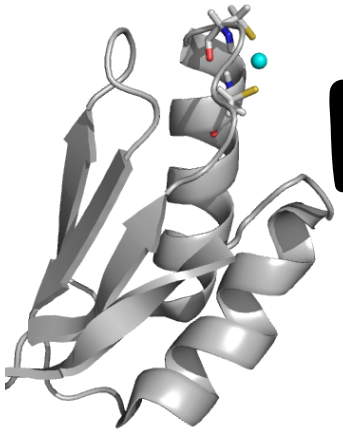
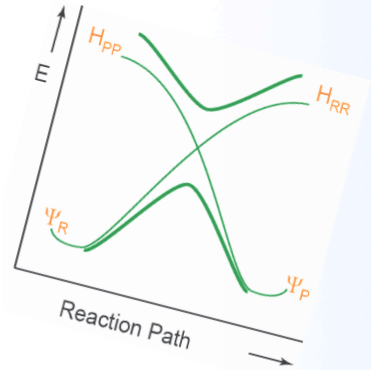


VB/MM Insights into Enzyme catalysis



Avital Shurki

Institute for Drug Research,

The Lise-Meitner Minerva Center for Computational Quantum Chemistry

The Hebrew University of Jerusalem

Acknowledgement

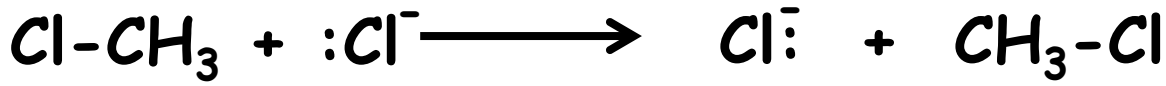
Hadar Crown
Avital Sharir-Ivry
Dr. Tamar Shnerb
Dr. Rajapandian Varatharaj
Moshe Amitay
Tamar Ansbacher
Dr. Hemant Kumar Srivastava

Collaborations:
Prof. Wei Wu

Interested post-docs are most welcome to the group to continue with this and other interesting projects

$$$ Israel Science Foundation (ISF)
Human Frontiers of Science Program (HFSP)
Alex Grass Center for Drug Design and Synthesis of Novel Therapeutics

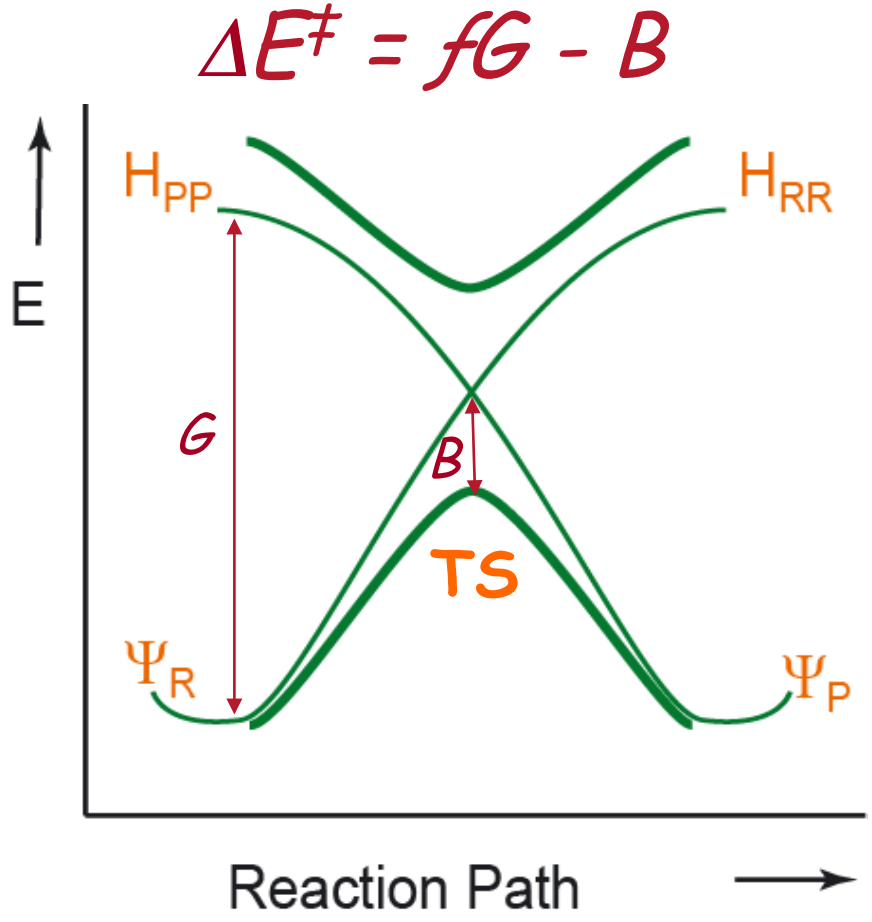
VB – Reactivity Tool



VB Structures

Ψ_R $Cl\cdot\cdot\cdot CH_3$ $:Cl^-$

Ψ_P $Cl:$ $CH_3\cdot\cdot\cdot Cl$



Empirical VB (EVB)

Advantages

- Simple picture of reactivity
- Relatively fast
- Comparative – reduces mistakes
- Calibration – reliable results
- Good & easy description of Reaction Coordinate

Disadvantages / Problems

- QM region - parameterization
- Difficult to handle more than two states
- Strong assumptions

ab initio VB/MM

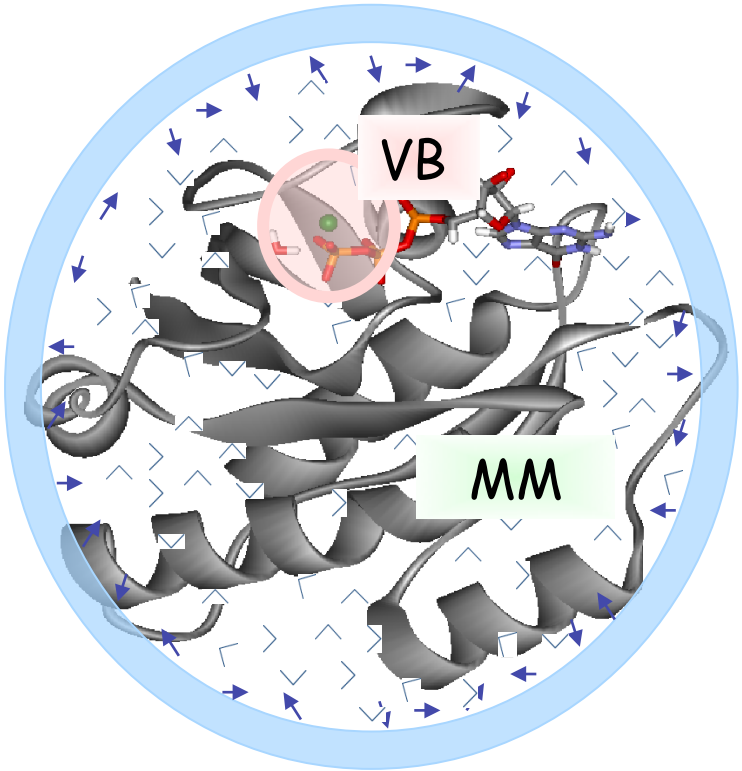
Ab-initio VB/MM

$$H_{VB/MM} = H(VB) + H(MM) + H(VB/MM)$$

Bonding

Electrostatic

✓VdW



HOW?

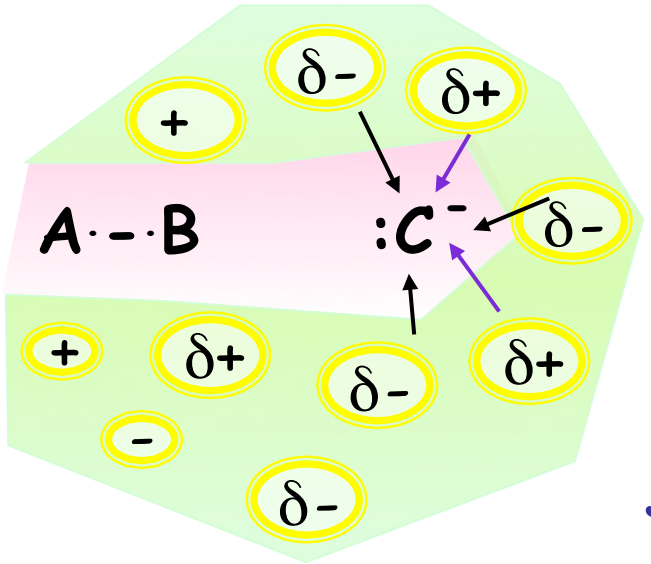
- **VdW** – classically
- **Electrostatic**:
  Mechanical embedding each VB structure
  → Wavefunction polarization (electrostatic embedding)
-

Shurki, Crown JPC B, 2005 109, 23638

ab initio VB/MM

VB structures – Electron localization

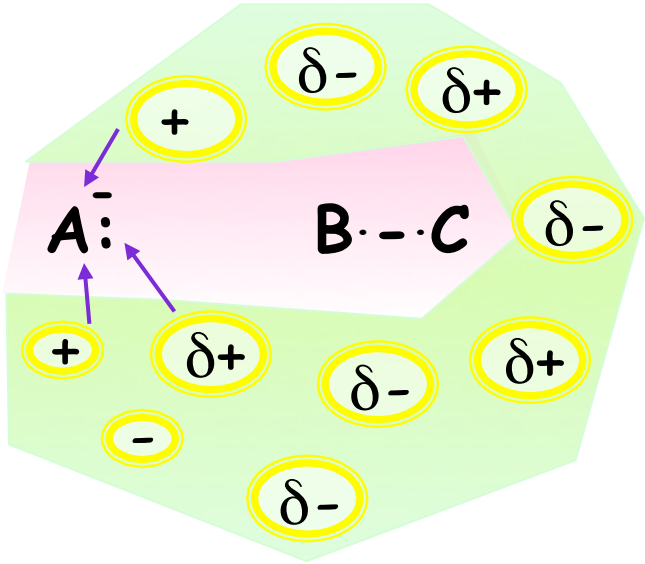
Ψ_R **A·–·B :C⁻**



Less

Stabilization

Ψ_P **A:⁻ B·–·C**

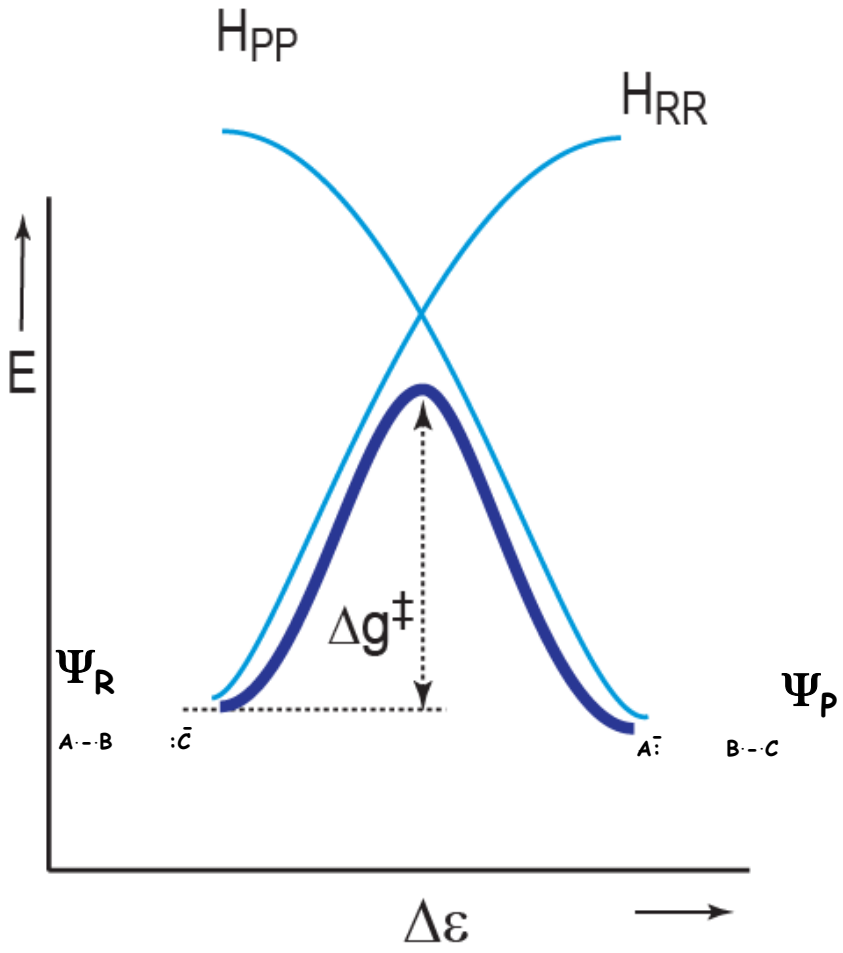


More

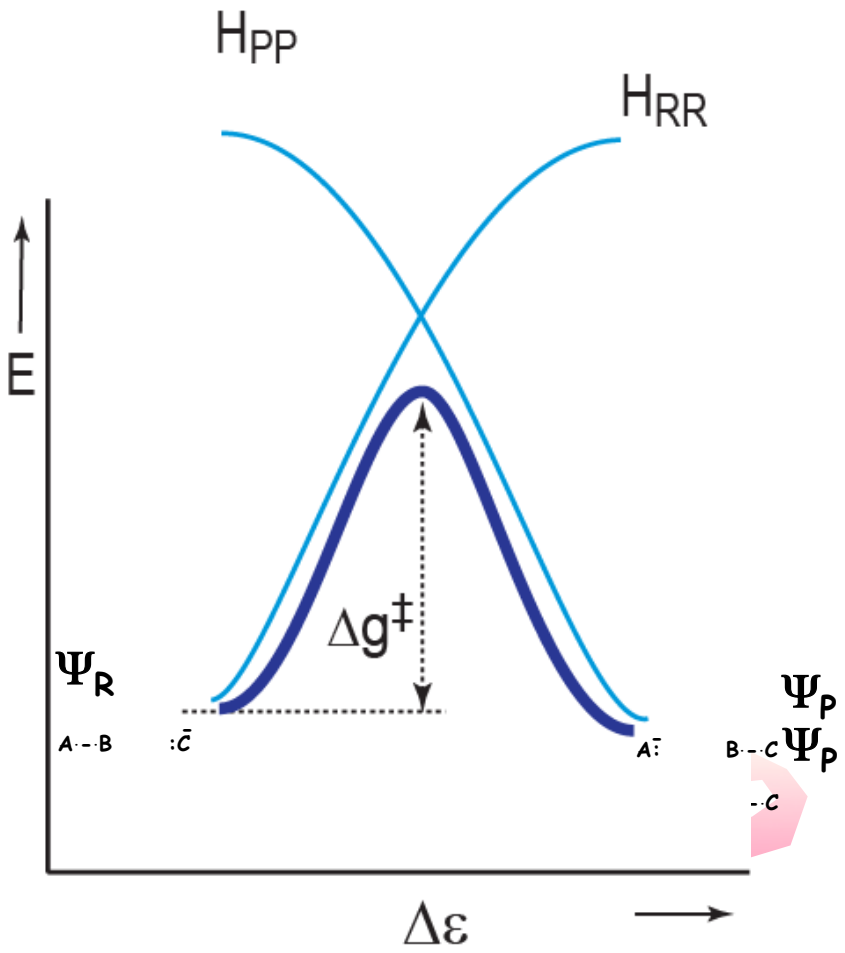
$$\Psi_{Total} = c_R\Psi_R + c_P\Psi_P$$

Mechanical Embedding with each VB structure separately should include most of the wavefunction polarization.

Gas



Protein



ab initio VB/MM

$$H_{VB/MM} = H(VB) + H(MM) + H(VB/MM)$$

Hii is the diabatic state energy:

$$H_{ii} = H_{ii}(VB) + H_{ii}^{\text{int}} + H(MM)$$

H_{ii}^{int} is calculated classically

How to calculate Hij ?

Assumption: Both overlap S_{ij} and reduced resonance integral, β_{ij} , are invariant to the environment.

$$\beta_{ij}^{gas} = \beta_{ij}^{env} \qquad S_{ij}^{gas} = S_{ij}^{env}$$

$$\beta_{ij} = H_{ij} - \tfrac{1}{2}\left(H_{ii} + H_{jj}\right)S_{ij}$$

$$H_{ij} = H_{ij}^{0}(VB) + \tfrac{1}{2}\left(H_{ii}^{\text{int}} + H_{jj}^{\text{int}}\right)S_{ij}$$

Solve new matrix,
Get new wavefuncetion

This formula will serve also in the general case

Shurki, Crown JPC B, 2005 109, 23638

ab initio VB/MM

New matrix is solved:

$$\begin{pmatrix} H_{11}^{0} + H_{11}^{\text{int}} & H_{12}^{0} + \tfrac{1}{2}\left(H_{11}^{\text{int}} + H_{22}^{\text{int}}\right)S_{12} \\ H_{21}^{0} + \tfrac{1}{2}\left(H_{11}^{\text{int}} + H_{22}^{\text{int}}\right)S_{21} & H_{22}^{0} + H_{22}^{\text{int}} \end{pmatrix}$$

New wavefunction, and energy are obtained:

$$E_{total} = E + H^{O}(MM)$$

Relax the environment accordingly and repeat

Finally, for the reaction profile:
use potential of mean force (PMF) combined with FEP/US

Shurki, Crown JPC B, 2005 109, 23638

ab initio VB/MM

Finally …

The ab –initio VB calculations of the reactive fragments utilized the program **XMVB**:

Lingchun Song, Wei Wu, Yirong Mo, Qianer Zhang, **XMVB** – an ab initio Non-orthogonal Valence Bond Program, Center of Theoretical Chemistry, Department of Chemistry, and State Key Laboratory for Physical Chemistry of Solid Surfaces, Xiamen University, Xiamen Fujain 36005, China.

The MM calculations as well as dynamics utilized the MOLARIS program with the ENZYMIX force-field:

Microscopic and Semimicroscopic Calculations of Electrostatic Energies in Proteins by the POLARIS and ENZYMIX Programs, F. S. Lee, Z. T. Chu, and A. Warshel, J. Comp. Chem. 14, 161 (1993).

Results: Li-F

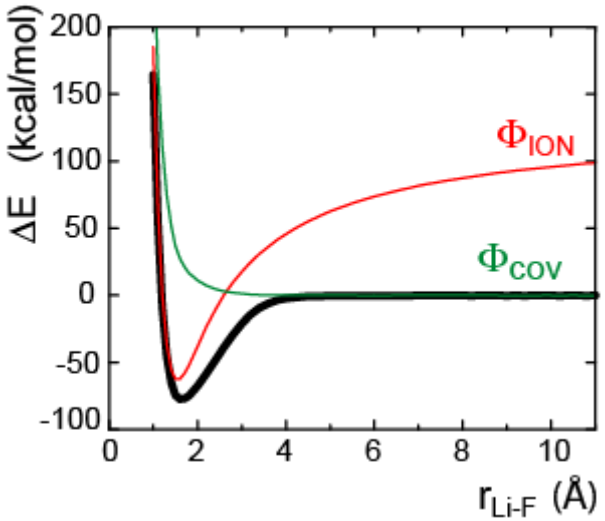
VB Structures:

Φ_{COV} Li·—·F ; Φ_{ION} Li^+ $:F^-$; ~~Φ_{ION} $Li:^-$ F^+~~

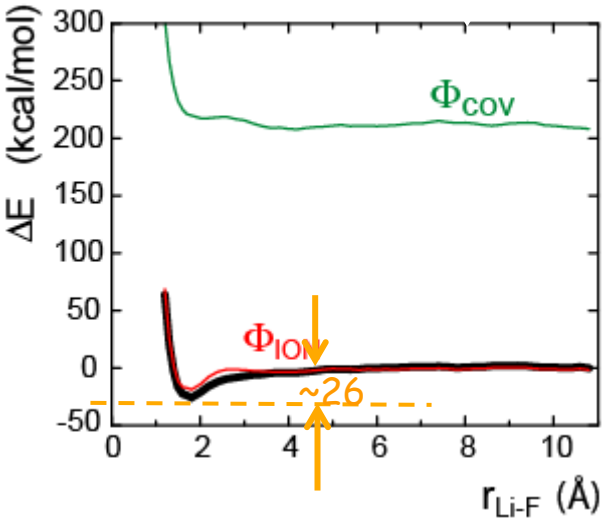
Energy Curves

6-31G* basis set
1S electrons frozen

Gas Phase



Solution



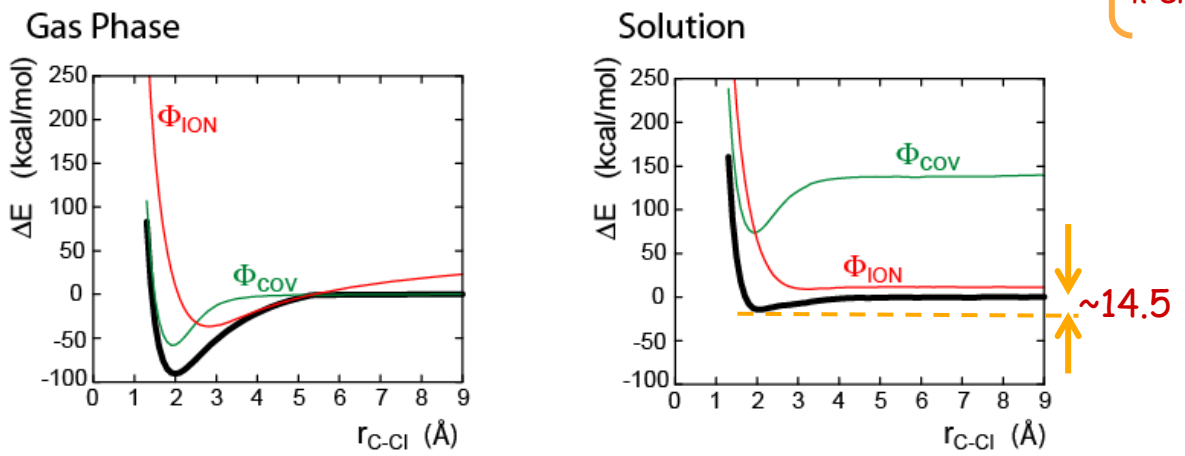
- correct description of dissociation into ions in solution
- Predicted dissociation of barrier ~26 kcal/mol (exp. ~5 kcal/mol)

Shurki, Crown JPC B, 2005 109, 23638

Results: Tertiary-Butyl Chloride (t-BuCl)

6-31G basis set
π electrons frozen

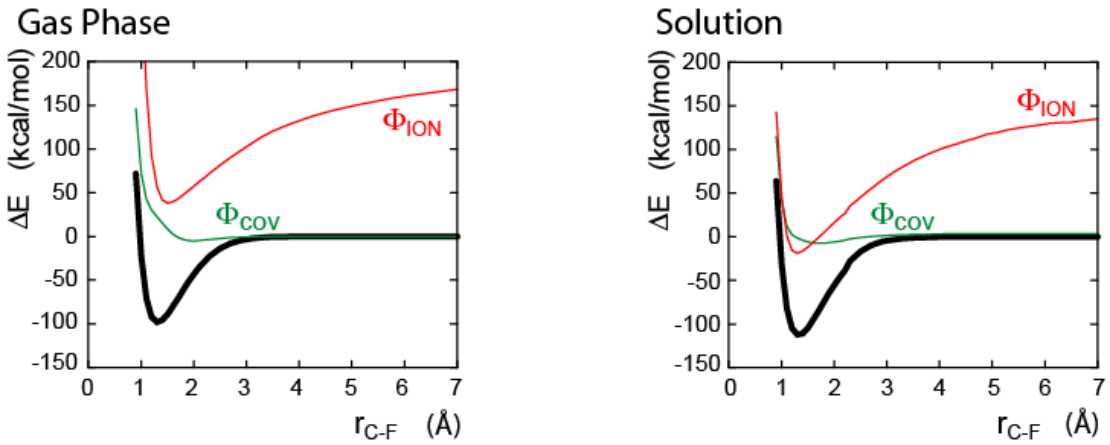
Energy Curves



- Dissociation into: radicals – vacuum vs ions - solution
- Predicted dissociation barrier of ~14.5 kcal/mol (Exp. ~19.5 kcal/mol)

Results: CH_3-F

Energy Curves



- Homolytic dissociation in both vacuum and solution
- Most of the bond energy comes from resonance

6-31G* basis set
Core electrons frozen

ab initio DE-VB/MM

Environment partial charges are included in the quantum Hamiltonian:

No need for the assumption regarding the overlap and the reduced resonance in case of an electrostatic environment:

$$\beta_{ij}^{gas} = \beta_{ij}^{env} \qquad S_{ij}^{gas} = S_{ij}^{env}$$

No Need!

New matrix is solved:

$$\begin{pmatrix} (H_{11}^0)' & (H_{12}^0)' \\ (H_{21}^0)' & (H_{22}^0)' \end{pmatrix}$$

New wavefunction, and energy are obtained:

$$E_{total} = E + H^O(MM)$$

Relax the environment accordingly and repeat

Use of potential of mean force PMF

Sharir-Ivry et al. JPC A, 2008, 112, 2489

Results: Identity S_N2 Reaction

Important VB Structures:

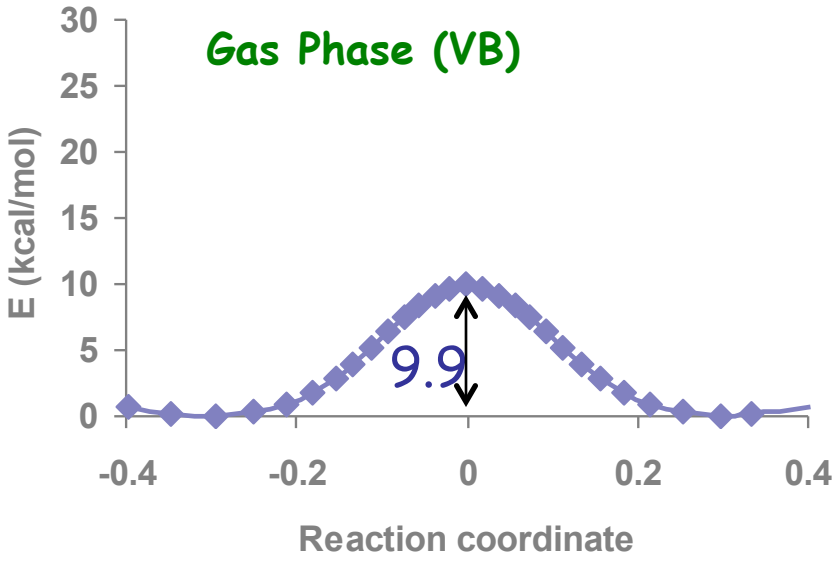
Φ_{covR} $Cl\cdot\!\!-\!\!\cdot CH_3$ $:Cl^-$

Φ_{covP} $Cl:^-$ $CH_3\cdot\!\!-\!\!\cdot Cl$

Φ_{ion} $Cl:^-$ CH^+_3 $Cl:^-$

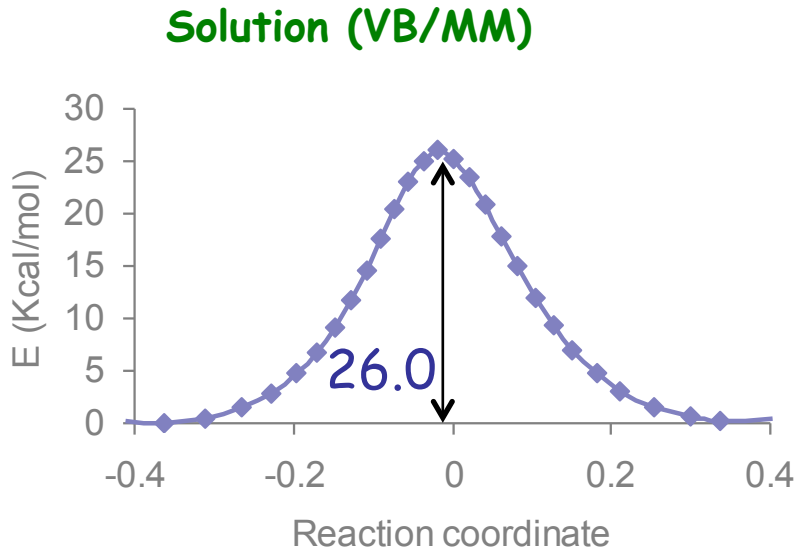
Use both VB/MM and DE-VB/MM

BOVB
6-31G* basis set
Core+π electrons frozen



Experimental
Gas ~10.2
Solution ~26.6

VB/MM predicts correctly the energetics



Sharir-Ivry et al. JPC A, 2008, 112, 2489

ab initio VB/MM

Mechanical Embedding

H_{ii}^{int} is calculated classically

$$\begin{pmatrix} H_{11}^{0} + H_{11}^{\text{int}} & H_{12}^{0} + \frac{1}{2}\left(H_{11}^{\text{int}} + H_{22}^{\text{int}}\right)S_{12} \\ H_{21}^{0} + \frac{1}{2}\left(H_{11}^{\text{int}} + H_{22}^{\text{int}}\right)S_{21} & H_{22}^{0} + H_{22}^{\text{int}} \end{pmatrix}$$

Requires the approximations: $\beta_{ij}^{gas} = \beta_{ij}^{env}$ $S_{ij}^{gas} = S_{ij}^{env}$

ab initio DE-VB/MM

Wavefunction Polarization

Environment partial charges are included in the quantum Hamiltonian:

H_{ii}^{int} is included in the quantum Hamiltonian

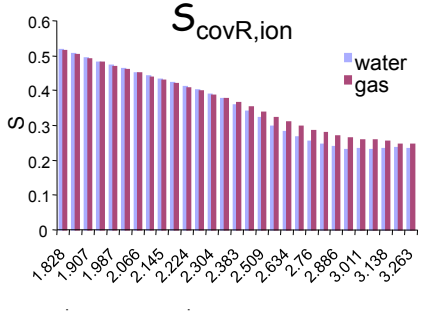
$$\begin{pmatrix} \left(H_{11}^{0}\right)' & \left(H_{12}^{0}\right)' \\ \left(H_{21}^{0}\right)' & \left(H_{22}^{0}\right)' \end{pmatrix}$$

in case of an electrostatic environment:

$\beta_{ij}^{gas} = \beta_{ij}^{env}$ $S_{ij}^{gas} = S_{ij}^{env}$ **No Need!**

Sharir-Ivry, Shurki, JPC B 2008 112, 12491

Examination of approximations regarding S_{ij} and β_{ij}



TS geometry (2.38Å)

	Gas	VB/MM	DE-VB/MM
	overlap		
$S_{covR,covP}$	0.124	Same as gas	0.118
$S_{covR,ion}$	0.376		0.359
$S_{covP,ion}$	0.349		0.359

Sharir-Ivry, Shurki, JPC B
2008, 112, 12491

- *Changes in overlap and reduced resonance are negligible*
- *The trends in the weights are kept ,VB/MM sufficient for wavefunction polarization*

The approximations in VB/MM seem reasonable

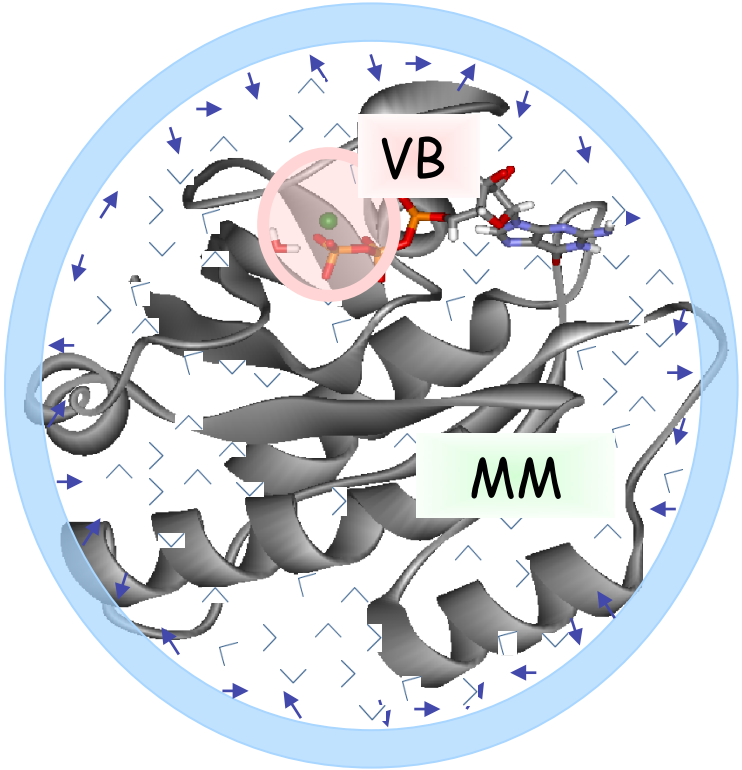
ab initio VB/MM

$$H_{VB/MM} = H(VB) + H(MM) + H(VB/MM)$$

Bonding

✓Electrostatic

✓VdW



HOW?

- **VdW** – classically
- **Electrostatic**:
  Mechanical embedding each VB structure
  → Wavefunction polarization (electrostatic embedding)
- **Bonding** – link atom scheme

Link Atom Scheme

Full treatment

$CH_3CH_2\text{-}COO^- + CH_3Cl \rightarrow CH_3CH_2\text{-}COOCH_3 + Cl^-$

QM

Link atom treatment

$CH_3CH_2\text{-}COO^- + CH_3Cl \rightarrow CH_3CH_2\text{-}COOCH_3 + Cl^-$

MM QM

Reaction barriers (kcal/mol)

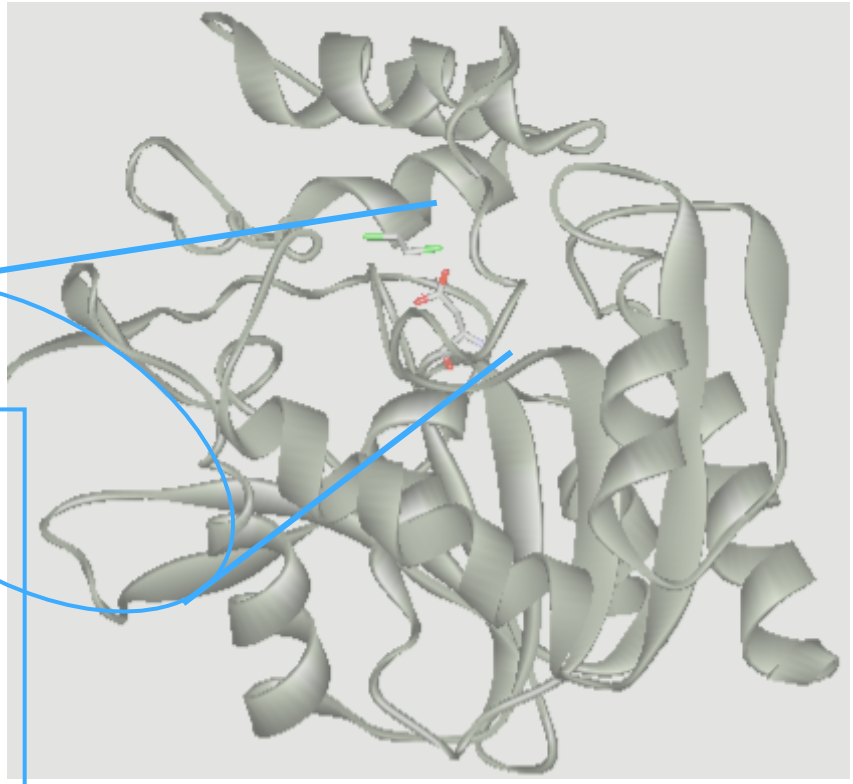
	$\Delta G_g^{\ddagger}$	$\Delta G_w^{\ddagger}$
Full treatment	11.9	18.2
Link atom treatment	11.5	18.7

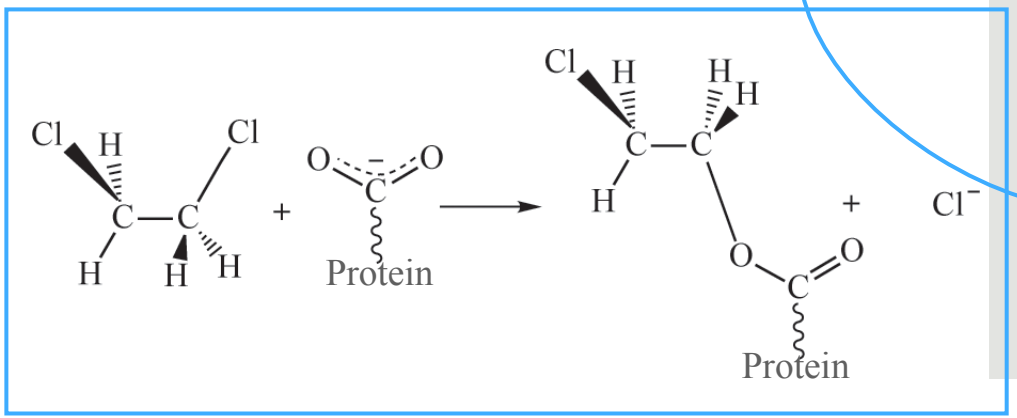
Barriers are similar for the two treatments
Link atom treatment is suitable

Sharir-Ivry, Shurki, JPC B 2010, 114, 2212

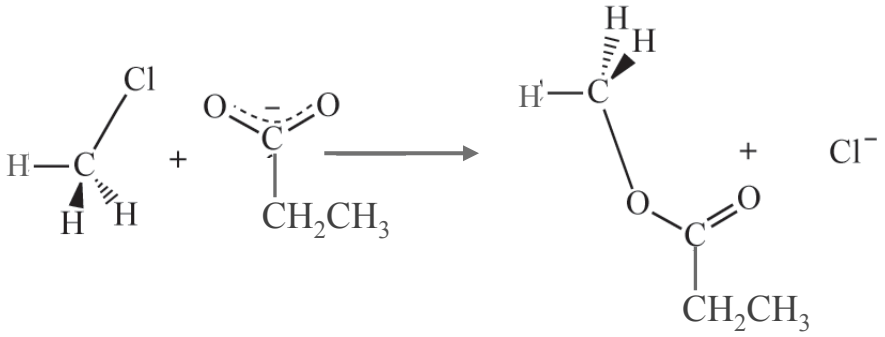
Link Atom Scheme

haloalkane dehalogenase (DhlA)

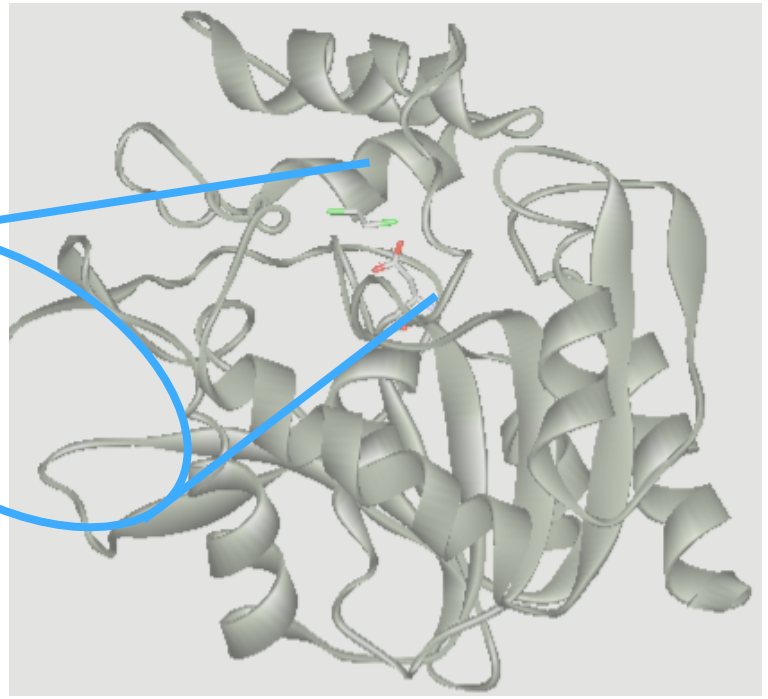




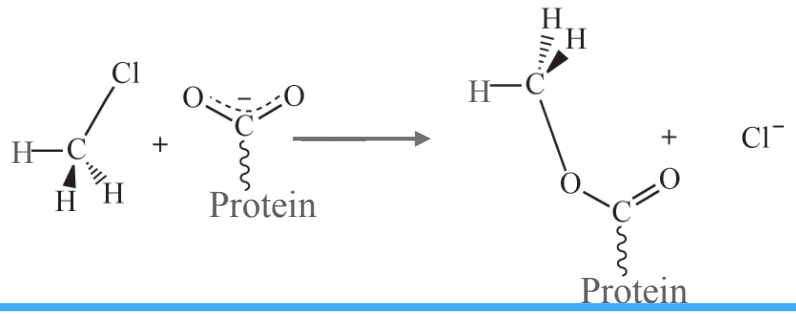
Our Model System



haloalkane dehalogenase (DhlA)



Our Model System of the Reactive Part



L-VBSCF
6-31G* basis set
Core+π electrons frozen

	Gas		Water		Protein
Exp	(18-28)	~7	(<26)	~-12	(<15)
VBSCF	13	7.1	20.1	-8.9	11.2

Low barriers (different experimental system and insufficient description of gas phase), yet the overall trend is correct

Solvation Energies (weighted)

		Reactants	TS	$\Delta_{(TS-R)}$	$\Delta\Delta_{(P-W)}$
water	CovR	-54	-17	37	
	Ion	-26	-49	-23	
protein	CovR	-54	-23	31	-6
	Ion	-37	-61	-24	-1
	Tot				**-8**

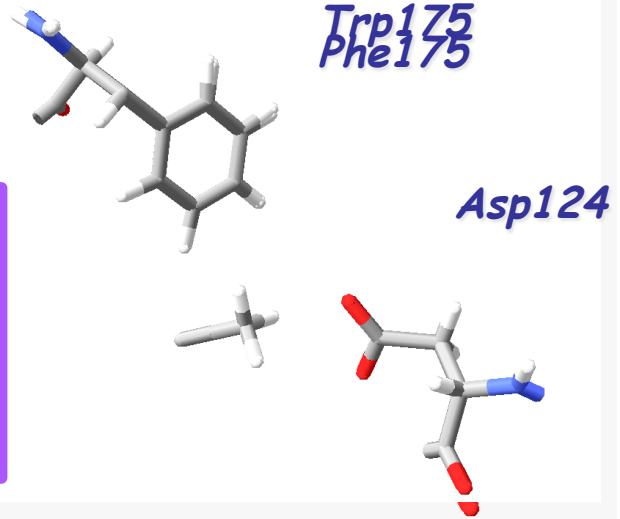
* Results based on one run and include VdW and intra electrostatic interaction.

- The stabilization in the protein is consistently larger than in water
- Water stabilize CovR in the reactants geometry much more than in the TS - leading to the increased barrier
- This differential stabilization considerably decreases in the protein

Sharir-Ivry, Shurki, JPC B 2010, 114, 2212

Understand mutations

W175F as a case study



Exp

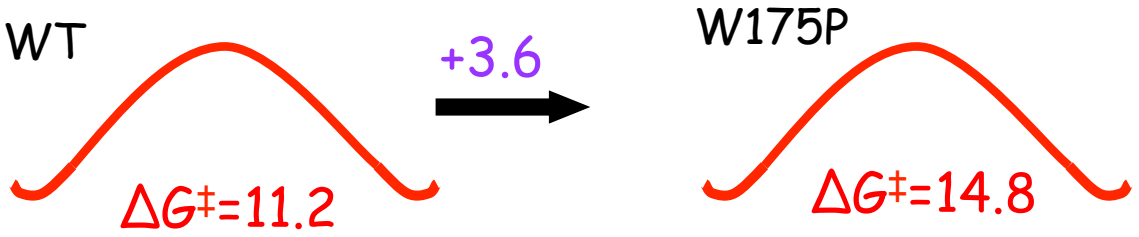
	wt	W175P
Units/mg	2.9	0.28

(Data corresponds to di-chloroethane as substrate)

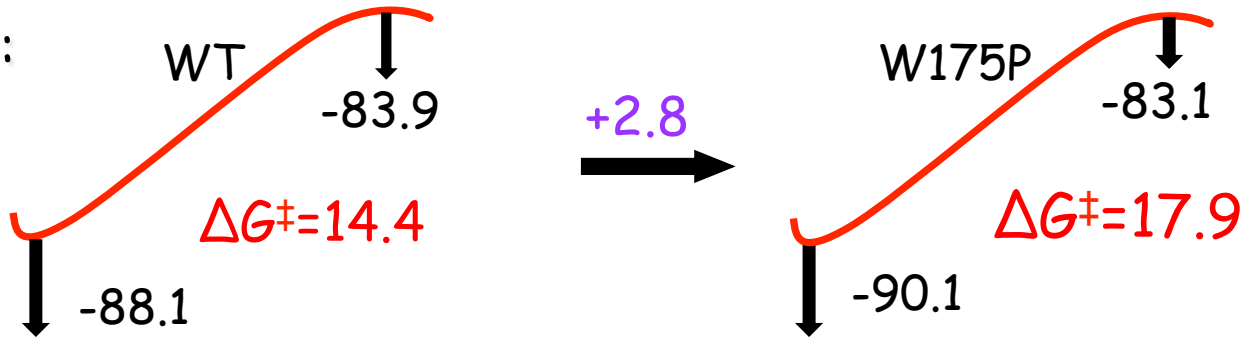
Observed anti-catalytic effect ≥3 kcal/mol!

Our calculations:

VB/MM-FEP:



LRA, solvation :



Understand mutations

Trp175	Φ_{covR}	Φ_{covP}	Φ_{ion}	Φ_{lb}	Tot
RS	-0.9	0.0	-2.1	0.0	-3.0
TS	-0.4	-1.3	-5.2	0.0	-6.9
catalytic effect	+0.5	**-1.3**	**-3.1**	0.0	**-3.9**

Trp 175 is catalytic due to better solvation in the TS

The main contribution to this effect comes from the higher TS stabilization of both Φ_{ion} and Φ_{covP}

Phe is not polar thus, ionic stabilization is decreased leading to decrease of catalytic effect.

Conclusions

- Two methods were presented: VB/MM and DE-VB/MM
- The approximations made for VB/MM were shown to be reasonable
- Mechanical embedding of each VB structure separately captures most of the environmental effect of wave-function polarization
- The method allows studies of reactions in solvents as well as enzymes and enables understanding of the effect of mutations on the reaction.

Acknowledgement

Hadar Crown
Avital Sharir-Ivry
Dr. Tamar Shnerb
Dr. Rajapandian V
Moshe Amitay
Tamar Ansbacher
Dr. Hemant Kumar Srivastava

Collaborations:
Prof. Wei Wu

Interested post-docs are most welcome to the group to continue with this and other interesting projects

$$$ Israel Science Foundation (ISF)
Human Frontiers of Science Program (HFSP)
Alex Grass Center for Drug Design and Synthesis of Novel Therapeutics

Understand mutations

Anti-catalytic effect of known mutants

	Calculated $\Delta\Delta g^{\ddagger(calc)}_{wt\rightarrow mut}$	Observed $\Delta\Delta g^{\ddagger(obs)}_{wt\rightarrow mut}$
wt	0.0	0.0
W125F	2.3	≤ 2.3
V226A	-2.2	0.7
W175Y	5.2	≤ 3.0
W175F	2.8	≤ 3.0
N148D	1.9	> 2.7
D-Mut	1.5	> 2.7

Calculated values from LRA using electrostatic

Agreement suggests that electrostatic indeed has a major role in catalysis.

residue	Tot-cat	$\Delta\Delta g_{sol}^{TS-RS}(\psi)$	$\Delta\Delta g_{sol}^{TS-RS}(\phi_{\mathrm{cov}}^{R})$	$\Delta\Delta g_{sol}^{TS-RS}(\phi_{\mathrm{cov}}^{P})$	$\Delta\Delta g_{sol}^{TS-RS}(\phi_{ion})$	$\Delta\Delta g_{sol}^{TS-RS}(\phi_{LB})$	$\Delta g_{sol}^{RS}(\psi)$
		Φ_{covR}	Φ_{covP}	Φ_{ion}	Φ_{lb}		RSsolv
W175	-3.85	0.53	**-1.26**	**-3.09**	-0.04	-2.97	
W125	-3.40	0.28	**-1.28**	**-2.36**	-0.05	-2.36	
V226	-1.02	-2.38	-3.40	0.58	-0.48	-1.00	
H54	0.25	**1.07**	-0.22	-0.57	-0.02	-2.77	

- VB analysis provides better understanding of the effects.

- without structural interference, dominant VB contribution remains also in the mutant.

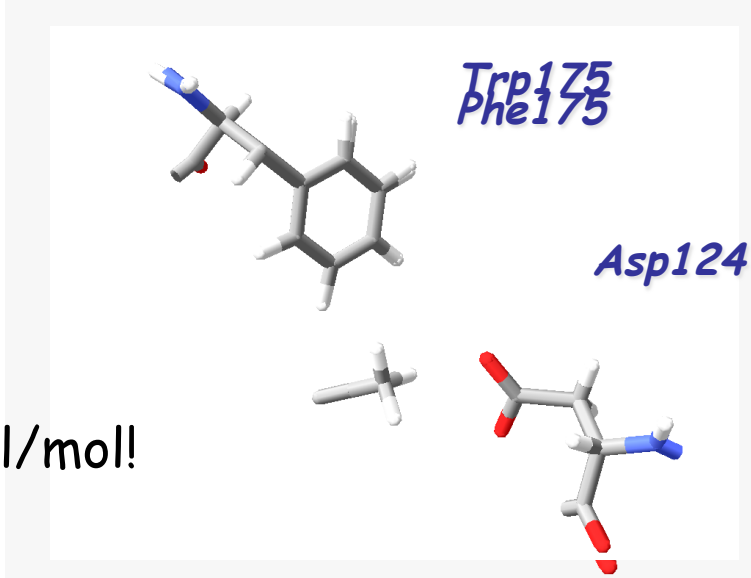
Understand mutations

W175F as a case study

	WT	W175P
Units/mg	2.9	0.28

(The data refers to the case of di-chloroethane as substrate)

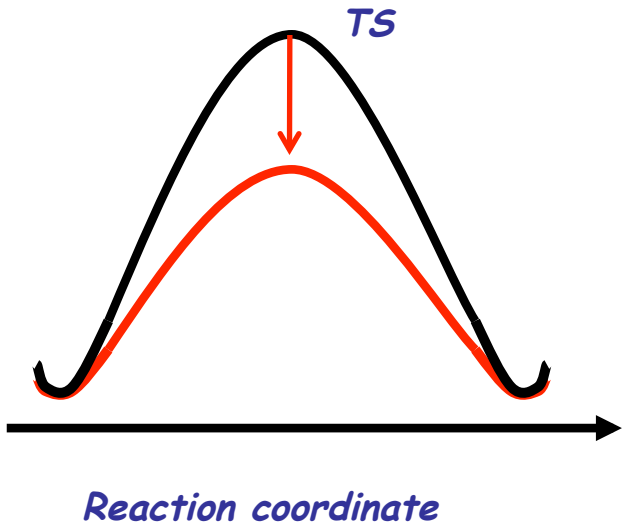
Observed anti-catalytic effect ≤ 2.3 kcal/mol!



Reverse direction: W175F → wt

We can now turn to study the specific contributions of residue 175!

Enzyme Design



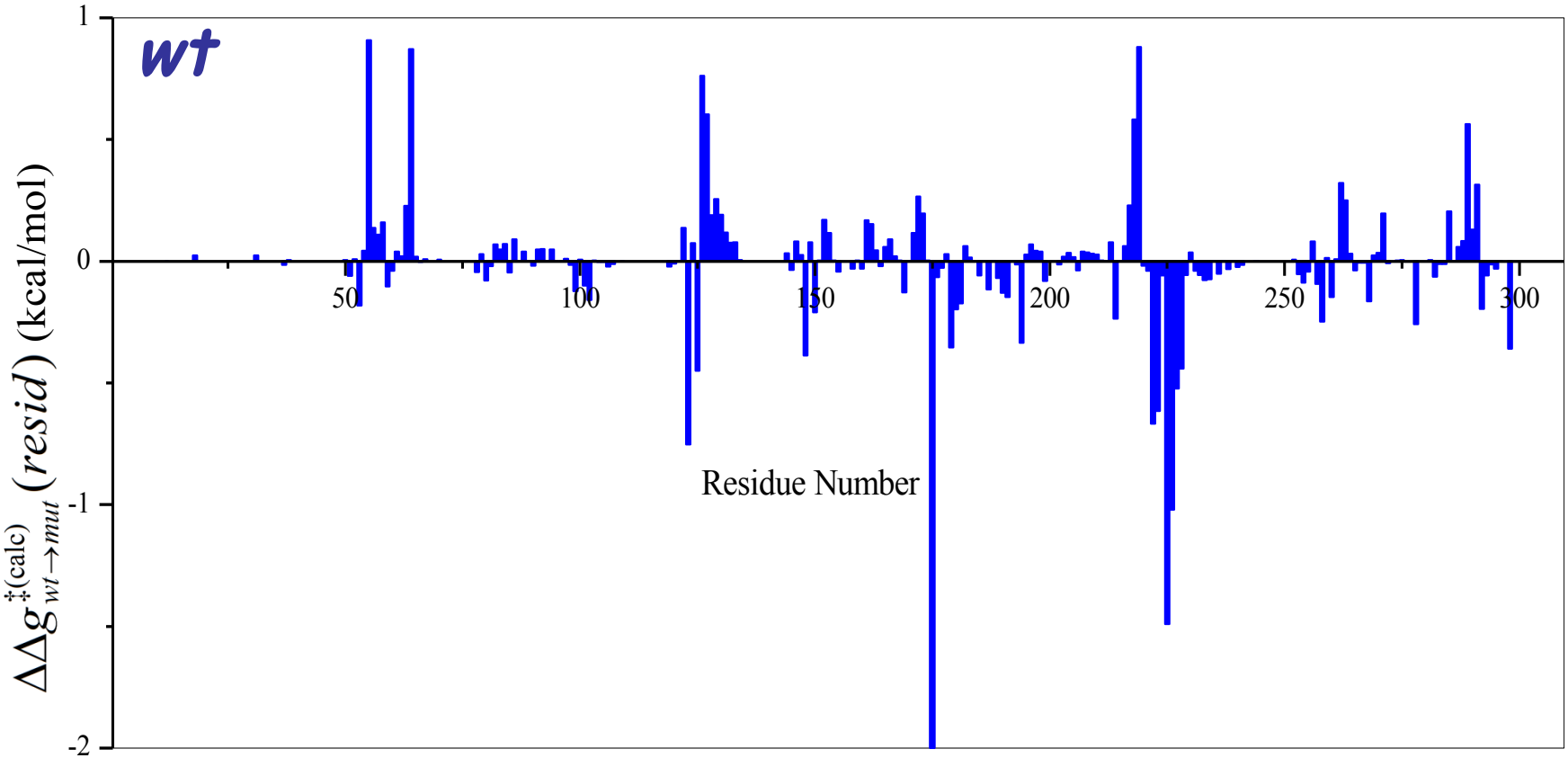
The challenge:
computational based enzyme design!

If electrostatics is important for catalysis
→ Identification of hot spots

Valence bond provides added insights

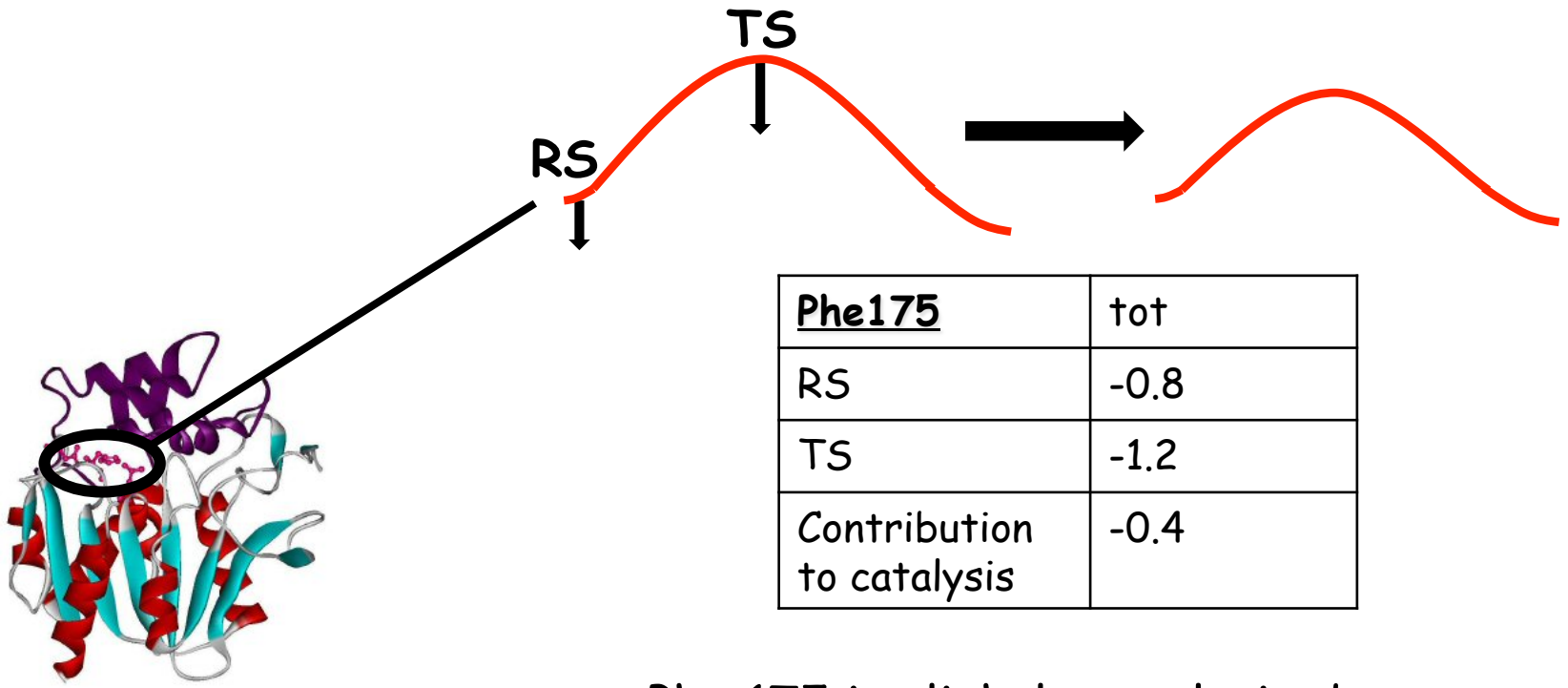
Scheme Validation

Contribution to differential solvation of particular residues



1. Classify the residue as catalytic, anti-catalytic or non-catalytic

Identify the residue's contribution to the solvation of the substrate in both RS and TS using LRA



Phe175	tot
RS	-0.8
TS	-1.2
Contribution to catalysis	-0.4

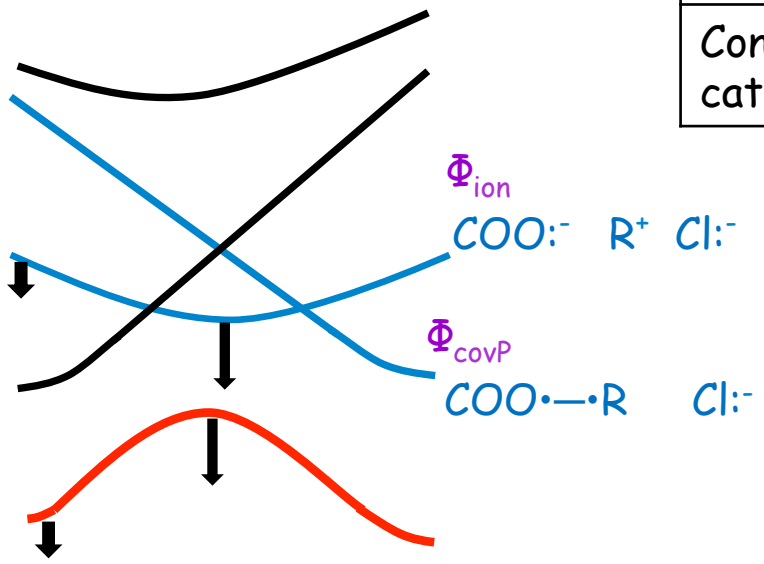
Phe 175 is slightly catalytic due to better solvation in the TS

2. Understand the origin of this catalytic effect

i. By decomposing the effect into the contributions from the various VB structures!

ii. Identify the VB structures that govern the overall effect.

Phe175	Φ_{covR}	Φ_{covP}	Φ_{ion}	Φ_{Ib}	Tot
RS	-0.3	0.0	-0.5	0.0	-0.8
TS	-0.1	-0.2	-0.9	0.0	-1.2
Contribution to catalysis	+0.2	-0.2	-0.4	0.0	**-0.4**



The main contribution to this effect comes from the higher TS stabilization of both Φ_{ion} and Φ_{covP}

Understand mutations

enhance ionic stabilization by increasing the residue polarity in the right direction - wt (W175)

Trp175	Φ_{covR}	Φ_{covP}	Φ_{ion}	Φ_{lb}	Tot
RS	-0.9	0.0	-1.6	0.0	-2.5
TS	-0.3	-1.1	-4.3	0.0	-5.7
catalytic effect	+0.6	-1.1	-2.7	0.0	**-3.2**

Effect of Φ_{ion} and Φ_{covP} increased and is still dominant

The main contribution to this effect comes from the higher TS stabilization of both Φ_{ion} and Φ_{covP}

3. *Understand/suggest mutations:*

a. enhance the effects of the leading VB structures if these are catalytic

b. decrease/change if it is anti-catalytic!

enhance ionic stabilization by increasing the residue polarity in the right direction - wt (W175)

Trp175	Φ_{covR}	Φ_{covP}	Φ_{ion}	Φ_{lb}	Tot
RS	-0.9	0.0	-1.6	0.0	-2.5
TS	-0.3	-1.1	-4.3	0.0	-5.7
catalytic effect	+0.6	-1.1	-2.7	0.0	**-3.2**

Effect of Φ_{ion} and Φ_{covP} increased and is still dominant

residue	Tot-cat	Φ_{covR}	Φ_{covP}	Φ_{ion}	Φ_{lb}	RSsolv
	$\Delta\Delta g_{sol}^{TS-RS}(\psi)$	$\Delta\Delta g_{sol}^{TS-RS}(\phi_{cov}^{R})$	$\Delta\Delta g_{sol}^{TS-RS}(\phi_{cov}^{P})$	$\Delta\Delta g_{sol}^{TS-RS}(\phi_{ion})$	$\Delta\Delta g_{sol}^{TS-RS}(\phi_{LB})$	$\Delta g_{sol}^{RS}(\psi)$
W175	-3.85	0.53	**-1.26**	**-3.09**	-0.04	-2.97
F175	-0.47	0.06	**-0.14**	**-0.38**	0.00	-0.50
Y175	-3.05	-0.26	**-0.71**	**-2.03**	-0.06	-0.99
W125	-3.40	0.28	**-1.28**	**-2.36**	-0.05	-2.36
F125	-0.43	0.34	**-0.23**	**-0.53**	-0.01	-1.03
V226	-1.02	-2.38	-3.40	0.58	-0.48	-1.00
A226	-1.04	-2.39	-3.43	0.56	-0.60	-0.98
H54	0.25	**1.07**	-0.22	-0.57	-0.02	-2.77
N54	-1.01	**-2.76**	0.02	1.72	0.00	7.36

- VB analysis provides better understanding of the effects.

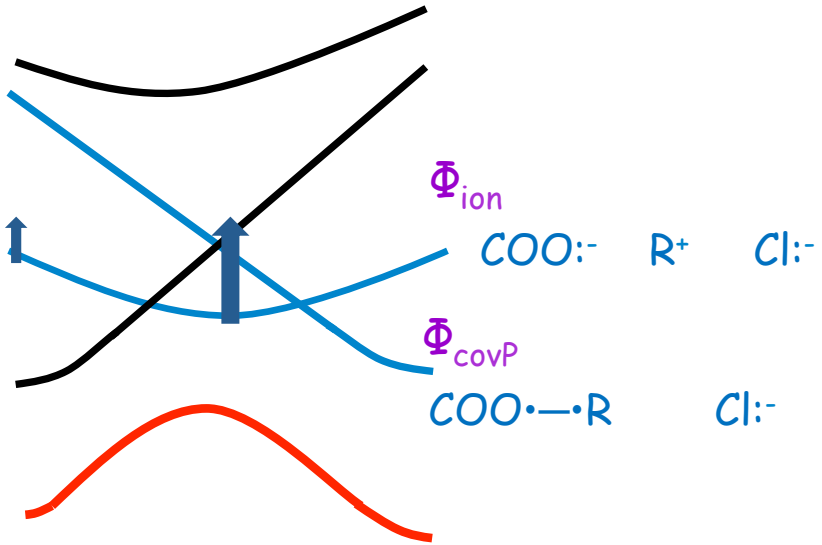
- without structural interference, dominant VB contribution remains also in the mutant.

Prediction Glu56

1. classify

	Glu56
RS	+2.3
TS	+2.5
catalytic effect	+0.2

Glu56 is slightly anti-catalytic



2. analyze, understand

Glu56	Φ_{covR}	Φ_{covP}	Φ_{ion}	Φ_{lb}	Tot
RS	+1.3	0.0	+1.0	0.0	+2.3
TS	+0.5	+0.3	+1.7	0.0	+2.5
catalytic effect	-0.8	+0.3	+0.7	0.0	**+0.2**

The main contribution to this effect is higher destabilization of ΦcovP and Φion in the TS

Prediction Glu56Gln

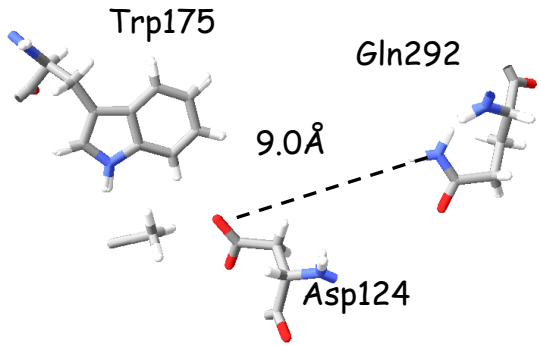
4. Suggest a mutation that will reduce and maybe even turn this destabilization interaction into stabilization, by e.g., removing the negative charge

Gln56	Φ_{covR}	Φ_{covP}	Φ_{ion}	Φ_{lb}	Tot
RS	-1.7	-0.1	-2.5	0.0	-4.3
TS	-0.6	-1.2	-4.0	0.0	-5.8
catalytic effect	+1.1	-1.1	-1.5	-0.0	**-1.5**

LRA calculations indeed predict catalytic trend,

with $\Delta\Delta g^{\ddagger(\mathrm{calc})}_{wt\rightarrow mut} = -6.5$

Prediction Gln292



1. classify

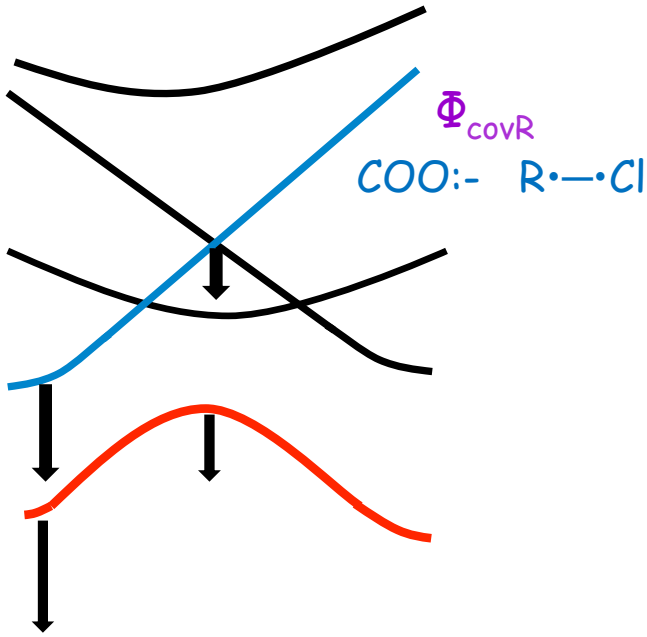
	Gln292
RS	-1.9
TS	-1.2
catalytic effect	+0.7

Gln 292 is slightly anti-catalytic

2. analyze, understand

Gln292	Φ_{covR}	Φ_{covP}	Φ_{ion}	Φ_{lb}	Tot
RS	-1.2	0.0	-0.7	0.0	-1.9
TS	-0.3	-0.1	-0.8	0.0	-1.2
catalytic effect	+0.9	-0.1	-0.1	0.0	**+0.7**

The main contribution to this effect is higher stabilization of ΦcovR in the RS

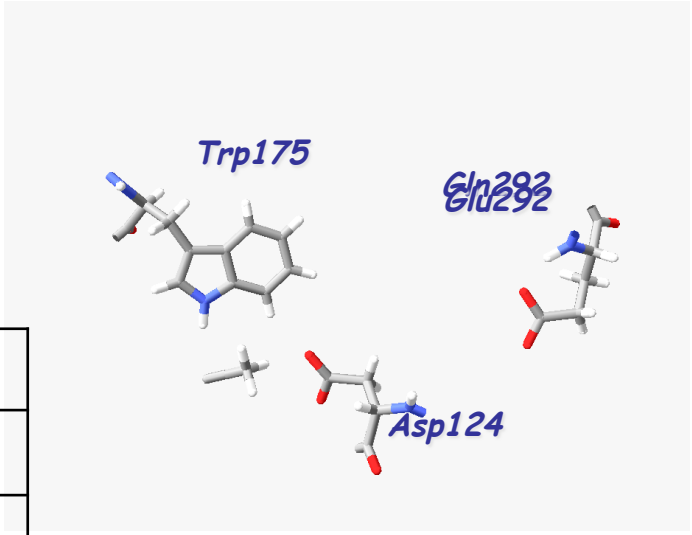


Prediction Gln292

4. Suggest a mutation that will destabilize ΦcovR by e.g., introducing negative charge

Φ_{covR} COO:- R•—•Cl

Glu292	Φ_{covR}	Φ_{covP}	Φ_{ion}	Φ_{lb}	Tot
RS	+12.7	0.0	+8.0	0.0	+20.7
TS	+4.4	+1.9	+4.0	0.2	+18.5
catalytic effect	-8.3	+1.9	+4.0	0.2	**-2.2**

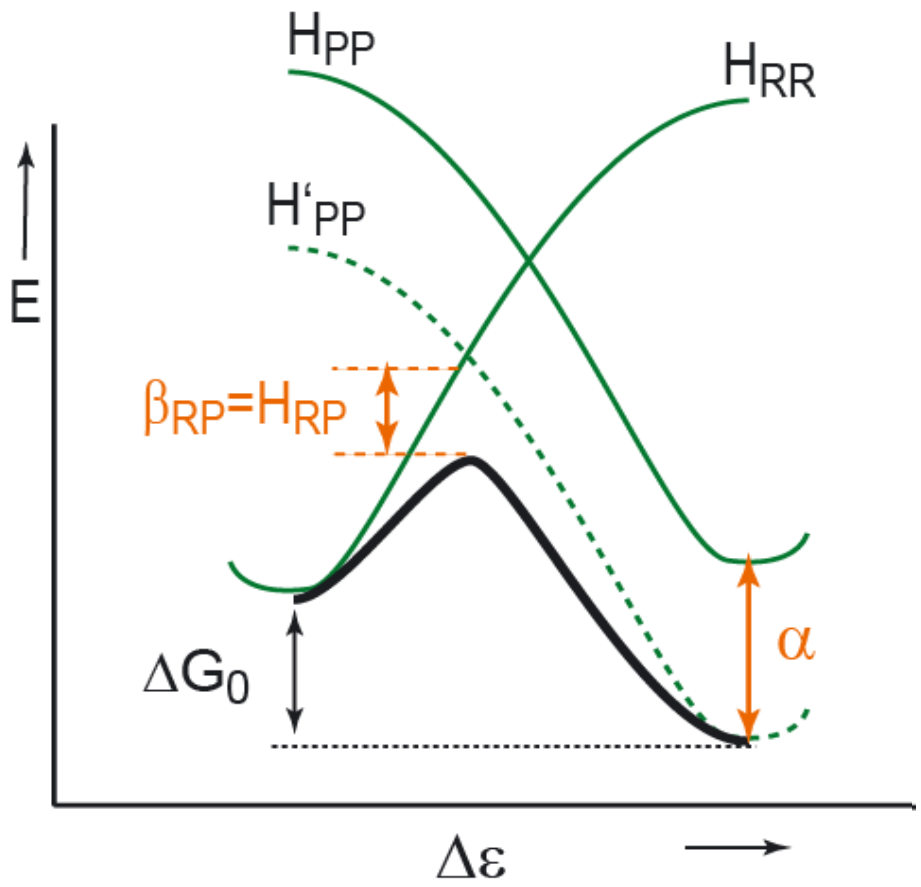


LRA calculations indeed predict catalytic trend,

with $\Delta\Delta g^{\ddagger(\mathrm{calc})}_{wt\rightarrow mut} = -1.0$

	Calculated $\Delta\Delta g^{\ddagger(calc)}_{wt \rightarrow mut}$	Observed $\Delta\Delta g^{\ddagger(obs)}_{wt \rightarrow mut}$
wt	0.0	0.0
W125F	2.3	≤ 2.3
V226A	-2.2	0.7
W175Y	5.2	≤ 3.0
W175F	2.8	≤ 3.0
N148D	1.9	> 2.7
D-Mut	1.5	> 2.7
predictions		
H54N	0.2	
E56Q	-4.4	
Q292E	-1.1	

Empirical VB (EVB)



Two independent classical states

Ψ_R **A·–·B** **:C⁻**

Ψ_P **A:⁻** **B·–·C**

Parameterize

- QM interactions

Assume

- Overlap $S_{RP} = \delta_{RP}$

Calibrate

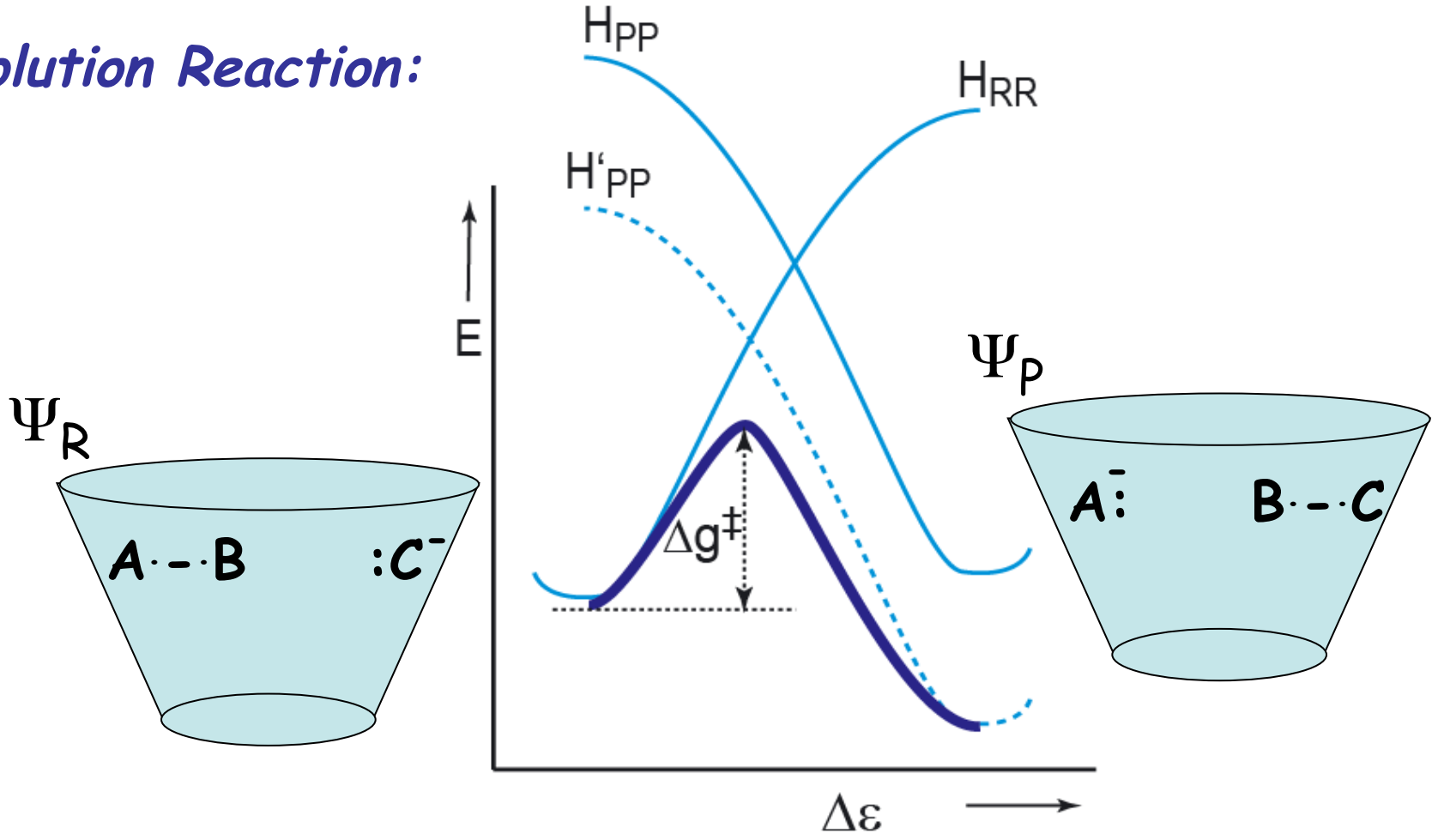
- Gas phase shift - α
- Resonance energy $H_{RP}=\beta_{RP}$

What can be studied ?

Empirical VB (EVB) – Comparative Studies

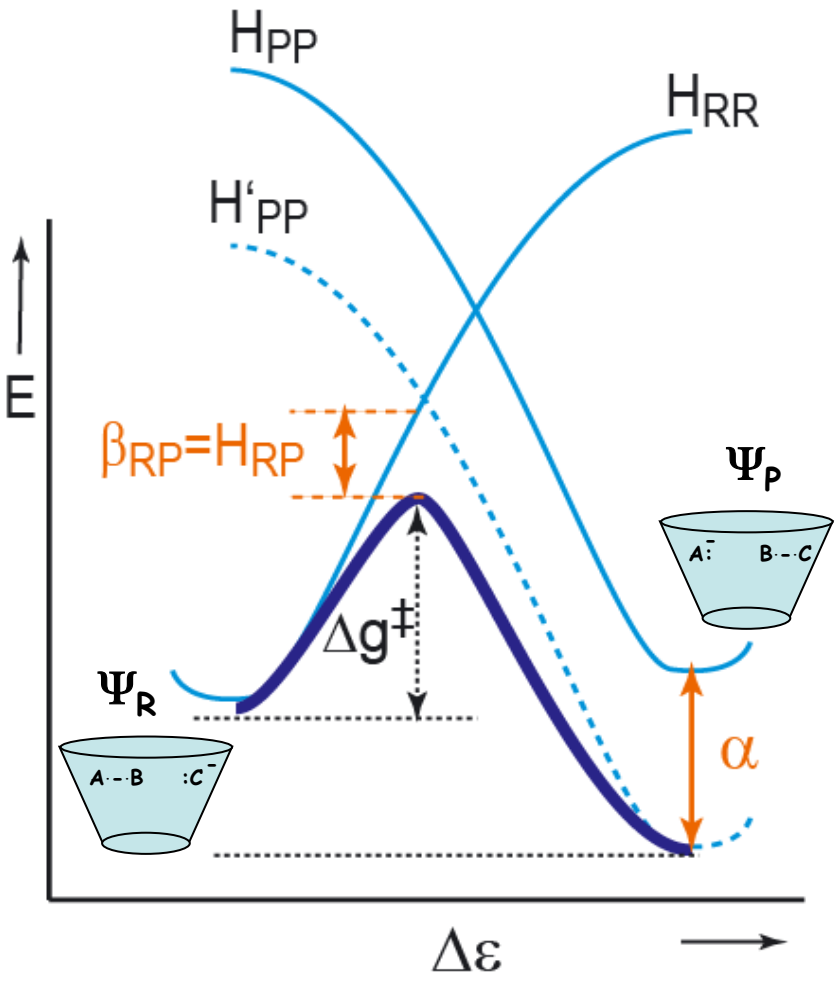
Use same parameters for different environments!

Solution Reaction:

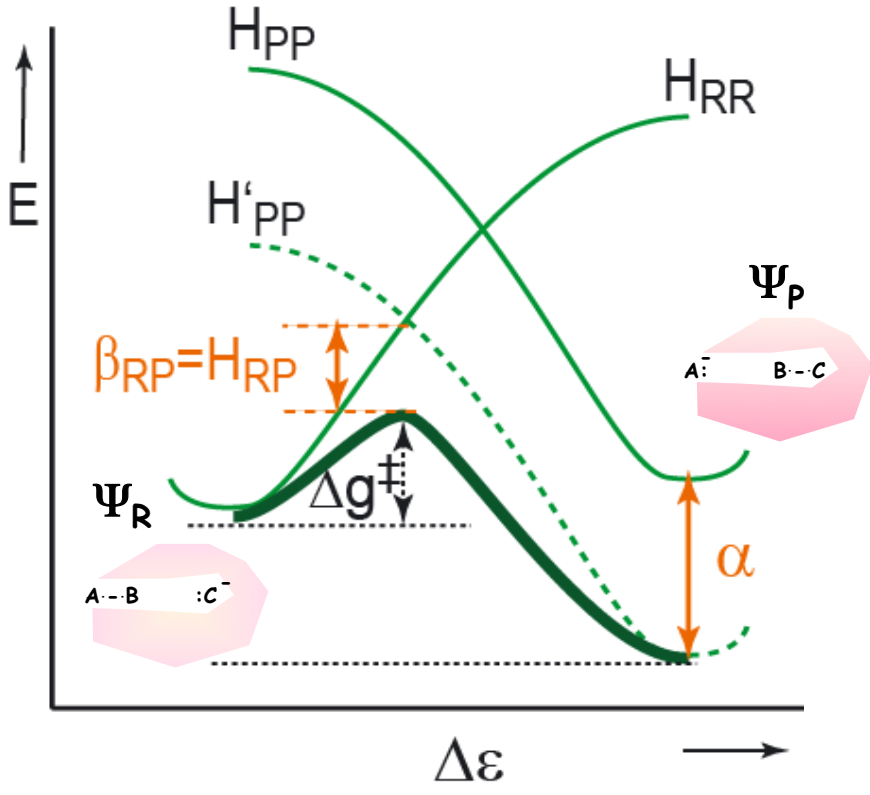


Two classical states which include the environment

Water

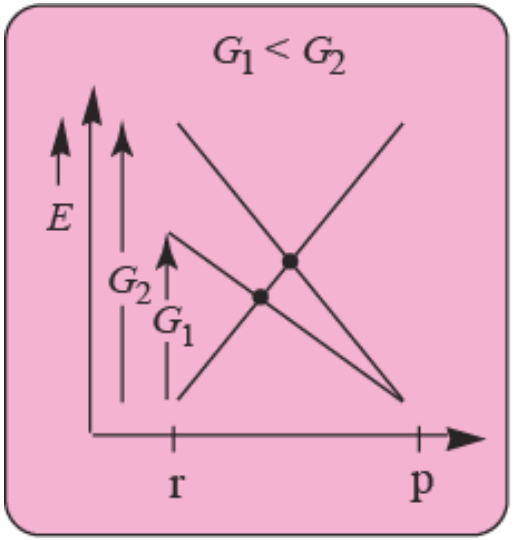


Protein

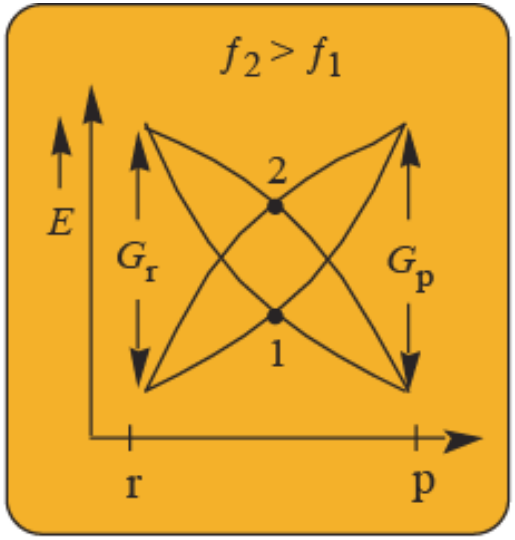


VB State Correlation Diagram (VBSCD)

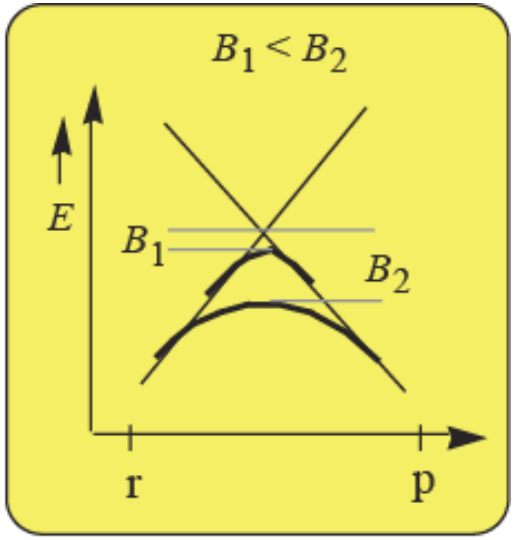
Factors governing reactivity:



$G = I_{X:-} - A_{R-X}$



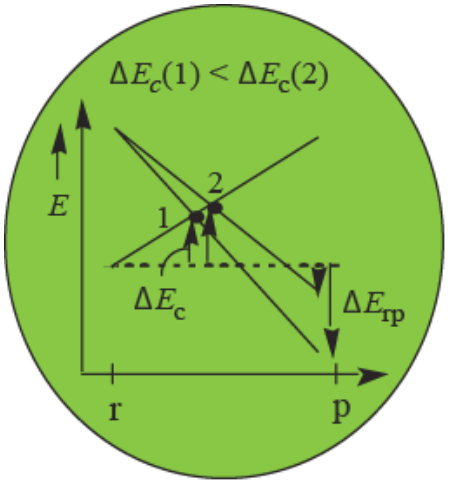
f : delocalization



B: Steriochemistry

$$\Delta E^{\ddagger} = f G - B$$

$$\Delta E^{\ddagger} = f_{av} G_r + F(\Delta E_{rp}) - B$$



Ionic contribution

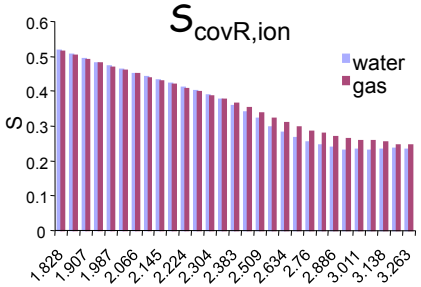
	Reactants	TS
Gas	0.36	0.53
Solution	0.34	0.61
Protein	0.38	0.59

The protein increased the contribution of the ionic structure in the reactants, increasing delocalization in the reactants and thus reducing the barrier compared to the solution.

Ionic (Reactant's Covalent) contribution

	Reactants	TS
Gas	0.36 (0.60)	0.53 (0.28)
Solution	0.34 (0.65)	0.61 (0.23)
Protein	0.38 (0.60)	0.59 (0.26)

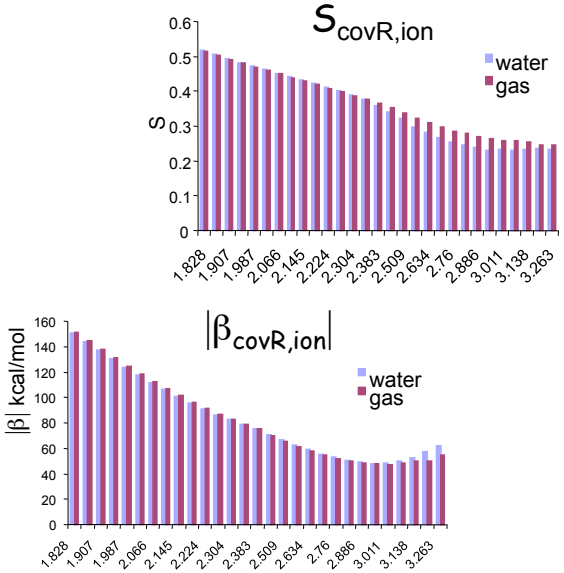
Examination of approximations regarding S_{ij} and β_{ij}



TS geometry (2.38Å)

	Gas	VB/MM	DE-VB/MM
	overlap		
$S_{covR,covP}$	0.124	Same as gas	0.118
$S_{covR,ion}$	0.376		0.359
$S_{covP,ion}$	0.349		0.359

Examination of approximations regarding S_{ij} and β_{ij}



TS geometry (2.38Å)

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overlap			
$S_{covR,covP}$	0.124	Same as gas	0.118
$S_{covR,ion}$	0.376		0.359
$S_{covP,ion}$	0.349		0.359
Reduced Resonance Integral			
$\beta_{covR,covP}$	-45.9	Same as gas	-45.1
$\beta_{covR,ion}$	-75.4		-75.6
$\beta_{covP,ion}$	-75.4		-75.4

- *Changes in overlap and reduced resonance are negligible*

The approximations in VB/MM seem reasonable

Examination of Wavefunction

$$Cl\text{-}CH_3 + Cl^- \longrightarrow Cl^- + CH_3\text{-}Cl$$

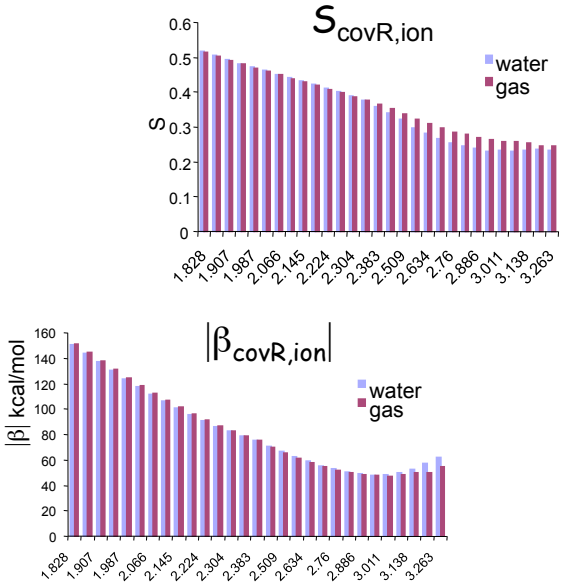
TS geometry (2.38Å)

	Gas	VB/MM	DE-VB/MM
weights			
w_{covR}	0.268	0.216	0.217
w_{covP}	0.268	0.219	0.222
w_{ion}	0.462	0.565	0.560

Changes in the weights are similar for the two methods

VB/MM seems sufficient to account for most of the wavefunction polarization

Examination of approximations regarding S_{ij} and β_{ij}



TS geometry (2.38Å)

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$S_{covR,covP}$	0.124	Same as gas	0.118
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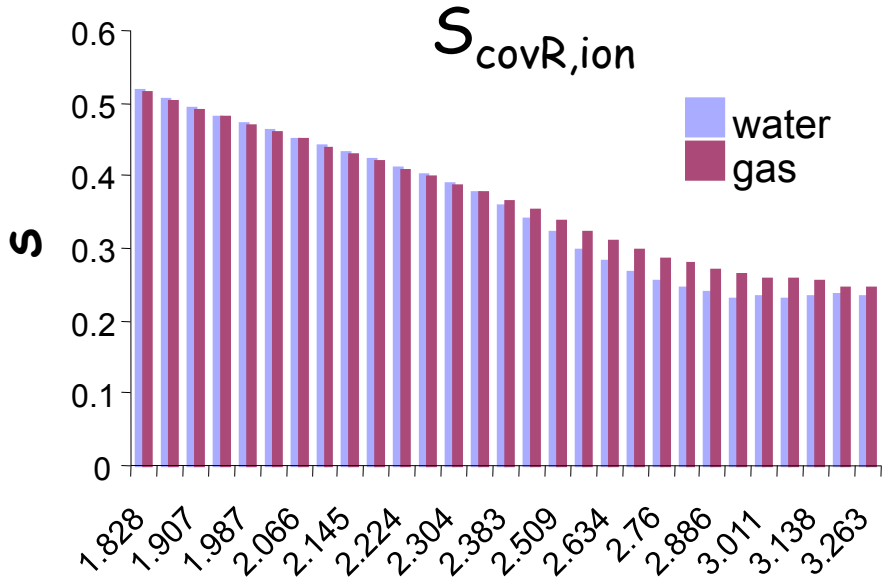
Reduced Resonance Integral			
$\beta_{covR,covP}$	-45.9	Same as gas	-45.1
$\beta_{covR,ion}$	-75.4		-75.6
$\beta_{covP,ion}$	-75.4		-75.4
weights			
φ_{covR}	0.268	0.216	0.217
φ_{covP}	0.268	0.219	0.222
φ_{ion}	0.462	0.565	0.560

- *Changes in overlap and reduced resonance are negligible*
- *The trends in the weights is kept*

The approximations in VB/MM seem reasonable

Examination of Approximations - overlap

$$Cl\text{-}CH_3 + Cl^- \longrightarrow Cl^- + CH_3\text{-}Cl$$



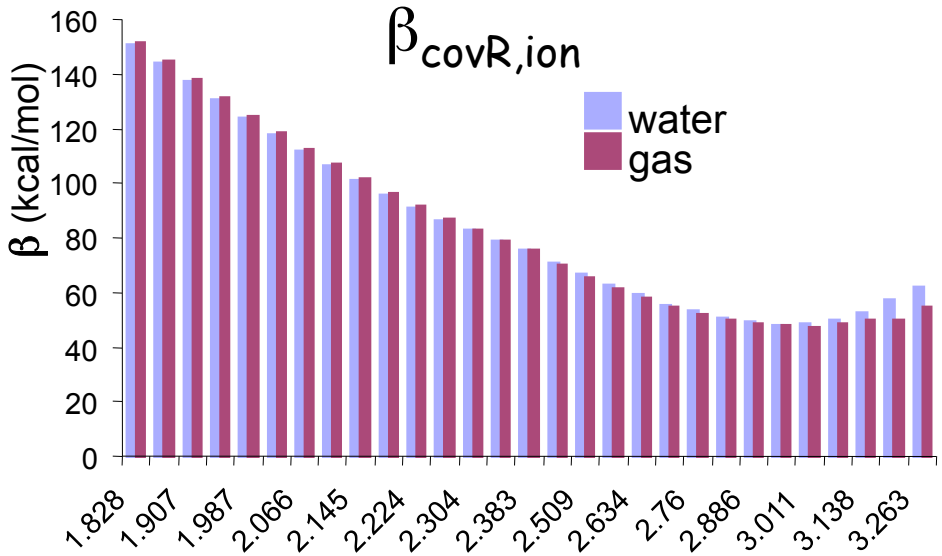
Changes in overlap are negligible

TS geometry (2.38Å)

	Gas	VB/MM	DE-VB/MM
	overlap		
$S_{covR,covP}$	0.124	Same as gas	0.118
$S_{covR,ion}$	-0.376		-0.359
$S_{covP,ion}$	-0.349		-0.359

Examination of Approximations – reduced resonance

$$Cl\text{-}CH_3 + Cl^- \longrightarrow Cl^- + CH_3\text{-}Cl$$



Changes in reduced resonance are negligible

TS geometry (2.38Å)

	Gas	VB/MM	DE-VB/MM
Reduced Resonance Integral			
$\beta_{covR,covP}$	-45.9	Same as gas	-45.1
$\beta_{covR,ion}$	75.4		75.6
$\beta_{covP,ion}$	75.4		75.4

Results: Li-F

Ionic Bond

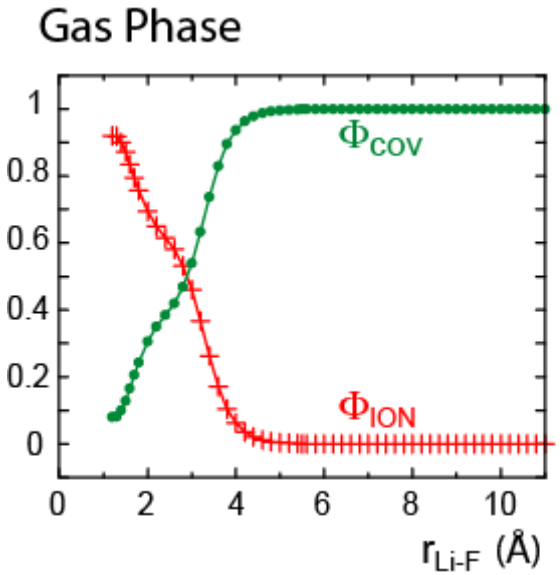
VB Structures:

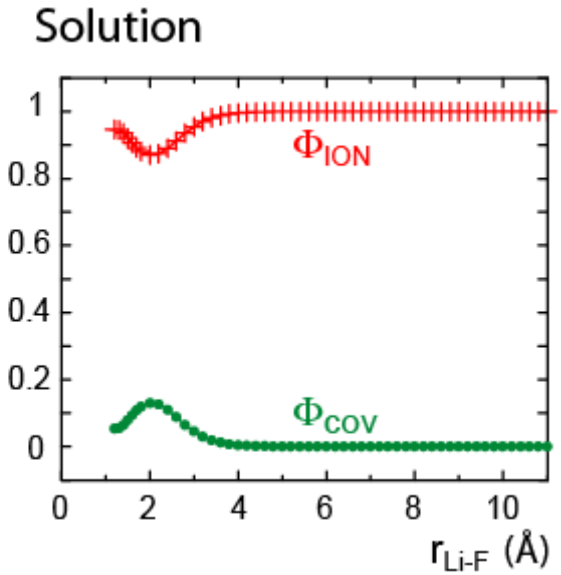
Φ_{COV} $Li\cdot\!\!-\!\!\cdot F$

Φ_{ION} $Li^+ :F^-$

~~Φ_{ION} $Li:^- F^+$~~

Weights:



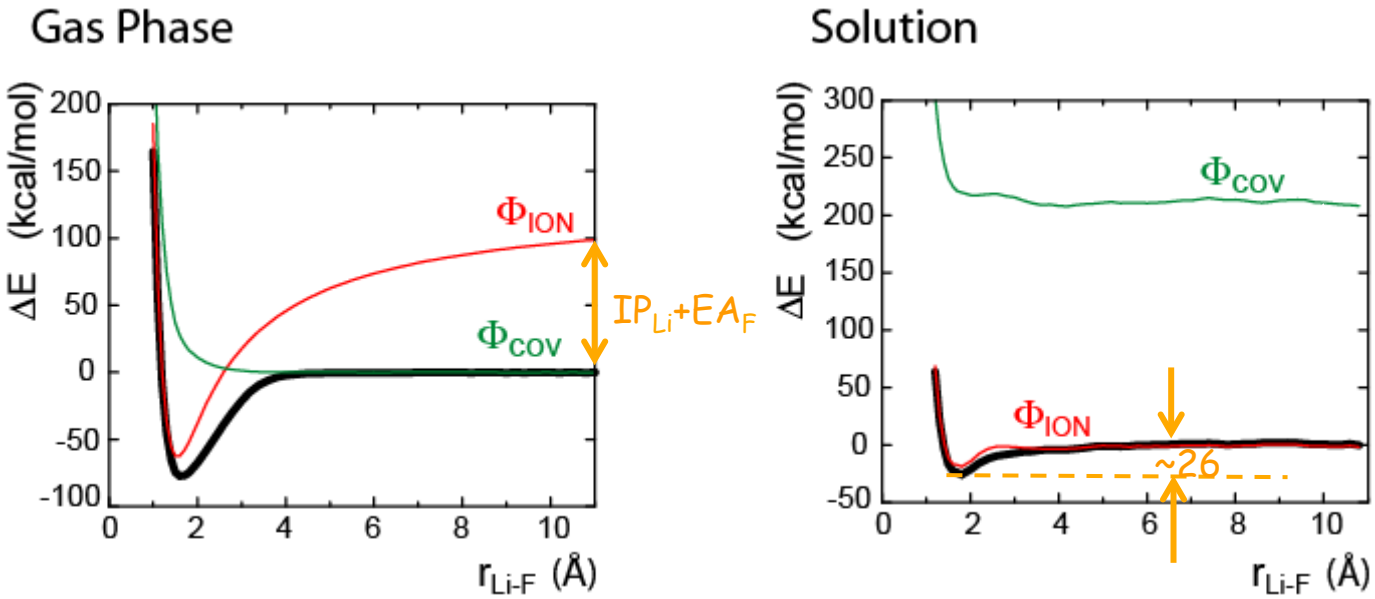


6-31G* basis set
1S electrons frozen

Results: Li-F

6-31G* basis set
1S electrons frozen

Energy Curves



- Predicted dissociation of barrier ~26 kcal/mol instead of ~5 kcal/mol probably due to mal gas phase behavior (VBPCM - 55/30 kcal/mol)
  exp $IP_{Li}+EA_F$ gives ~45 kcal/mol and not ~100 kcal/mol as calculated
- Bond length shifts from 1.6Å to 1.8Å in solution
- Adiabatic state stabilizes ~210 kcal/mol relative to the covalent state at long distance in agreement with ~220 kcal/mol solvation of the ions

Tertiary-Butyl Chloride: t-BuCl

Polar Covalent Bond → Vacuum → Radicals

Polar Covalent Bond → Solution → Ions

$Cl\text{-}C(CH_3)_3 + Cl^- \longrightarrow \boxed{Cl^- + (CH_3)_3C^+ + Cl^-} \longrightarrow Cl^- + C(CH_3)_3\text{-}Cl$

S_N1 mechanism in solution

Relevant VB Structures:

Φ_{COV} $Cl\cdot\!\!-\!\!\cdot C(CH_3)_3$

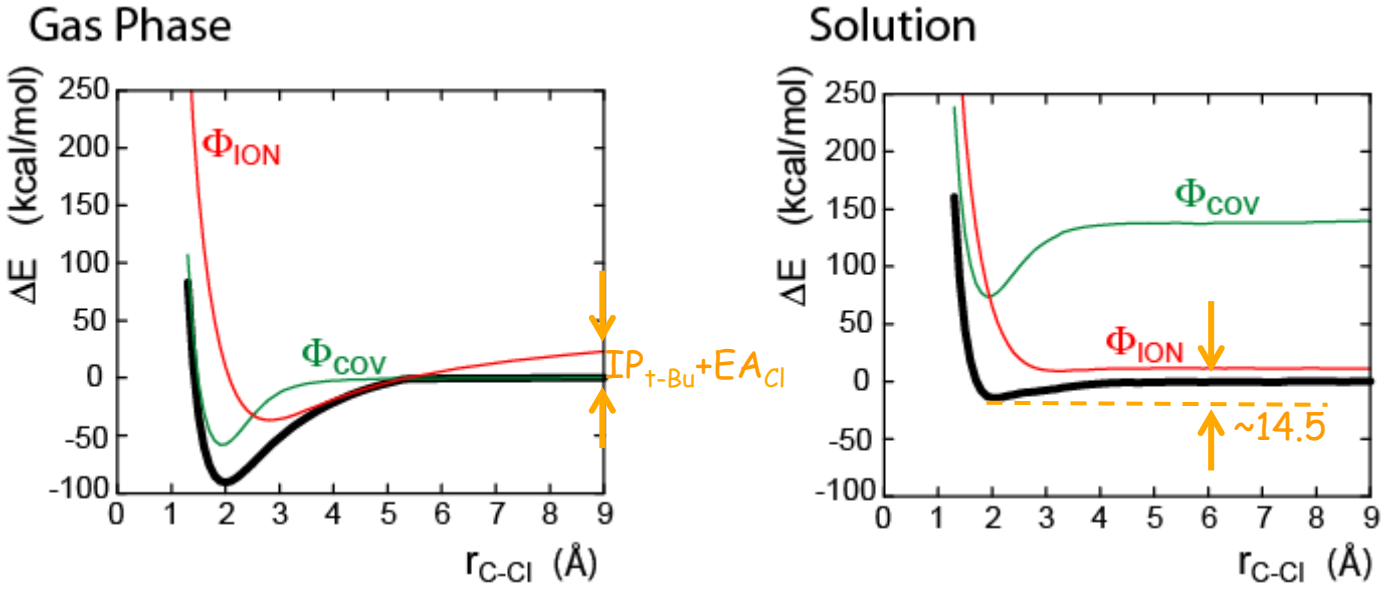
Φ_{ION1} $Cl:^-\ (CH)_3C^+$

~~Φ_{ION2} $Cl^+\ (CH)_3C:^-$~~

Results: t-BuCl

Energy Curves

6-31G basis set
π electrons frozen



- Dissociation into: radicals – vacuum vs ions - solution

- Predicted dissociation barrier of ~14.5 kcal/mol instead of ~19.5 kcal/mol (VBPCM 27.8 kcal/mol)
- Absence of ion pair formation
  again, probably due to mal gas phase behavior

($IP_{t\text{-}Bu}+EA_{Cl}$ gives ~84 kcal/mol and not ~56 kcal/mol as calculated)

CH_3-F

Polar Covalent Bond $\xrightarrow[\text{Solution}]{\text{Vacuum}}$ Radicals

Relevant VB Structures:

Φ_{COV} $F\cdot\!\!-\!\!\cdot CH_3$

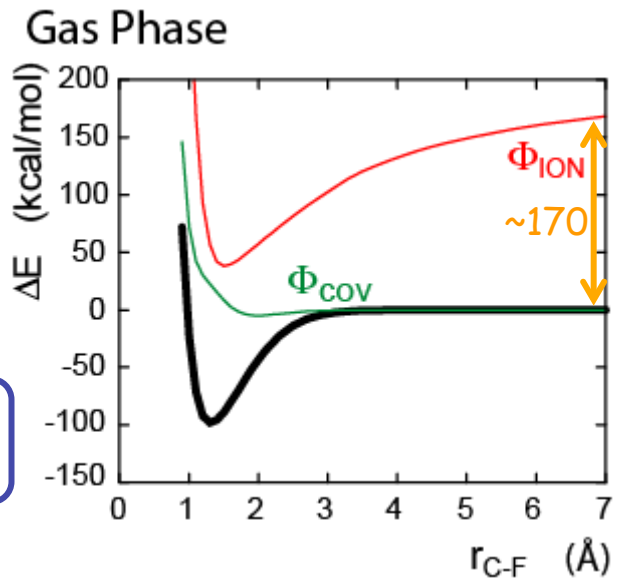
Φ_{ION1} $F:^- \; CH_3^+$

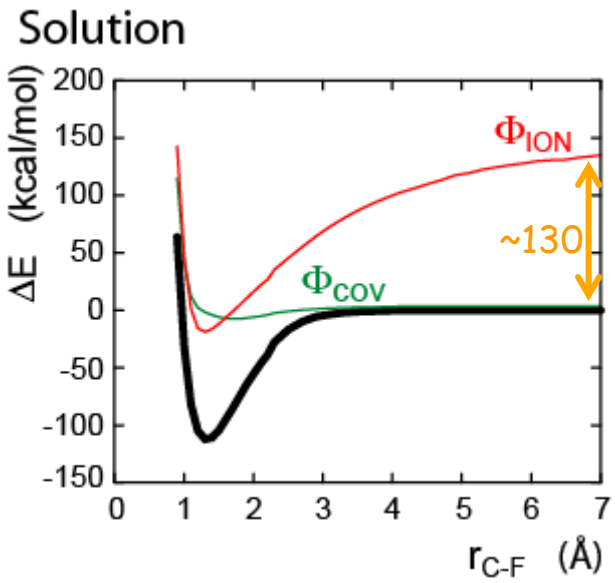
~~Φ_{ION2} $F^+ \; CH_3:^-$~~

CH_3-F

Energy Curves

6-31G* basis set
Core electrons frozen





- Homolytic dissociation in both vacuum and solution
- Most of the bond energy comes from resonance
- Ionic stabilization of 50 kcal/mol – equilibrium
  40 kcal/mol – long distance
- Expected small ionic-covalent gap in solution

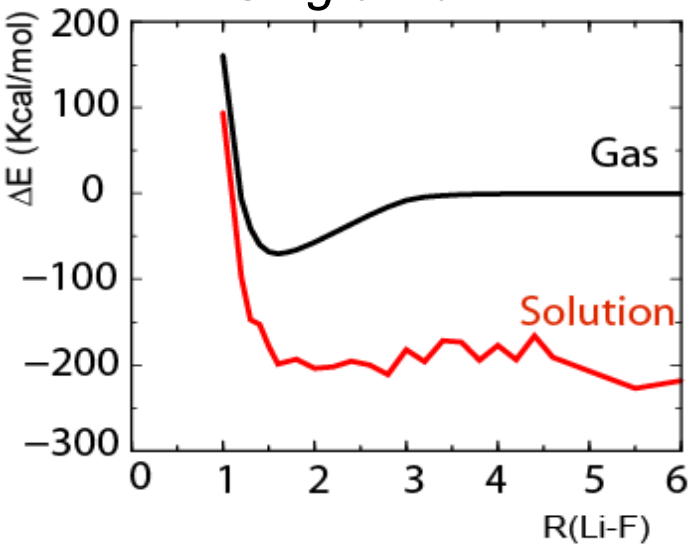
(IP_{CH_3} + EA_F π ~149 kcal/mol = Solvation[CH_3^+]+ Solvation[F^-])

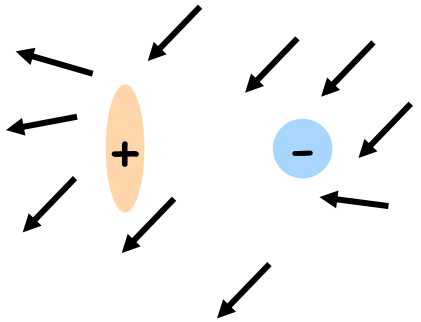
- Mal gas phase description – basis set
- Mean field description of the solution configurations.
- Insufficient sampling

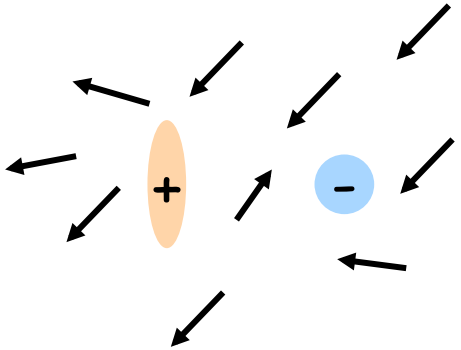
The importance of enough Sampling

Taken from calculations without the E_{MM}

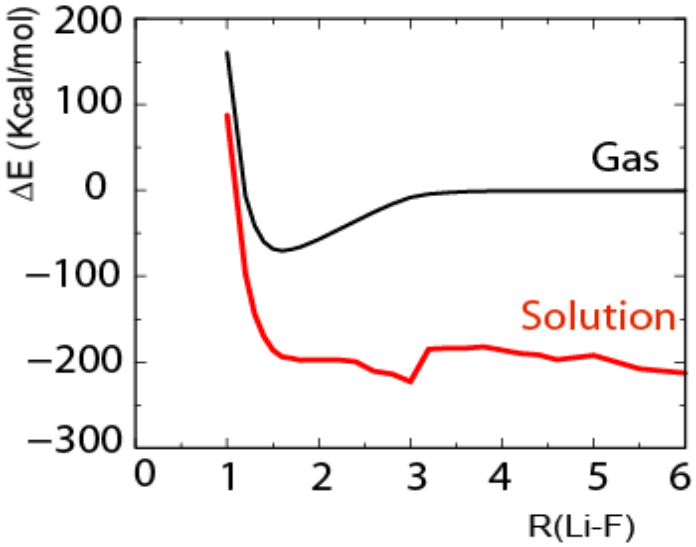
Single Run

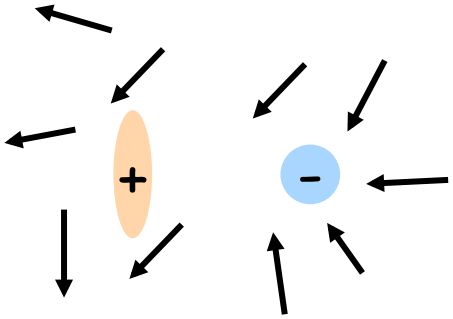






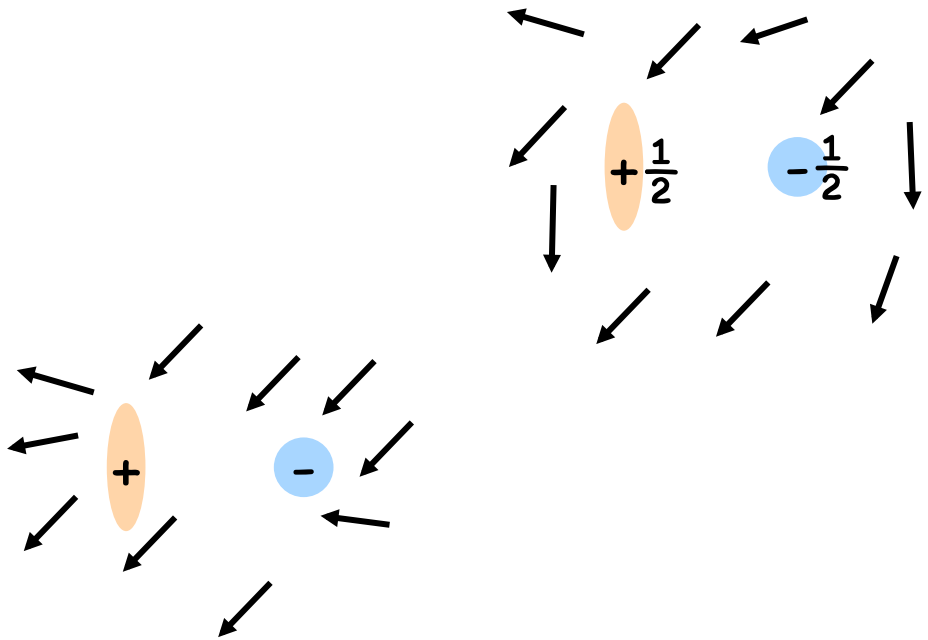
Average over 30 configurations



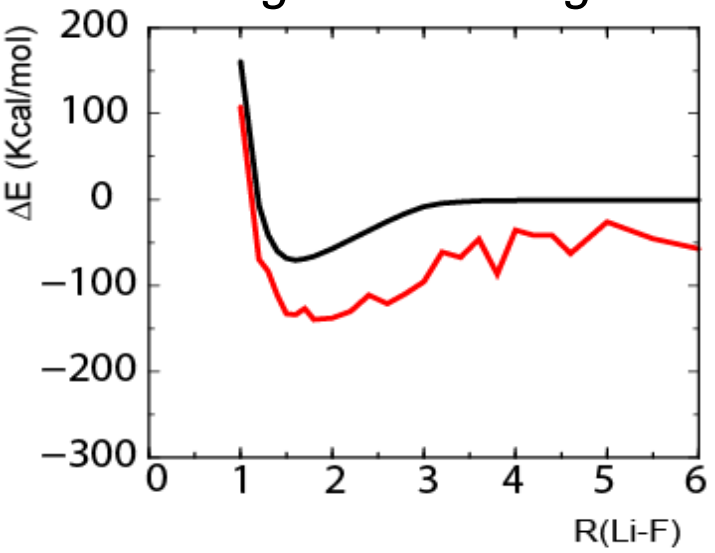


The importance of correct Sampling

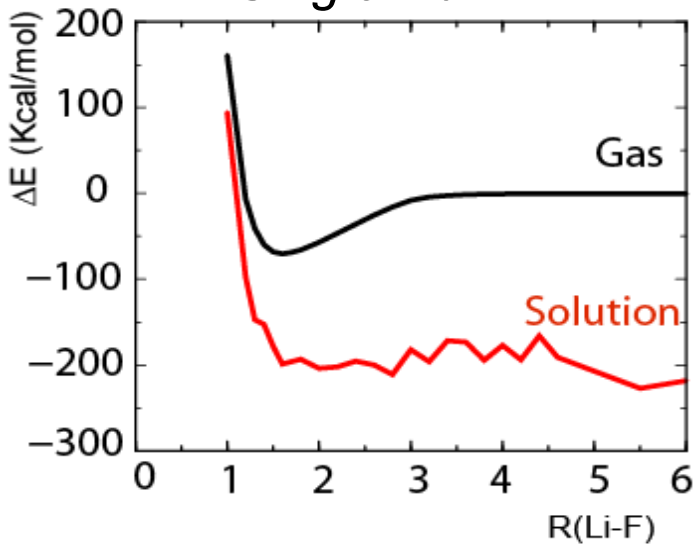
Taken from calculations without the E_{MM}



Wrong water configuration



Single Run



$\Psi \rightarrow \frac{1}{2}(\Phi_{ION}+\Phi_{COV})$

Water relaxed with

$\Psi \rightarrow$ mainly Φ_{ION}

ab initio VB/MM

Finally …

The ab –initio VB calculations of the reactive fragments utilized the program **XMVB**:

Lingchun Song, Wei Wu, Yirong Mo, Qianer Zhang, **XMVB** – an ab initio Non-orthogonal Valence Bond Program, Center of Theoretical Chemistry, Department of Chemistry, and State Key Laboratory for Physical Chemistry of Solid Surfaces, Xiamen University, Xiamen Fujain 36005, China.

These values were incorporated into MOLARIS whose MM calculations were utilized

Microscopic and Semimicroscopic Calculations of Electrostatic Energies in Proteins by the POLARIS and ENZYMIX Programs, F. S. Lee, Z. T. Chu, and A. Warshel, J. Comp. Chem. 14, 161 (1993).

Solvation Energies (weighted)

Old values

		Reactants	TS	$\Delta_{(TS-R)}$
water	CovR	-54	-17	37
	Ion	-26	-49	-23
	Tot	**-80**	**-73**	**7**
protein	CovR	-54	-23	31
	Ion	-37	-61	-24
	Tot	**-92**	**-93**	**-1**

- The stabilization in the protein is consistently larger than in water
- Water stabilize CovR in the reactants geometry much more than in the TS - leading to the increased barrier

Solvation Energies (weighted)

Old values

		Reactants	TS	$\Delta_{(TS-R)}$	$\Delta\Delta_{(P-W)}$
water	CovR	-54	-17	37	
	Ion	-26	-49	-23	
	Tot	**-80**	**-73**	**7**	
protein	CovR	-54	-23	31	-6
	Ion	-37	-61	-24	-1
	Tot	**-92**	**-93**	**-1**	**-8**

- The stabilization in the protein is consistently larger than in water
- Water stabilize CovR in the reactants geometry much more than in the TS - leading to the increased barrier
- This differential stabilization considerably decreases in the protein