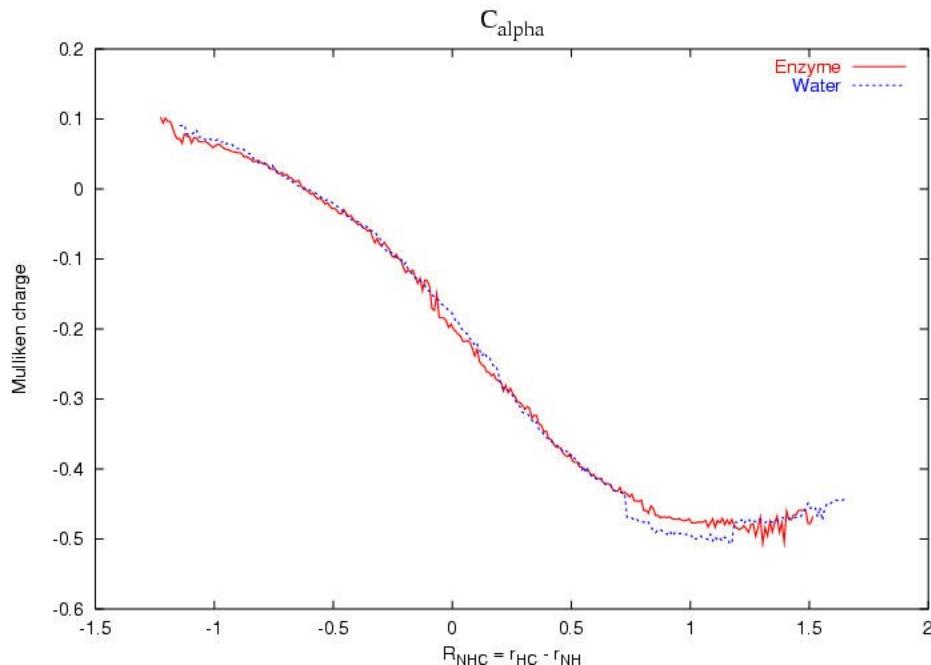
Atomic Charges



substrate + Lys166

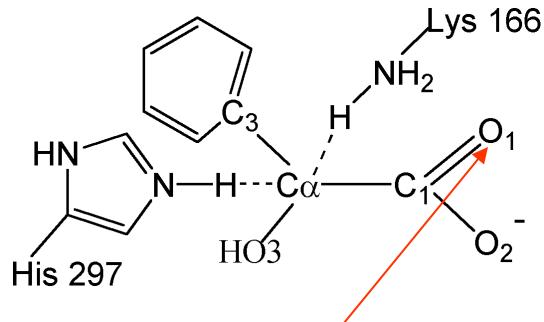
The enzyme does not subtract charge from the C_α

substrate + His297



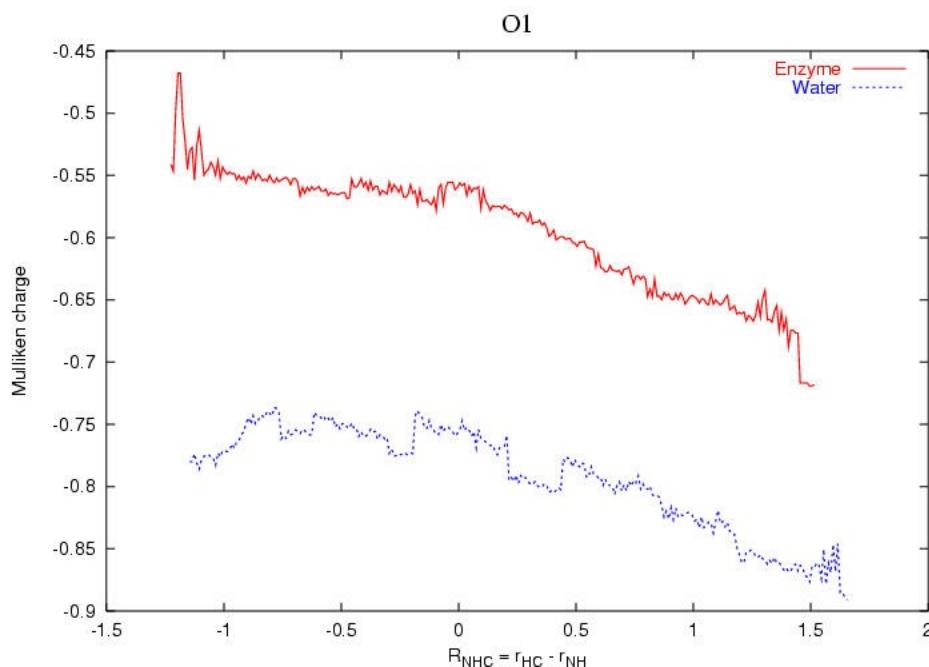
PMF: mechanism. Comparison with uncatalyzed reaction

Atomic Charges

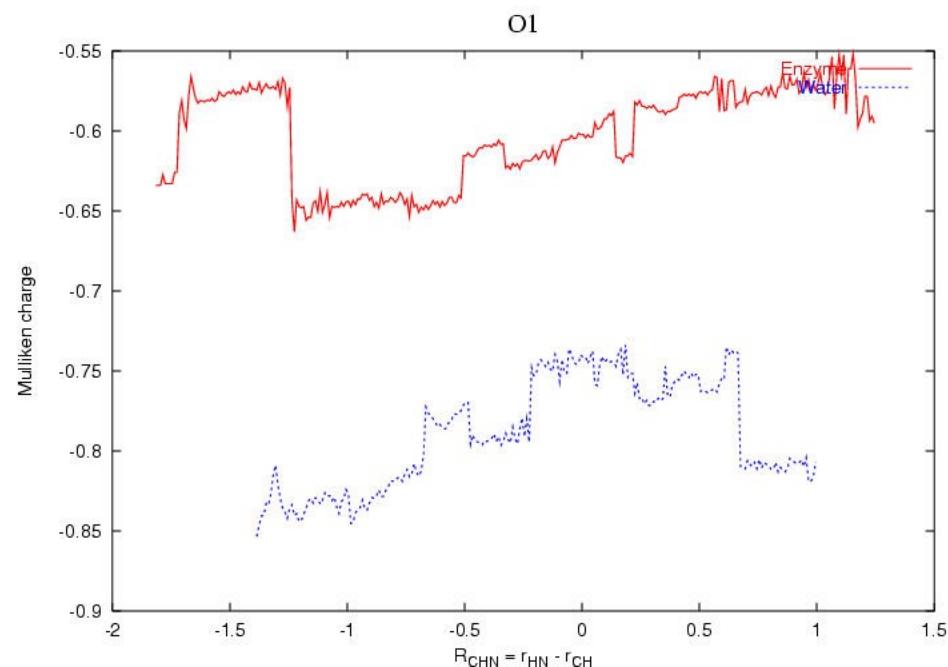


The symmetry of the carboxylic group is different but not correlated to the reaction: **Mg role**

substrate + Lys166

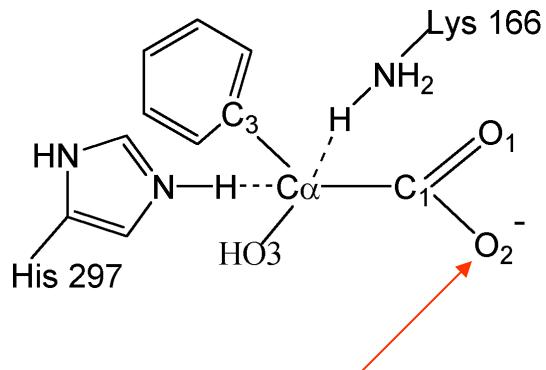


substrate + His297



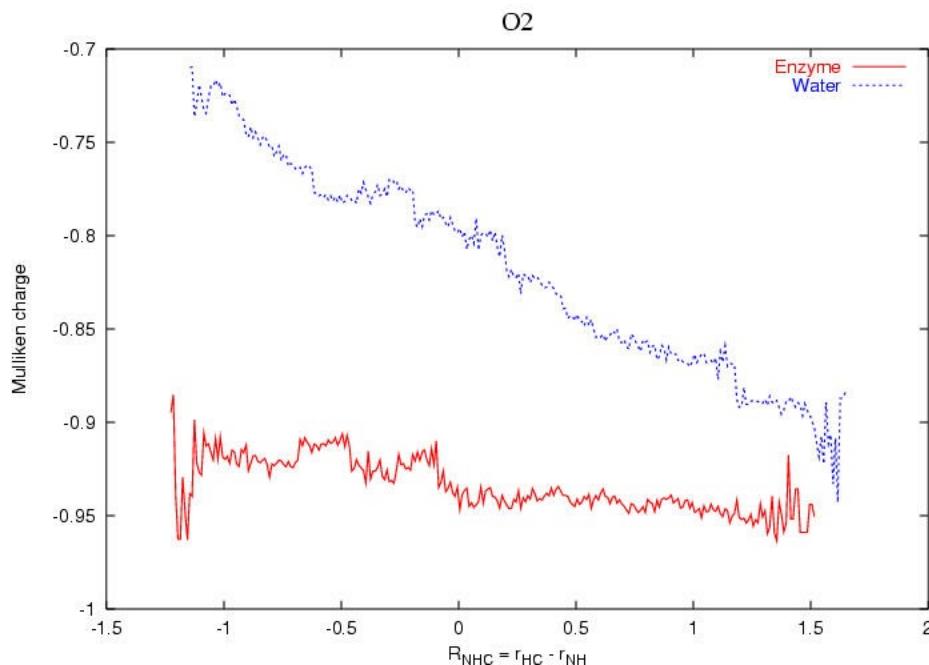
PMF: mechanism. Comparison with uncatalyzed reaction

Atomic Charges

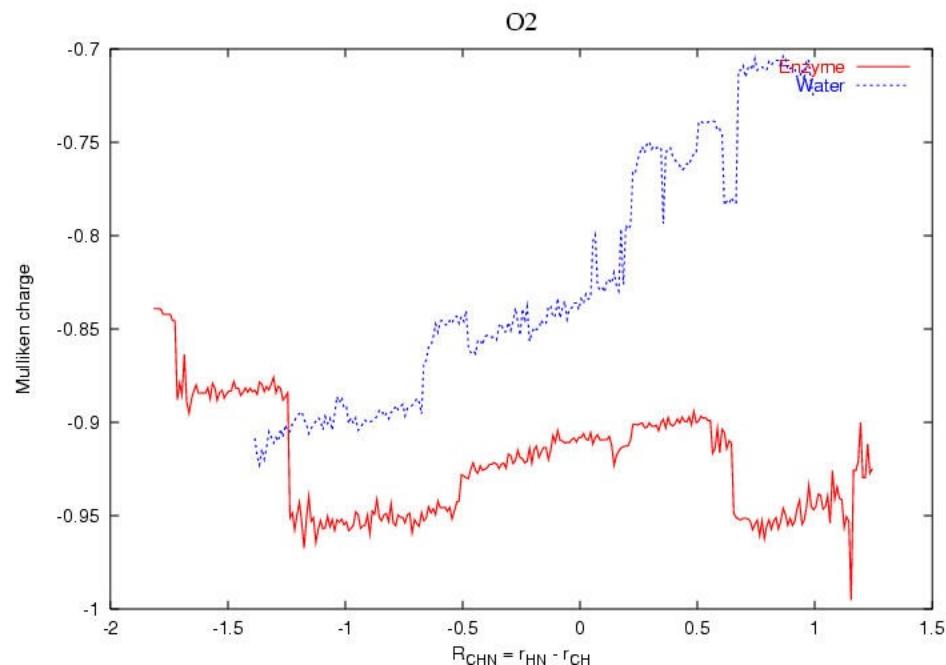


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substrate + Lys166

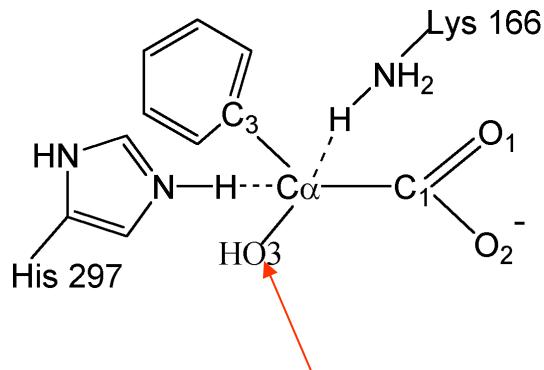


substrate + His297



PMF: mechanism. Comparison with uncatalyzed reaction

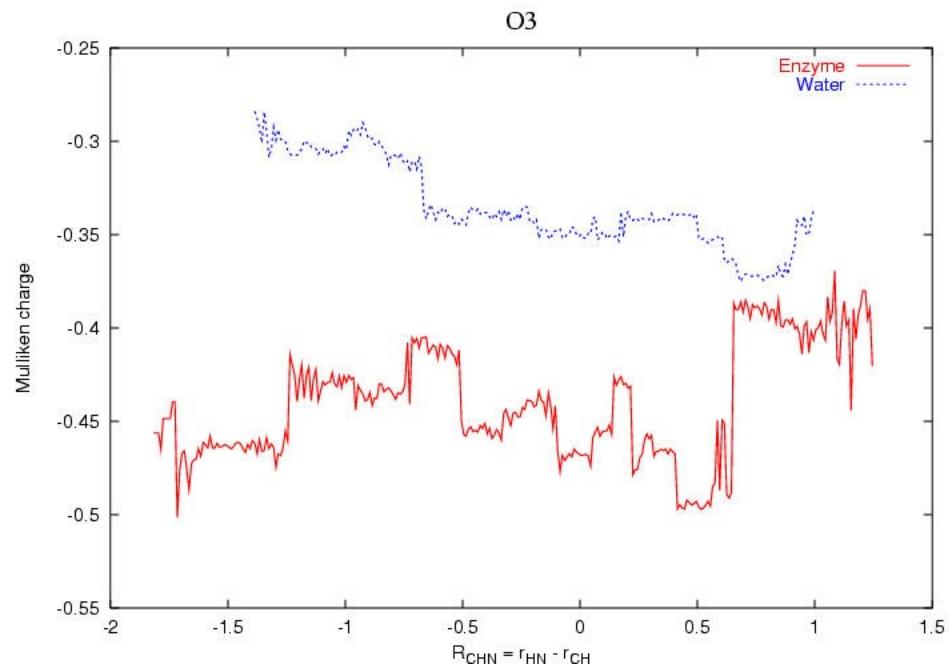
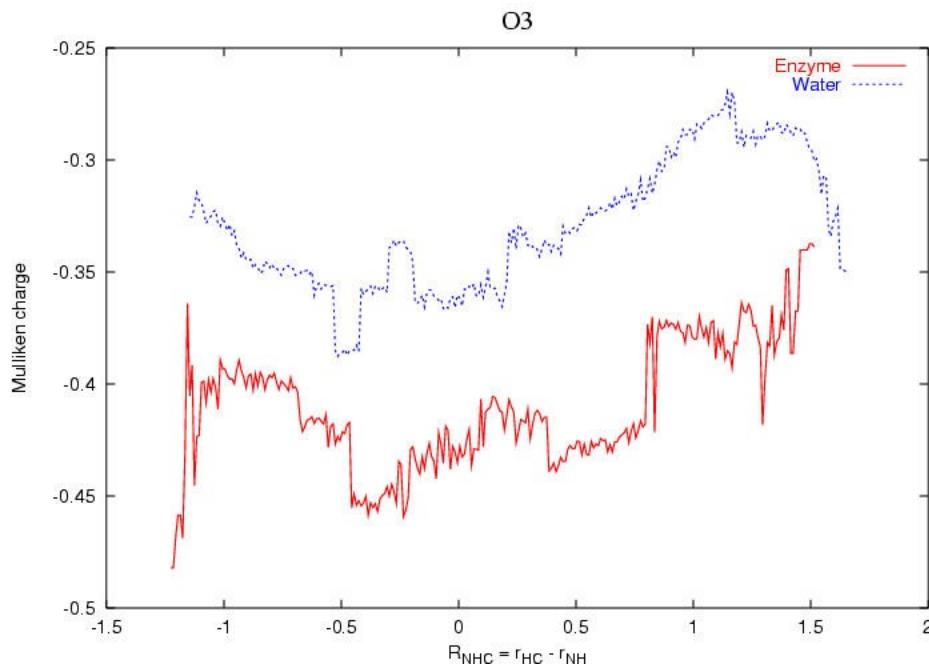
Atomic Charges



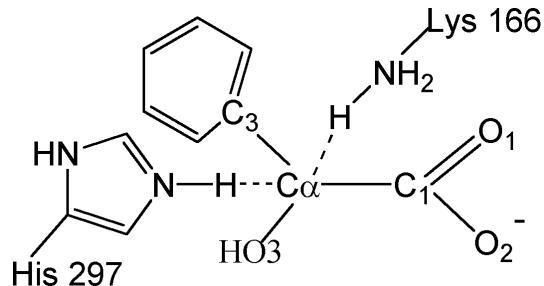
The OH group in the enzyme takes more charge: **Mg role**

substrate + Lys166

substrate + His297



PMF: mechanism. Comparison with uncatalyzed reaction



Charge and bond order analysis: Some differences can be seen between enzyme and the uncatalyzed reaction

But the difference in the energy barrier comes probably from the electrostatic stabilization of the enzyme:

Mg, Glu270, Ser139, Asp195, Glu220...

Conclusions: Mandelate Racemase



The concerted mechanism is the most favourable

No anionic stable intermediate exists, but a TS

MR catalyzes the reaction in both directions($S \Rightarrow R$, $R \Rightarrow S$)
at “similar” rate, being chemically symmetric

Some residues are important to stabilize unstable structures

Conclusions: Methodology

The location of saddle points is a valuable task to design an appropriate reaction coordinate

The difference between the MEP and Free Energy Path:

The energy barrier can be very different (this is not the case)

The geometry is conceptually different

...But some characteristics of the reaction remain!

Conclusions: Methodology

Acc. Chem. Res. 2001, 34, 72–79

Elucidating the Nature of Enzyme Catalysis Utilizing a New Twist on an Old Methodology: Quantum Mechanical-Free Energy Calculations on Chemical Reactions in Enzymes and in Aqueous Solution

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OREOLA DONINI,[†] MIKAEL PERAKYLA,[†]
ROB STANTON,[†] AND DIRK BAKOWIES[§]

Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, California 94143

JOURNAL OF CHEMICAL PHYSICS

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Are these transition etc

too restricted methods:
QM(1 valley)/MM

Acc. Chem. Res. 1989, 22, 184–189

Free Energy Calculations: A Breakthrough for Modeling Organic Chemistry in Solution

WILLIAM L. JORGENSEN

22 FEBRUARY 2000

Free energy calculation on enzyme reactions with an efficient iterative procedure to determine minimum energy paths on a combined *ab initio* QM/MM potential energy surface

Yingkai Zhang, Haiyan Liu, and Weitao Yang

Department of Chemistry, Duke University, Durham, North Carolina 27708

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Reaction path potential for complex systems derived from combined *ab initio* quantum mechanical and molecular mechanical calculations

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(Received 16 February 2004; accepted 9 April 2004)

Conclusions: Methodology

Simulation of activation free energies in molecular systems

Eyal Neria

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Strasbourg, France 67000*

(Received 18 September 1995; accepted 29 March 1996)

too restricted methods:
Cartesian coordinates Rc

Calculation of the potential of mean force using molecular dynamics with linear constraints: An application to a conformational transition in a solvated dipeptide

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(Received 6 April 1990; accepted 15 June 1990)

The reaction coordinate must include all the relevant degrees of freedom. They are localized in few bonds and angles but may not be trivial even in some proton transfer reactions. It should be a compromise:

reduced number of dof to avoid coordinates that do not belong to the path,
and general enough to account all the possible reactive conformations



...the end