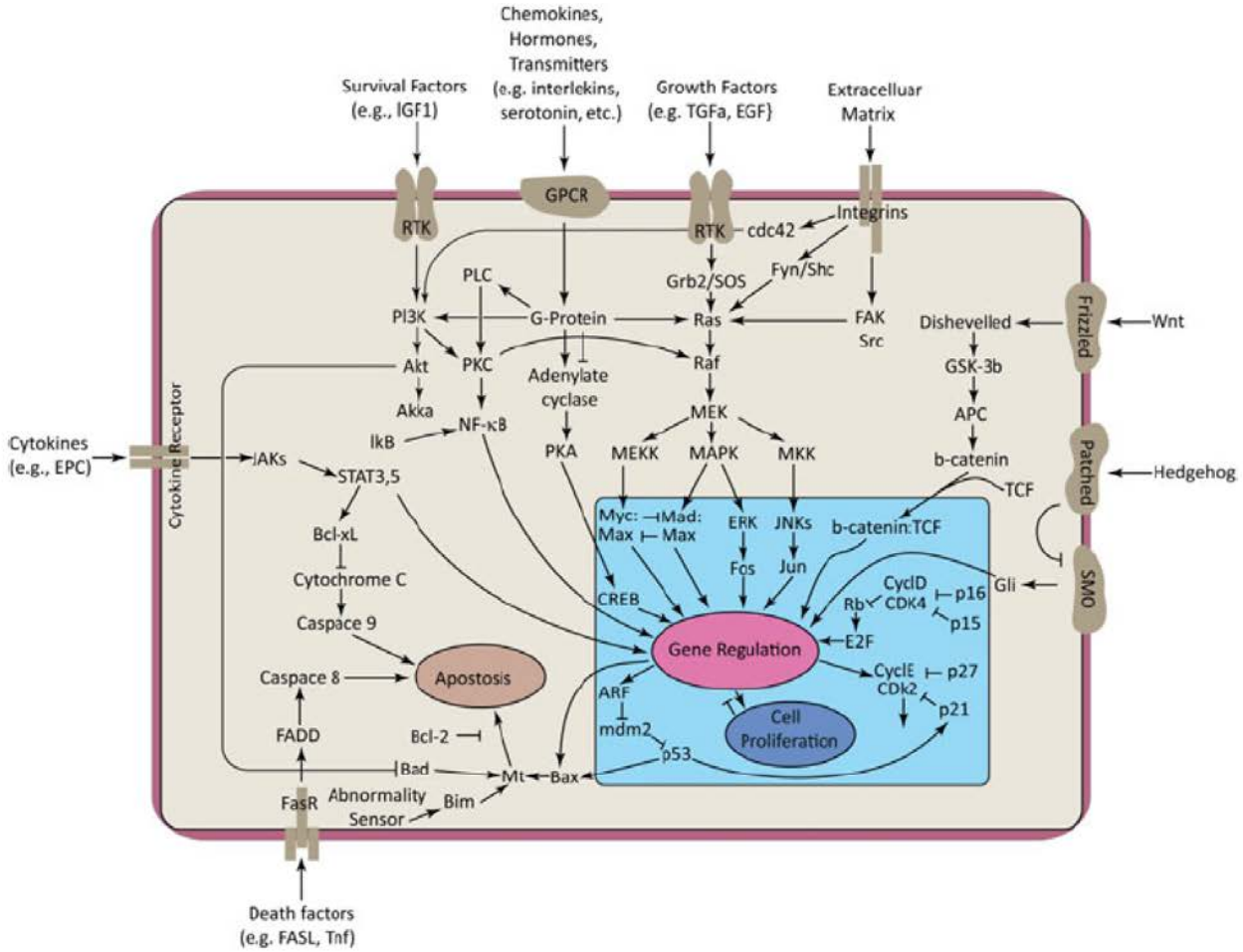
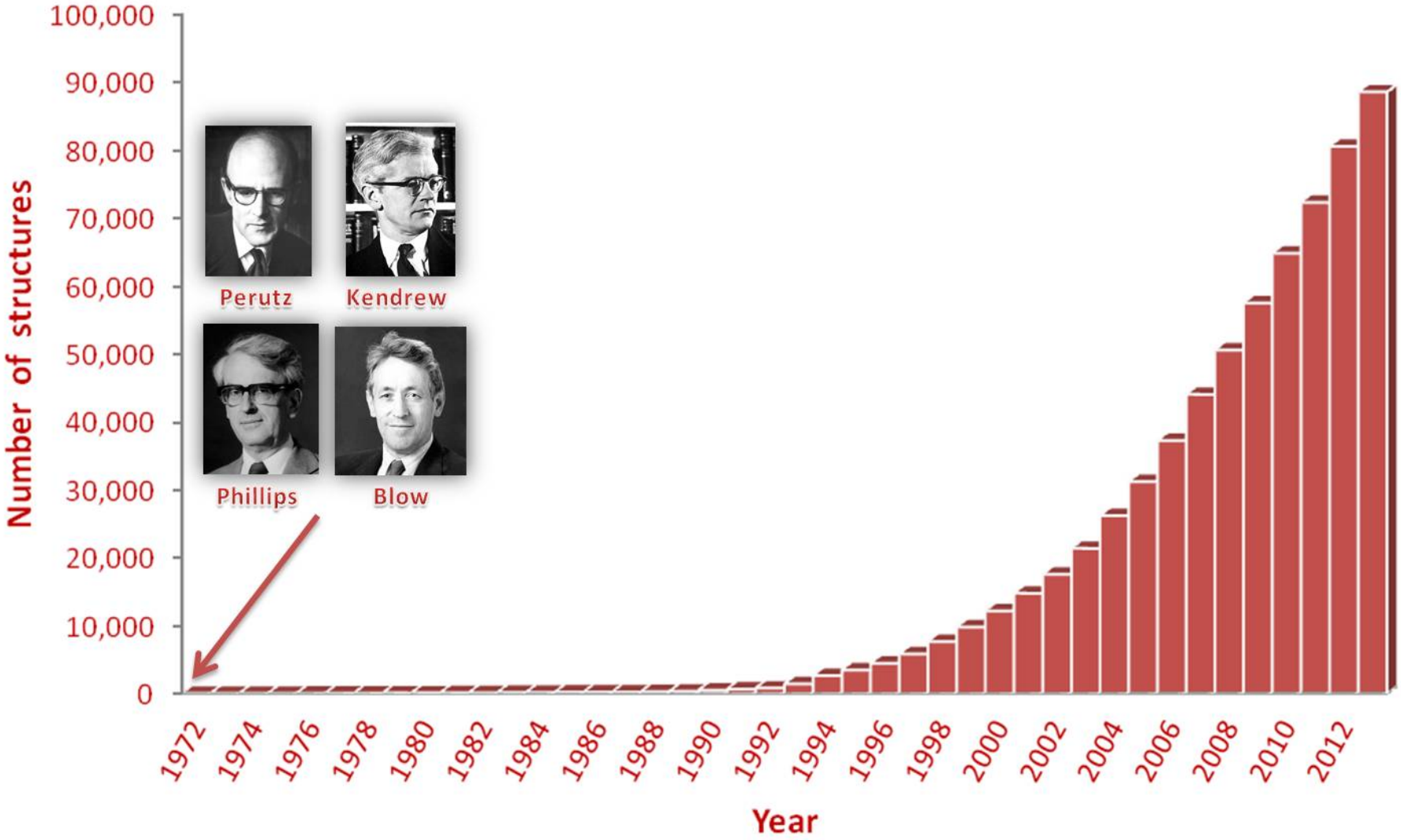
Computer Simulations of Biological Functions; From Enzymes to Molecular Machines

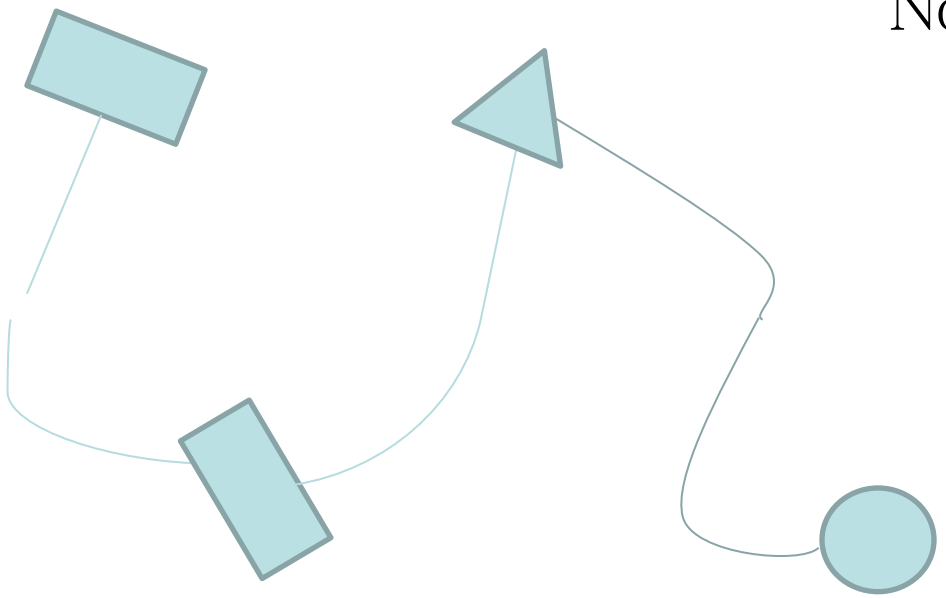
Sending information (Signals) in the cell



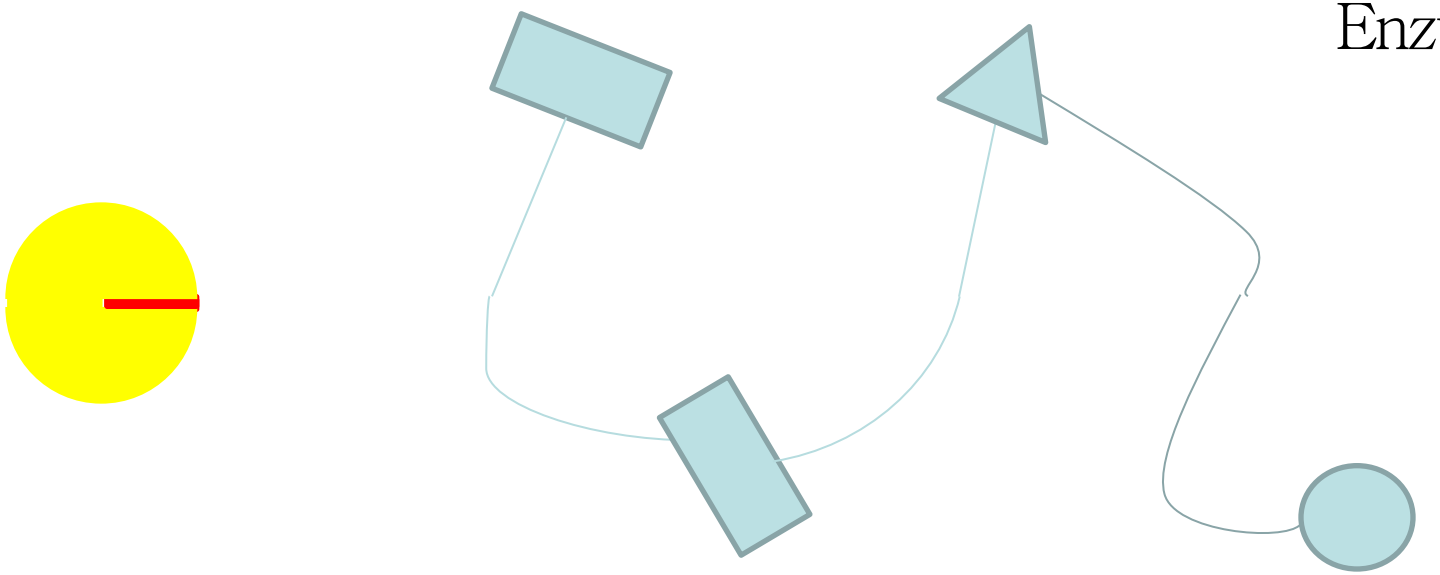
Yearly Growth of Protein Structures



No Enzyme

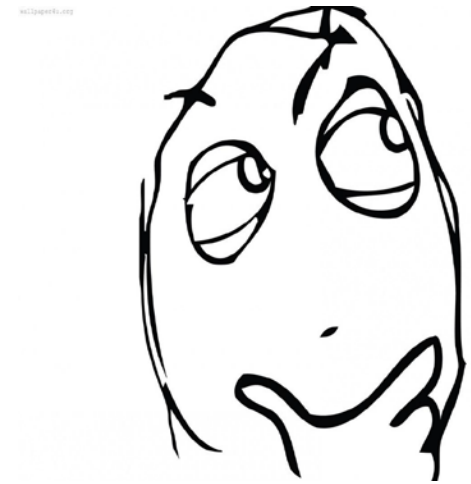


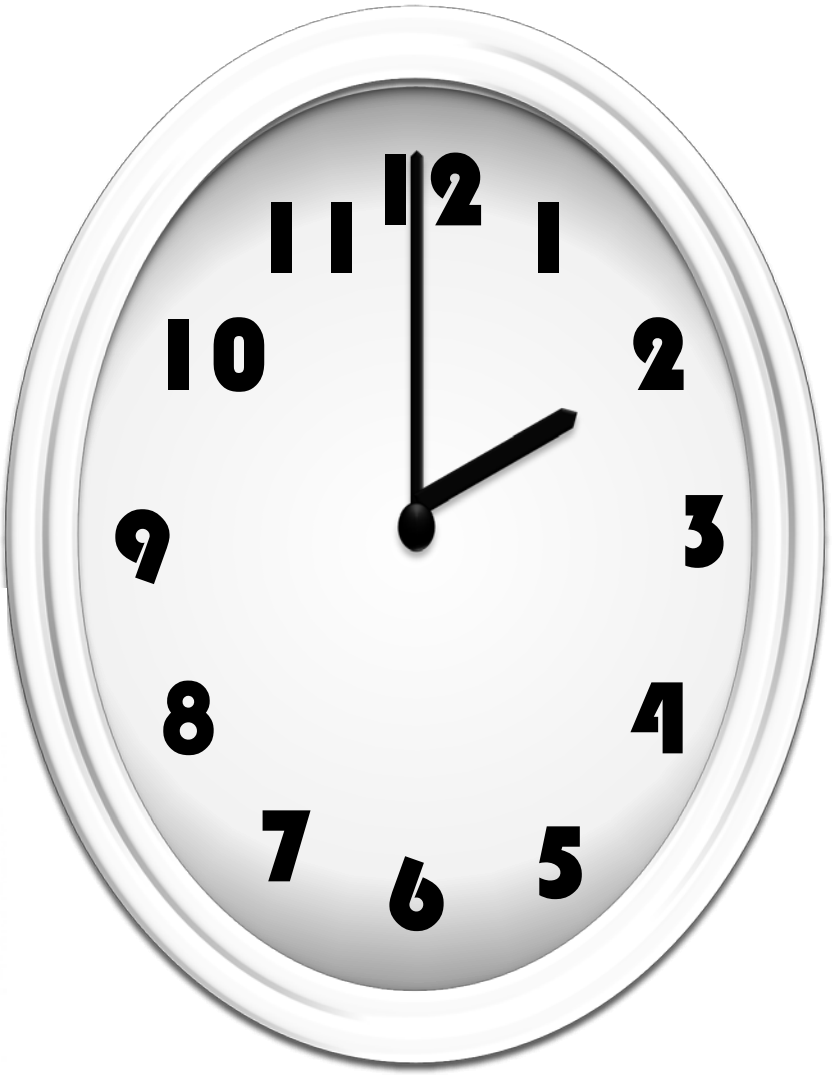
Enzyme



HUMAN
ERROR.
AGAIN.

How does that work?





Biochemistry –Discovers the clock
Crystallography –Shows all the parts
Single molecules– Determines how fast the wheels rotate

Abstract

Israel J. of Chem. Proceeding of the 34 Meeting Vol 4 1966

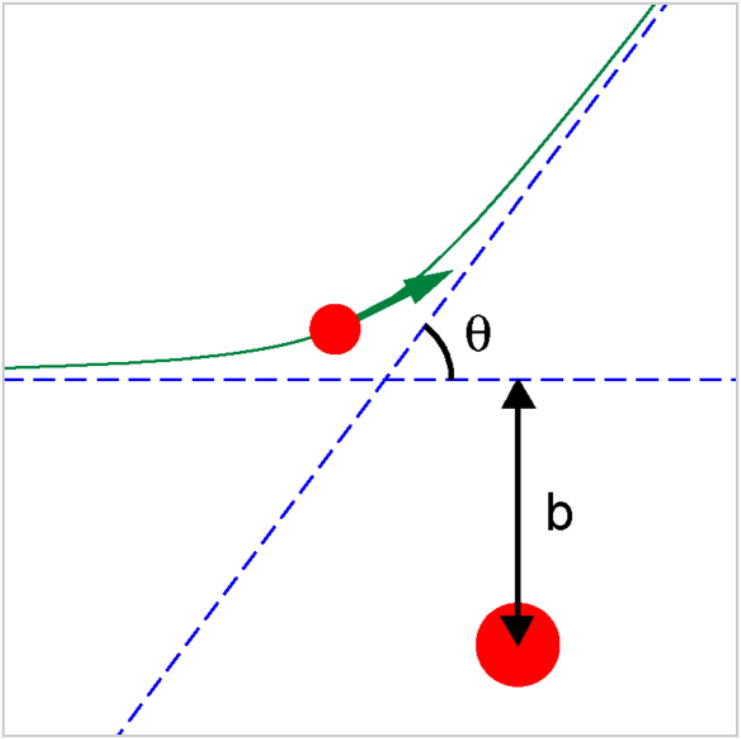
"On the interaction of chymotrypsin with ionized Substrate"

A. Varshel and Y Shalitin

During undergraduate work concluded that
(since external salts have very small effect)
electrostatic is unlikely to be important

Impact parameter

Asymptotic solution for enzymes (Thecnion 1965)-Eventially EVB



Impact parameter *b* and scattering angle θ.

Lifson (Nir David)——Warshel (Sde Nahom) about 3 km distance

1968-1970 Weizmann Institute

THE JOURNAL OF CHEMICAL PHYSICS VOLUME 49, NUMBER 11 1 DECEMBER 1968

Consistent Force Field for Calculations of Conformations, Vibrational Spectra, and Enthalpies of Cycloalkane and *n*-Alkane Molecules

S. LIFSON AND A. WARSHEL
Department of Chemical Physics, Weizmann Institute of Science, Rehovot, Israel

Consistent Force Field Calculations. II. Crystal Structures, Sublimation Energies, Molecular and Lattice Vibrations, Molecular Conformations, and Enthalpies of Alkanes

ARIEH WARSHEL AND SHNEIOR LIFSON
Department of Chemical Physics, Weizmann Institute of Science, Rehovot, Israel
(Received 2 February 1970)

Around 20 years latter

Consistent Force Field for Calculation of Vibrational Spectra and Conformations of Some Amides and Lactam Rings

A. WARSHEL, M. LEVITT, AND S. LIFSON

Refinement of Protein Conformations using a Macromolecular Energy Minimization Procedure

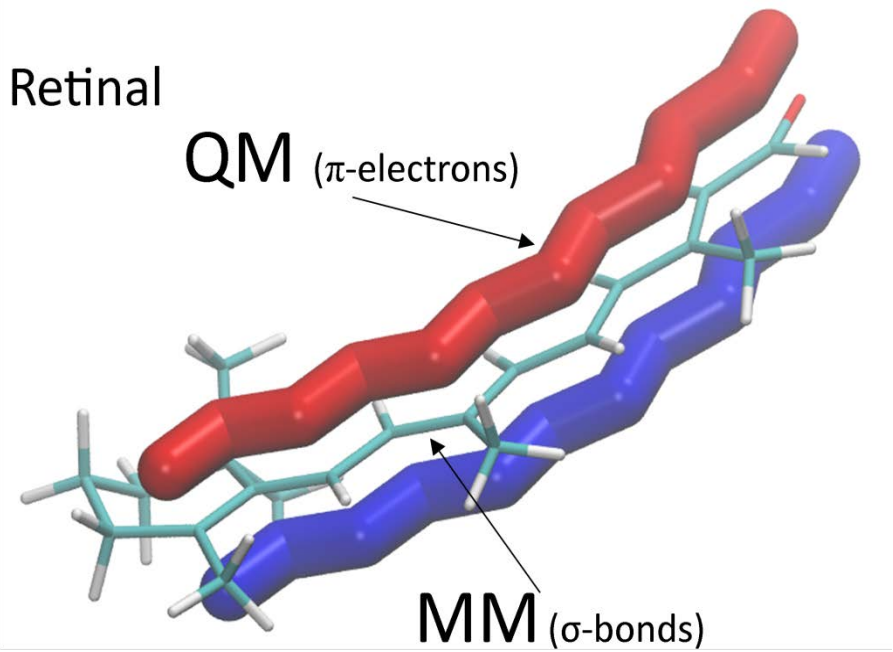
MICHAEL LEVITT AND SHNEIOR LIFSON
Weixmann Institute of Science
Rehovot, Israel

Early development of the general Cartesian Force field and programm (1966-69)

Energy , Structure and vibrations of general molecules and molecular crystals

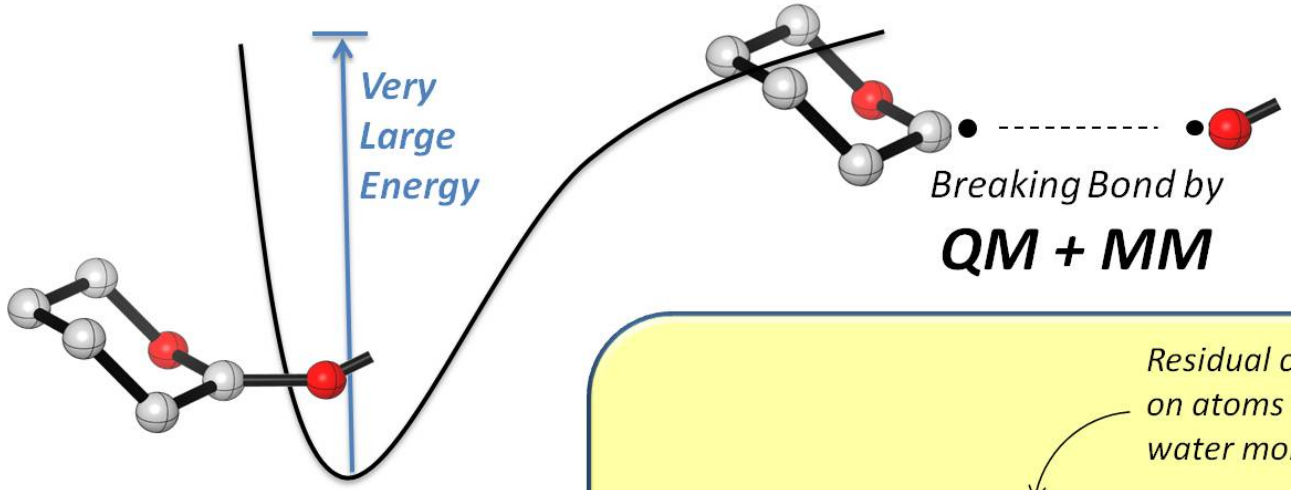
A. Warshel & M. Karplus, *J. Am. Chem. Soc.*, 1972

QM(MO)+MM



Back to Enzymes

Adding the environment to the quantum mechanics(QM) part



Breaking Bond by

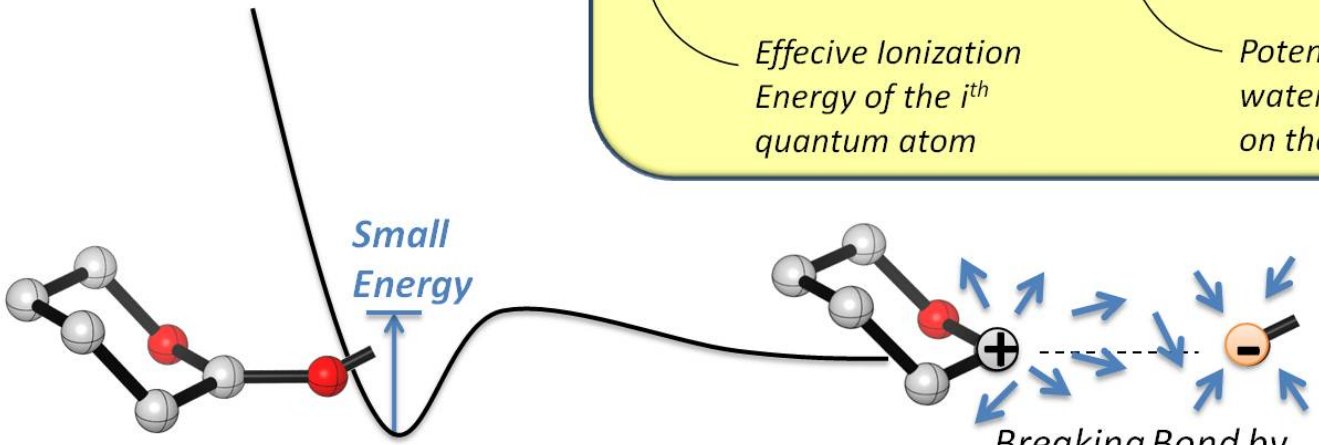
QM + MM

Residual charge on atoms of water molecules

$$H_{ii} = H_{ii}^{0} - \sum_{j} Q_j / R_{ij}$$

Effecive Ionization Energy of the i^{th} quantum atom

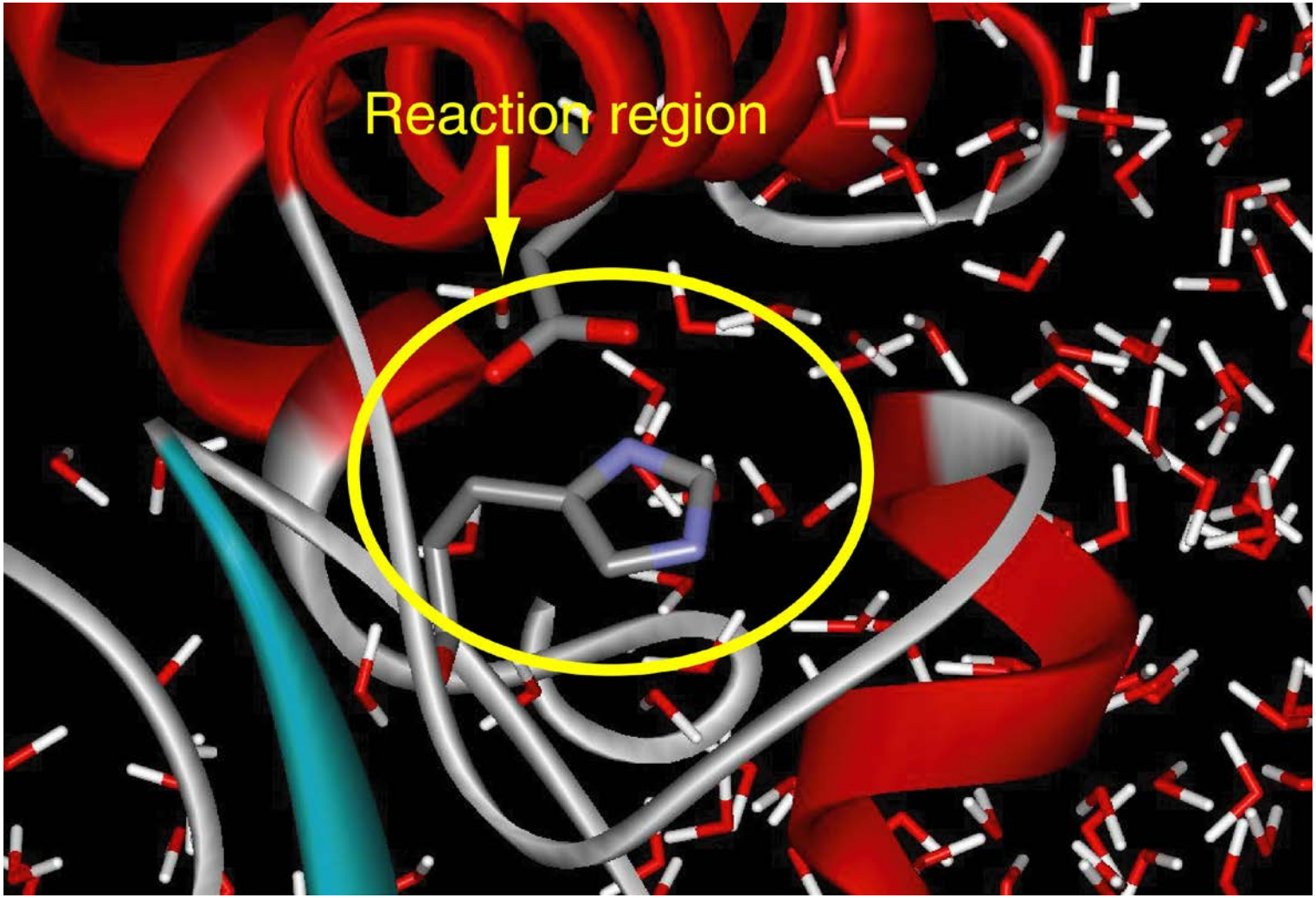
Potential from water molecules on the i^{th} atom

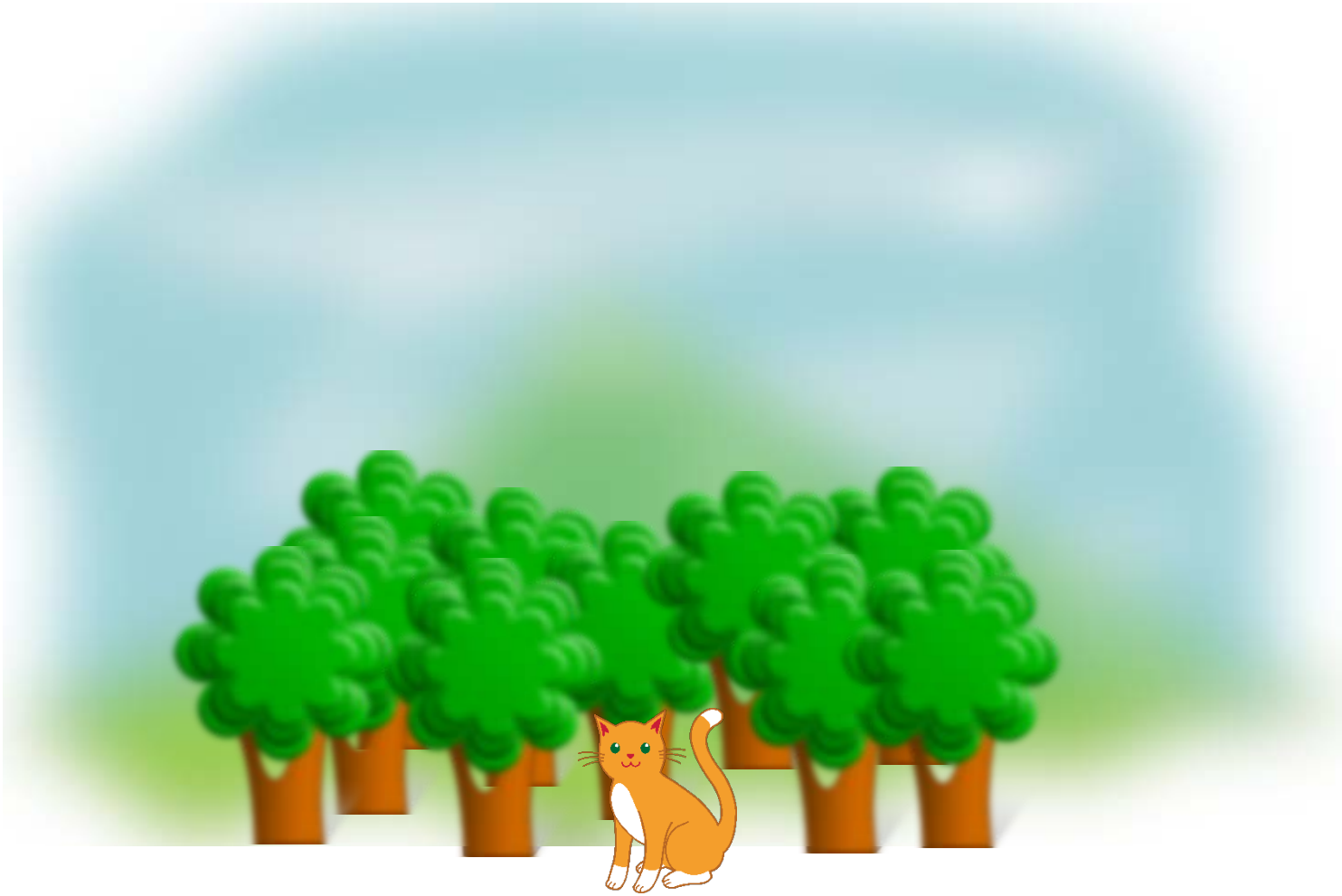


Breaking Bond by

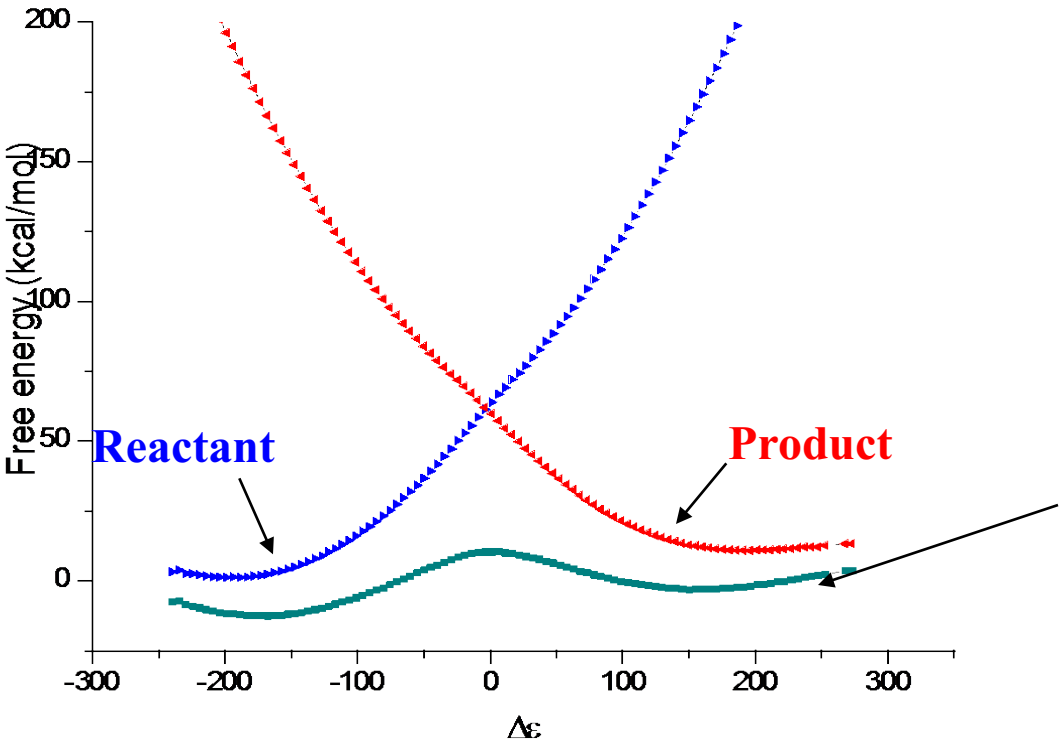
QM/MM

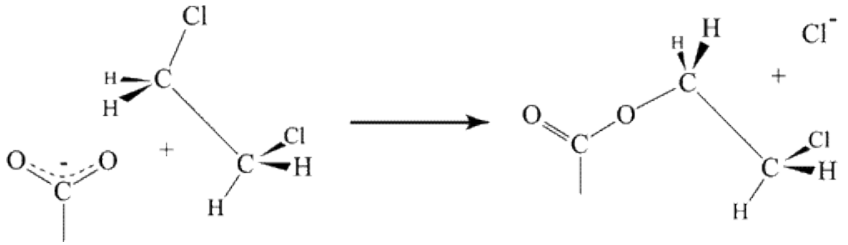
QM/MM: To study enzymatic reactions, we divide the system in two parts (Warshel & Levitt, JMB 1976)





The Empirical Valence Bond (EVB) method (JACS 1980)





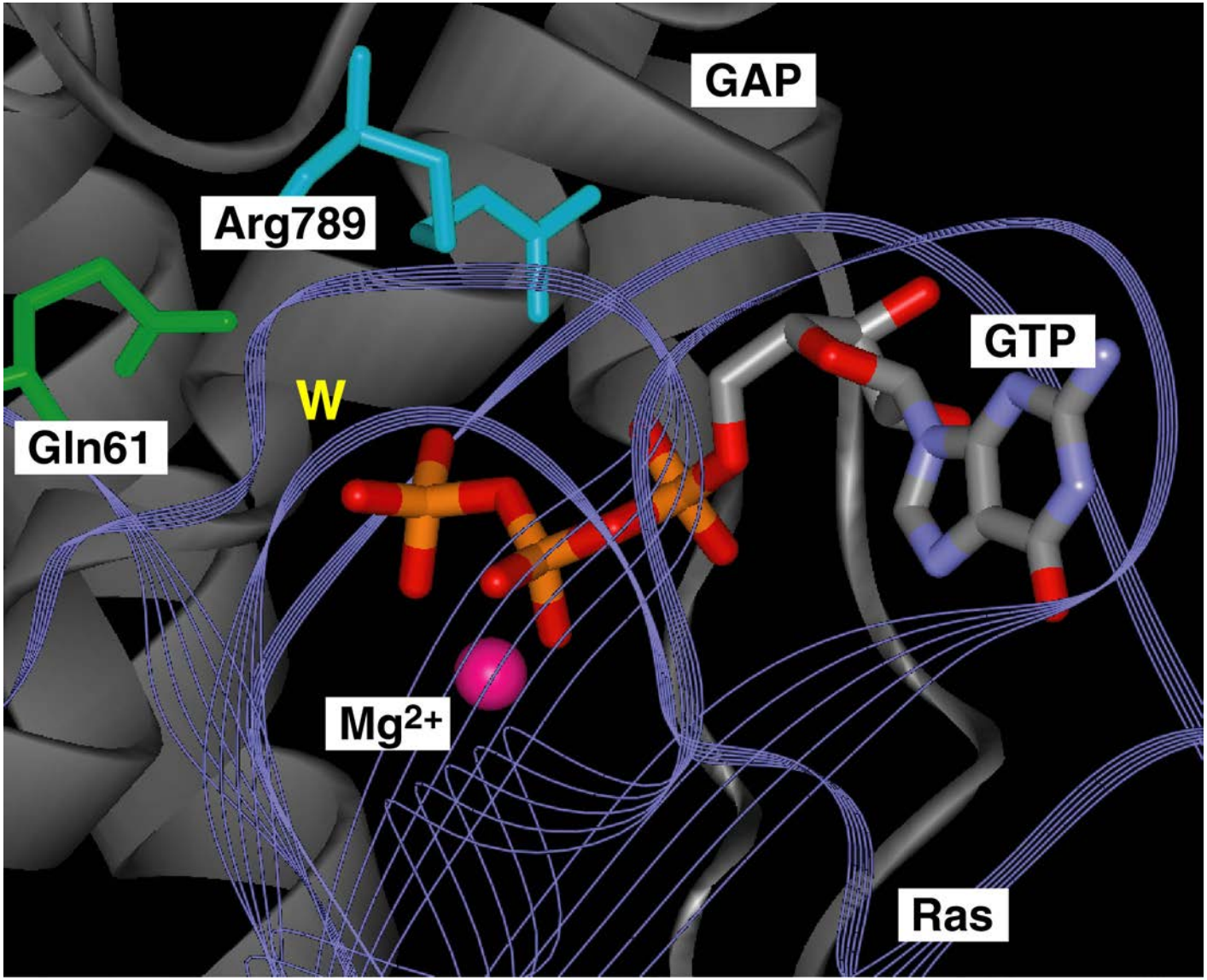
Reactant:
Force field-like functions describing the reactants' bonding pattern

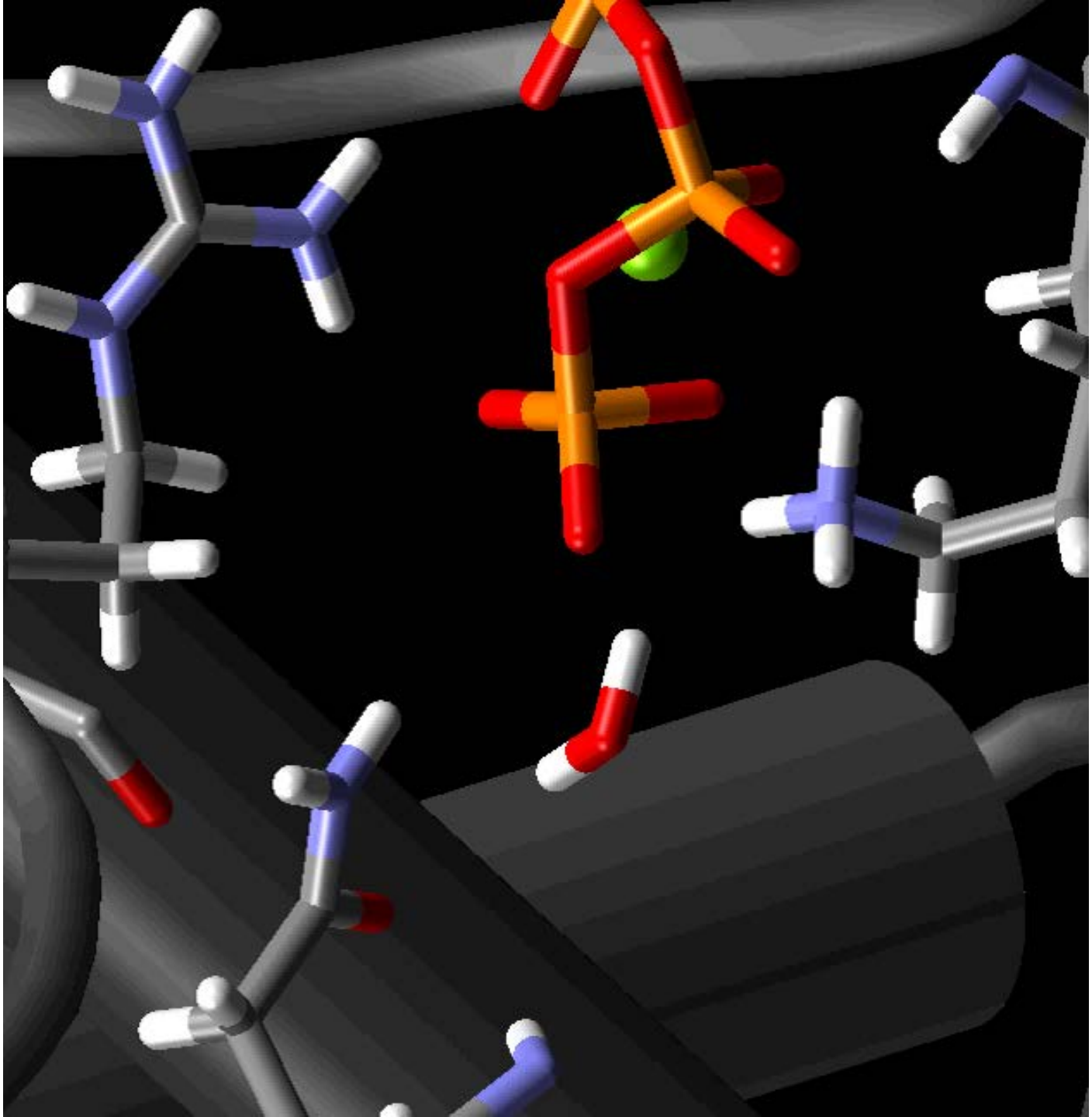
Product:
Force field-like functions describing the products' bonding pattern

Ground State:
Eigenvalue of 2x2 Hamiltonian built from Reactant and Product energies and Off-diagonal function (H_{12}).

$$H = \begin{pmatrix} \varepsilon_{react} & H_{12} \\ H_{12} & \varepsilon_{prod} \end{pmatrix}$$

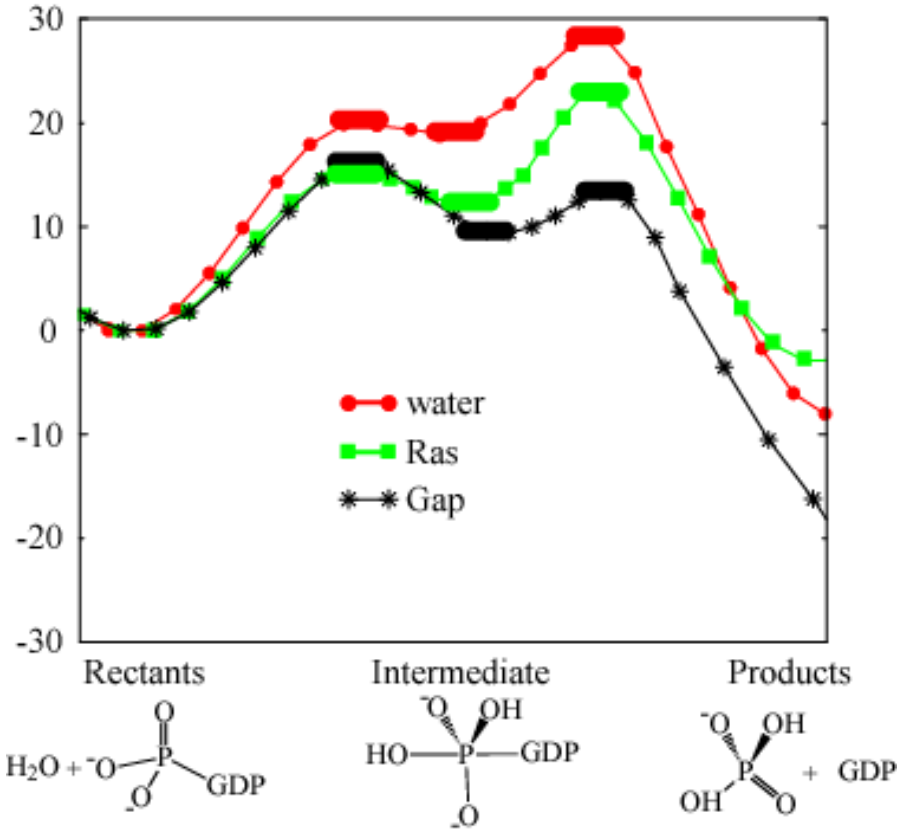
The Ras/GAP complex catalyzes GTP hydrolysis



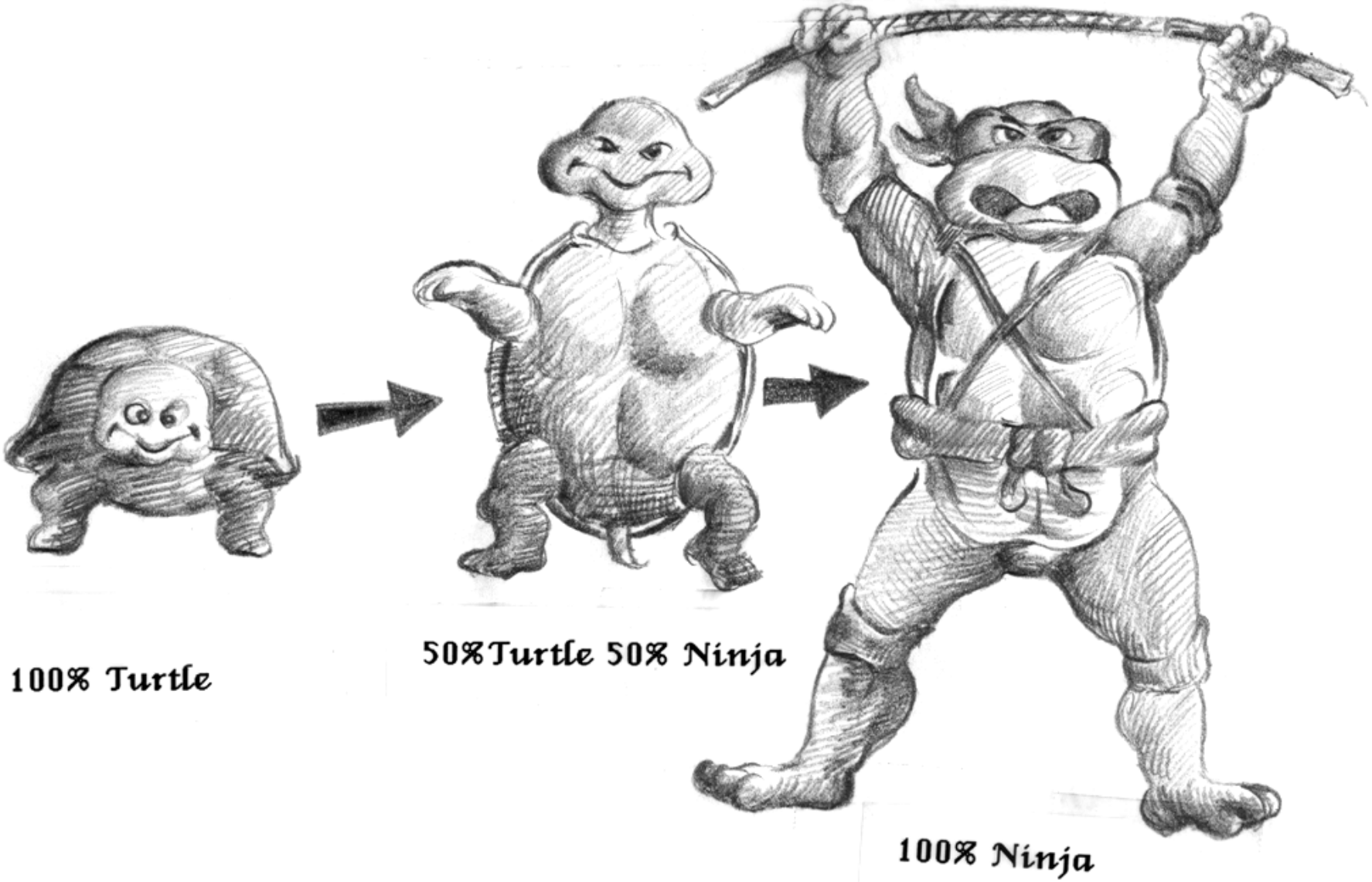


	Calc	Exp
Water	27.9	*(27.5)*
Ras	23.2	23.1, 22.2
RasGap	16.1	15.9

Good Agreement between calculation and experiment



How the Turtle is mutated into the Ninja by the Free Energy Perturbation method

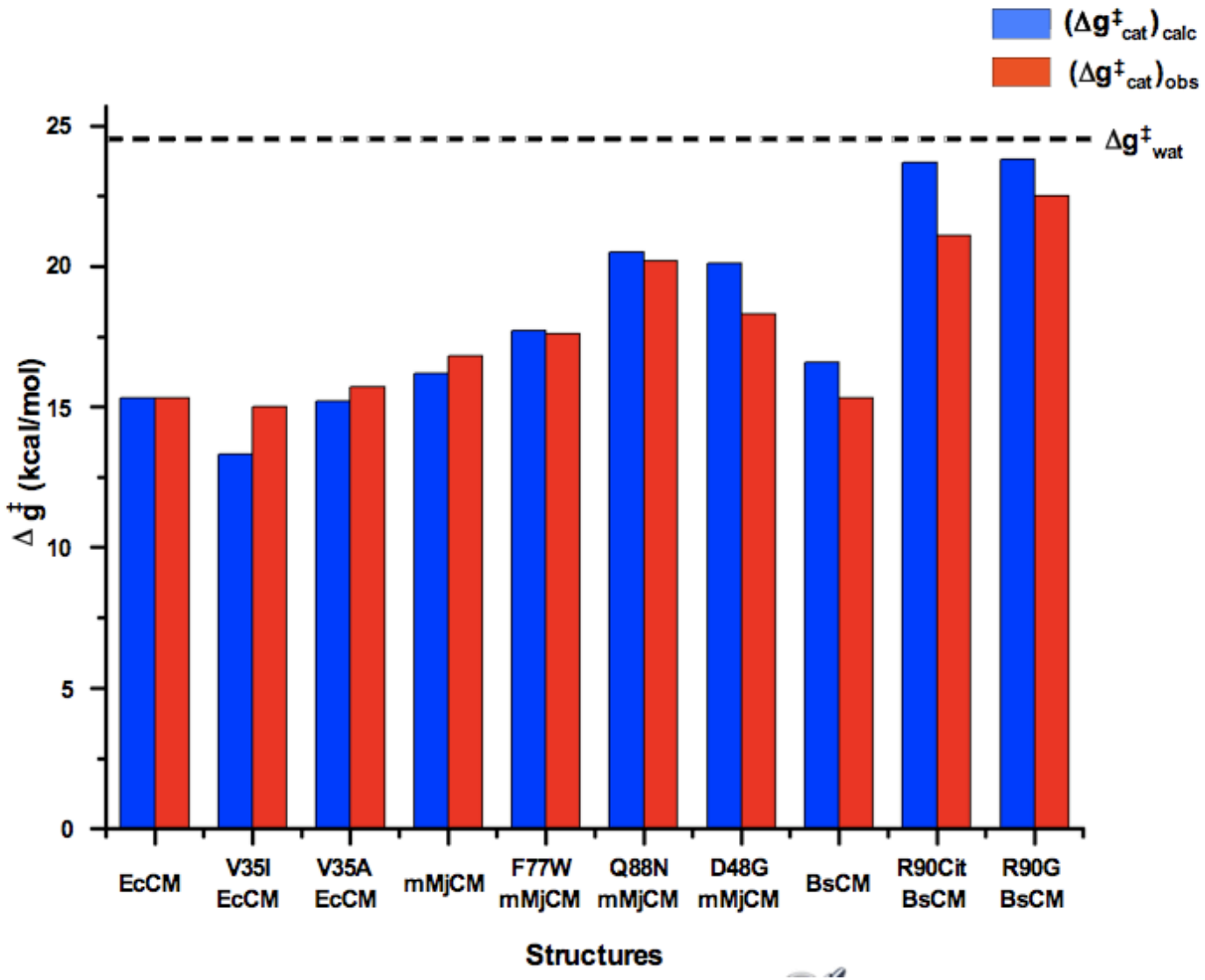


100% Turtle

50%Turtle 50% Ninja

100% Ninja

M. Roca, A. Vardi-Kilshtain and A. Warshel,
Biochemistry, 48, 3046-3056 (2009). Calculating effect of mutations

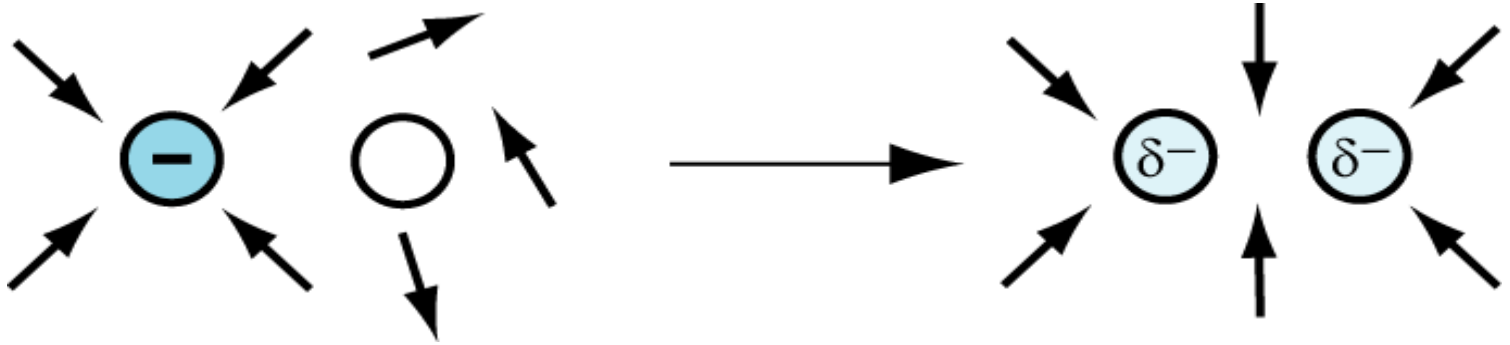


Warshel , PNAS (1978)

- The secret of Enzyme catalysis is electrostatic preorganization

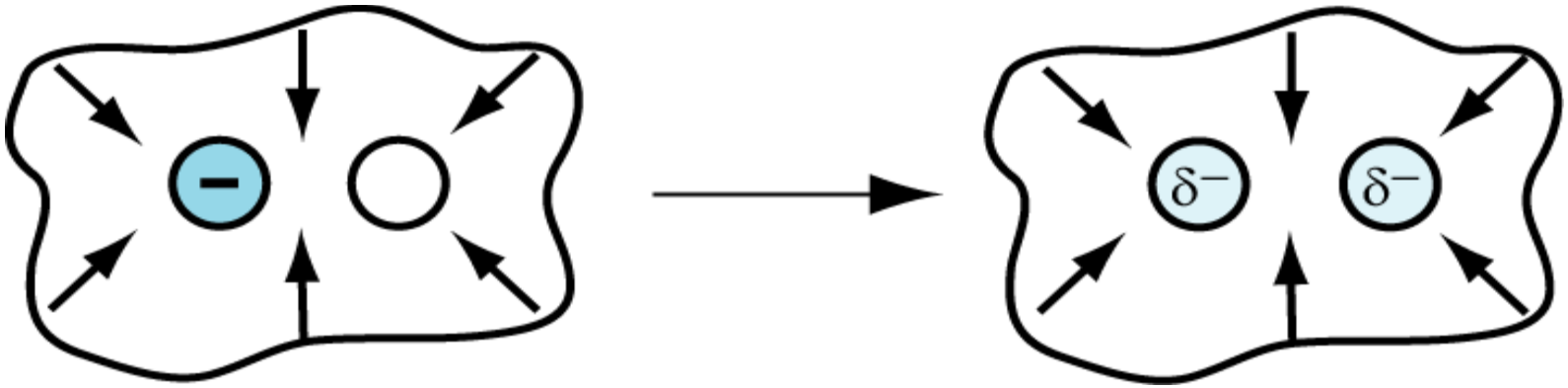
Reaction in water

Spend a large amount of energy rotating the water molecules



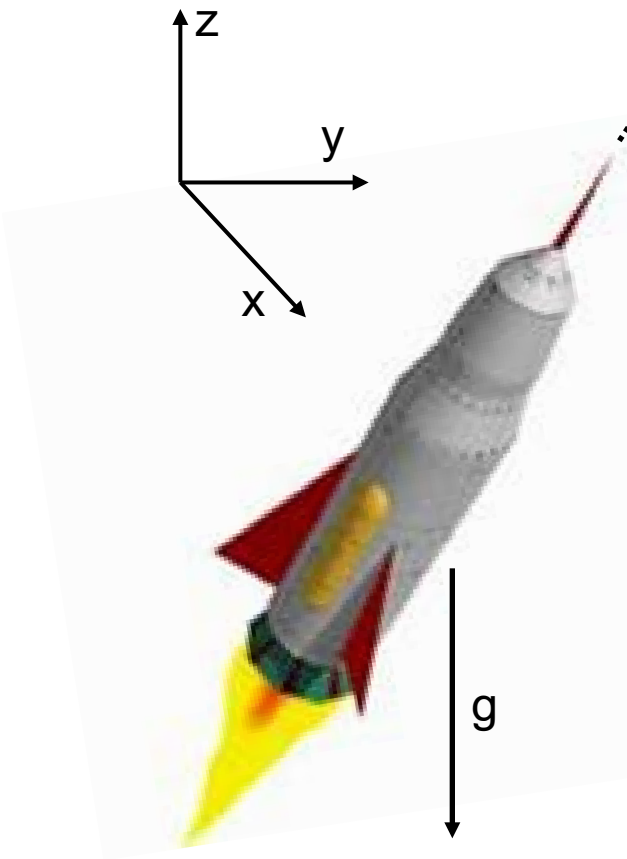
Reaction in protein

The protein polar groups and charges are already pointing in the correct direction



Bridging time scales and length scales

For short time scales can use direct MD simulations to determine the exact time dependence on an atomistic level



$$T = \frac{1}{2} m(\dot{x}^2 + \dot{y}^2 + \dot{z}^2)$$

$$V = mgz$$

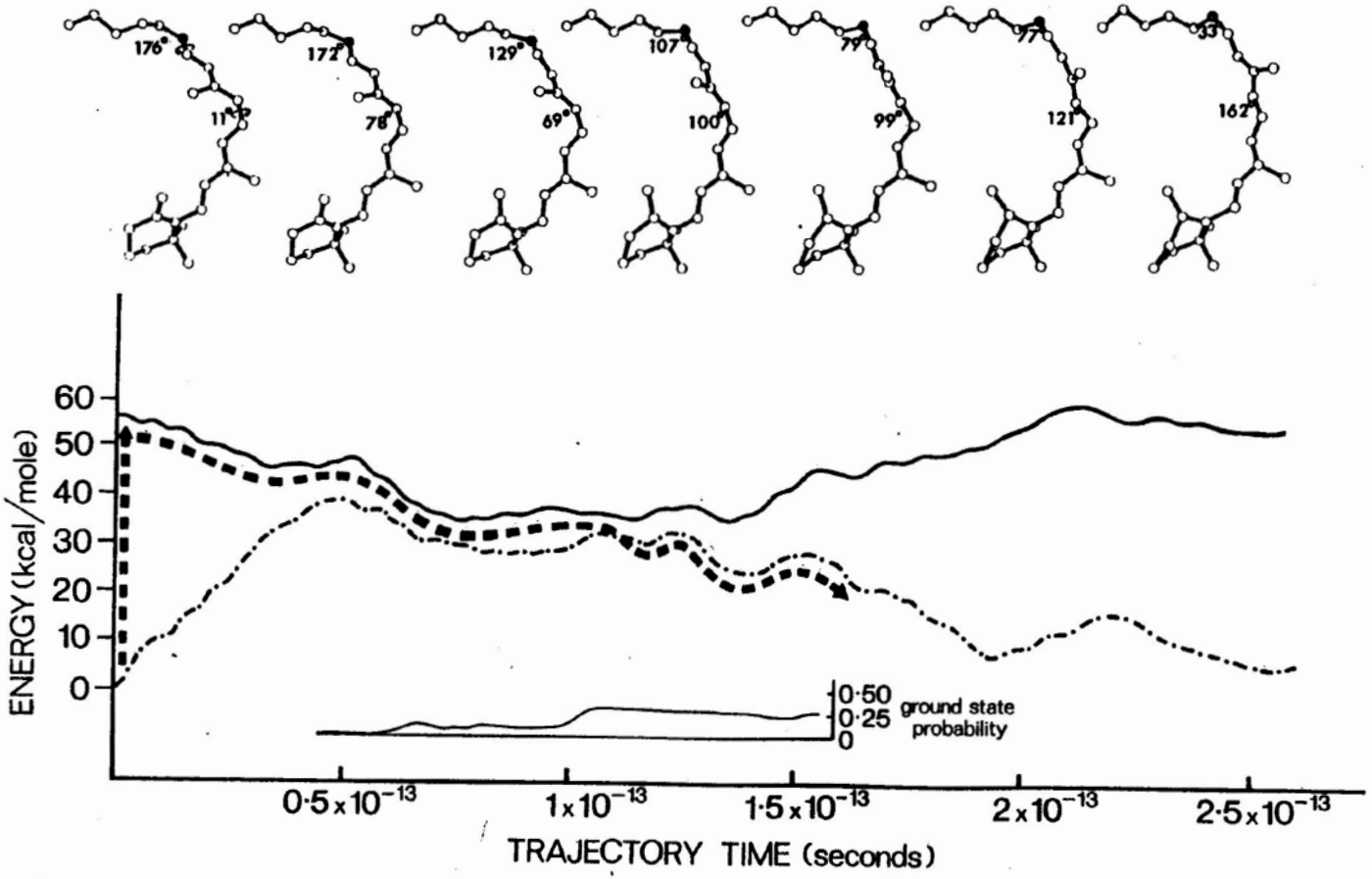
$$m\ddot{z} = -\frac{\partial V}{\partial z} = -mg$$

$$x(t) = x_0 + \dot{x}_0 t$$

$$z(t) = z_0 + \dot{z}_0 t - \frac{1}{2} g t^2$$

The Dynamics
of the First Step
in the
Vision Process
© 1980 Arieh Warshel

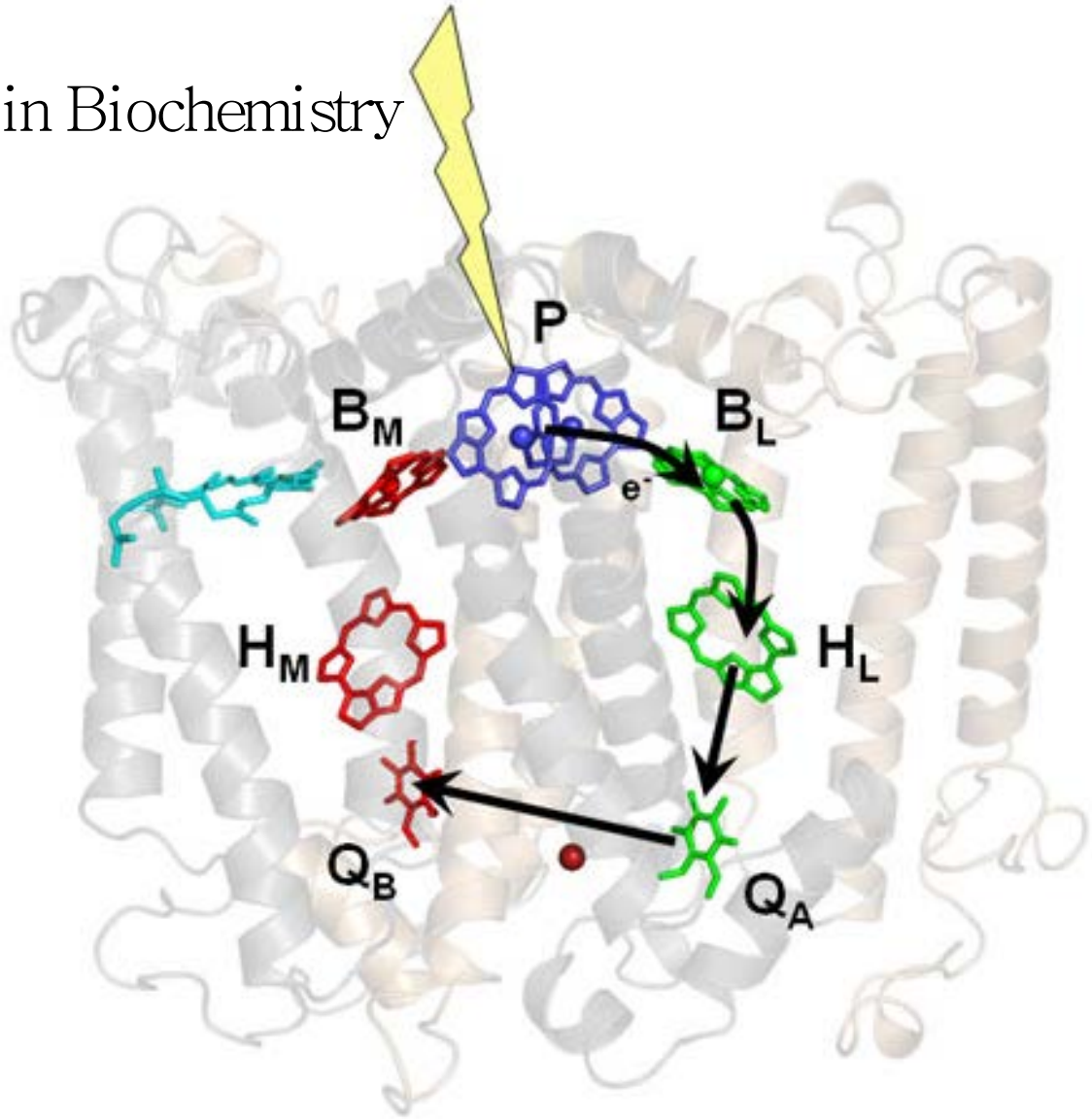
Warshel, Nature, 260, 679 (1976)



$$a_0(\tau) = \int_0^{\tau} \left[\langle \Psi_0 \mid \frac{\partial \Psi_1}{\partial t} \rangle a_1(t) \exp\left\{ -(i/\hbar) \int_0^t (E_1 - E_0) \mathrm{d}t' \right\} \right] \mathrm{d}t$$

Photosynthetic Reaction Centers

Simulated in Biochemistry
1988



What about reproducing the structural changes and their time dependence and long time dynamics

Needs Free Eenrgy landscape and and efficient approach

Coarse Grained (CG) approaches

Very Early CG

Computer Simulation of Protein Folding
Michael Levitt and Arieh Warshel, *Nature* (1975) **253**, 694-698

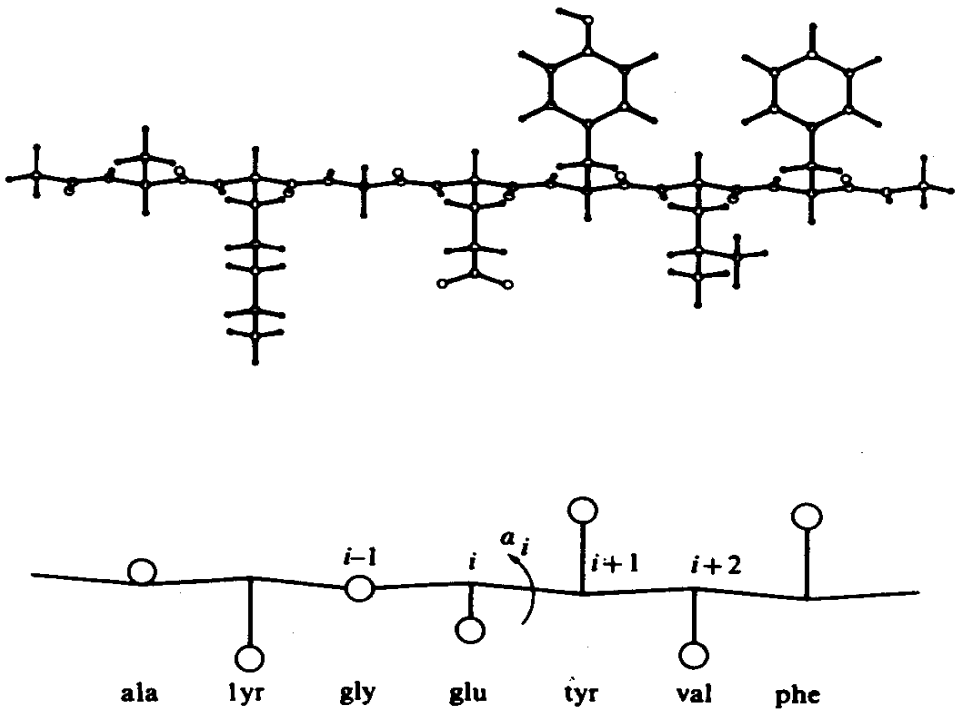
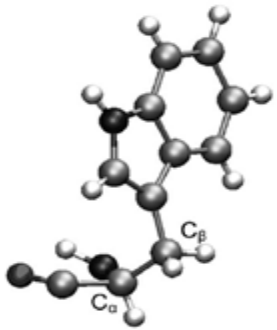
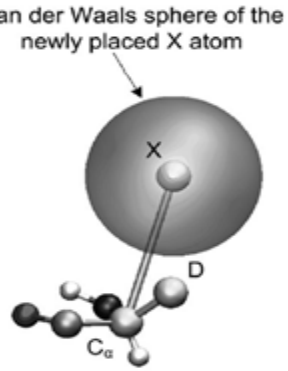


Fig. 1 Relationship between the simplified model of protein structure introduced here and the real all-atom structure of proteins. The two reference points for each residue in the simplified model correspond to the centroid of the side chain and the C^{α}. Each residue is only allowed one degree of freedom: the torsion angle α between the 4 successive C^{α}s of residues $(i-1, i, i+1, i+2)$. All the side chains of a given type have the same simplified geometry. The bond lengths, bond angles, and torsion angles used to define the geometry of the simplified molecule were taken as the average values found in eight protein conformations, though they could just as well have been taken from amino-acid model compounds.

Improved Coarse Grained Model

PROTEINS , 78, 1212–1227 (2010)
***Ann Rev Phys Chem** 62, 41-64 (2011)*

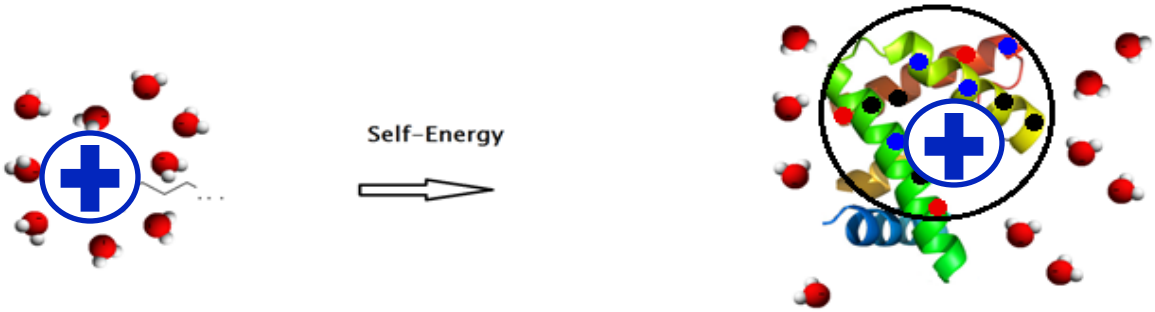




Now focused on better treatment of electrostatics free energy

Mainly self energy (solvation) and charge-charge interaction

$$\Delta G_{self} = \sum_i \left(U_{self}^{np}\left(N_i^{np}\right) + U_{self}^{p}\left(N_i^{p}\right) + U_{self}^{mem}\left(N_i^{mem}\right) \right)$$



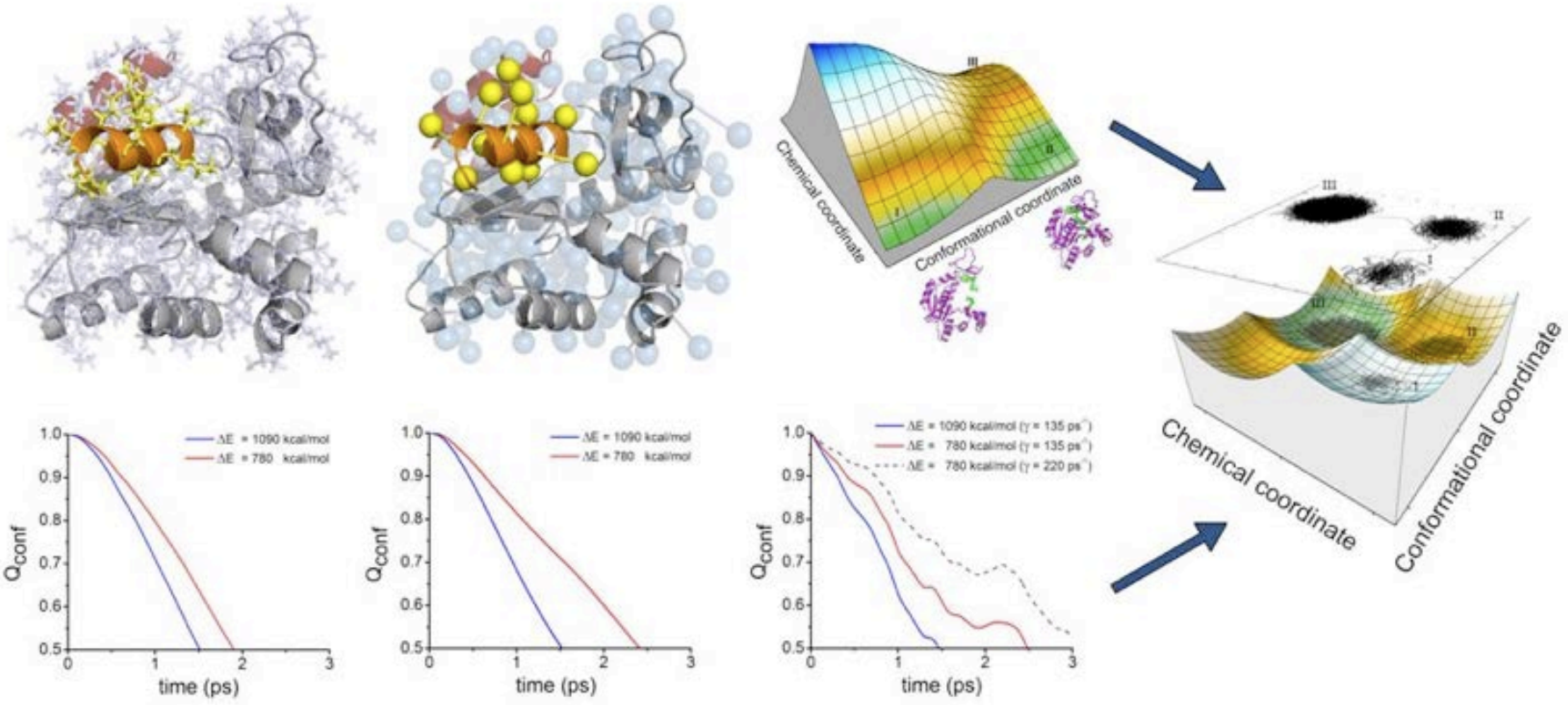
- Nonpolar residues
- Polar residues
- Ionizable residues

- Long time simulations

Newtonian Dynamics

Brownian Dynamics

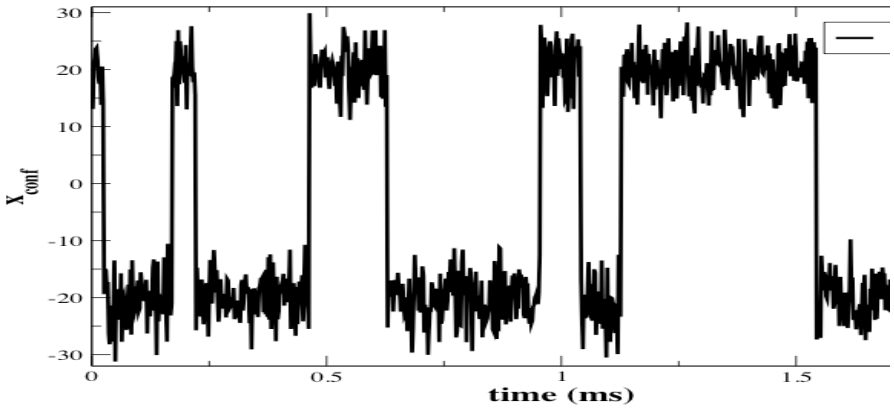
The Renormalization Model

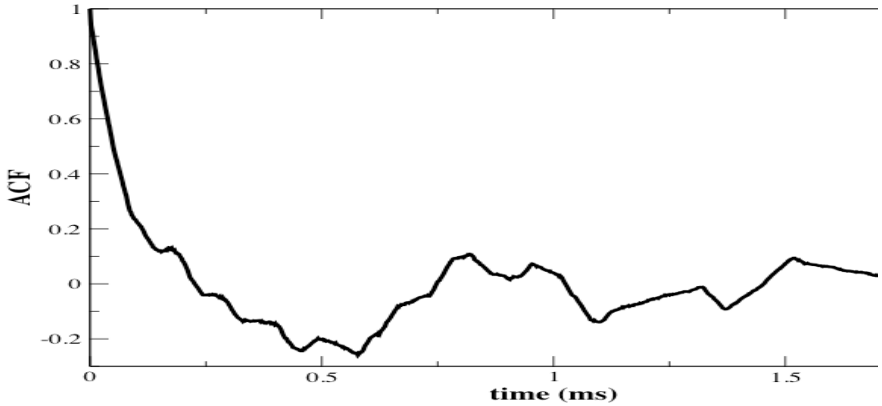


Long time dynamics, conform. coordinate

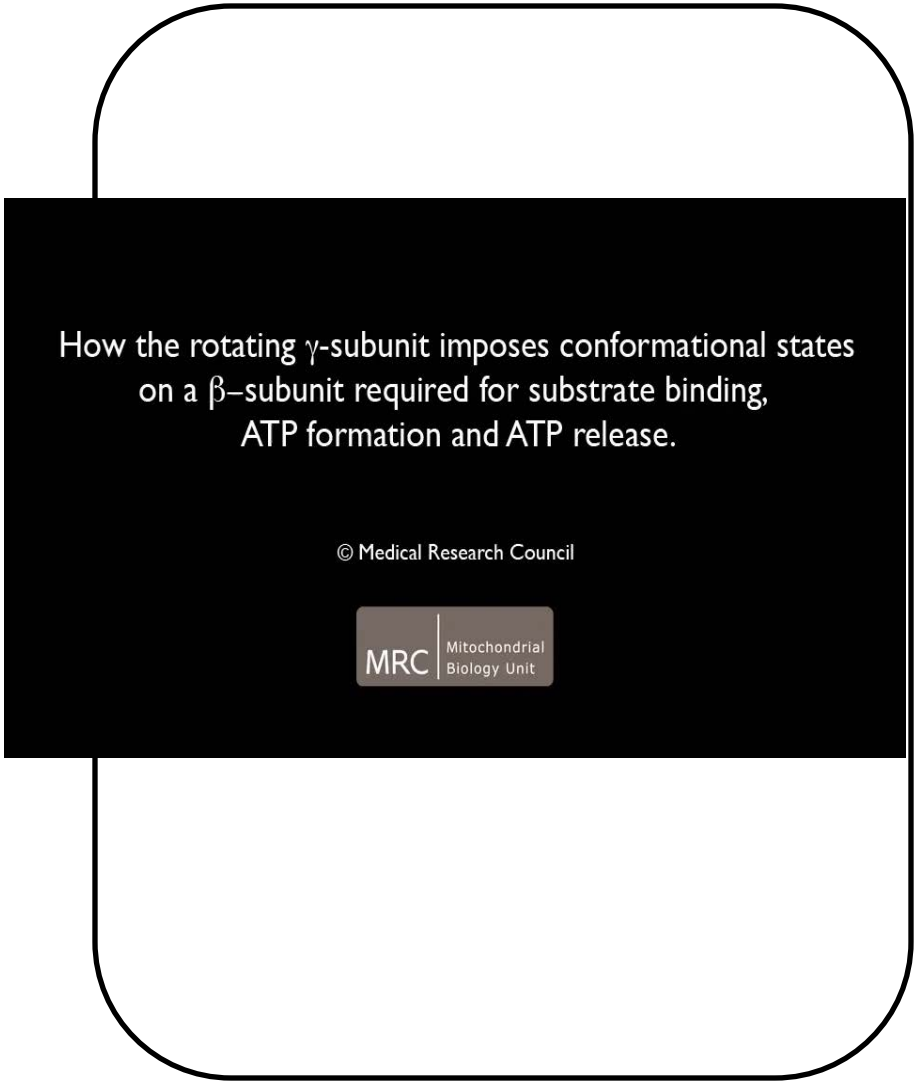
$x_{conform}(t)$

autocorrelation function

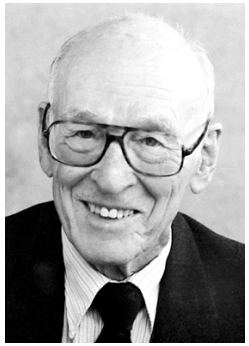
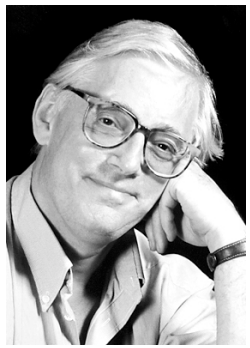
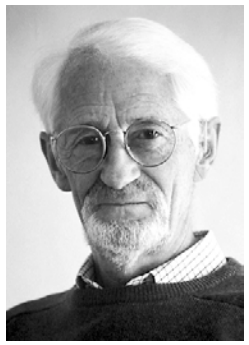




F_1F_0-ATP synthase – The smallest rotary motor



The 1997 Nobel Prize in Chemis

F_1F_0 are two coupled rotary motors; an ATPase and an ion-pump

In presence of right ion-gradient
F_0 transports ion across the membrane and F_1 synthesizes ATP

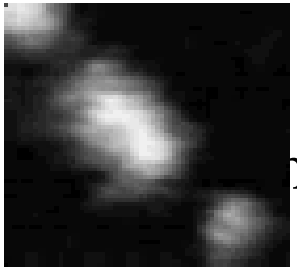
In the opposite direction ATP hydrolysis occurs in F_1, while
F_0 acts as an ion-pump

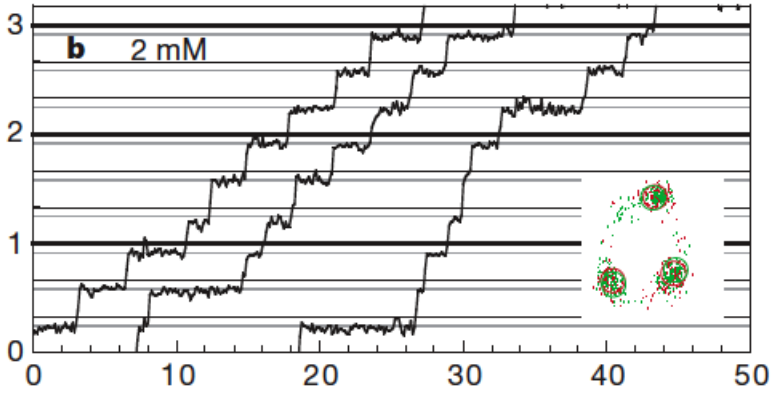
Mechano-Chemical Coupling between the central stalk and the catalytic dimers in F_1

Each 120° rotation of the Stalk broken in 80° and 40° steps by the Catalytic Dwell

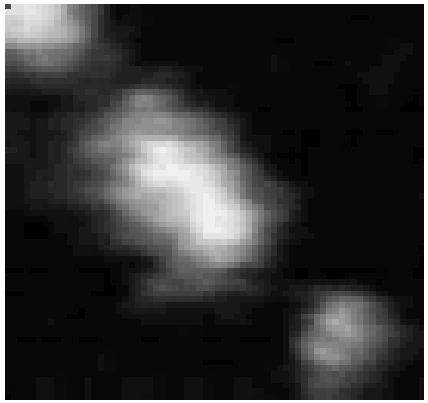
Sequence of Events

Ligand Binding → 80° rotation → ATP catalysis → 40° rotation →



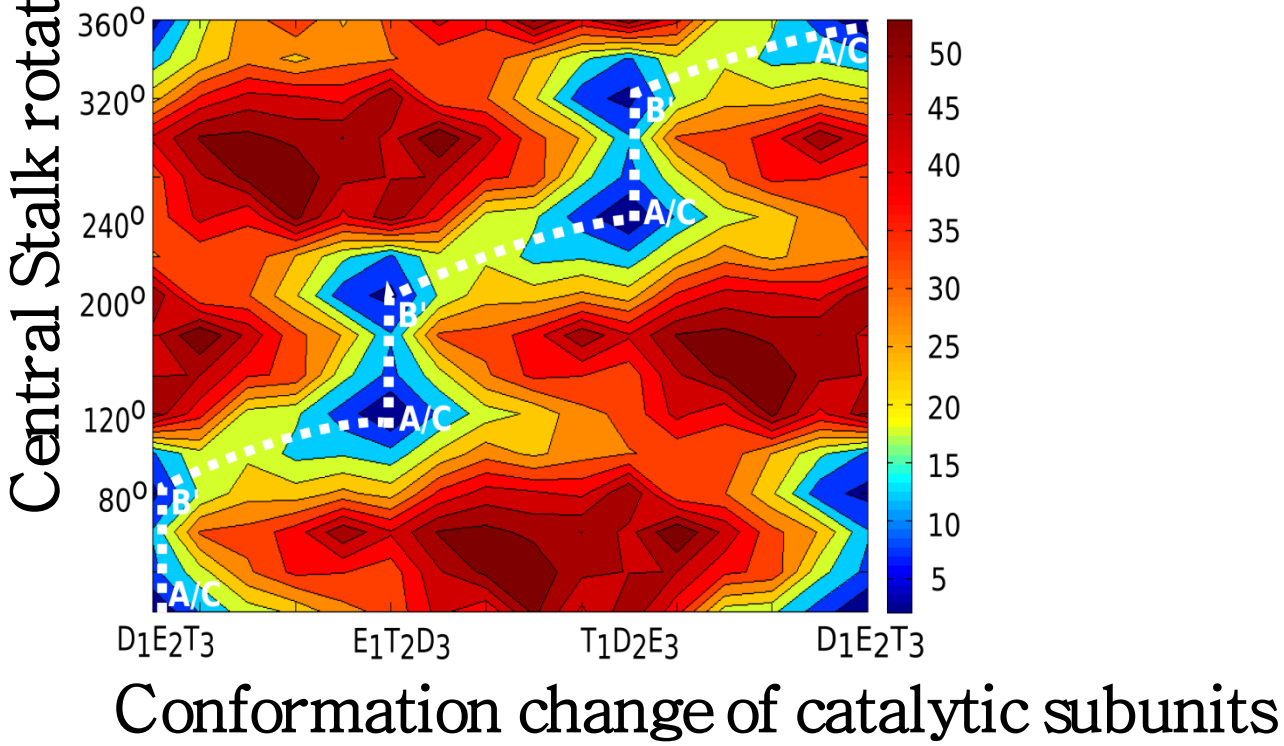


Yasuda, R. et. al., Nature, 2001.



The CG electrostatic free energy for the 360° rotation of central stalk and catalytic subunit conformation changes.

S. Mukherjee and A.Warshel, Proc. Natl. Acad. Sci. USA ,108, 20550–20555 (2011)



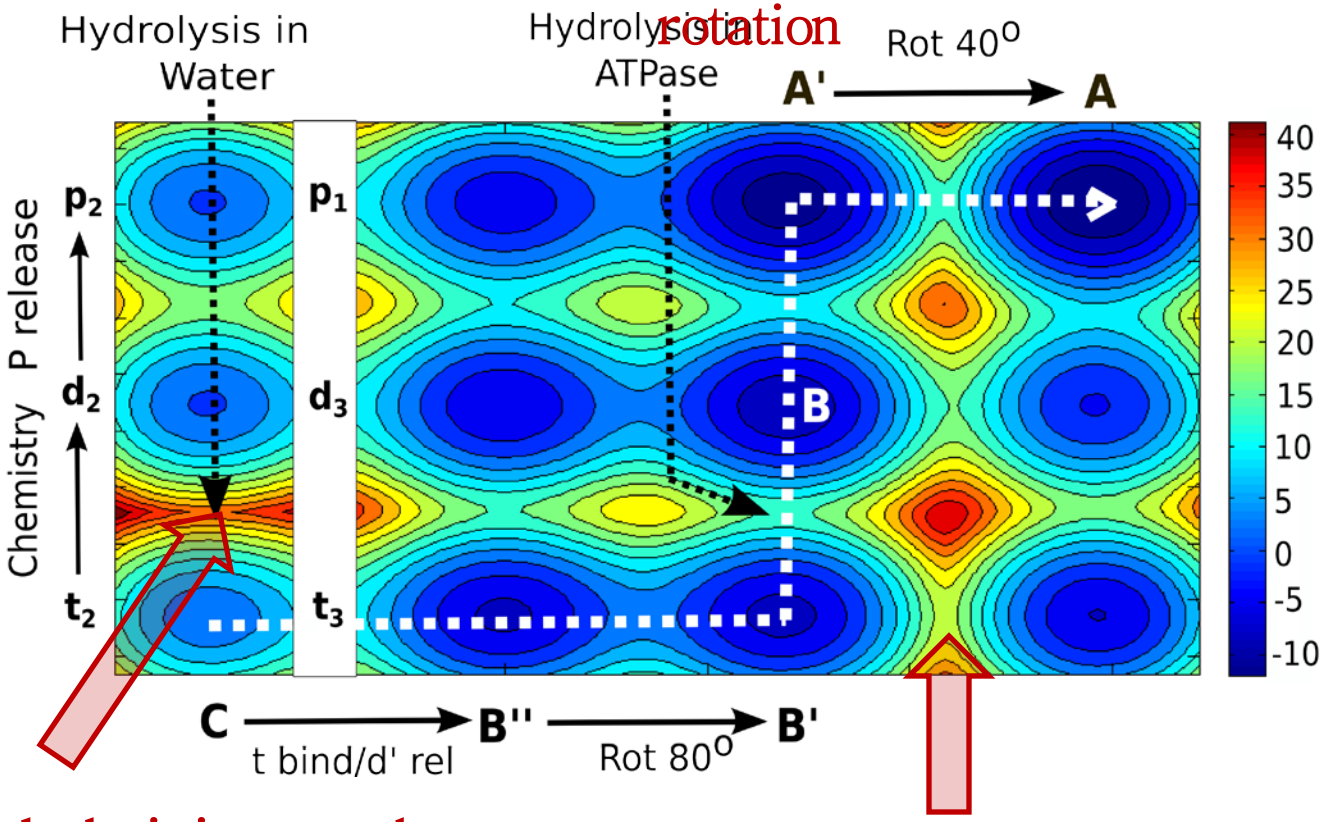
The least energy path clearly shows the 80° /40° substeps.

The 80° rotation has small electrostatic barrier.

The 40° rotation and conformation change of catalytic subunits has

Simplified surface of F_1-ATPase function shows the coupling of ATP hydrolysis with central stalk rotation

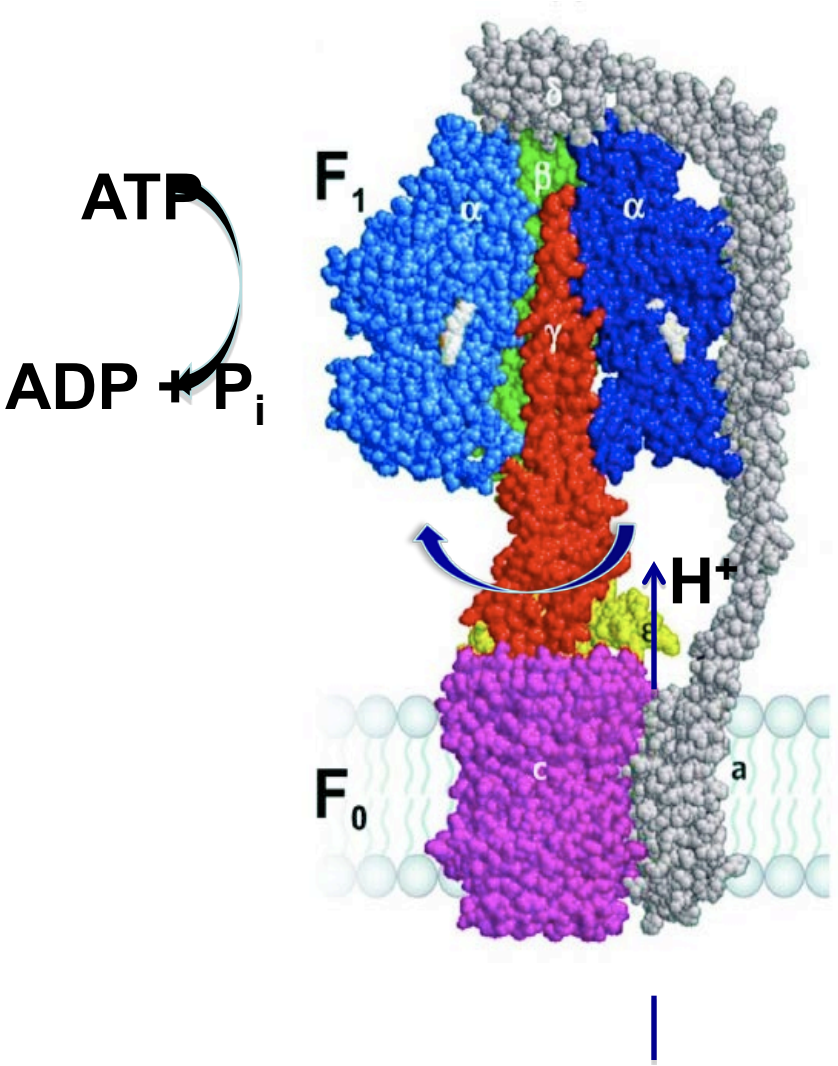
The functional surface reveals why catalysis occurs after 80° rotation



ATP hydrolysis in water has very high barrier and will need months to occur

High barrier of 40° rotation and catalytic subunit changes bias the system towards ATP hydrolysis

F1F0-ATP synthase – The smallest rotary motor



F1F0 are two coupled ATPase and ion-pump

Consists of a rotary motor and a stator portion

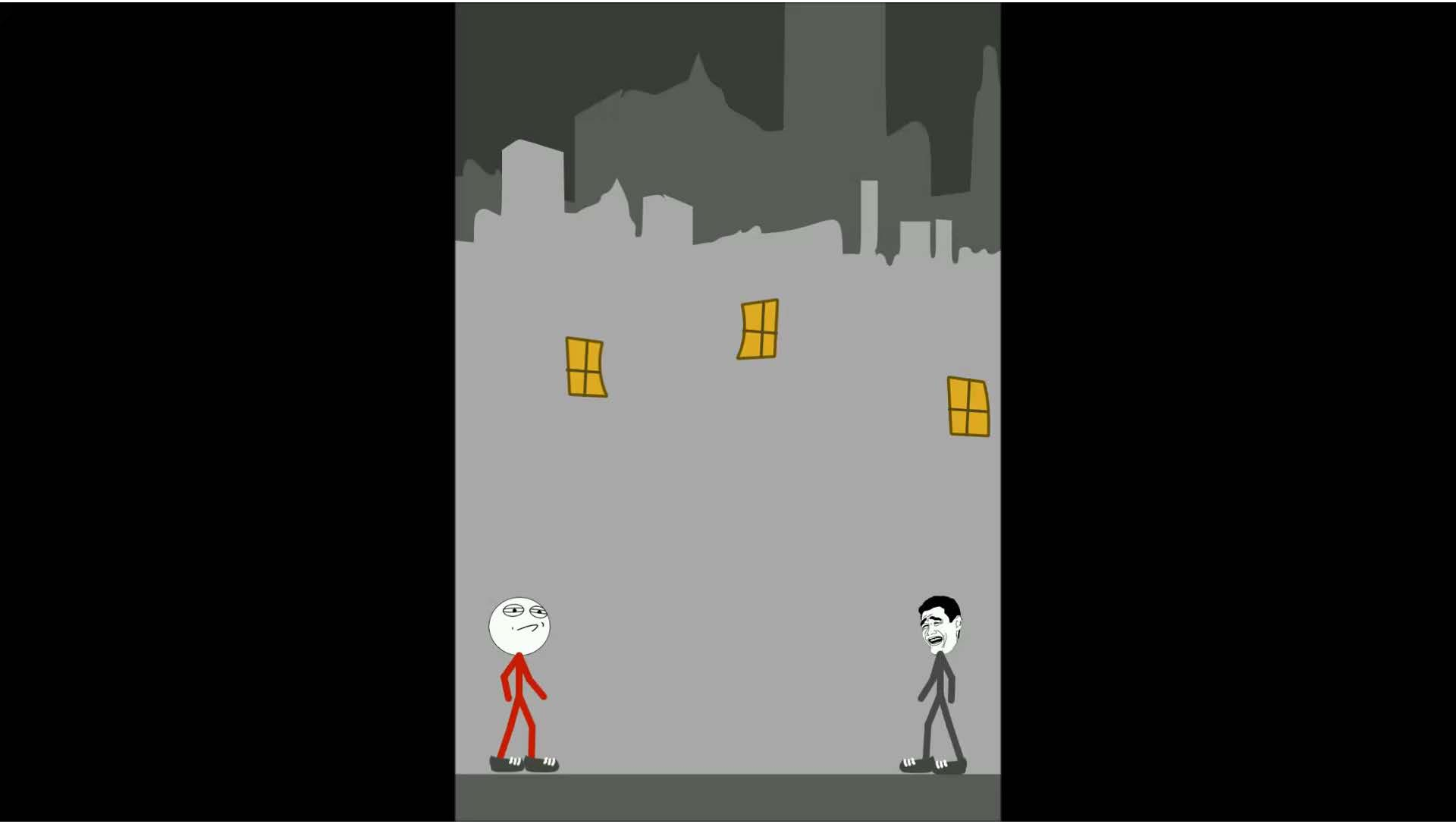
In presence of right ion-gradient across the membrane ATP synthesis occurs in the F1

In the opposite direction ATP hydrolysis occurs while the F0 acts as an ion-pump

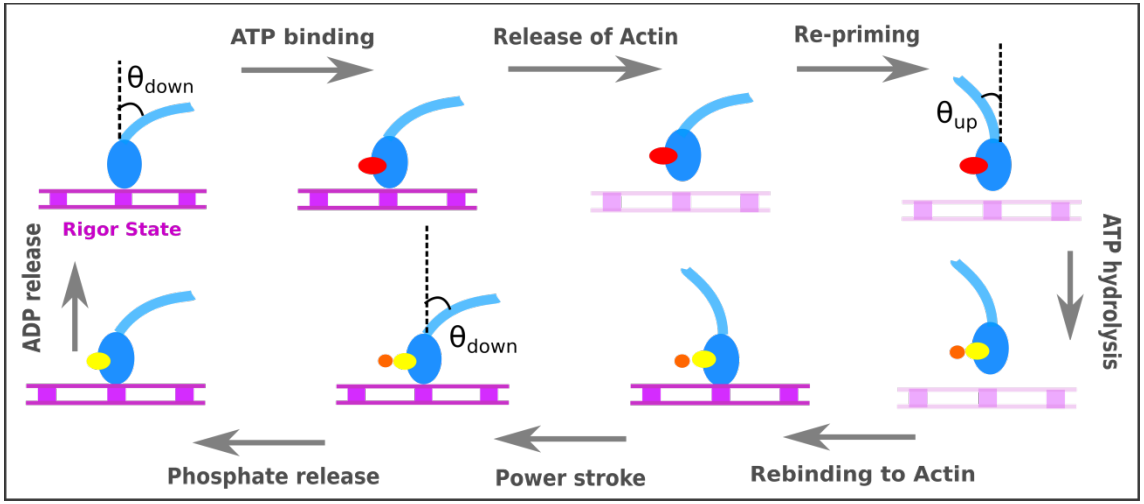
What drives unidirectional walking motion of myosin V on actin filaments

Almost no backsteps as myosin V walks over actin filament

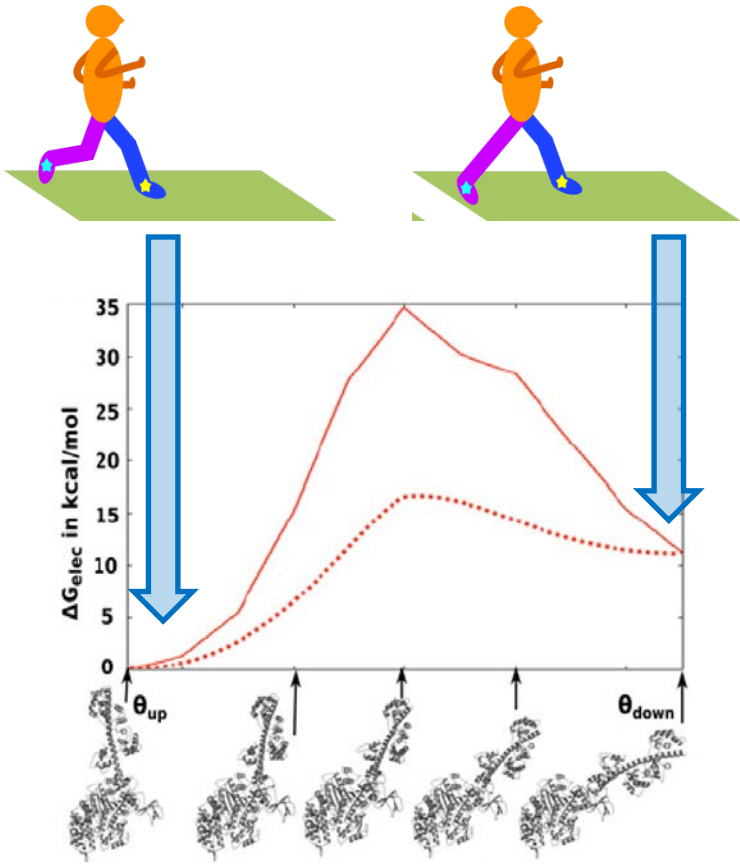
It is hard to understand unidirectional movement, even in our daily life !!!



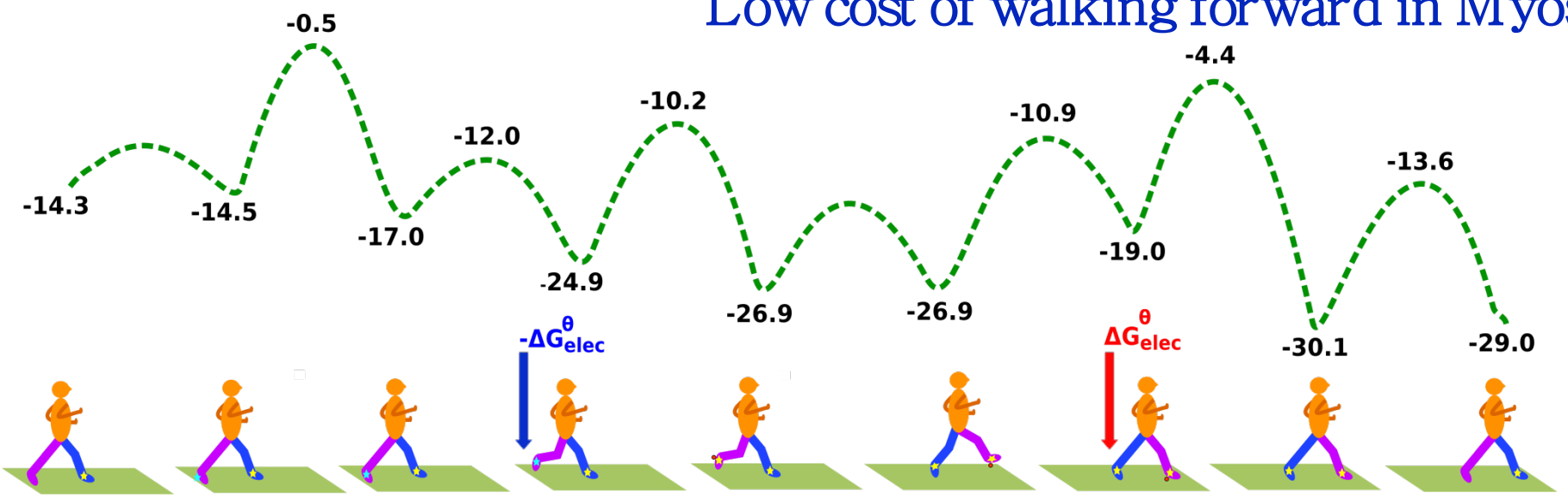
CG energetics of a single leg as it bends (changes conformation)



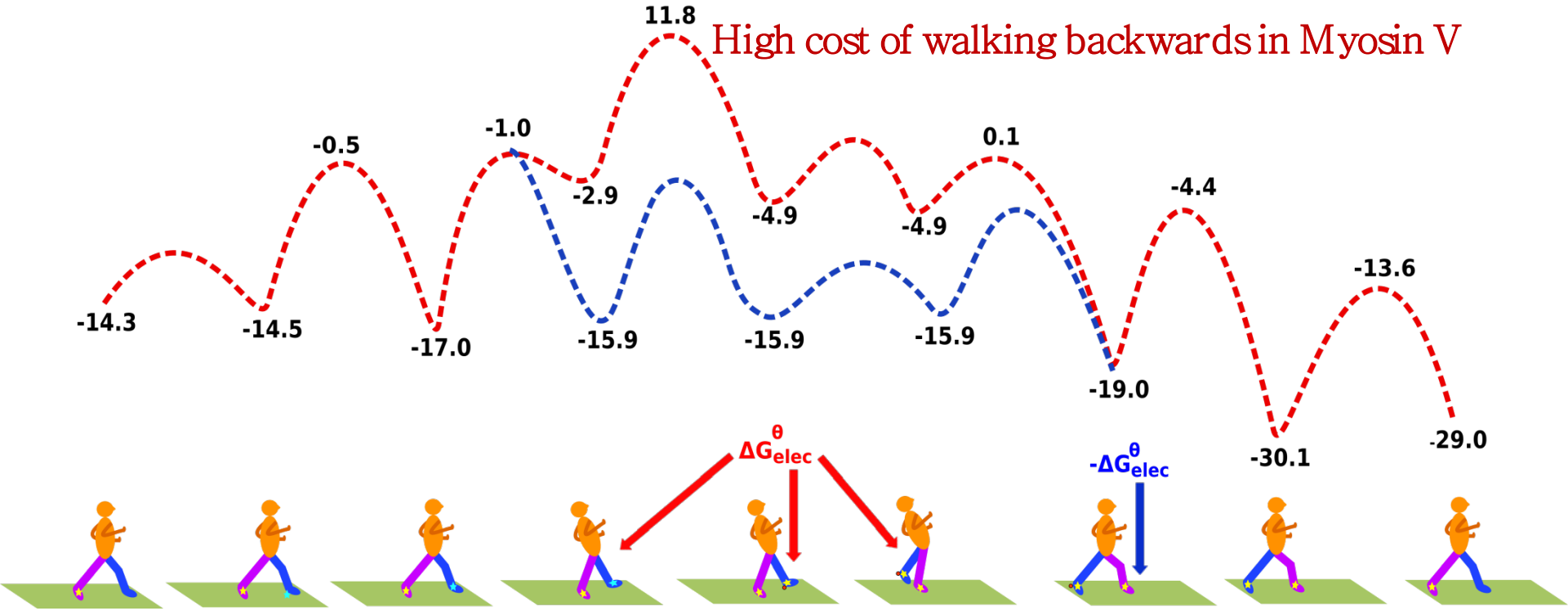
Schematic functional cycle of myosin V single leg



Low cost of walking forward in Myosin



High cost of walking backwards in Myosin V

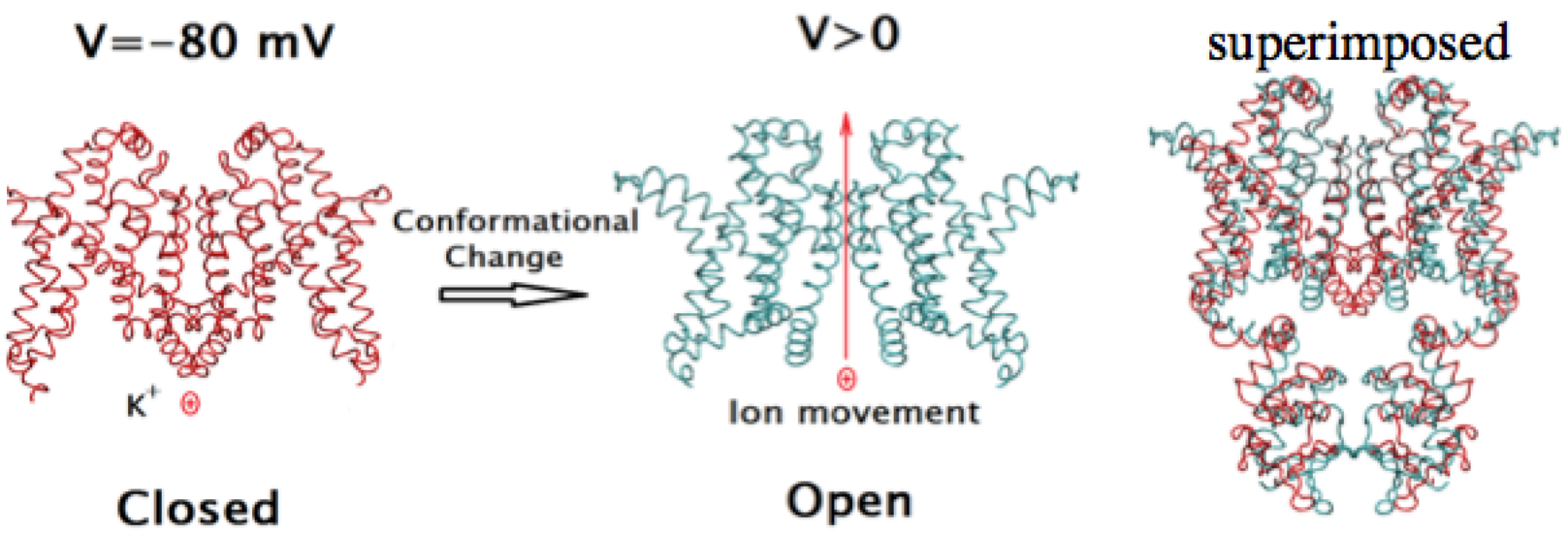


Voltage-activated Ion Channel

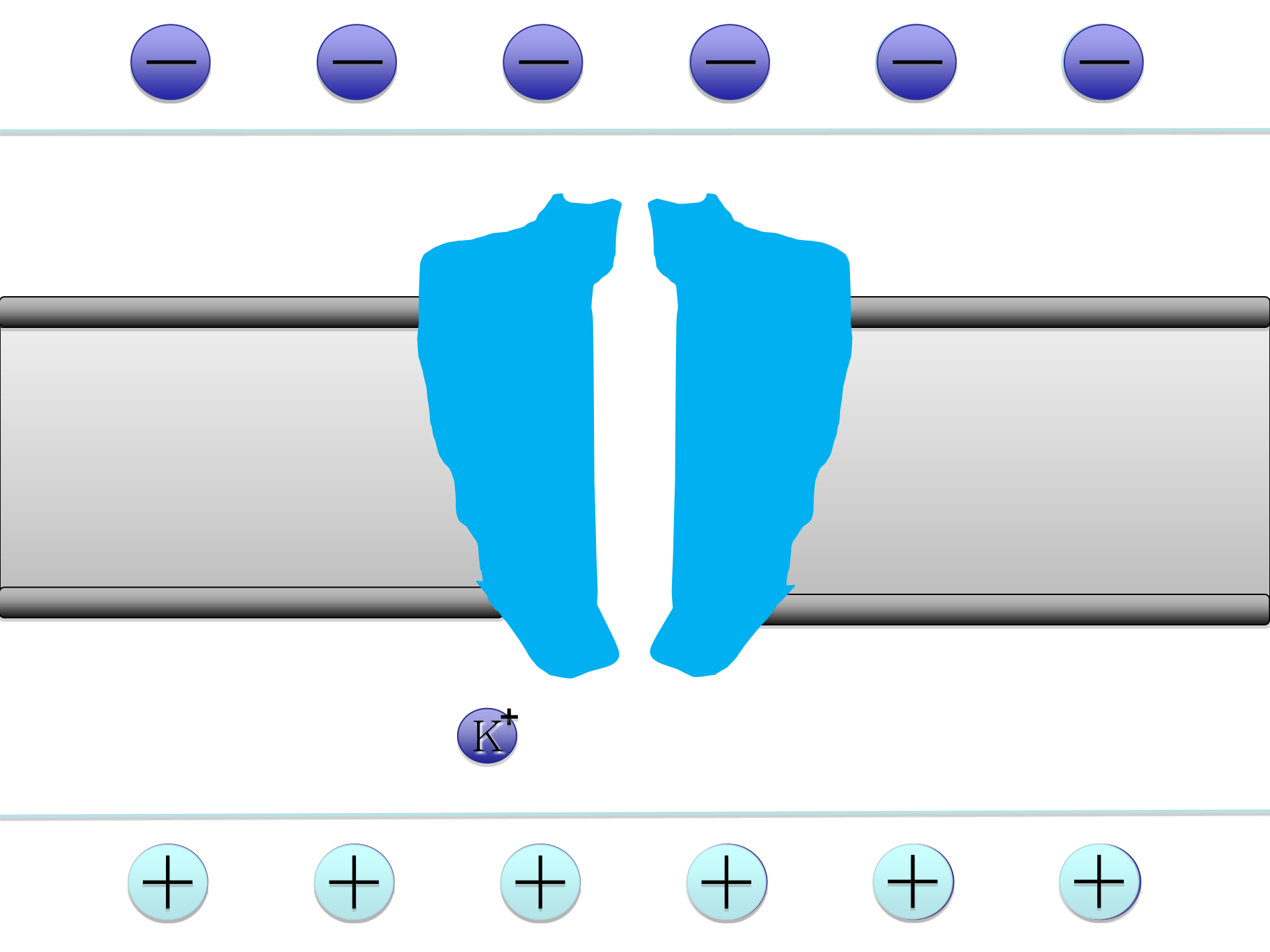
Roderick MacKinnon

Part of the structure

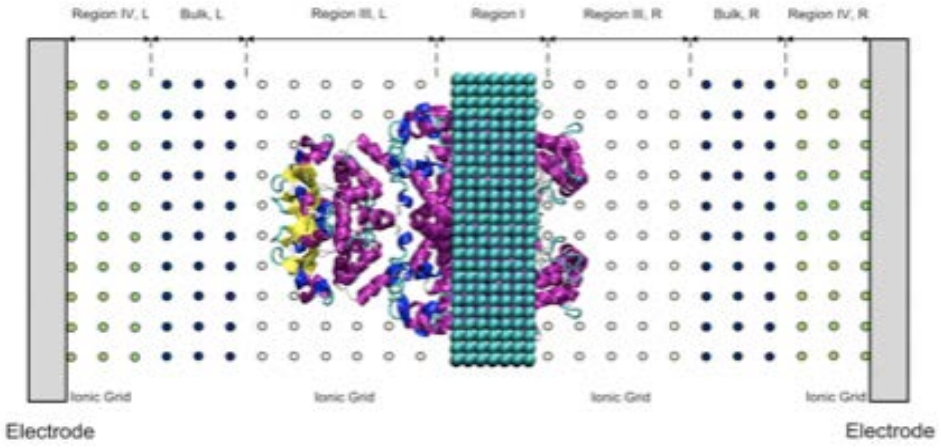
Open, Closed Structures superimposed



Kv1.2 channel: A delayed rectifier subtype of shaker potassium channels that is selectively inhibited by a variety of scorpion venoms


K+

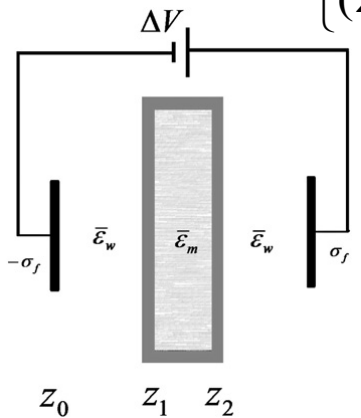
Ionic Strength Effect and External Potential



$$q_i^g = q_i^+ + q_i^- \qquad q_i^\pm = \frac{\alpha^\pm N_{box}^\pm e^{\mp\beta\phi_i}}{A^\pm} = \frac{Q_{box}^\pm e^{\mp\beta\phi_i}}{A^\pm}$$

$$\varphi_i = 332\sum_j \frac{q_j^P}{\varepsilon_{eff}^{gp} r_{ij}} + 332\sum_{k\neq i} \frac{q_k^g}{\varepsilon^{wat} r_{ik}} + V_i^{ext}$$

$$V_{ext}^i = V_{ideal}^i = \begin{cases} (Z - Z_0)\cdot D_0 / \varepsilon_{wat} & Z < Z_1 \\ (Z_1 - Z_0)\cdot D_0 / \varepsilon_{wat} + (Z - Z_1)\cdot D_0 / \varepsilon_{mem} & Z_1 \le Z \le Z_2 \\ (Z_1 - Z_0)\cdot D_0 / \varepsilon_{wat} + (Z_2 - Z_1)\cdot D_0 / \varepsilon_{mem} + (Z - Z_2)\cdot D_0 / \varepsilon_{wat} & Z > Z_2 \end{cases}$$



For protein-containing systems
Need to approximate

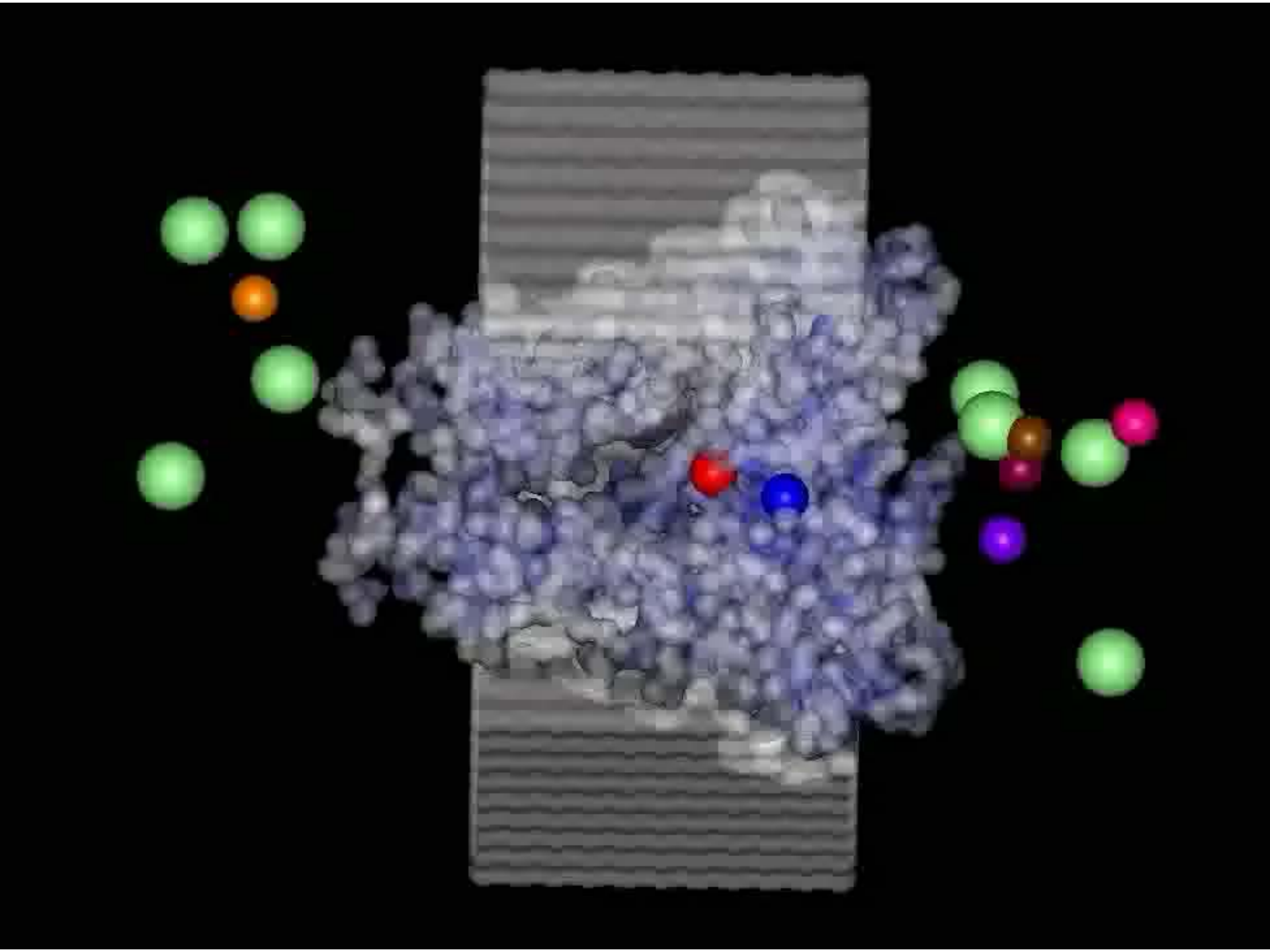


Photo: U. Montan

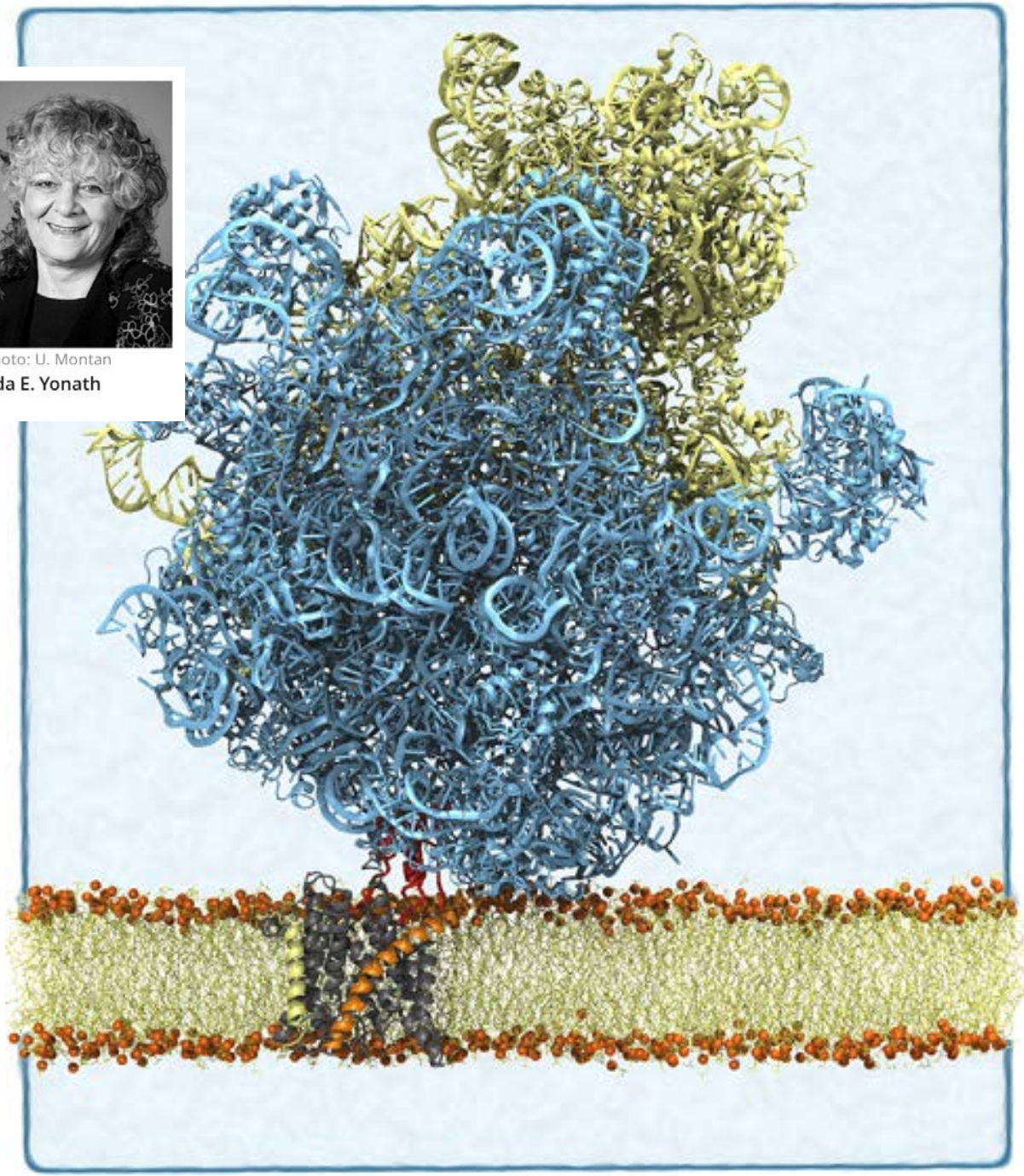
Venkatraman Ramakrishnan

Photo: U. Montan

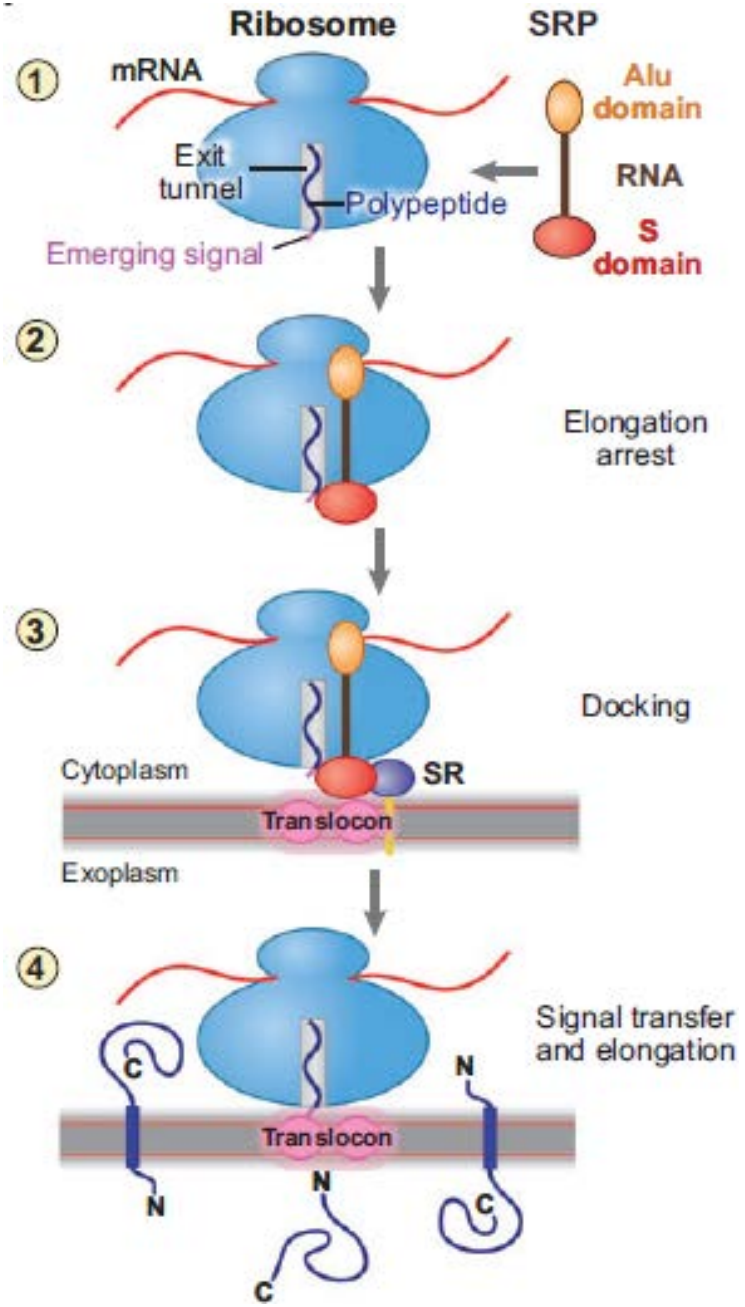
Thomas A. Steitz

Photo: U. Montan

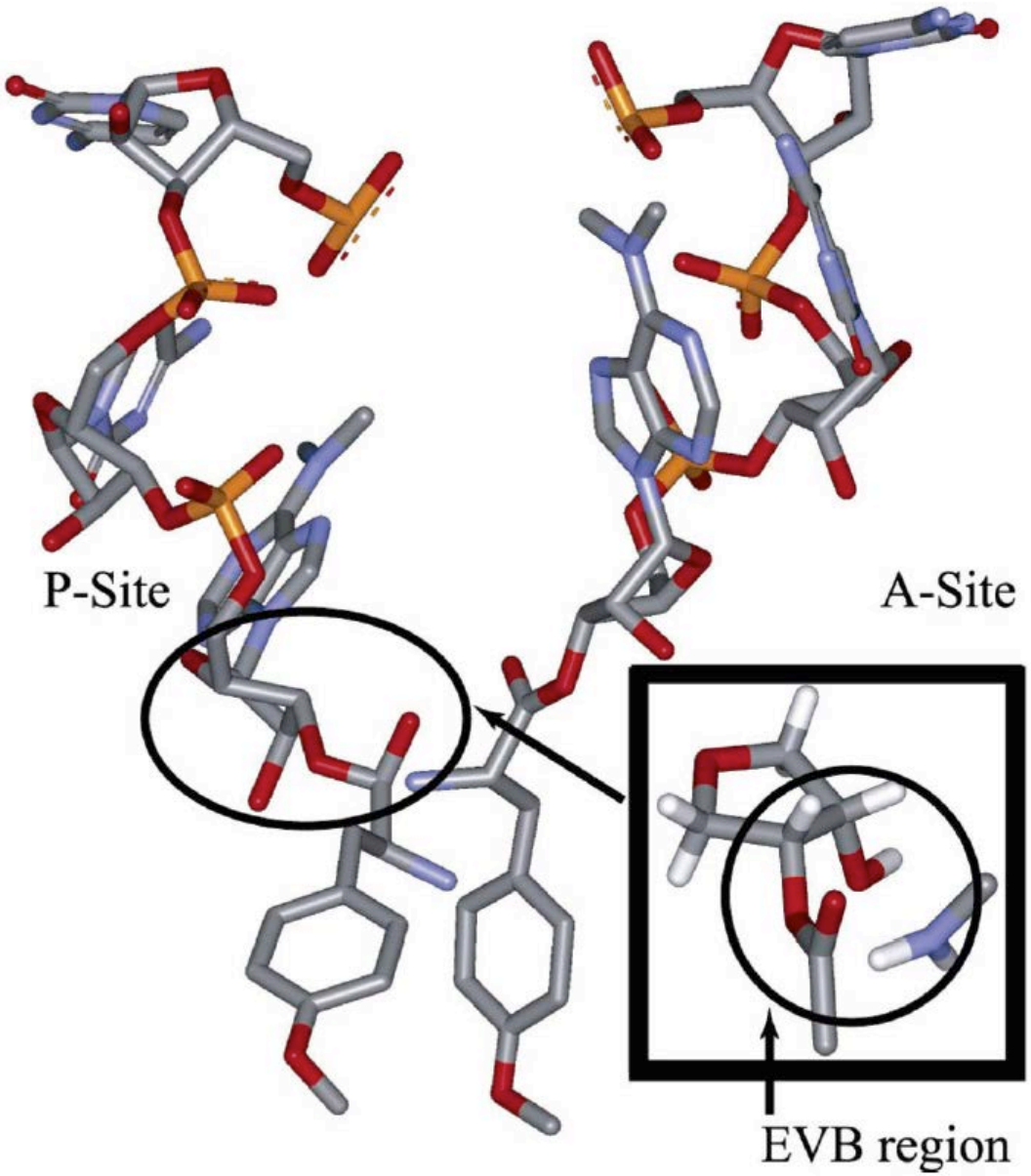
Ada E. Yonath

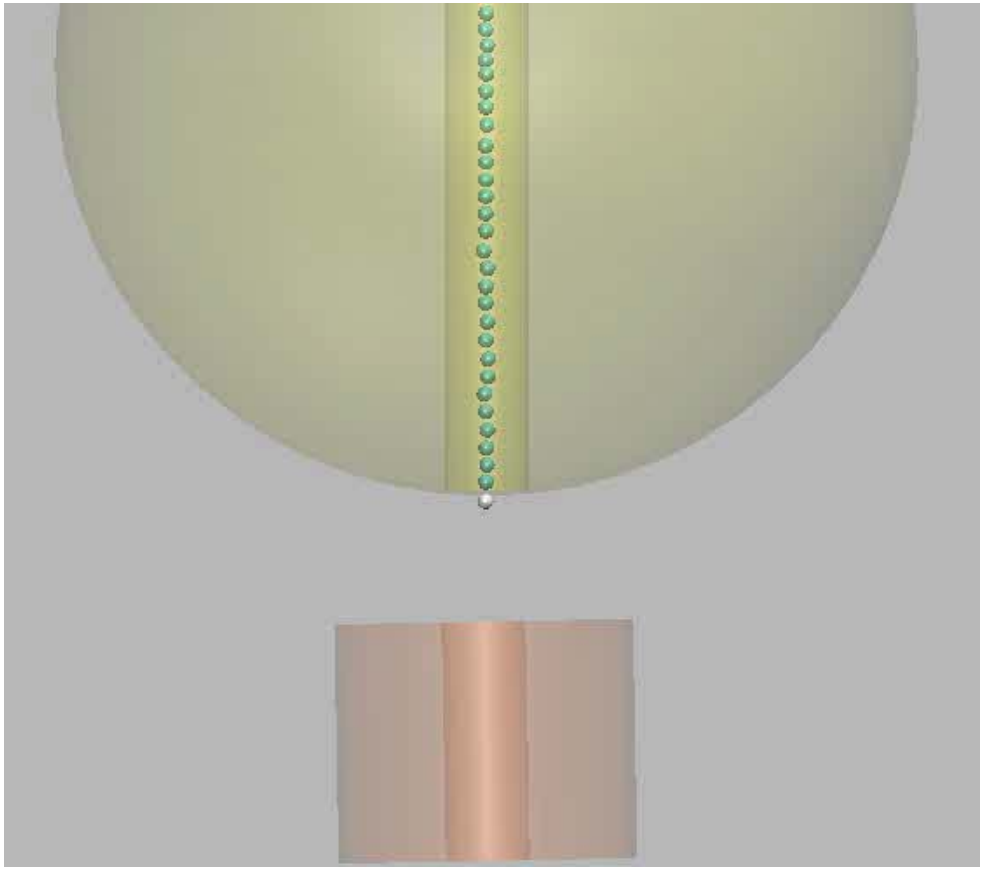


Translocon and Ribosome Coupling

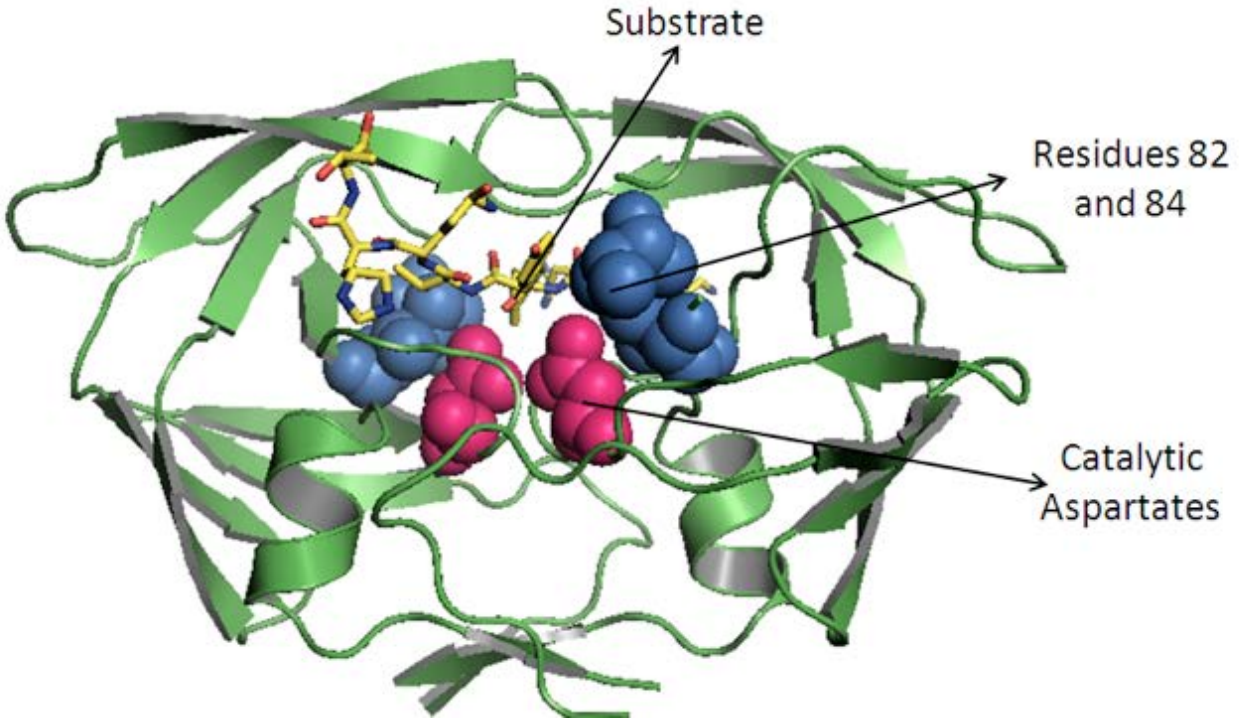


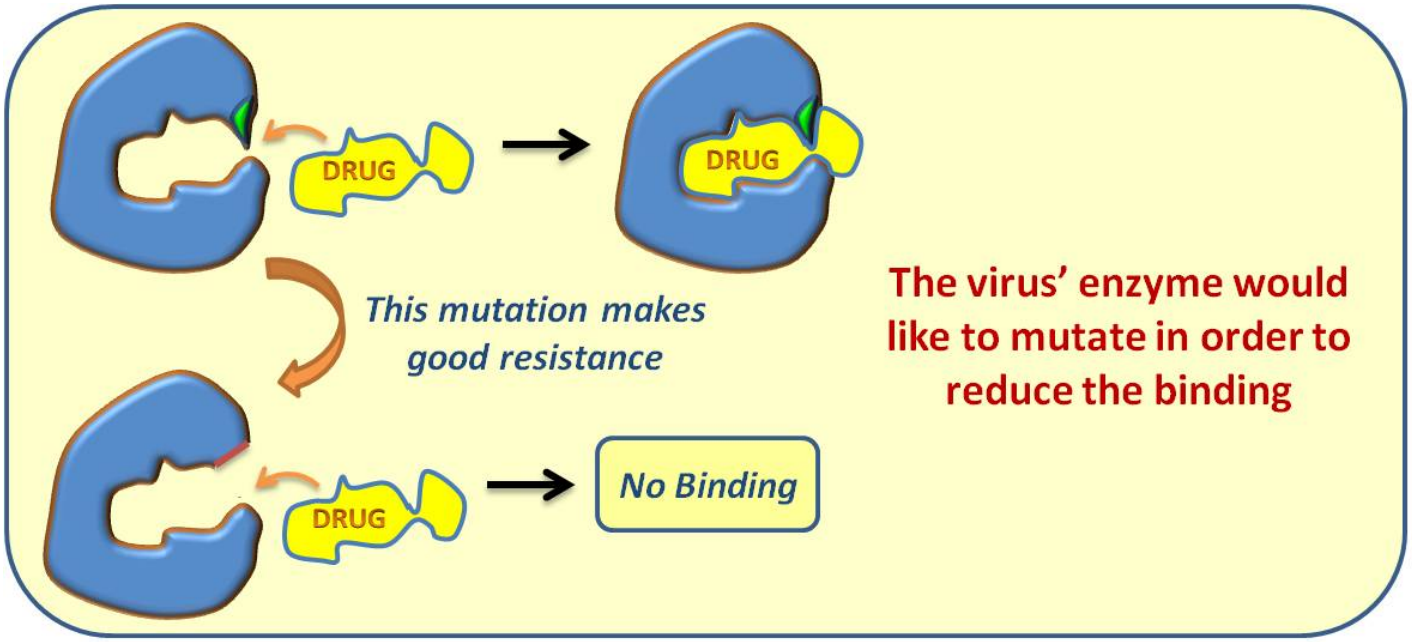
White and von Heijne, Annu. Rev. Biophys., 2008

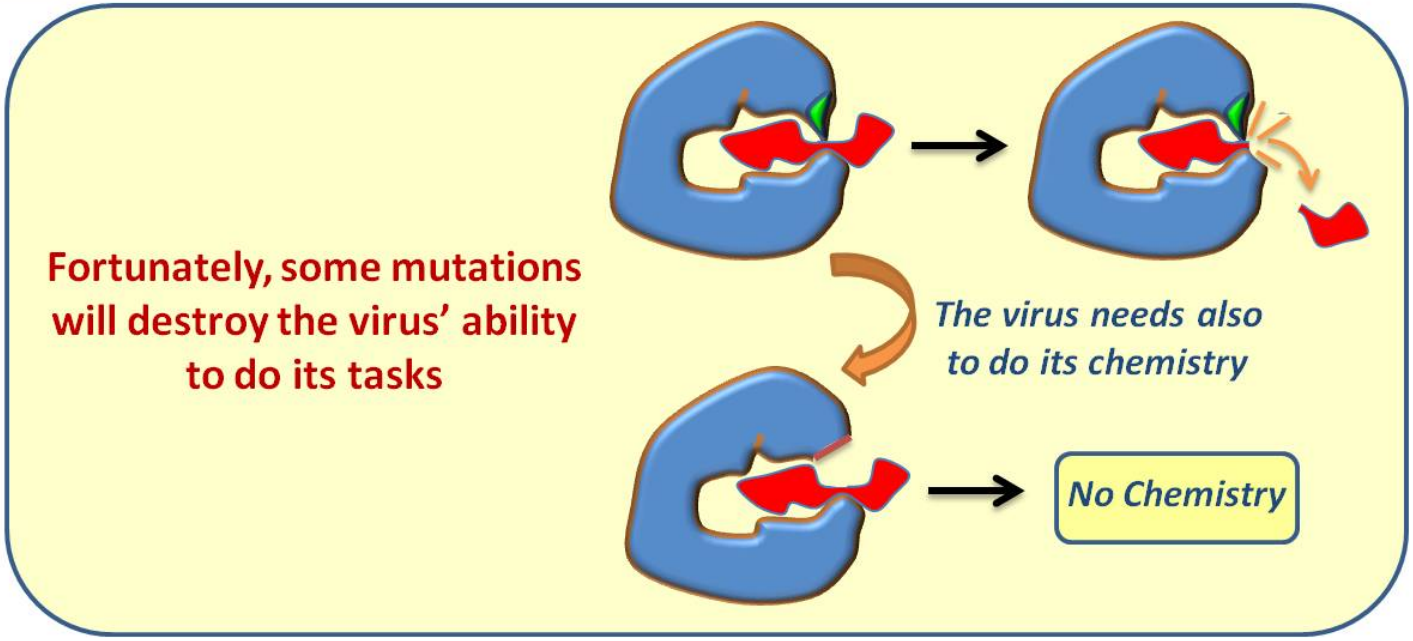

P-Site
A-Site
EVB region



Drug Resistance

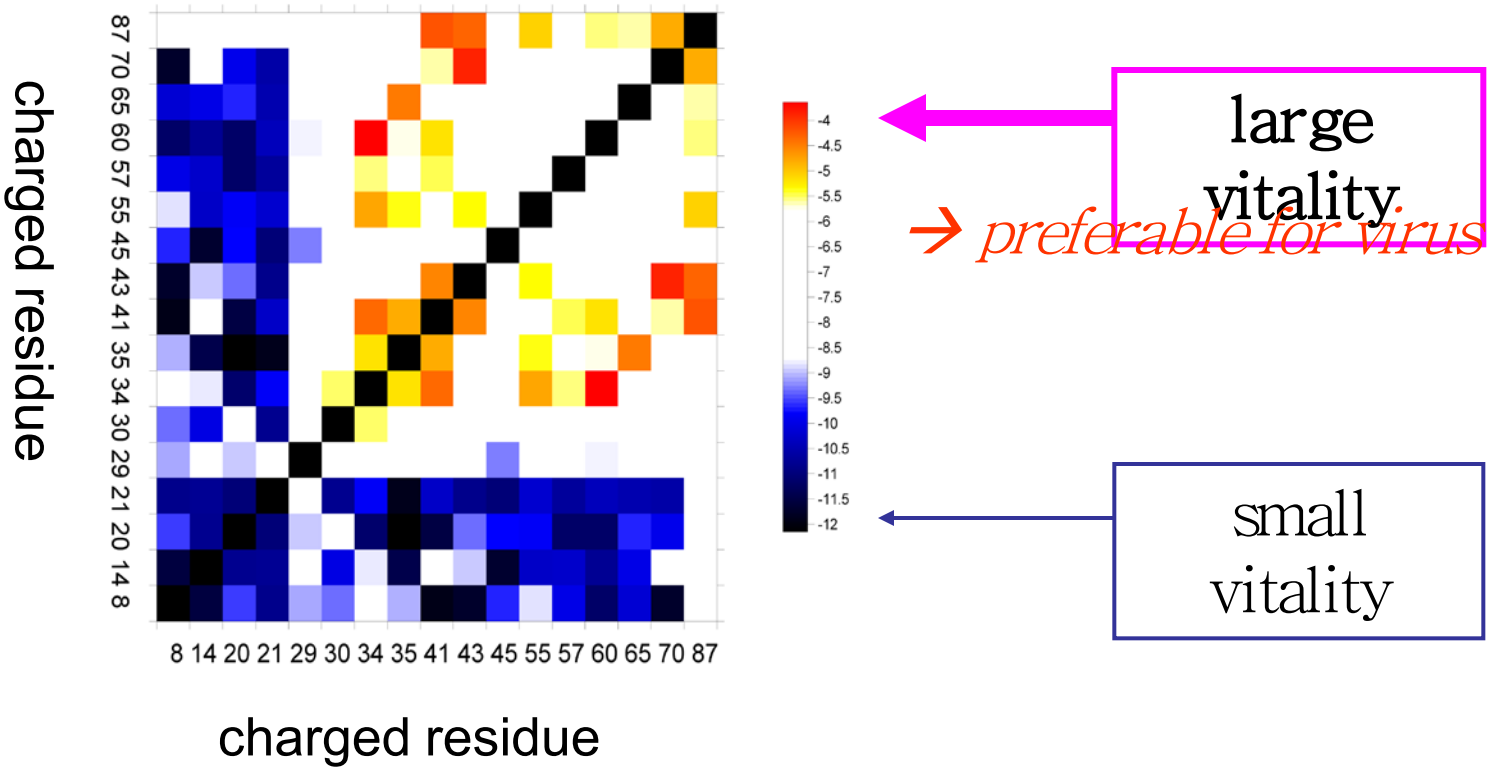



DRUG
DRUG
This mutation makes good resistance
DRUG
No Binding
The virus' enzyme would like to mutate in order to reduce the binding


Fortunately, some mutations will destroy the virus' ability to do its tasks
The virus needs also to do its chemistry
No Chemistry

Vitality diagram for double mutant

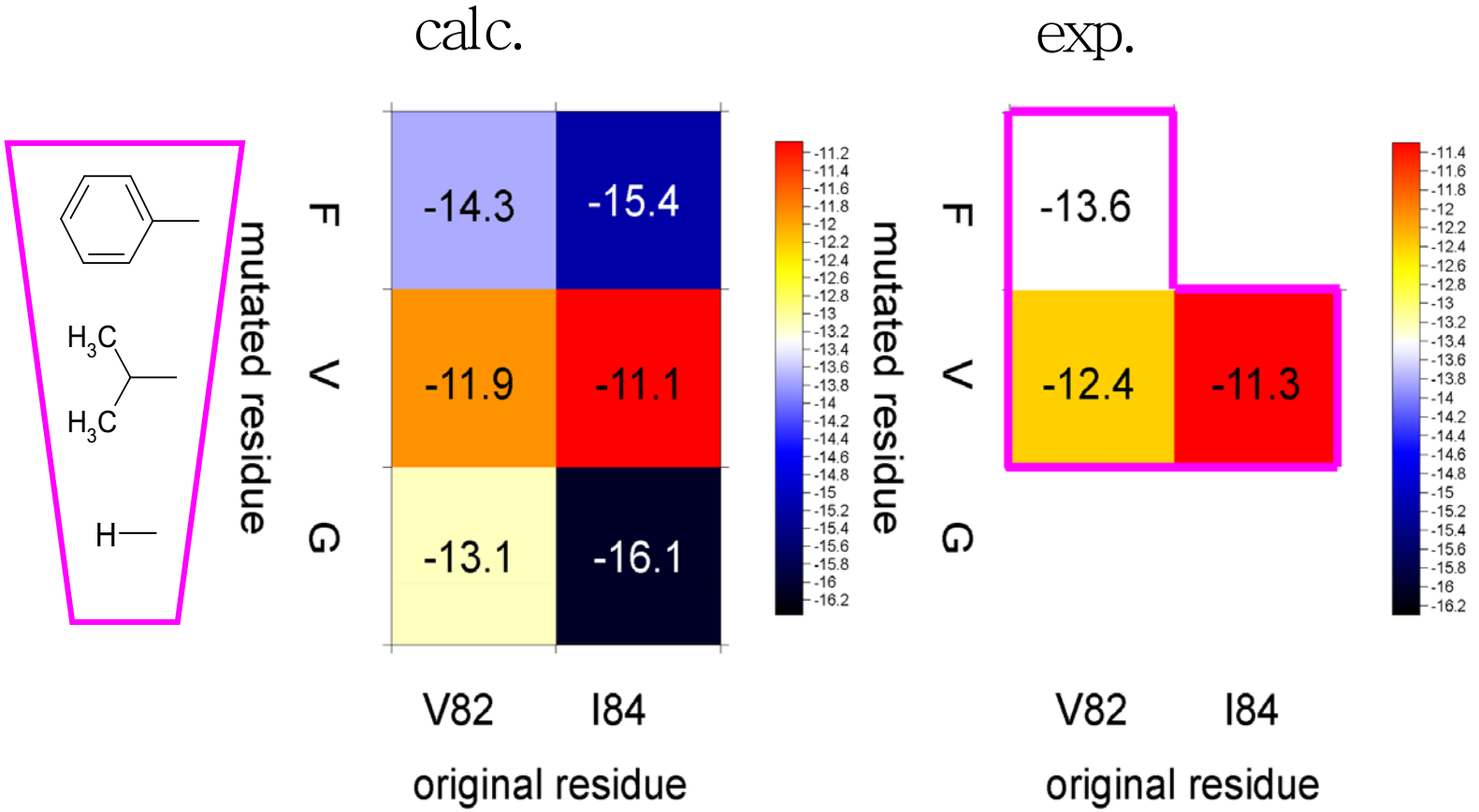
$$-\Delta\Delta G_{bind}^{N\to M}(TS)+\Delta\Delta G_{bind}^{N\to M}(drug)$$



Ishikita & Warshel (2007)

Influence of the size of nonpolar residue on ***vitality value***

$$-\Delta\Delta G_{bind}^{N\rightarrow M}(TS)+\Delta G_{bind}(drug)$$

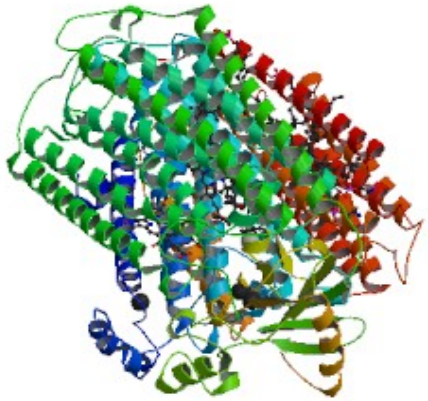


Ishikita & Warshel (2007)

STRUCTURE-FUNCTION RELATIONSHIP

STRUCTURE

FUNCTION



Missing link

ENERGY

SOLVATION BY PROTEIN + WATER

MAINLY ELECTROSTATIC!

A.Vardi
A.Dryga
M.Clark
A.Adamczyk
A.Reymer
A.Rychkova
S. Braun-Sand
M.Bohac
A.Papazyan
F.Lee
B.Messer
B.Kormos
M. Frushicheva
C.Deakyne
Y.J.Chien
E.Rosta
T. Wesolowski
F.Sussman
R.Matute
G. Naray-Szabo
G.Hong
G.King
H.Y.Woon
H.Liu
H.Ishikita
Z.T.Chu
I.Kim
I.Kupchenko
I.Muegge
J.Florian
J.Mavri
J.K.Hwang
J.Lameira
J.Cao
J.Aqvist
J.Villa
J.Bertran
L.Manna
M.Roca
M.K.Singh
M.Klahn
M.Strajbl
M.Olsson
A.Burykin
A.Shurki
H.Tao
M.Kosloff
A.Churg
M.Kato
M.Fuxreiter
N.Vaidehi
N.Singh
N.Plotnikov
N.Schutz
N. Li
P.Sharma
P.Schopf
P. Oelschlaeger
P.Varnai
R.Langen
R.P.Bora
A.Pisliakov
R.Mueller
R.Weiss
R.Rucker
E.Chudyk
S.Mukherjee
L.Kamerlin
M.Fothergill
S.Vicatos
S.Russel
S. Chakrabarty
S.Schweins
T.Glennon
M. de Caceres
V.Luzhkov
W.Thompson
A.Yadav
Y.Sham
Y.Xiang
S.Kuwajima
M.Haranczyk
J.Bentzien
J.Na
M.Mills

Acknowledgements

A.Adamczyk
J.Aqvist
J.Bentzien
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M.Bohac
R.P.Bora
S.Braun-Sand
A.Burykin
J.Cao
S.Chakrabarty
Z.T.Chu
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A.Churg
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A.Dryga
J.Florian
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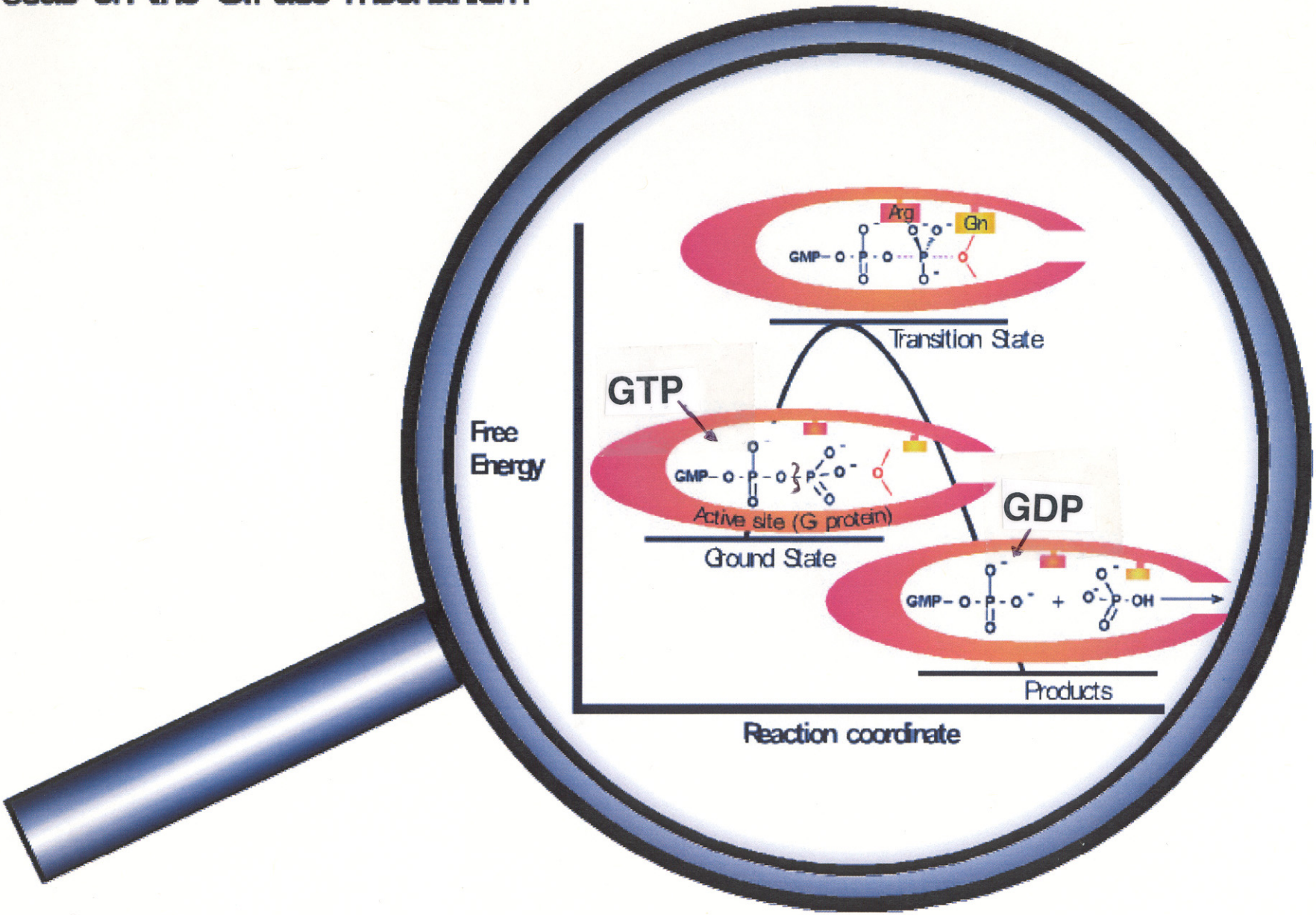
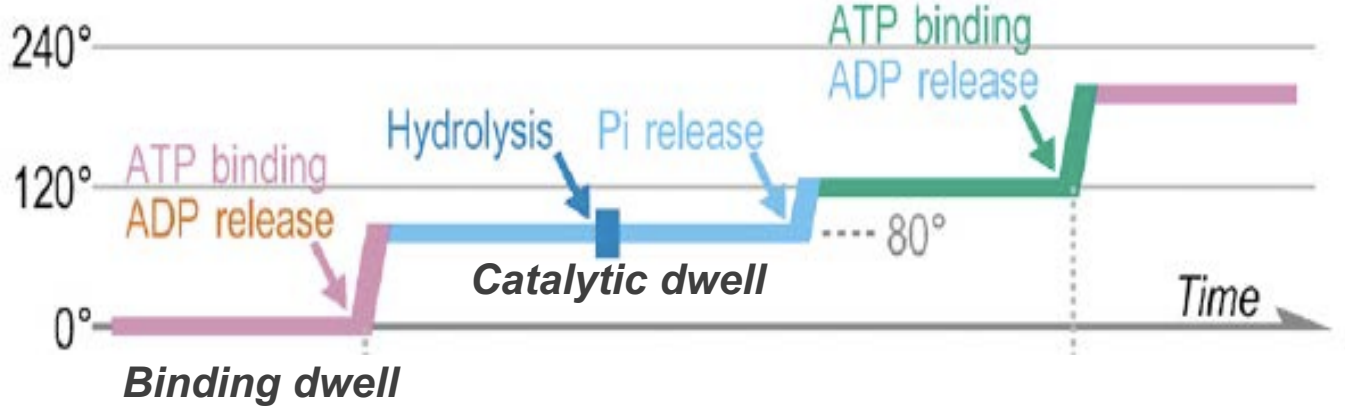
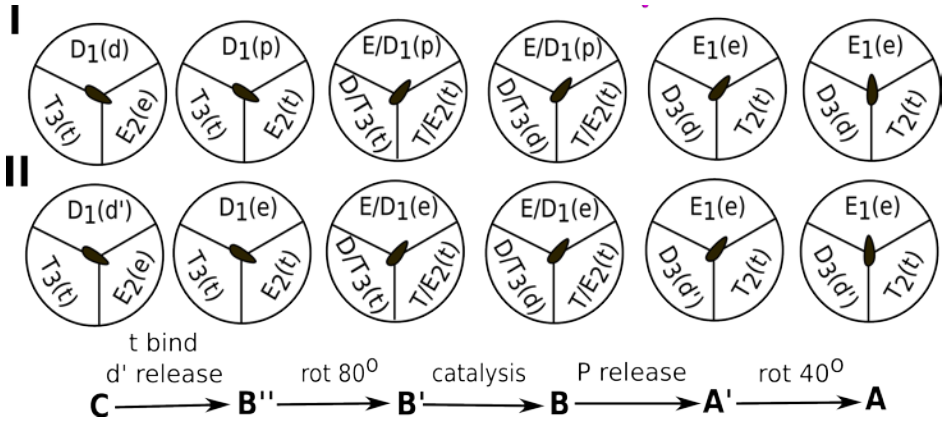
M.Frushicheva
M.Fuxreiter
T.Glennon
M.Haranczyk
G.Hong
J.-K.Hwang
H.Ishikita
C-Y.Jen
L.Kamerlin
M.Kato
I.Kim
M.Kato
I.Kim
G.King
M.Klahn
B.Kormos
M.Kosloff
I.Kupchenko
S.Kuwajima
J.Lameira
R.Langen
F.Lee
N.Li
H.Liu
V.Luzhkov
L.Manna
R.Matute
J.Mavri
B.Messer
M.Mills
I.Muegge
R.Mueller
S.Mukherjee
J.Na
G.Naray-Szabo
P.Oelschlaeger
M.Olsson
A.Papazyan
A.Pisliakov
N.Plotnikov
A.Reymer
M.Roca
E.Rosta
R.Rucker
S.Russel
A.Rychkova
P.Schopf
N.Schutz
T.Schweins
Y.Sham
P.Sharma
A.Shurki
M.K.Singh
N.Singh
M.Strajbl
F.Sussman
H.Tao
W.Thompson
N.Vaidehi
A.Vardi
P.Varnai
S.Vicatos
J.Villa
R.Weiss
T.Wesolowski
H.Y.Woon
Y.Xiang
A.Yadav

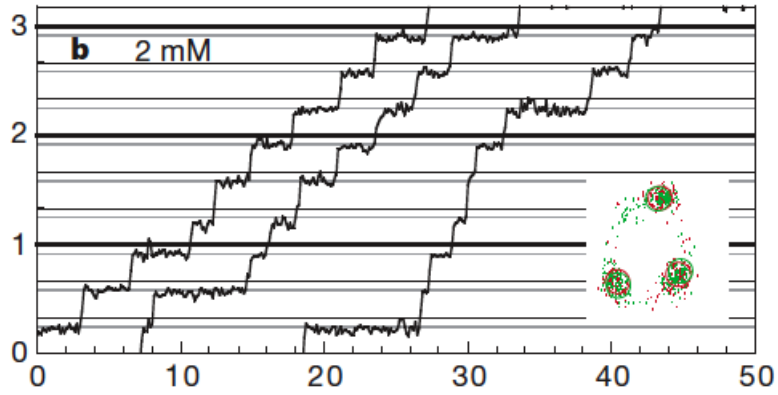
Focus on the GTPase mechanism



Mechano-Chemical Coupling between γ and α/β dimers :
As revealed by numerous single-molecule studies



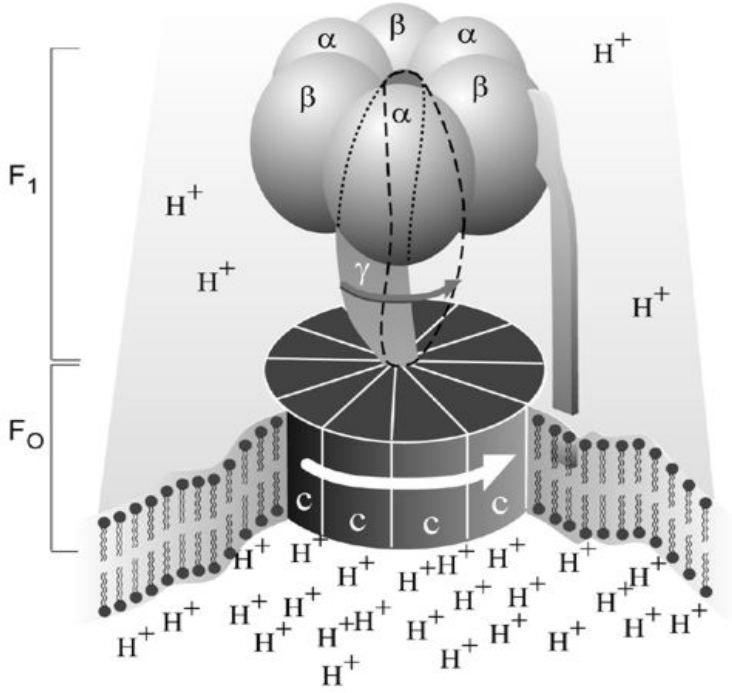


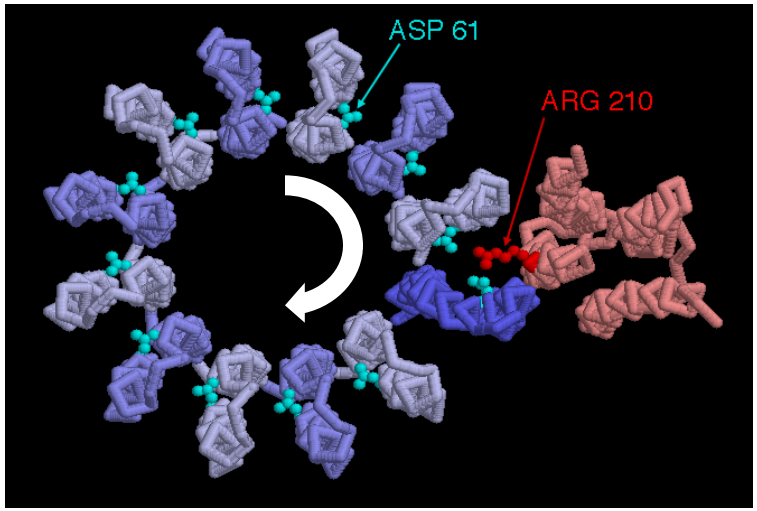


Shimo-Kon, R. et. al., Biophys. J., 2010.

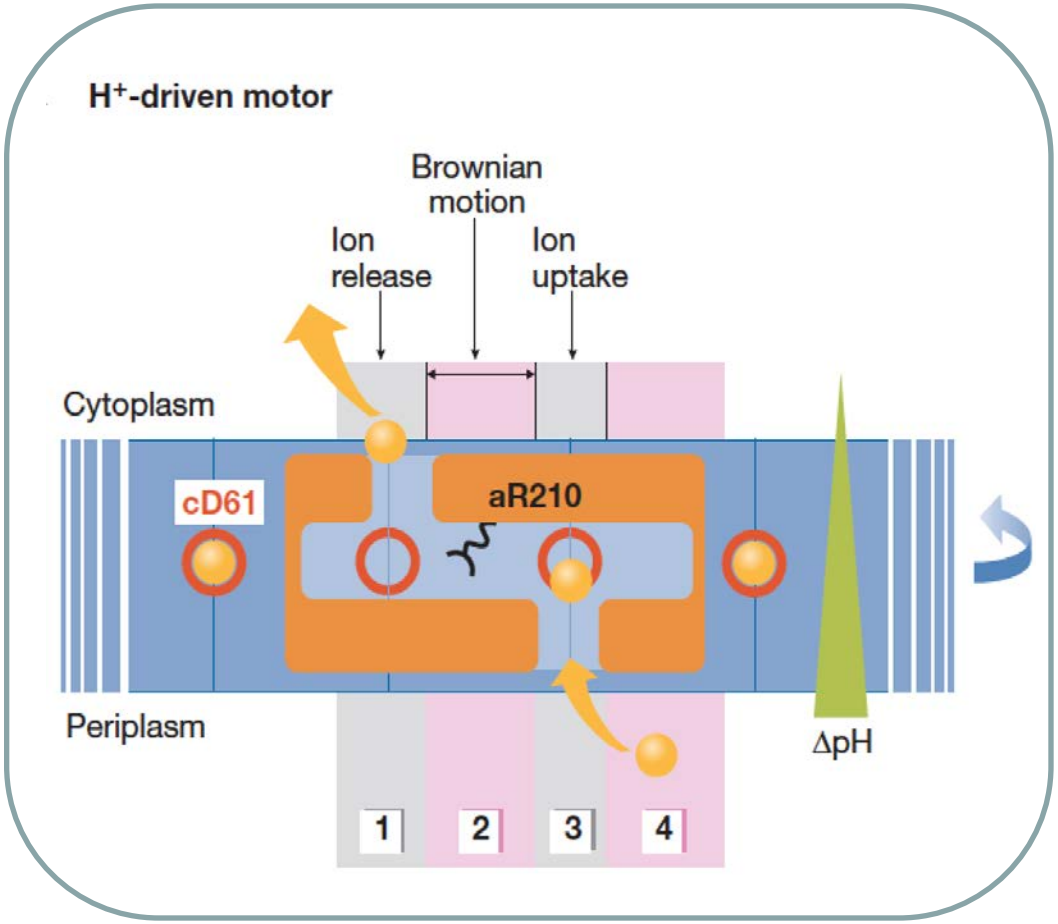
Yasuda, R. et. al., Nature, 2001.

Understanding the action of proton driven F_0 motor



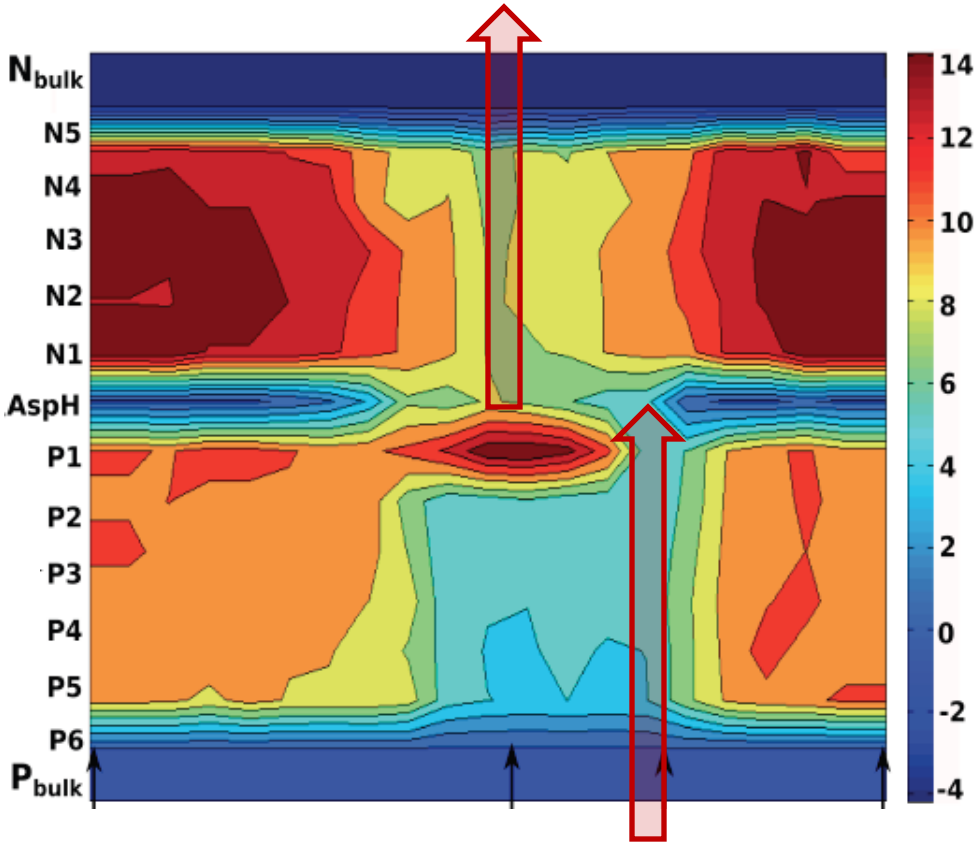


A 2D schematic model of
F_0 rotation coupled to ion transport



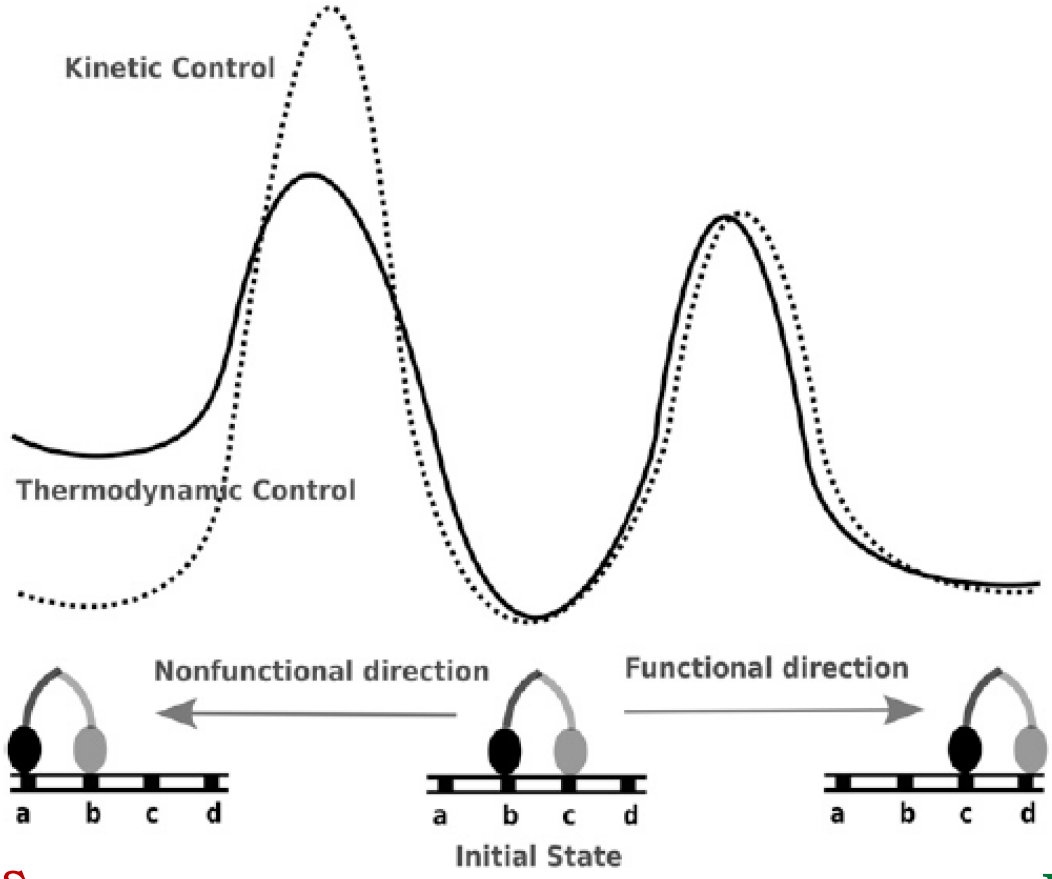
The CG electrostatic free energy for F_0 rotation coupled to proton transport across membrane

Release of protons to the high pH side



Uptake of protons from the low pH side

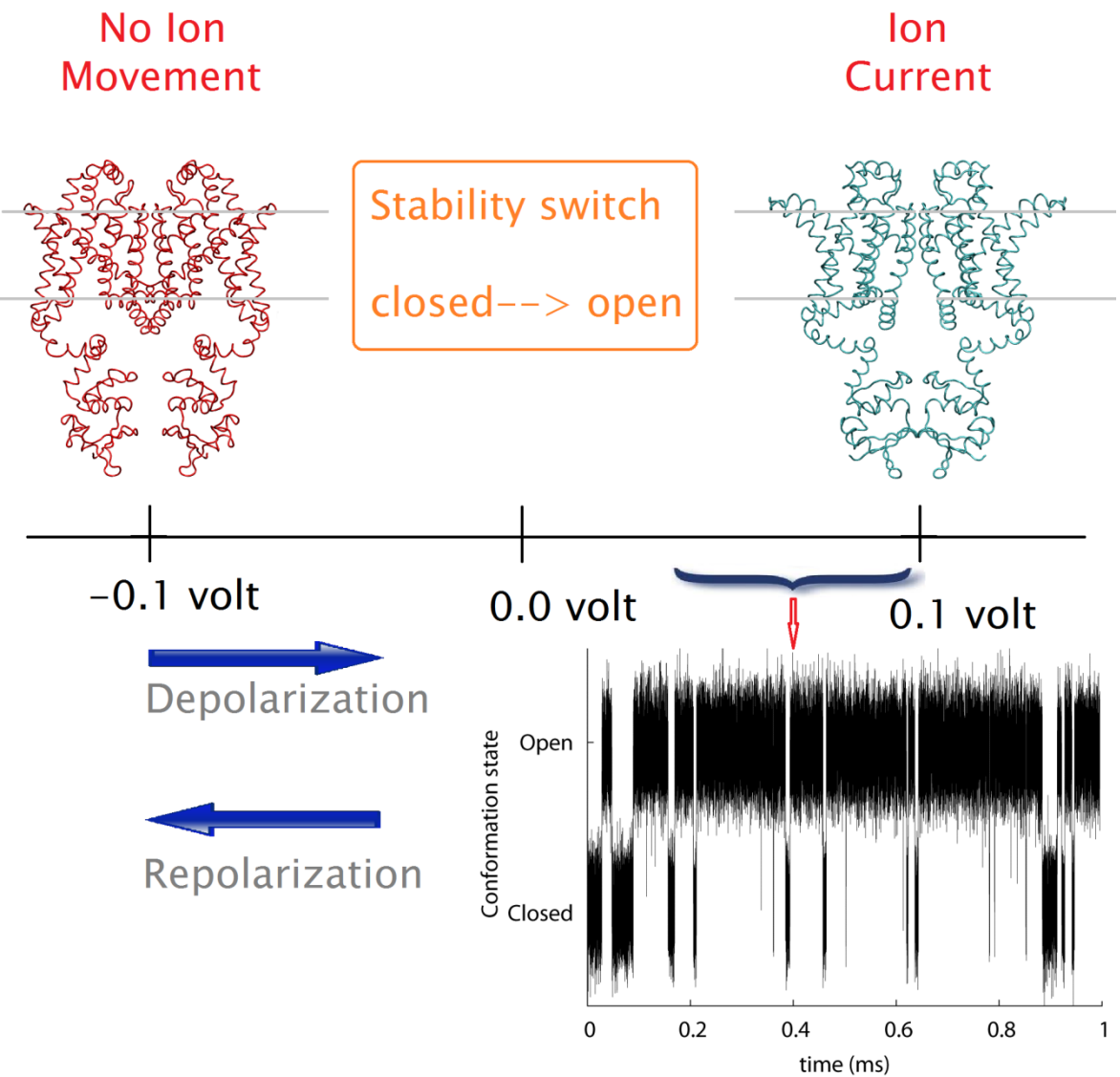
Free energy surface that can explain unidirectional walking motion



Backward steps are obstructed by higher barriers or free energies

Forward steps are preferred by lower barriers or free energies

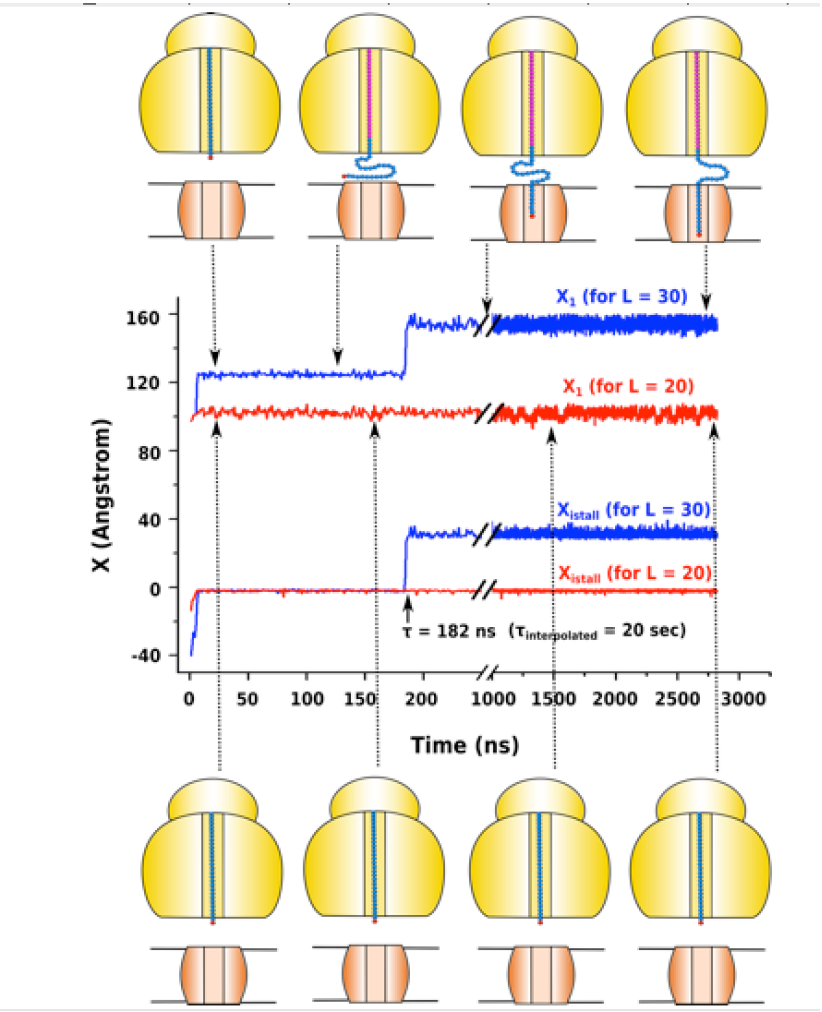
Free Energy of the System



The free energy at different regions of the conformation/voltage landscape

Voltage (volt)	Closed (kcal)	Open (kcal)
-0.2	**-203**	**-189**
0.0	**-201**	**-200**
+0.2	**-202**	**-218**

Dryga A, Chakrabarty S, Vicatos S, & Warshel A (2011) Realistic simulation of the activation of voltage gated ion channels. *PNAS* (in press)


X (Angstrom)
160
120
80
40
0
-40
0
50
100
150
200
1000
1500
2000
2500
3000
Time (ns)
X1 (for L = 30)
X1 (for L = 20)
Xistall (for L = 30)
Xistall (for L = 20)
τ = 182 ns (τinterpolated = 20 sec)

A General Cycle For Moving between Different Multiscale Leveles

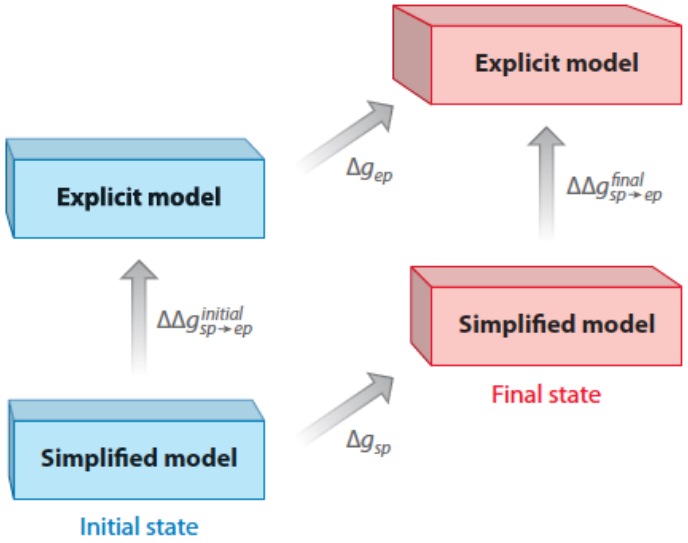
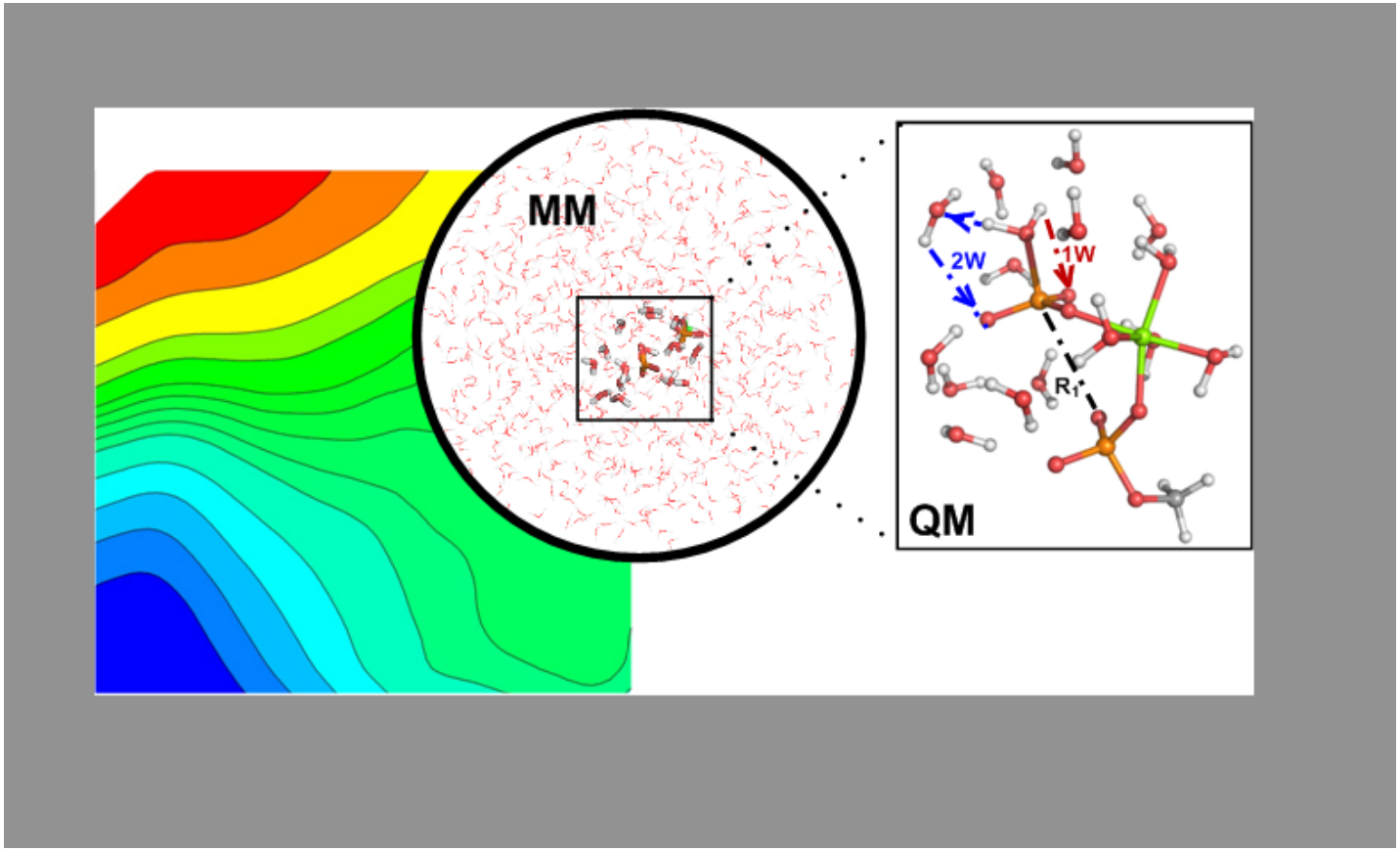
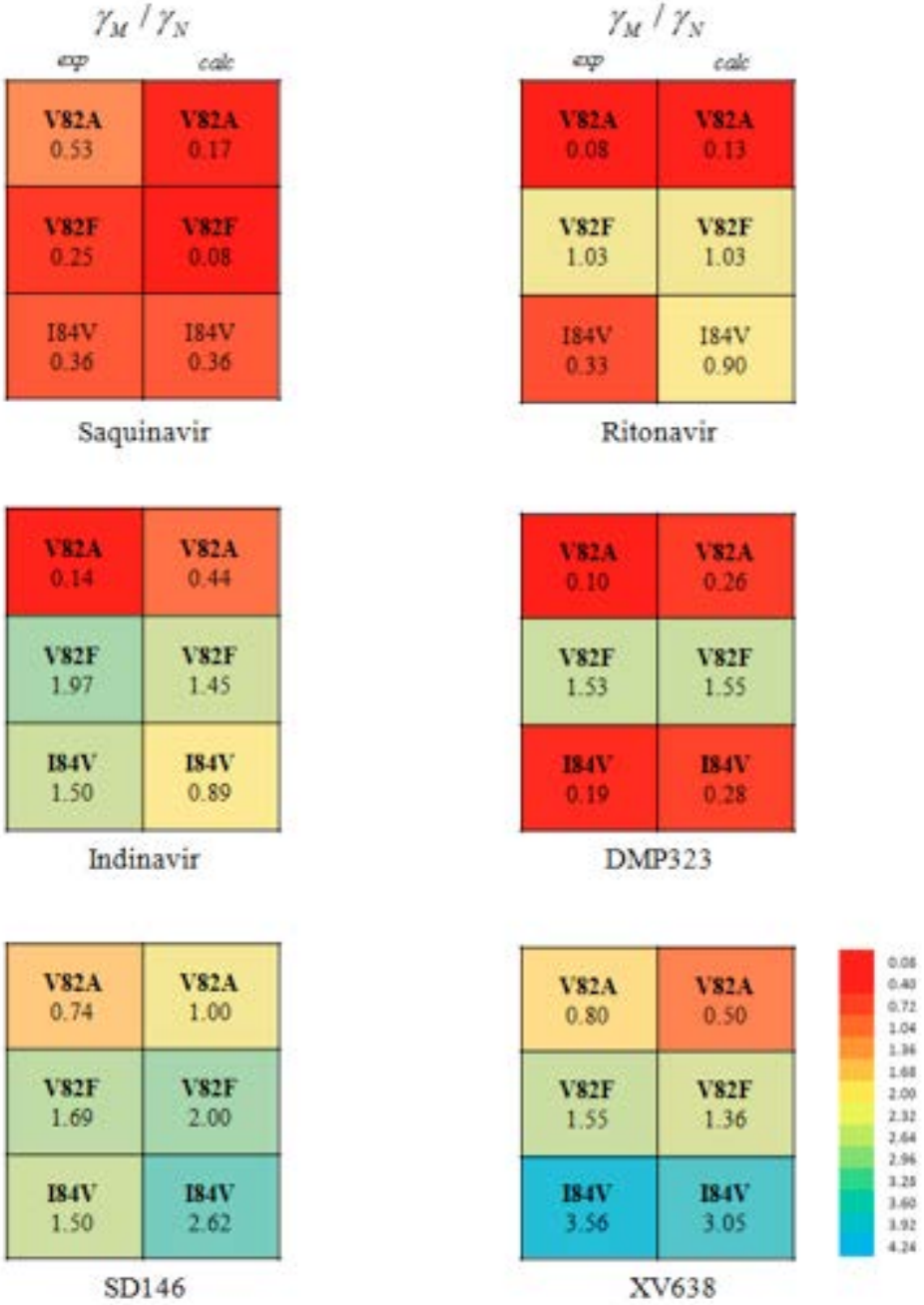


Figure 2

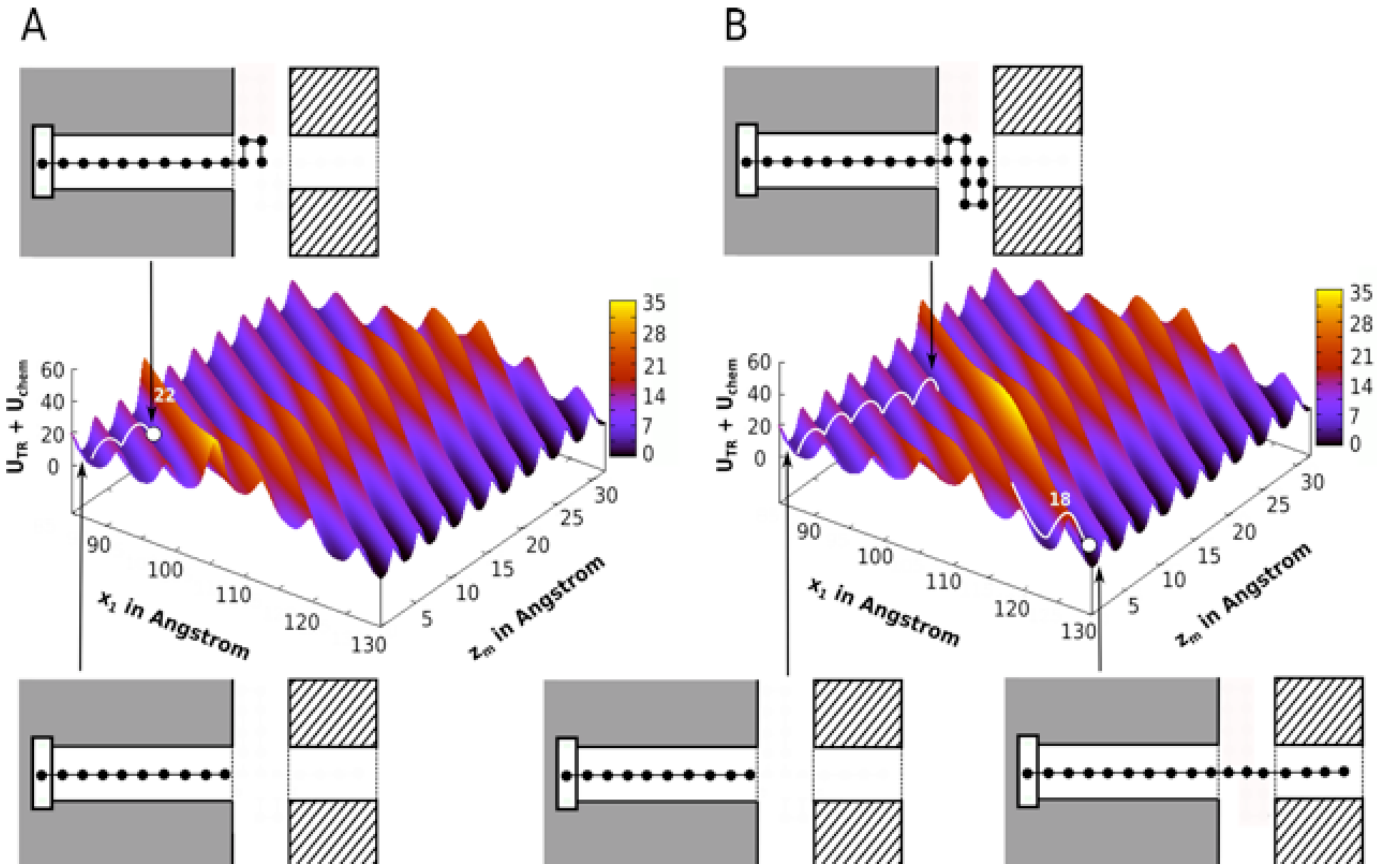
The thermodynamic cycle used to calculate the change (Δg_{ep}) in free energy for a generic process in an explicit system. Having calculated the free-energy change of the corresponding simplified model, Δg_{sp}, umbrella sampling can be used to calculate the free-energy change $\Delta\Delta g_{sp\to ep}$ for the initial and final states to obtain Δg_{ep}.

JPC 2013
Systematic QM(ai)/MM PMFs

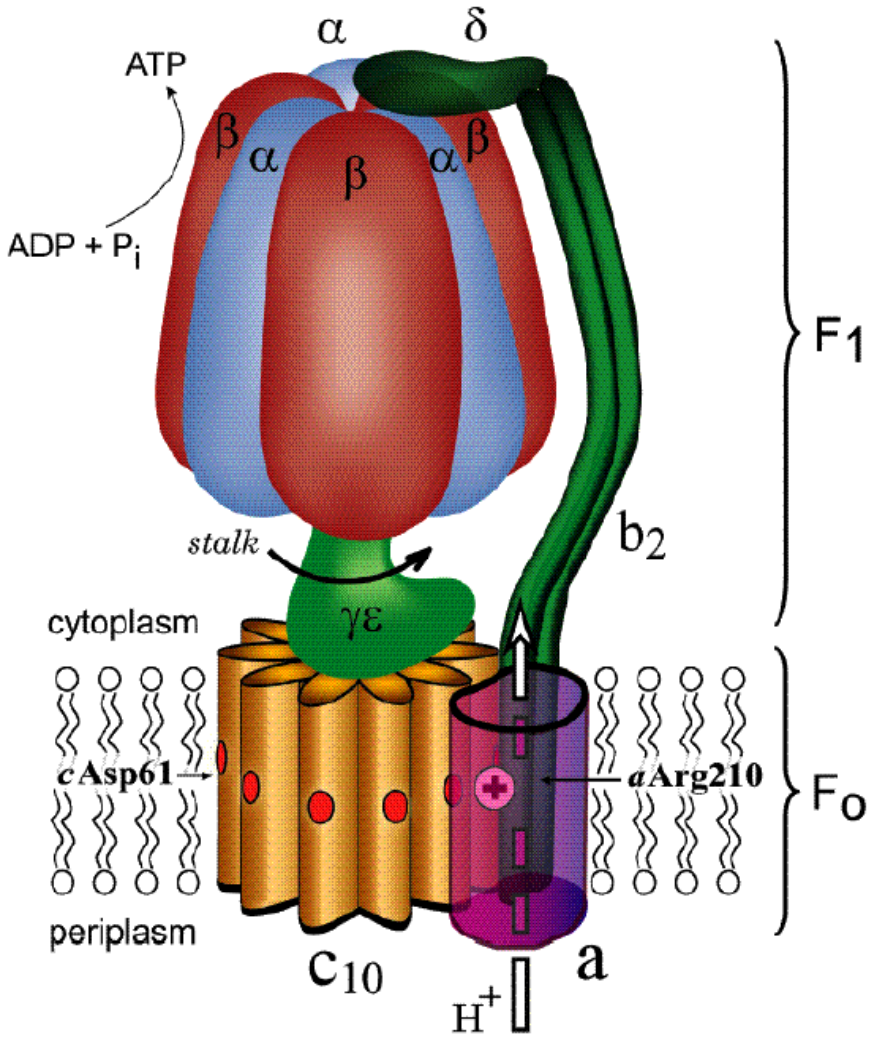



γ_M / γ_N
exp
calc
V82A 0.53
V82A 0.17
V82F 0.25
V82F 0.08
I84V 0.36
I84V 0.36
Saquinavir
γ_M / γ_N
exp
calc
V82A 0.08
V82A 0.13
V82F 1.03
V82F 1.03
I84V 0.33
I84V 0.90
Ritonavir
V82A 0.14
V82A 0.44
V82F 1.97
V82F 1.45
I84V 1.50
I84V 0.89
Indinavir
V82A 0.10
V82A 0.26
V82F 1.53
V82F 1.55
I84V 0.19
I84V 0.28
DMP323
V82A 0.74
V82A 1.00
V82F 1.69
V82F 2.00
I84V 1.50
I84V 2.62
SD146
V82A 0.80
V82A 0.50
V82F 1.55
V82F 1.36
I84V 3.56
I84V 3.05
XV638
0.08
0.40
0.72
1.04
1.36
1.68
2.00
2.32
2.64
2.96
3.28
3.60
3.92
4.24

PNAS 2013


A
B
$U_{TR} + U_{chem}$
60
40
20
0
22
18
90
100
110
120
130
x_1 in Angstrom
5
10
15
20
25
30
z_m in Angstrom
35
28
21
14
7
0

Adenosine Triphosphate (ATP) Synthase



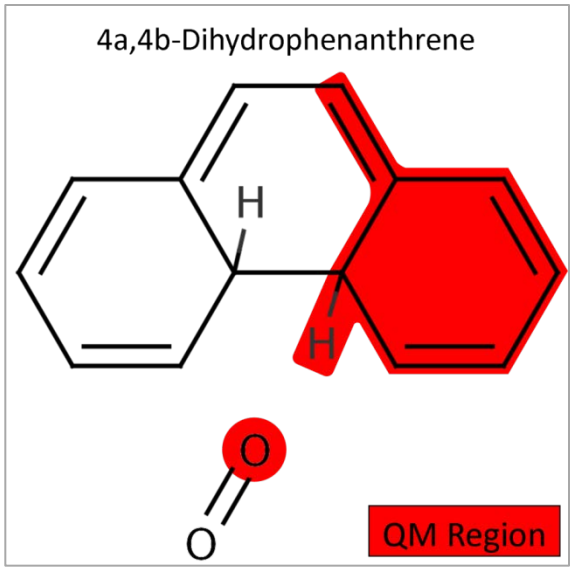
Rotary catalysis: Two protein motors coupled via common central stalk γδ

Solvent exposed F_1 unit ($\alpha_3\beta_3\gamma\delta\varepsilon$): central stalk rotation causes conformational changes in catalytic sites, driving ATP synthesis

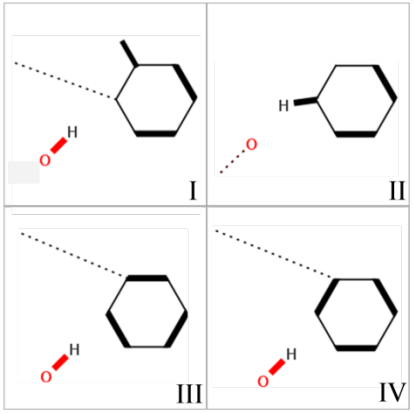
Transmembrane F_o unit (ab_2c_{10}): converts proton motive force into mechanical rotation of central stalk

A. Warshel & A. Bromberg, *J. Chem. Phys.*, 1970

QM(VB)
+MM



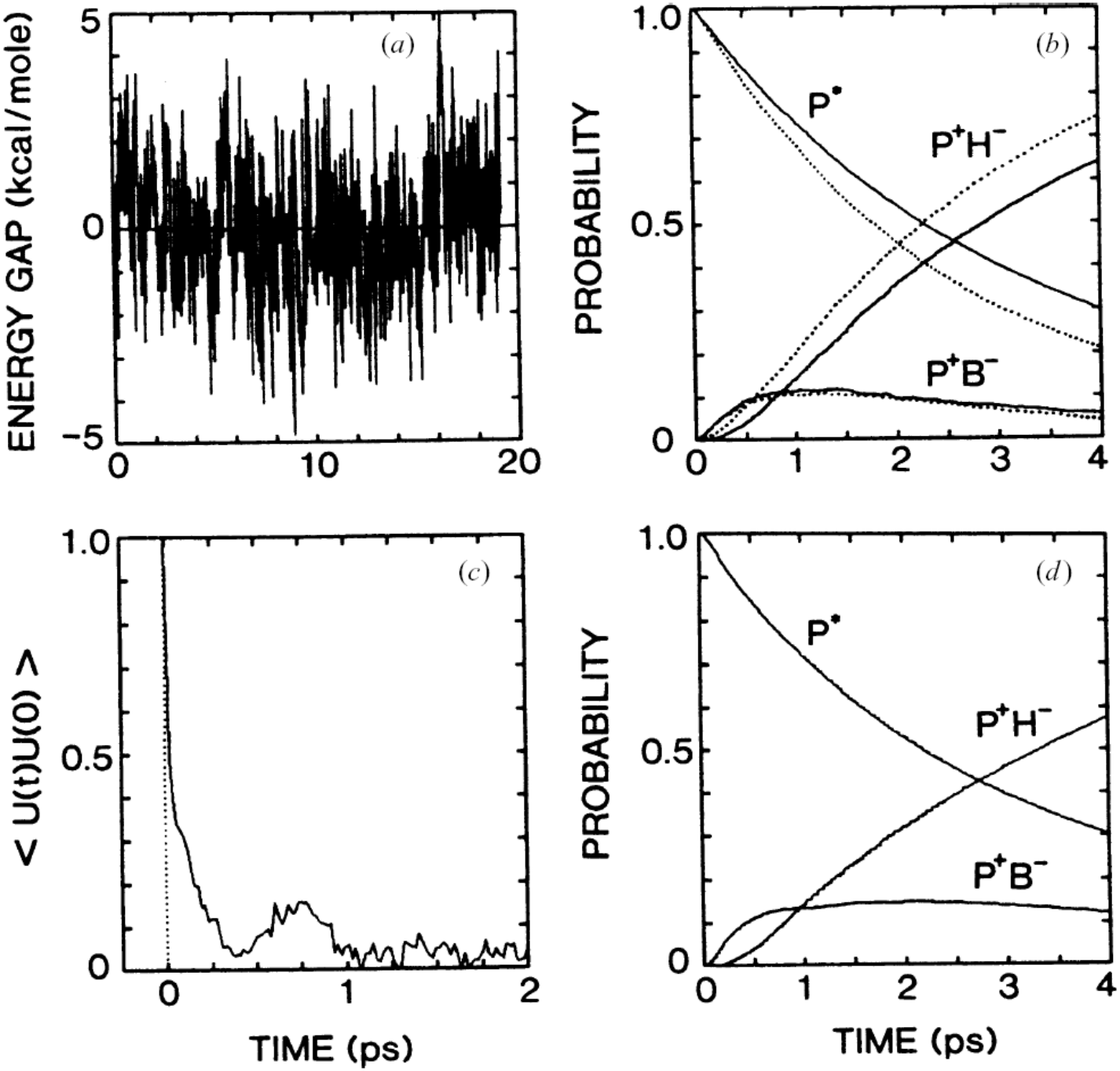
$$\mathbf{H} = \begin{pmatrix} 2^5\left[Q+\beta+\delta_2-\frac{1}{2}(\alpha+\gamma)\right] & -2^4[Q+\alpha+\beta+\delta_2-2\gamma] & -2^2\left[Q+\beta+\delta_2+6\delta_1-\frac{1}{2}(\alpha+\gamma)\right] & -2^4\left[Q+\beta+\delta_2+\frac{3}{2}\delta_1-\frac{1}{2}(\alpha+\gamma)\right] \\ -2^4[Q+\alpha+\beta+\delta_2-2\gamma] & 2^5\left[Q+\alpha+\delta_2-\frac{1}{2}(\beta+\gamma)\right] & 2[Q+\alpha+\beta+\delta_2+6\delta_1-2\gamma] & 2^3\left[Q+\alpha+\beta+\delta_2+\frac{3}{2}\delta_1-2\gamma\right] \\ -2^2\left[Q+\beta+\delta_2+6\delta_1-\frac{1}{2}(\alpha+\gamma)\right] & 2[Q+\alpha+\beta+\delta_2+6\delta_1-2\gamma] & 2^5\left[Q+\beta+\frac{3}{2}\delta_1-\frac{1}{2}(\alpha+\gamma+\delta_2)\right] & 2^3\left[Q+\beta+6\delta_1-\frac{1}{2}(\alpha+\gamma+\delta_2)\right] \\ -2^4\left[Q+\beta+\delta_2+\frac{3}{2}\delta_1-\frac{1}{2}(\alpha+\gamma)\right] & 2^3\left[Q+\alpha+\beta+\delta_2+\frac{3}{2}\delta_1-2\gamma\right] & 2^3\left[Q+\beta+6\delta_1-\frac{1}{2}(\alpha+\gamma+\delta_2)\right] & 2^5\left[Q+\beta+\frac{3}{2}\delta_1-\frac{1}{2}(\alpha+\gamma+\delta_2)\right] \end{pmatrix}$$



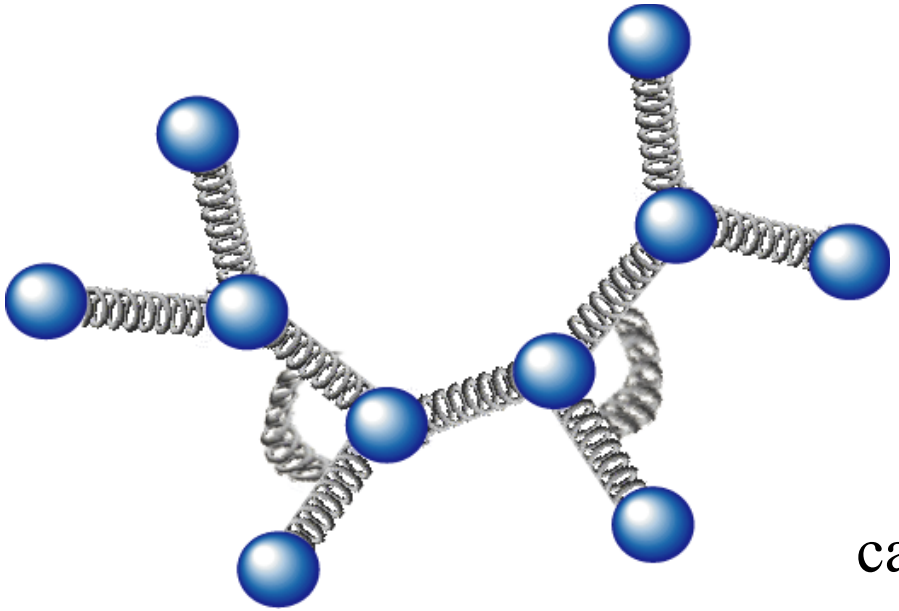
Dashed lines represent an electron removed to infinity.
Paired electrons are represented by heavy lines.

A. Warshel & M. Karplus, *J. Am. Chem. Soc.*, 1972

QM(MO)
+MM

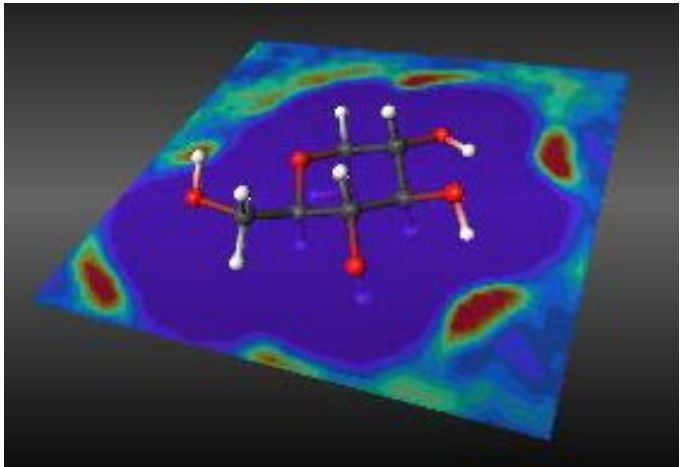

(a)
ENERGY GAP (kcal/mole)
5
0
−5
0
10
20
(b)
PROBABILITY
1.0
0.5
0
P*
P+H−
P+B−
0
1
2
3
4
(c)
< U(t)U(0) >
1.0
0.5
0
0
1
2
TIME (ps)
(d)
PROBABILITY
1.0
0.5
0
P*
P+H−
P+B−
0
1
2
3
4
TIME (ps)

Molecular Mechanics



cannot study chemistry

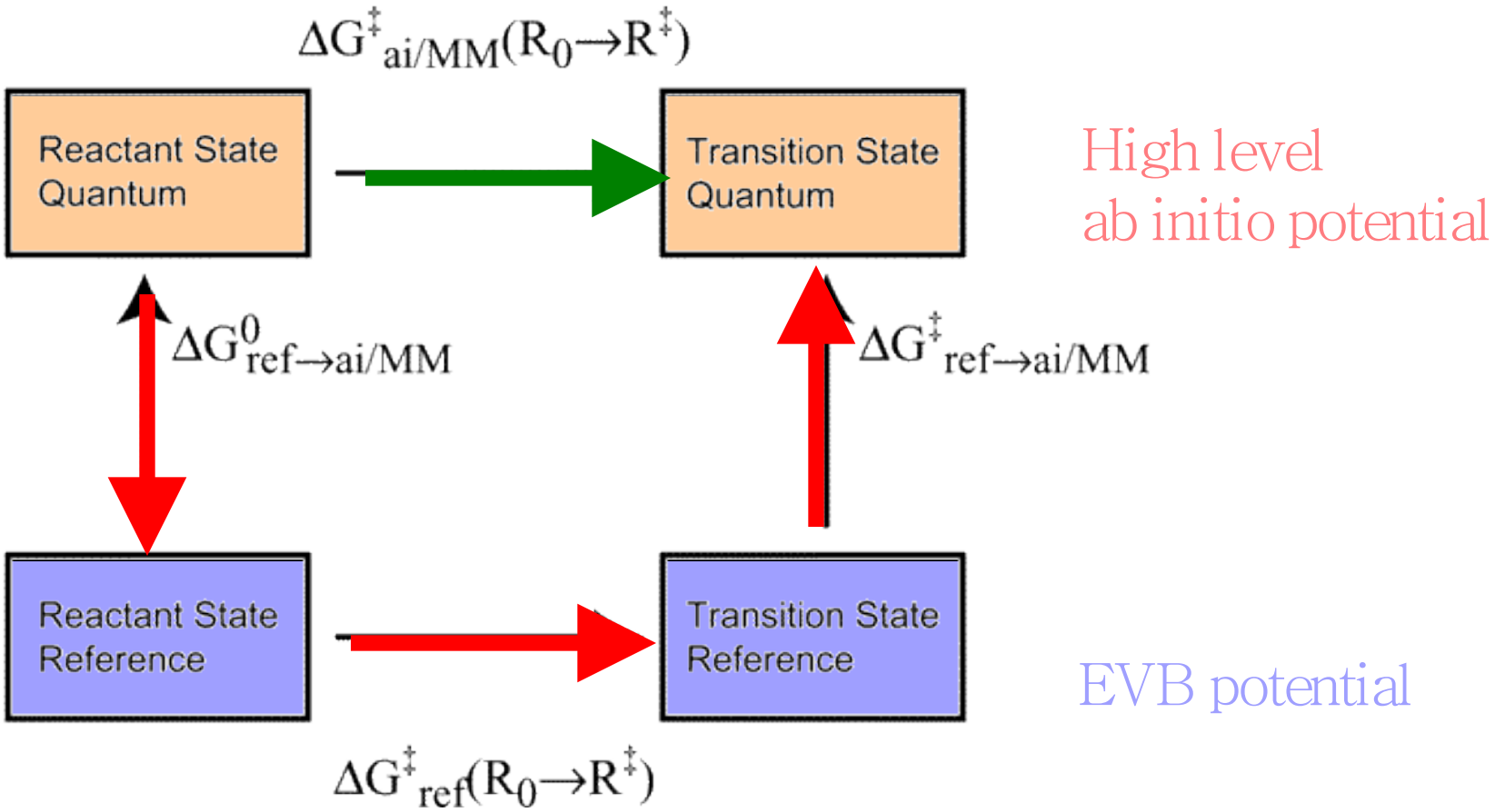
Quantum Mechanics



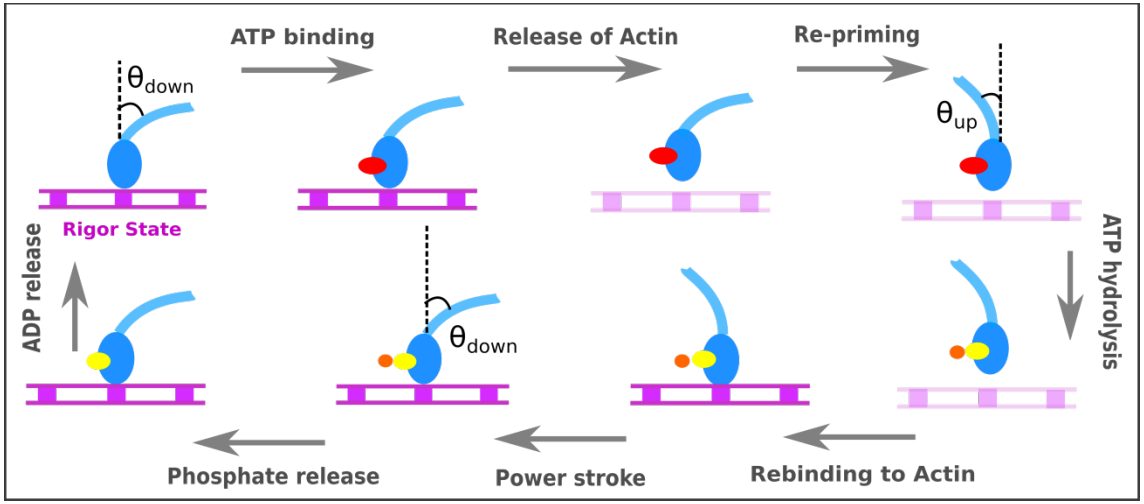
very expensive

Our Pradynamics（since 1992 with different names）

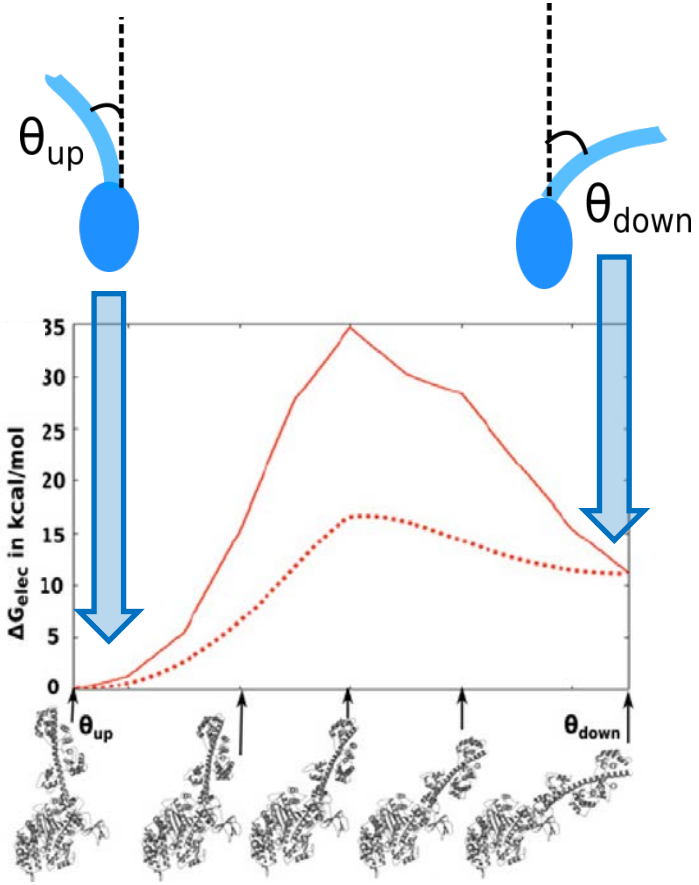
A General Thermodynamic CycleFor MultiScale Modeling

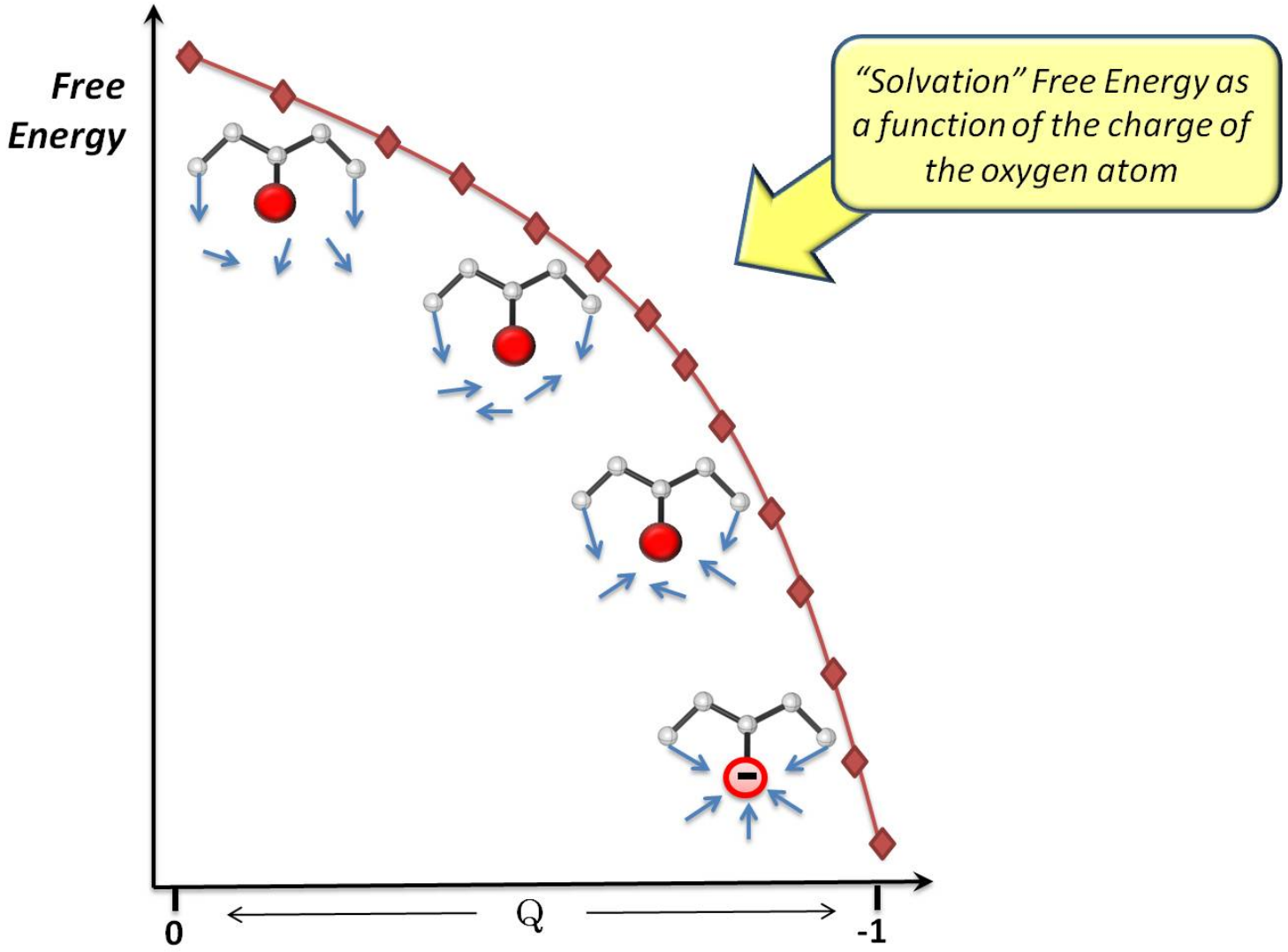
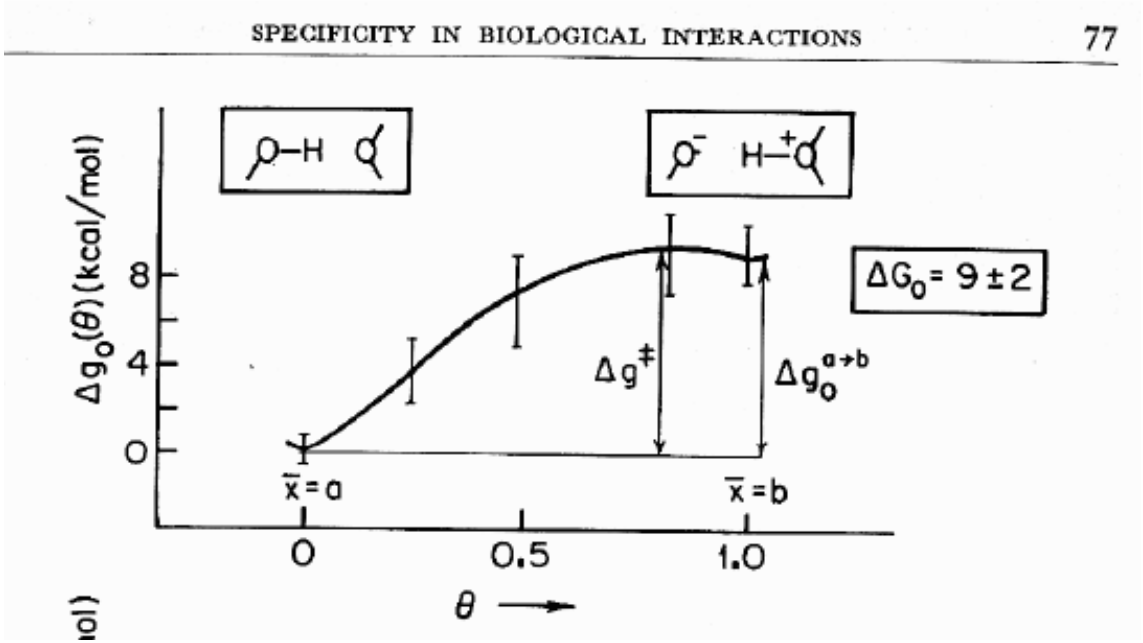


CG energetics of a single leg as it bends (changes conformation)



Schematic functional cycle of myosin V single leg




Free Energy
"Solvation" Free Energy as a function of the charge of the oxygen atom
0
Q
-1

Pontifical Academy
1983
Free energy perturbation in enzyme catalysis