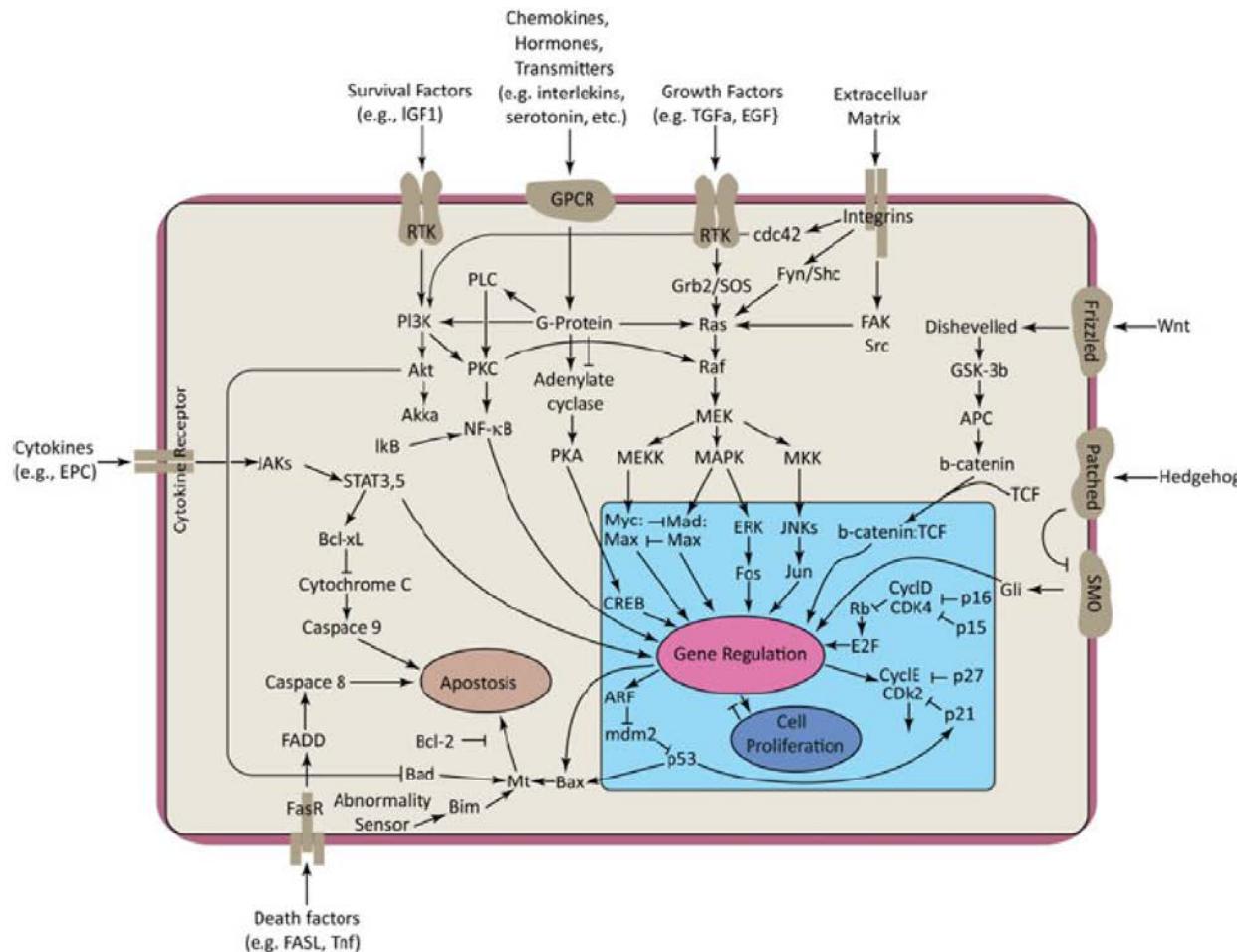


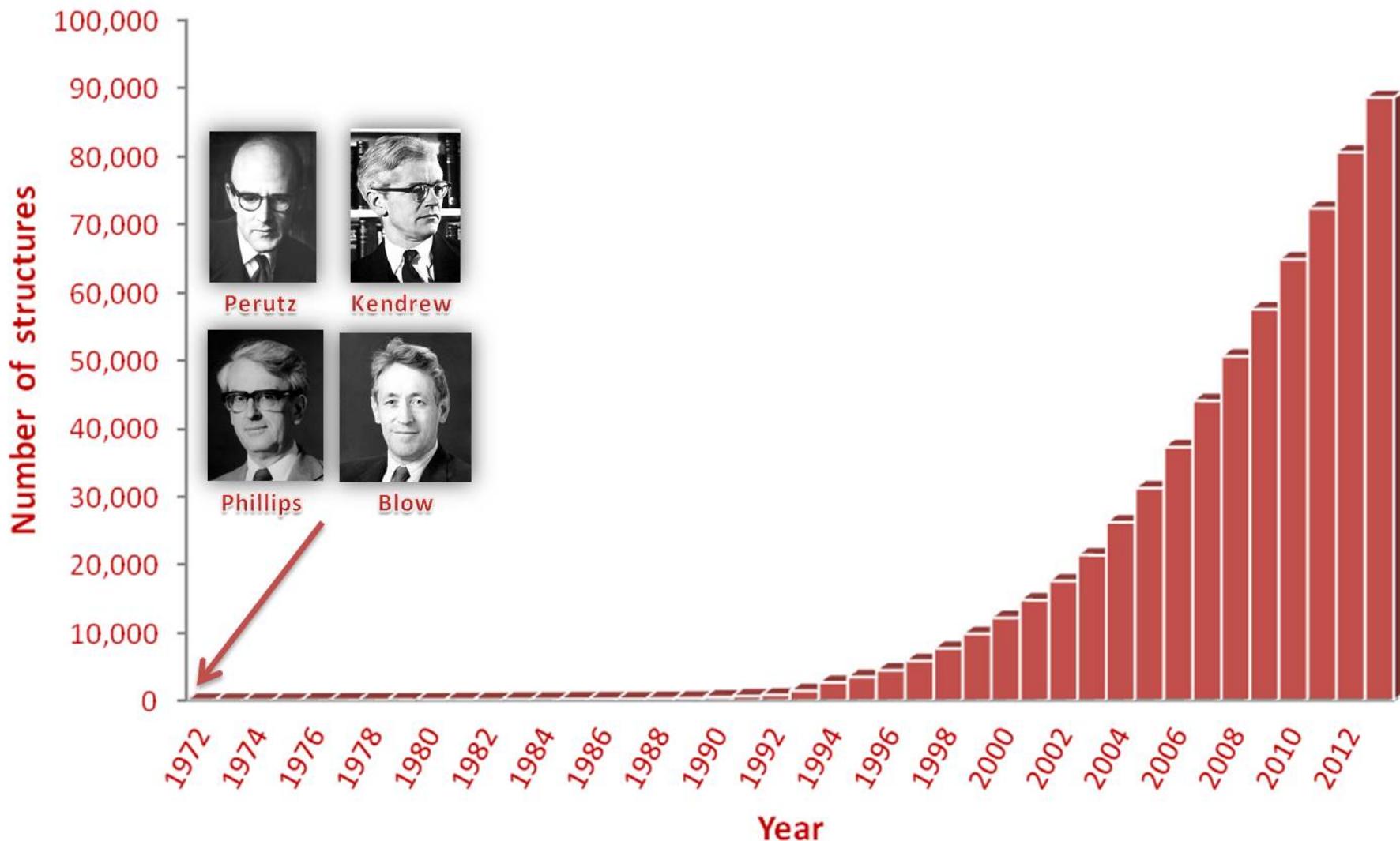
Computer Simulations of Biological Functions ; From Enzymes to Molecular Machines

Sending information (Signals) in the cell

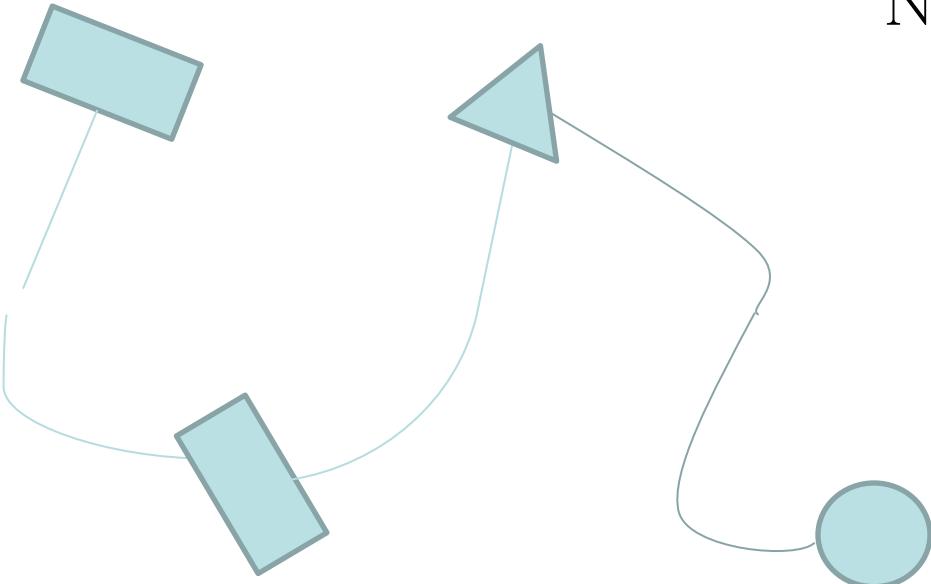
Why nature really chose phosphate 87



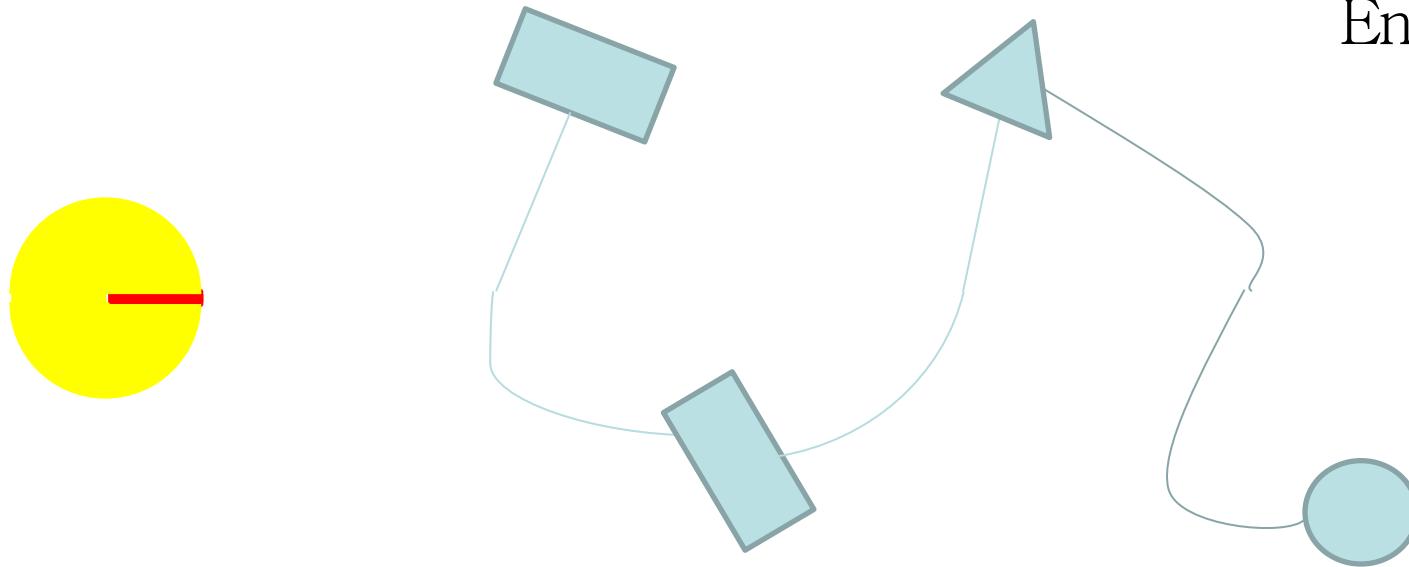
Yearly Growth of Protein Structures

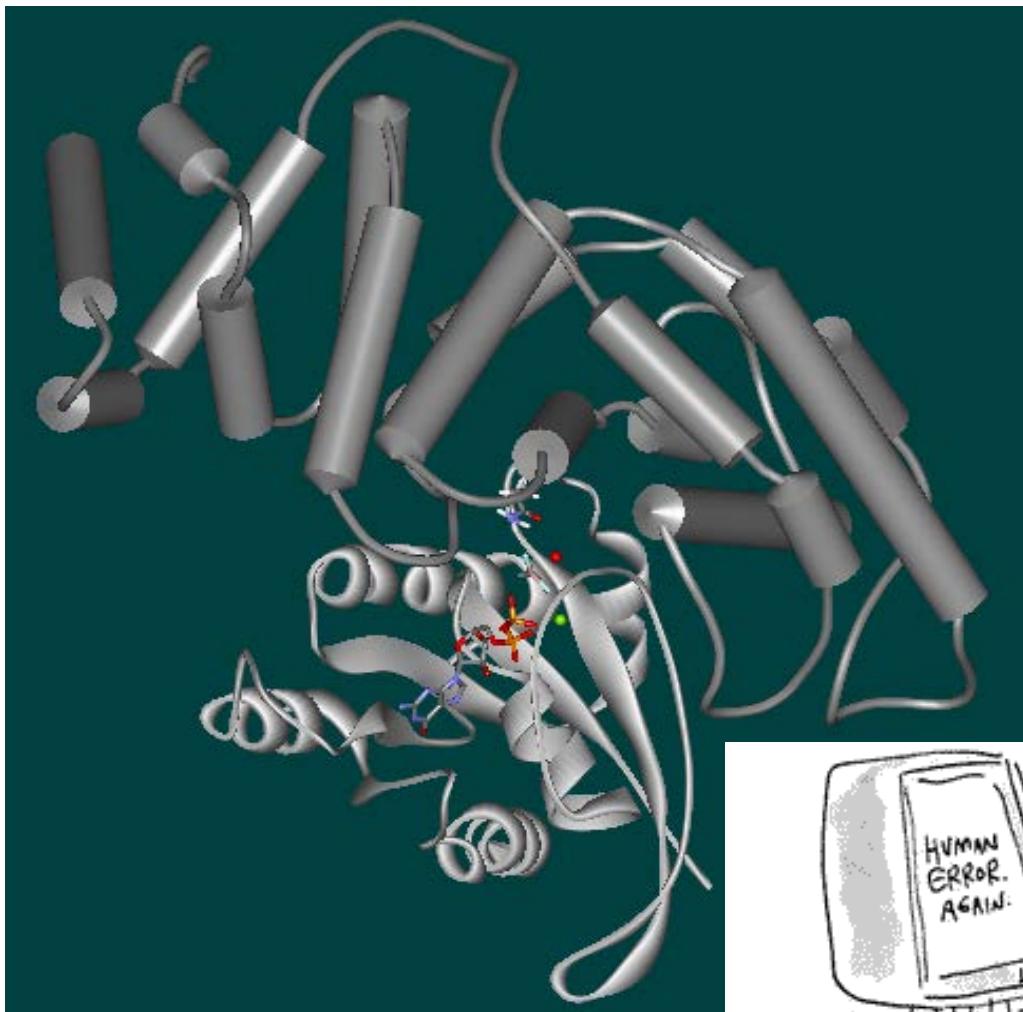


No Enzyme

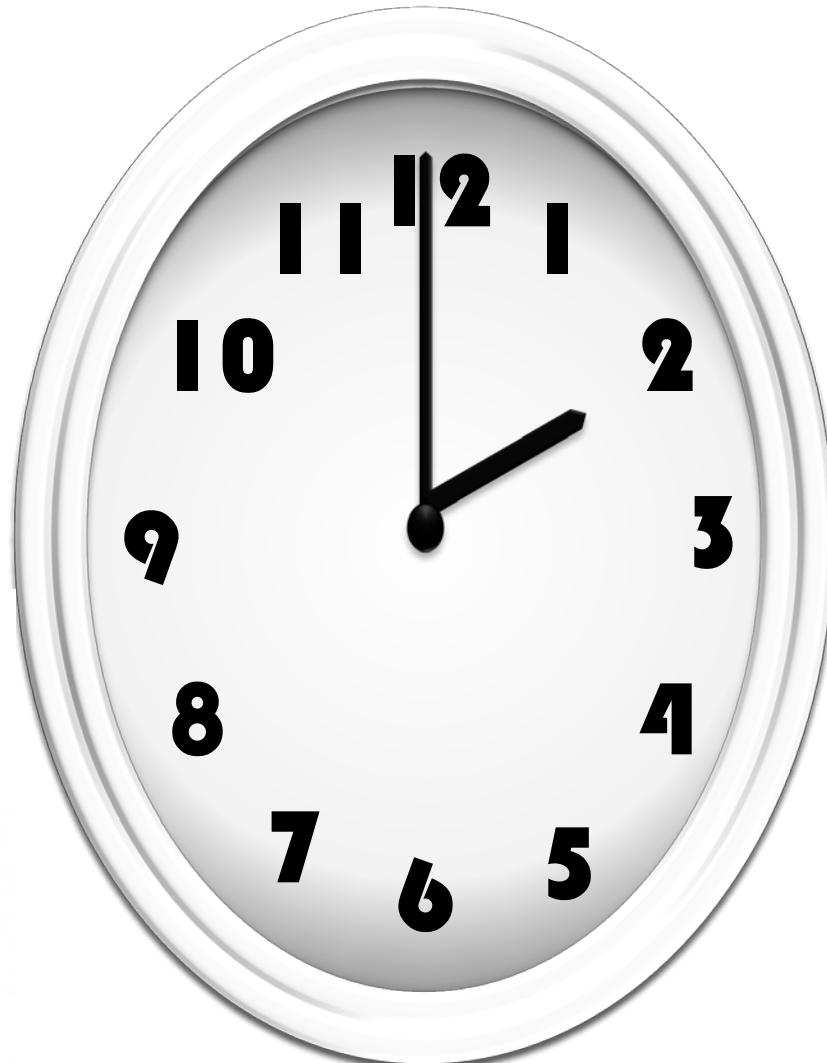


Enzyme





**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

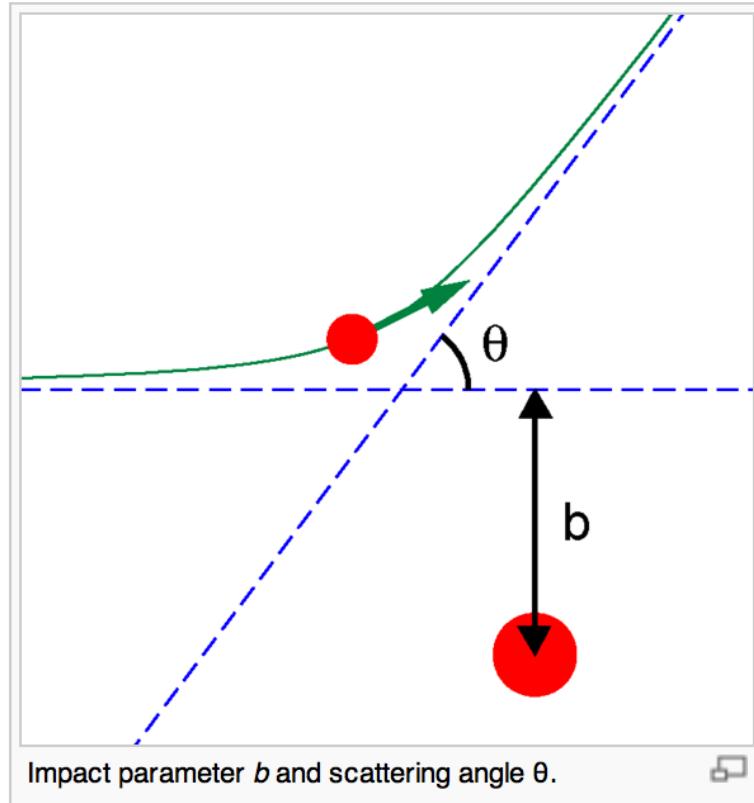
“On the interaction of chymotrypsin with ionized Substrate”

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)-Eventually EVB





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

*Weizmann Institute of Science
Rehovot, Israel*

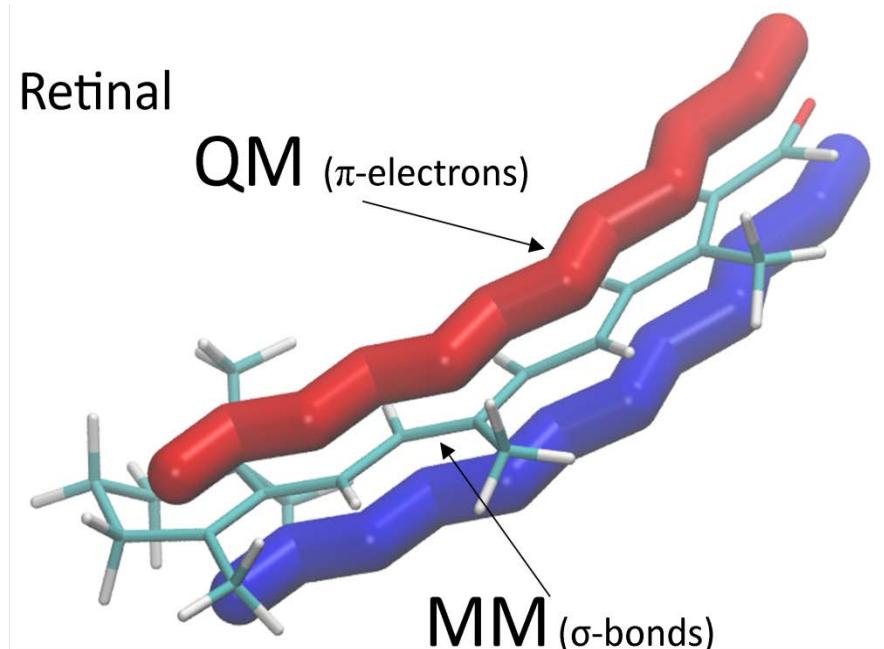


Early development of the general Cartesian Force field
and program (1966-69)

Energy , Structure and vibrations of general molecules
and molecular crystals

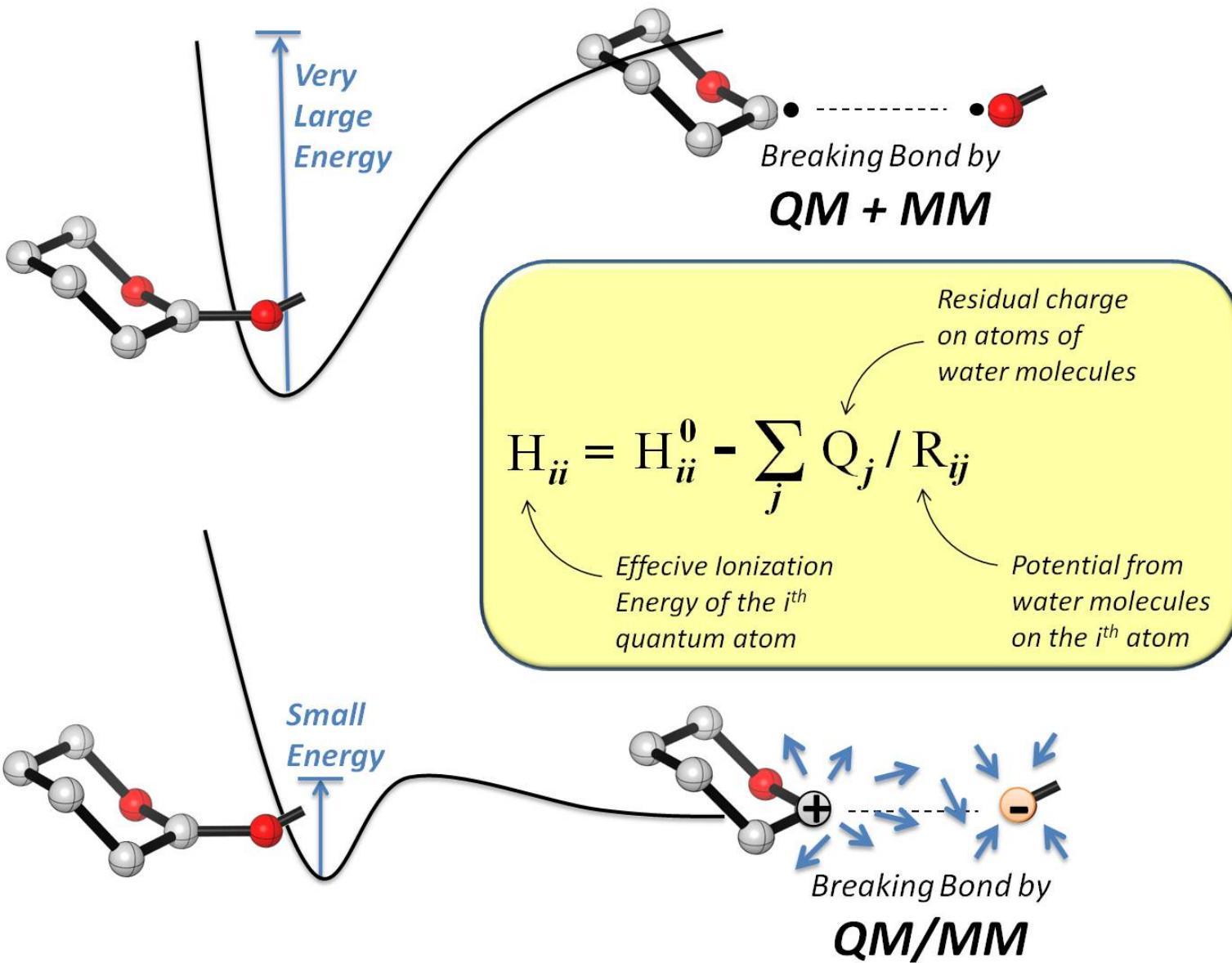
A. Warshel & M. Karplus, *J. Am. Chem. Soc.*, 1972

$QM(MO) + M$
M

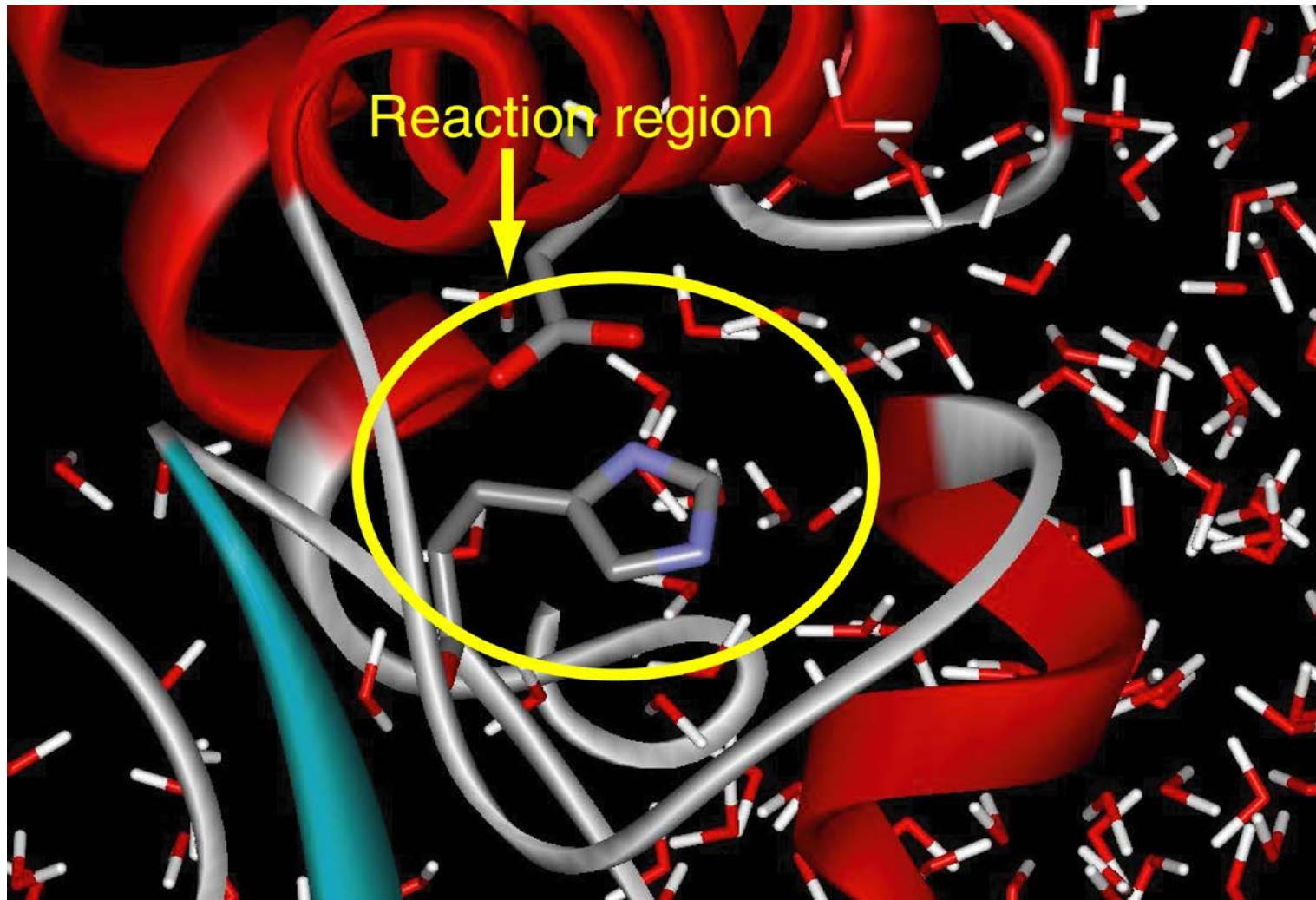


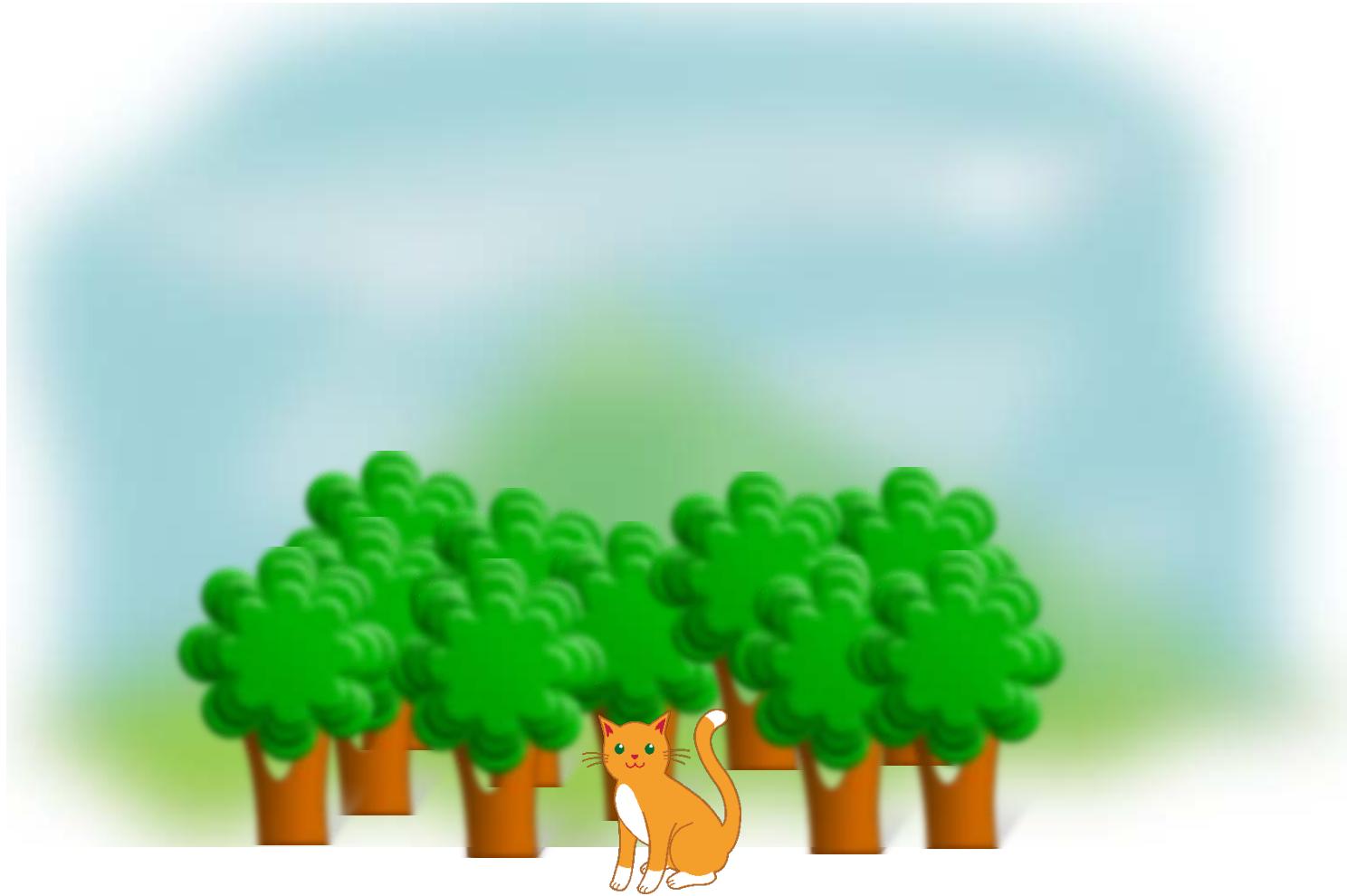
Back to Enzymes

Adding the environment to the quantum mechanics(QM) part

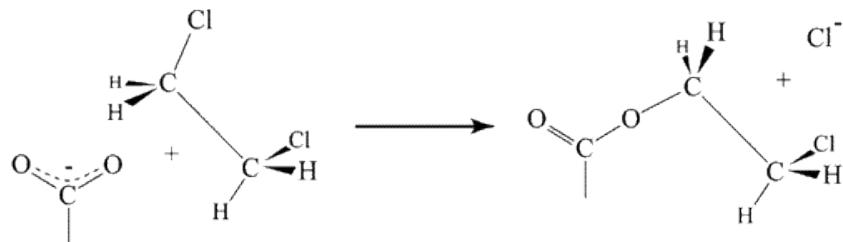
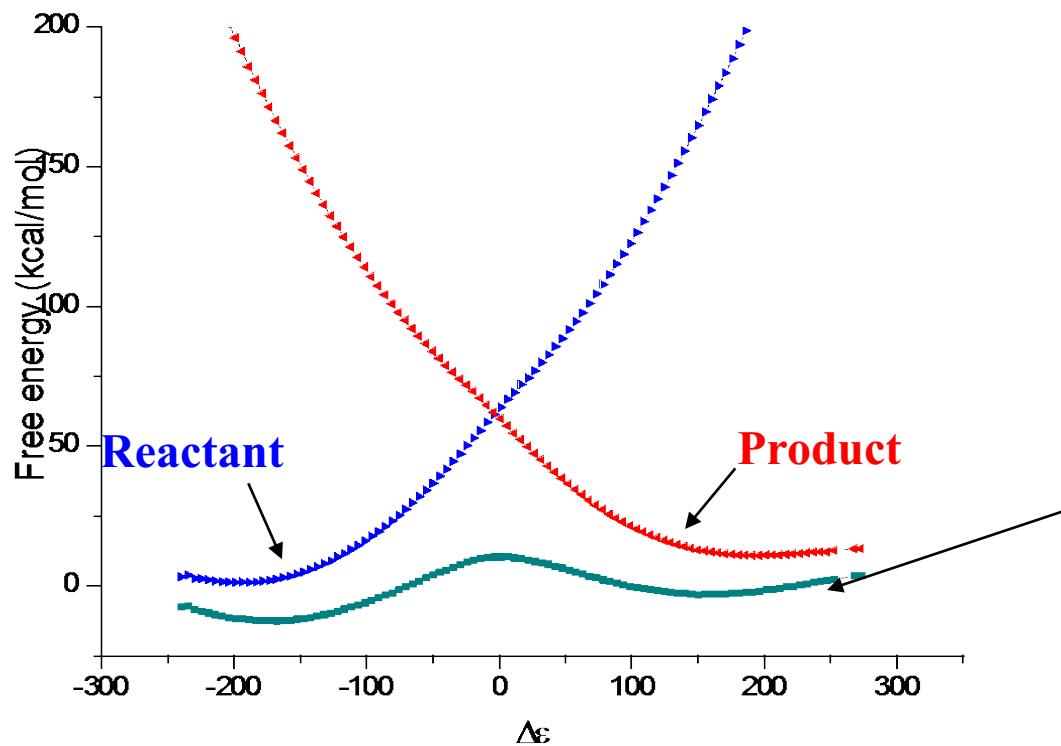


QM/MM: To study enzymatic reactions, we divide the system in two parts (Warshel & Levitt, JMB 1976)





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



Reactant:

Force field-like functions
describing the reactants'
bonding pattern

Product:

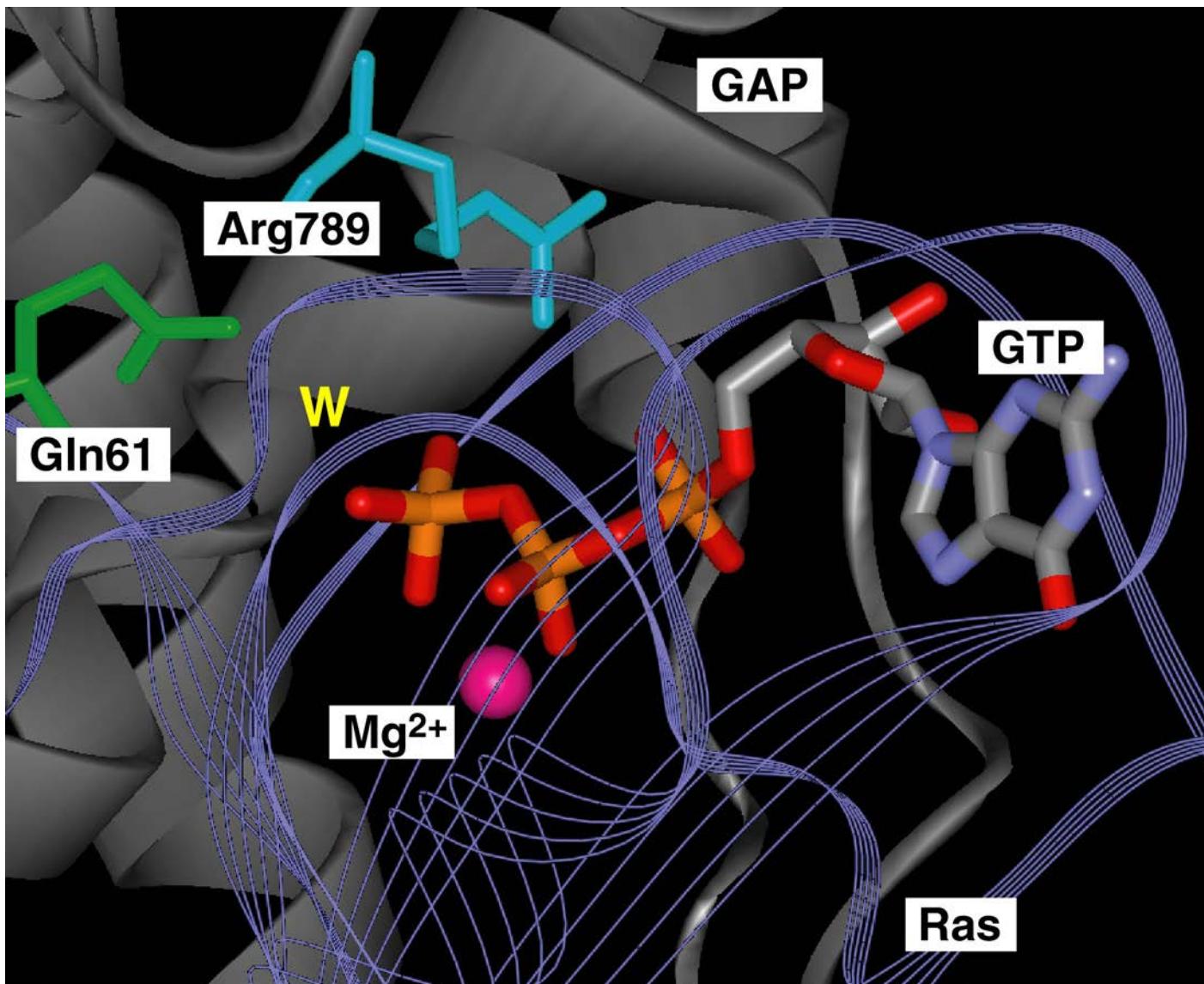
Force field-like functions
describing the products'
bonding pattern

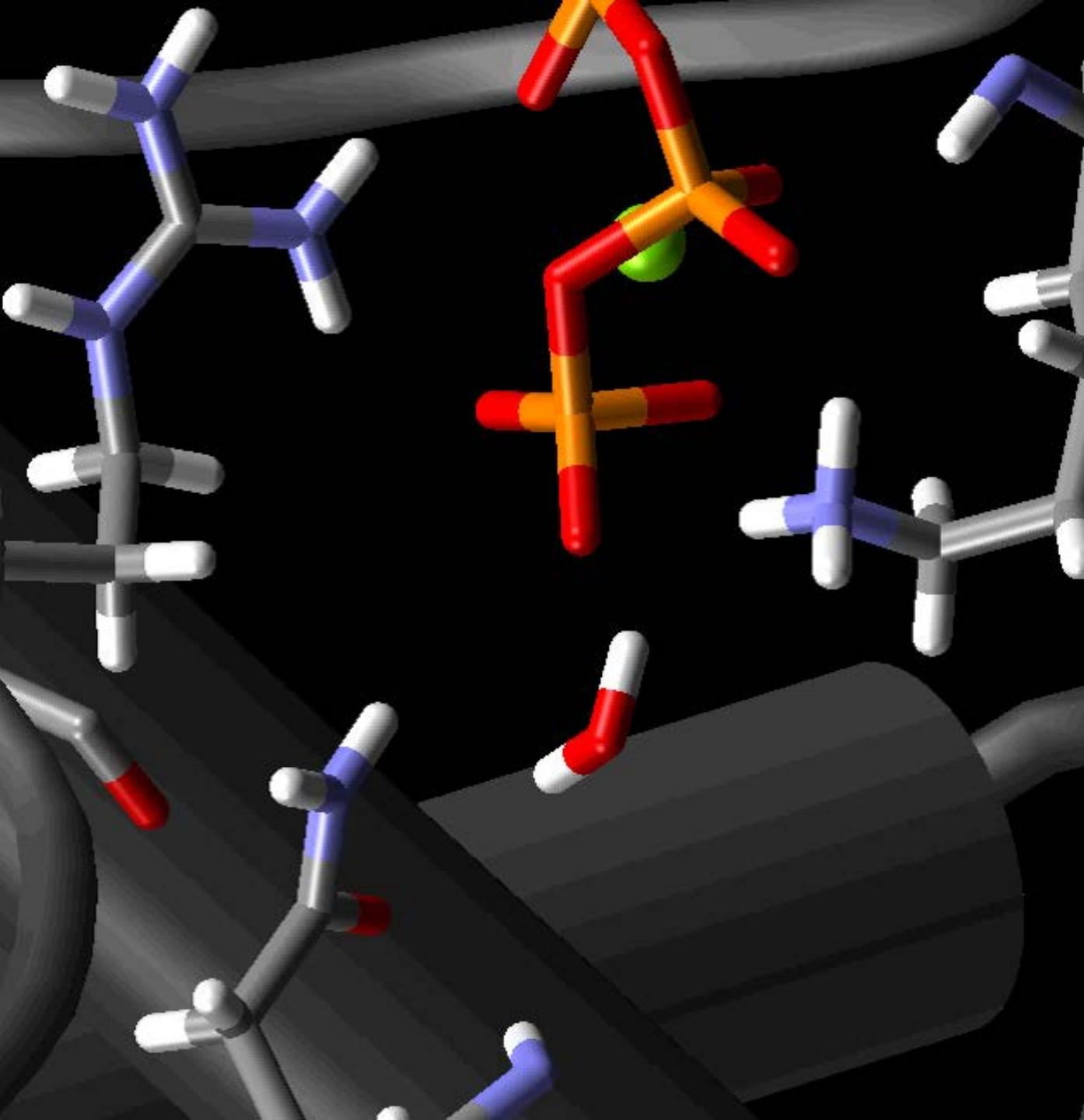
Ground State:

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

$$H = \begin{pmatrix} \mathcal{E}_{react} & H_{12} \\ H_{12} & \mathcal{E}_{prod} \end{pmatrix}$$

The Ras/GAP complex catalyzes GTP hydrolysis

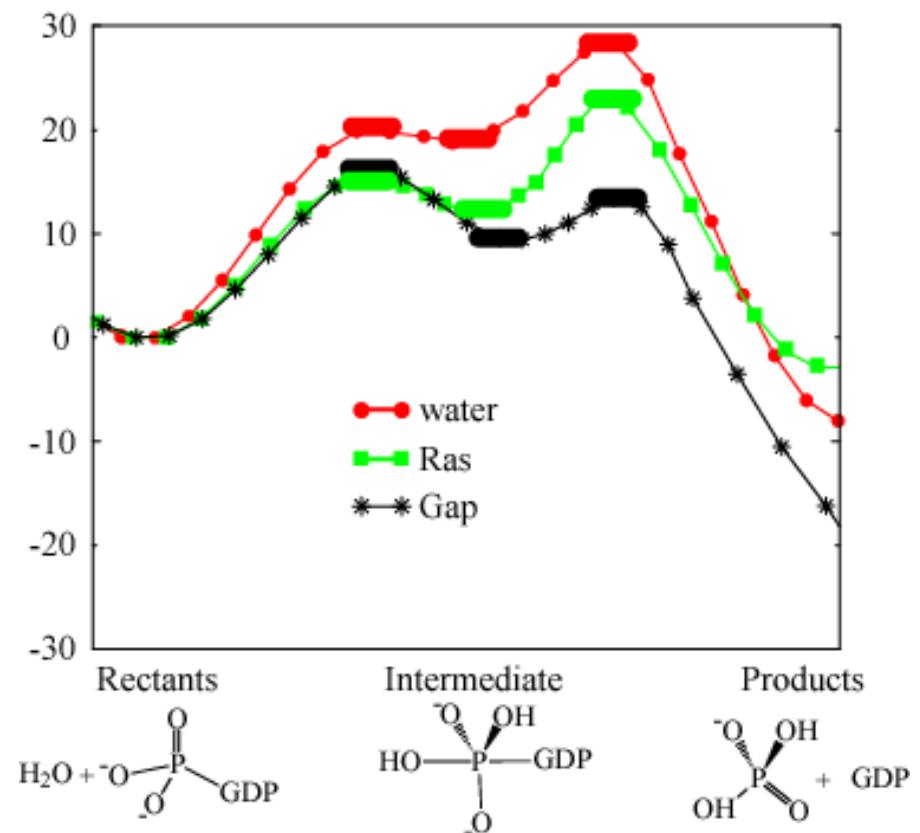




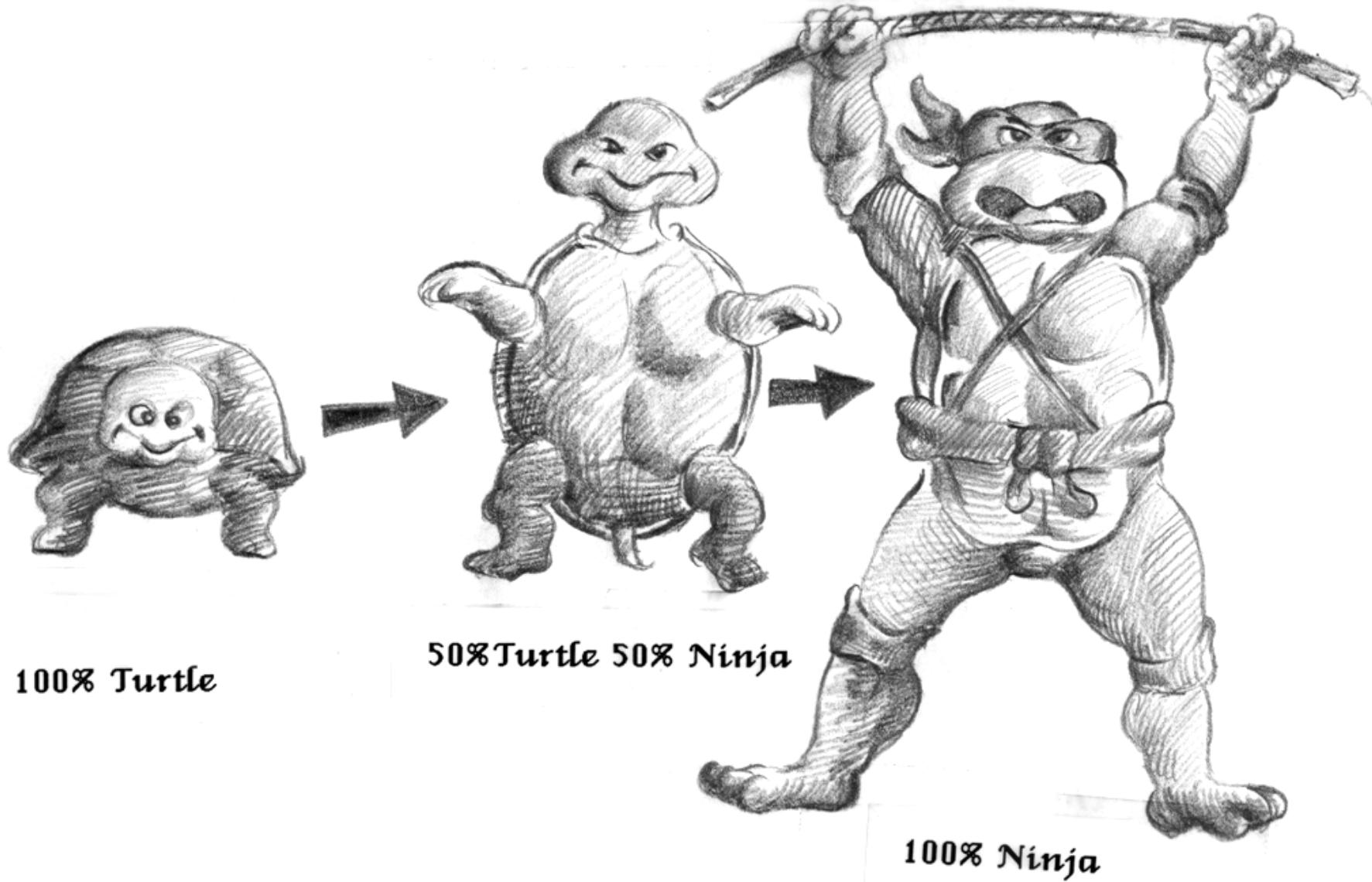
Calc Exp

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

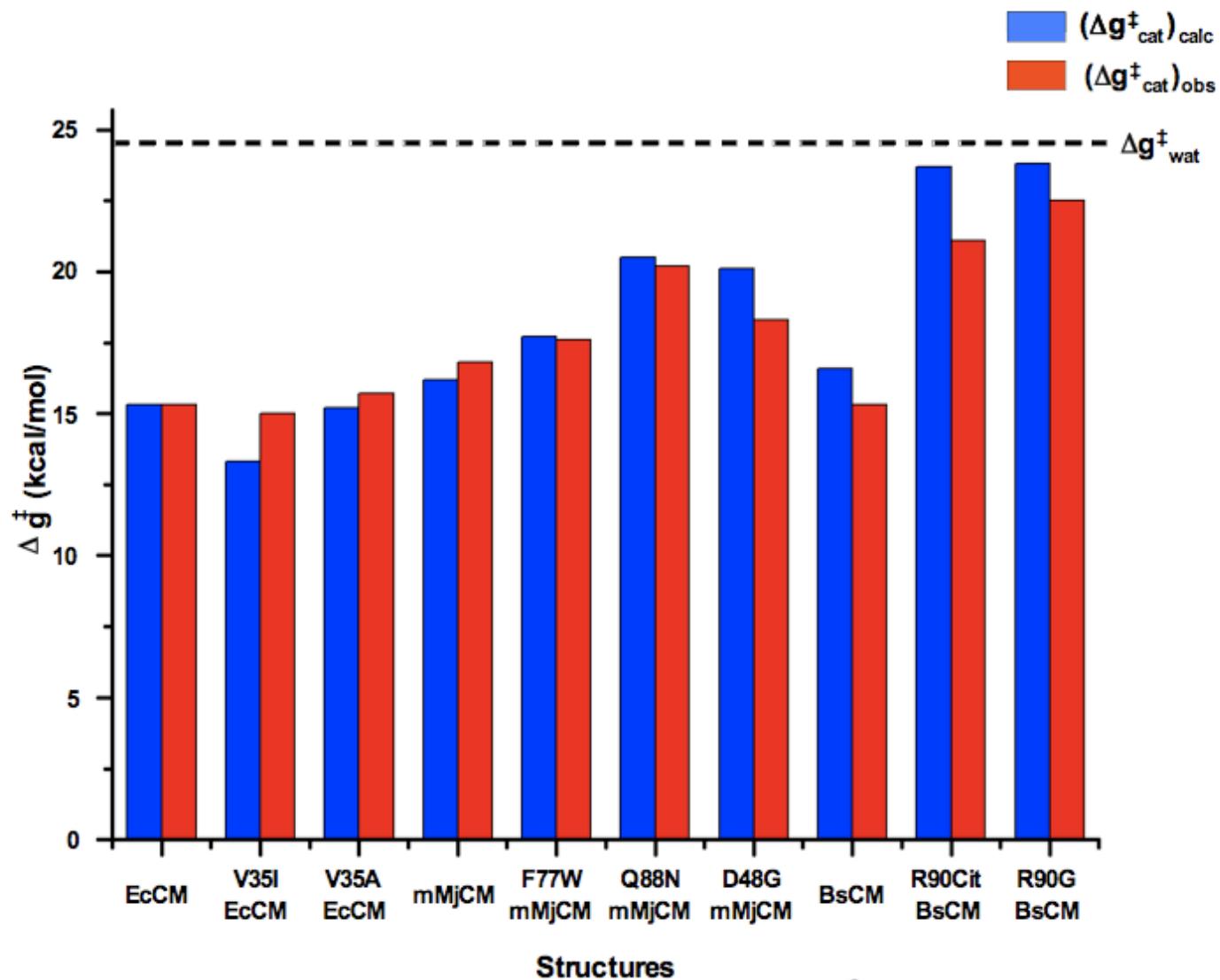
Good Agreement between
calculation and experiment



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



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

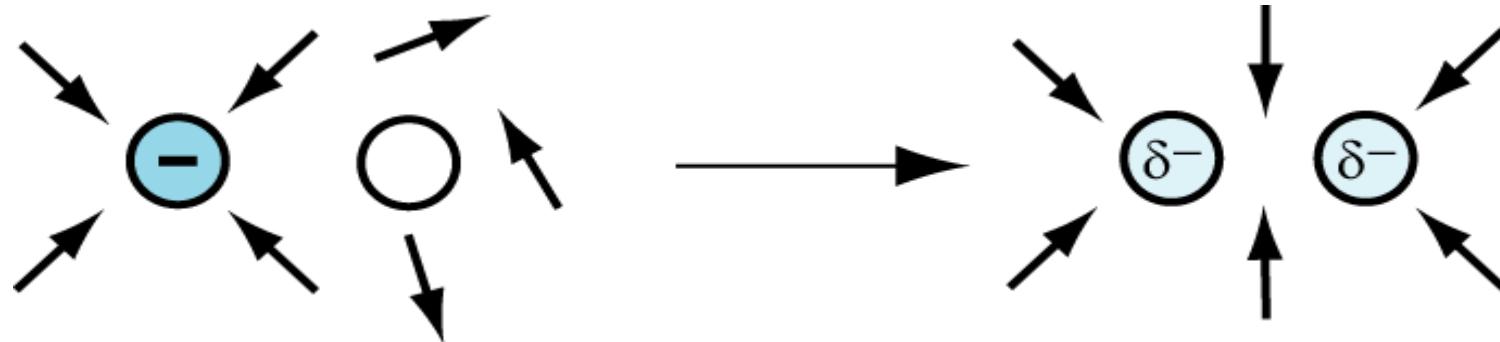


Warshel , PNAS (1978)

- The secret of Enzyme catalysis
is electrostatic preorganization

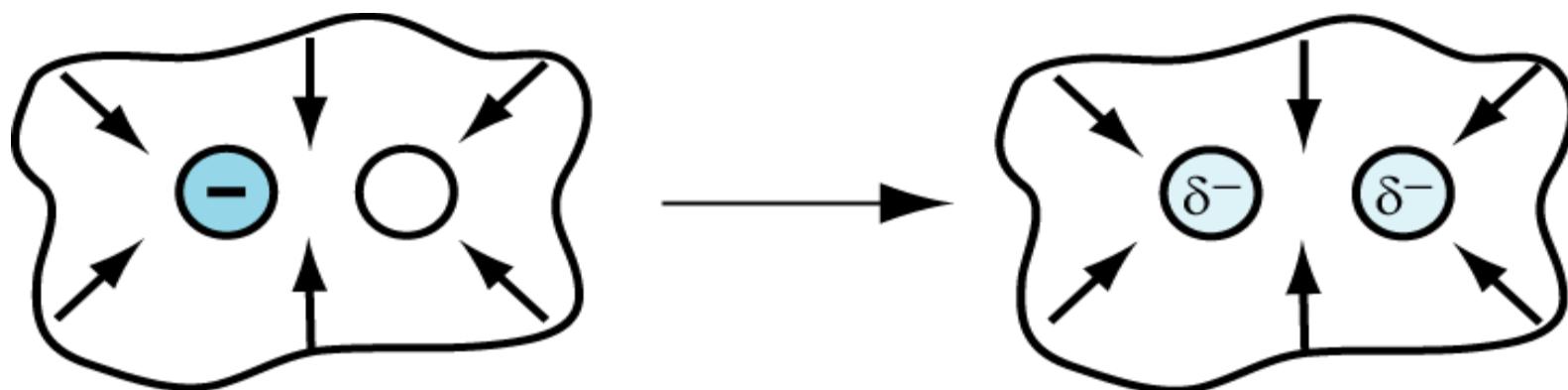
Reaction in water

Spend a large amount of energy rotating the water molecules



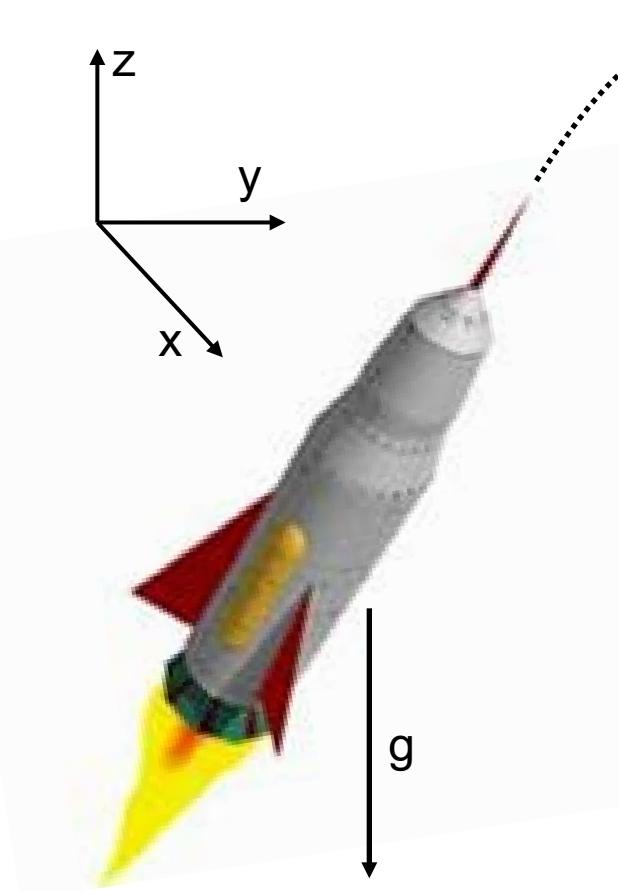
Reaction in protein

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



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

$$V = mgz$$

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

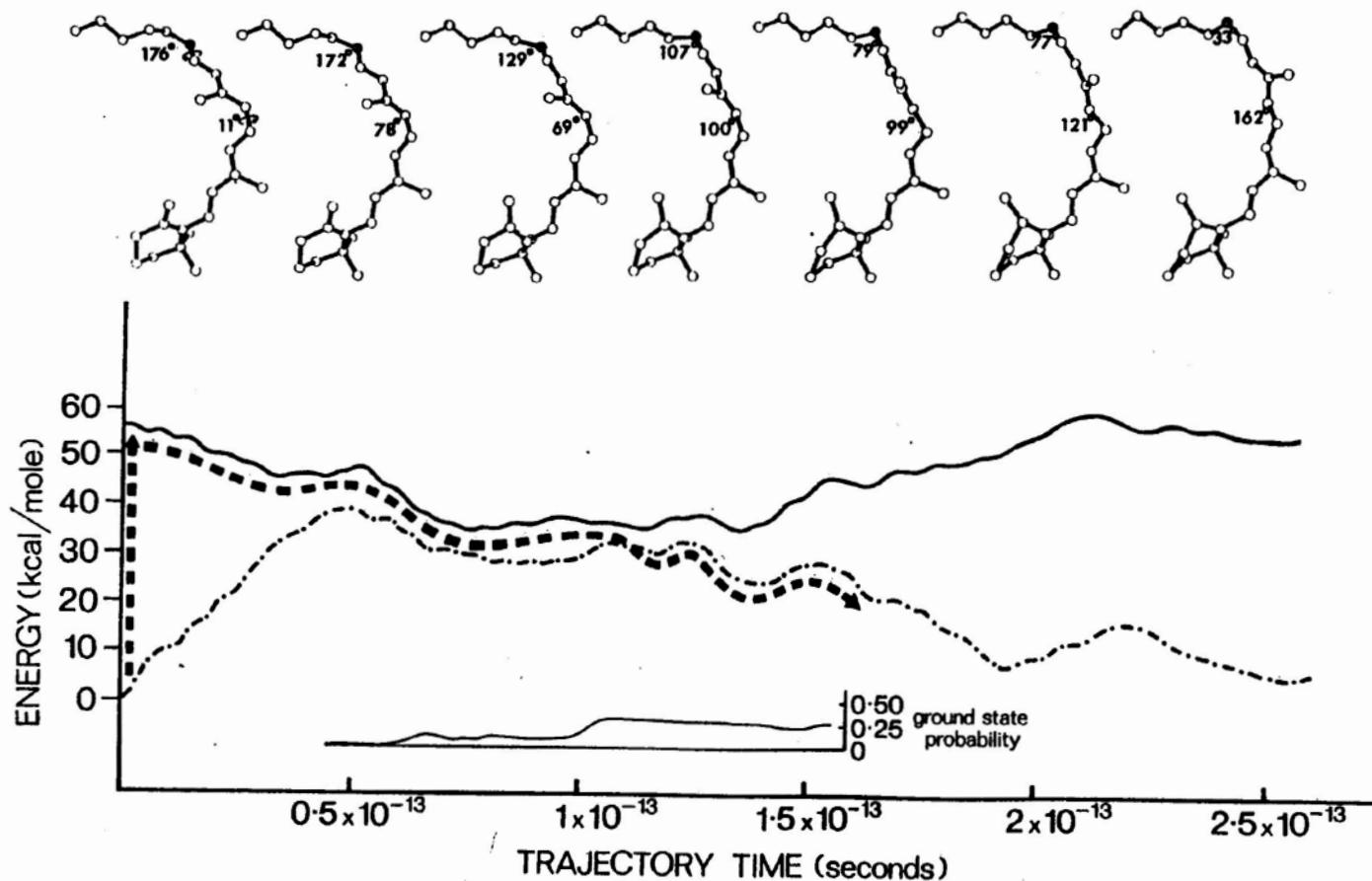
$$x(t) = x_0 + \dot{x}_0 t$$

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

The Dynamics
of the First Step
in the
Vision Process

© 1980 Arieh Warshel

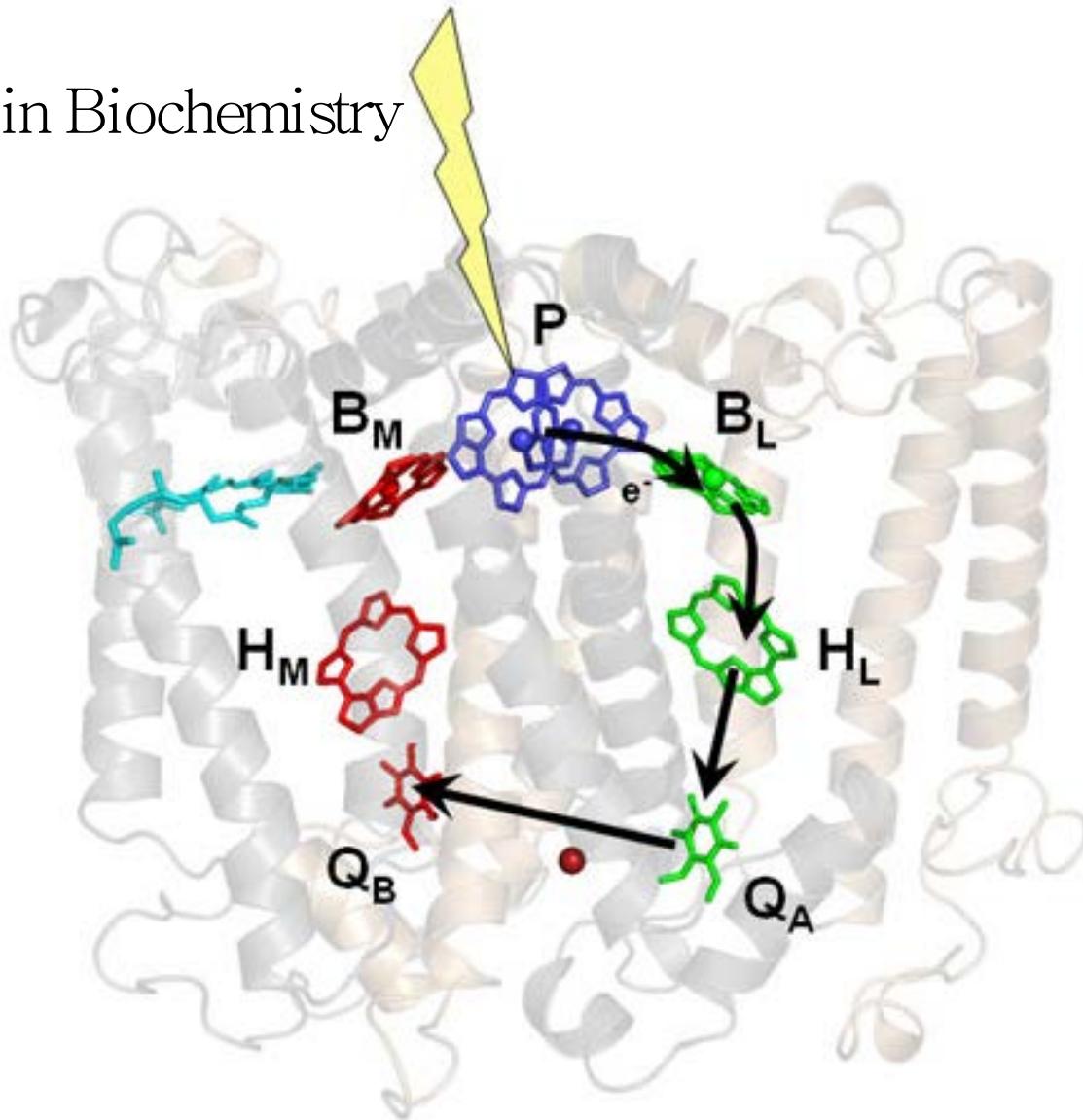
[Warshel, Nature, 260, 670 (1976)]



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

Photosynthetic Reaction Centers

Simulated in Biochemistry
1988



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

Needs Free Energy landscape 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

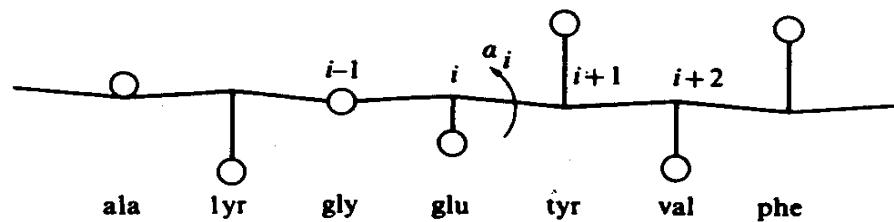
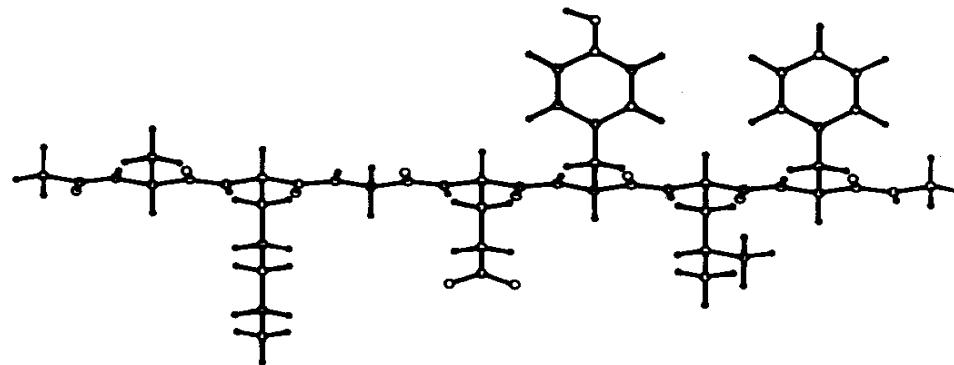
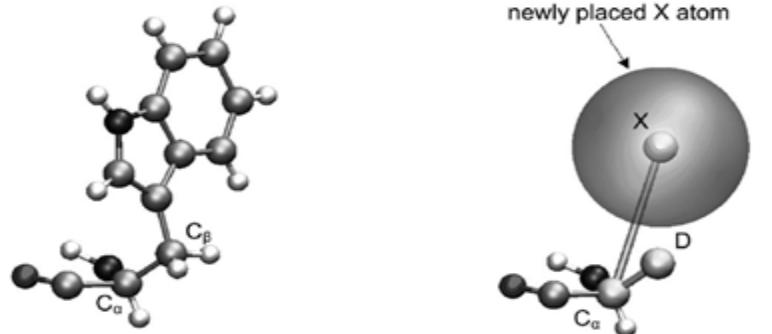


Fig. 1 Relationship between the simplified model of protein structure introduced here and the real all-atom structure of proteins. The two reference points for each residue in the simplified model correspond to the centroid of the side chain and the C^a. Each residue is only allowed one degree of freedom: the torsion angle α between the 4 successive C^as 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

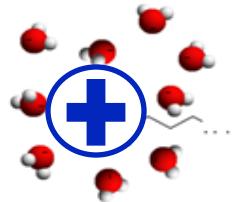
PROTEINS , 78, 1212–1227 (2010)
Ann Rev Phys Chem 62, 41-64 (2011)



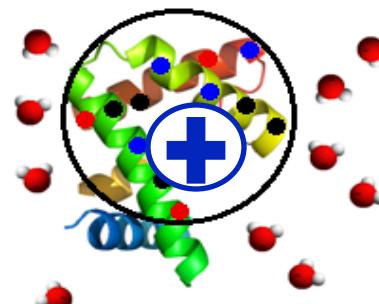
Now focused on better treatment of electrostatics free energy

Mainly self energy (solvation) and charge-charge interaction

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



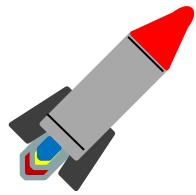
Self-Energy
→



- Nonpolar residues
- Polar residues
- Ionizable residues

- Long time simulations

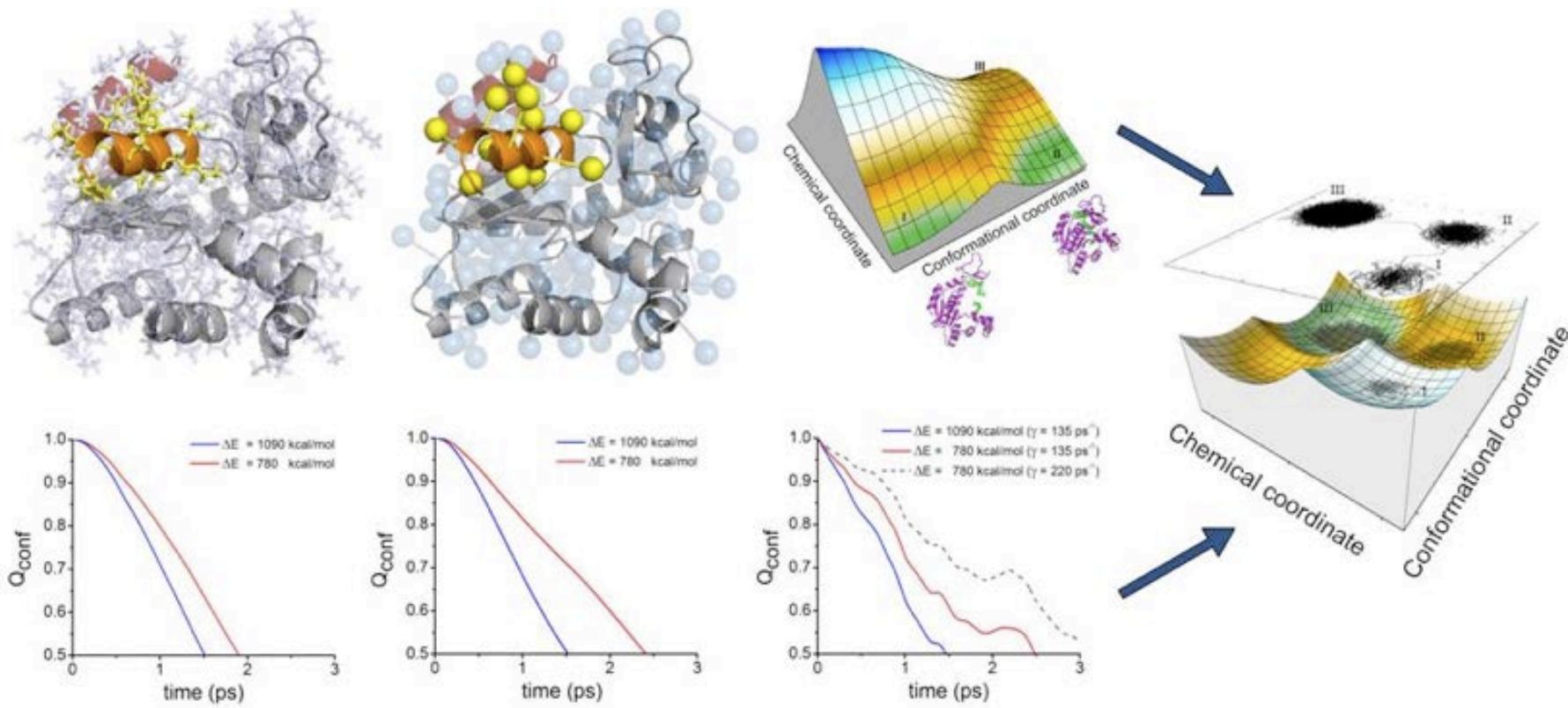
Newtonian Dynamics



Brownian Dynamics

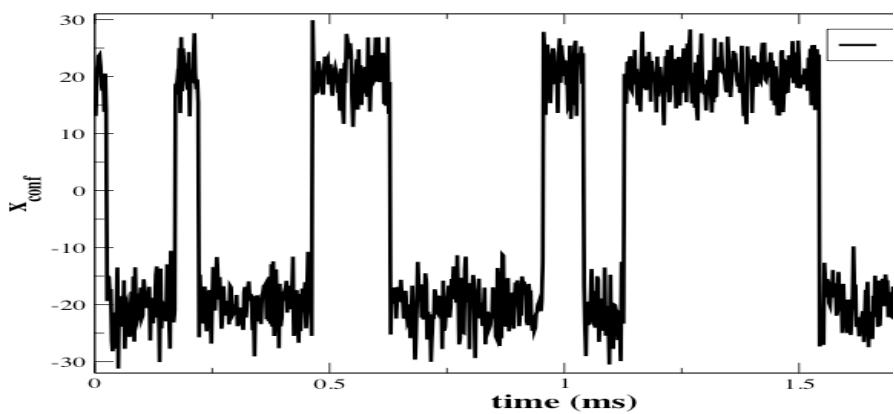


The Renormalization Model

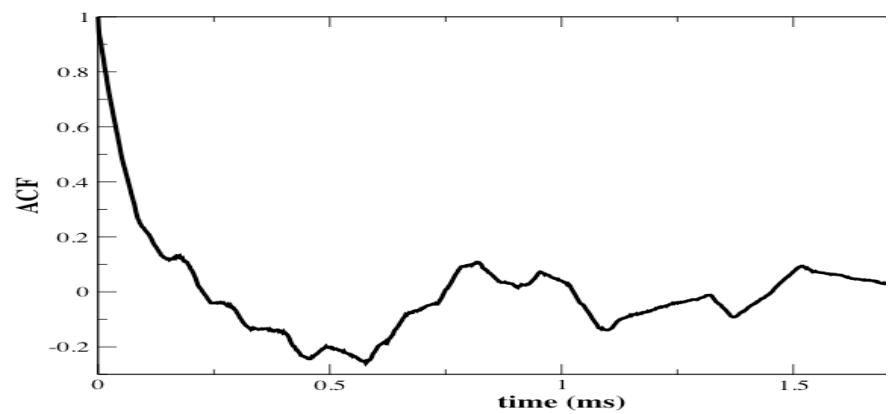


Long time dynamics, conform. coordinate

$x_{\text{conform}}(t)$



autocorrelation function

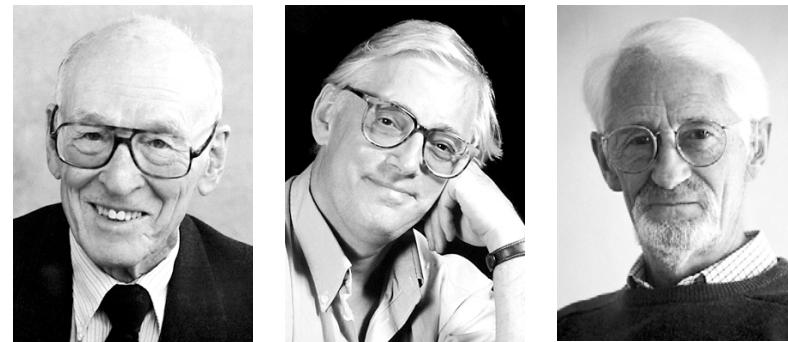


F₁F₀-ATP synthase – The smallest rotary motor

The 1997 Nobel Prize in Chemistry

How the rotating γ -subunit imposes conformational states
on a β -subunit required for substrate binding,
ATP formation and ATP release.

© Medical Research Council



F₁F₀ are two coupled rotary motors; an ATPase and an ion-pump

In presence of right ion-gradient
F₀ transports ion across the membrane
and F₁ synthesizes ATP

In the opposite direction ATP hydrolysis
occurs in F₁, while
F₀ acts as an ion-pump

Mechano-Chemical Coupling between the central stalk and the catalytic dimers in F₁

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

Sequence of Events

Ligand Binding



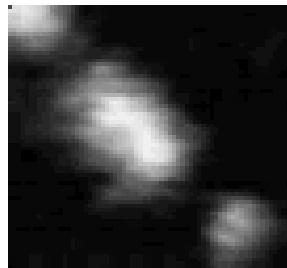
80° rotation



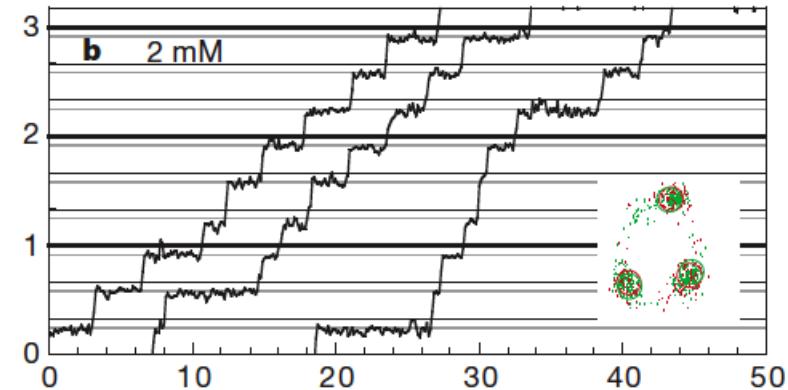
ATP catalysis



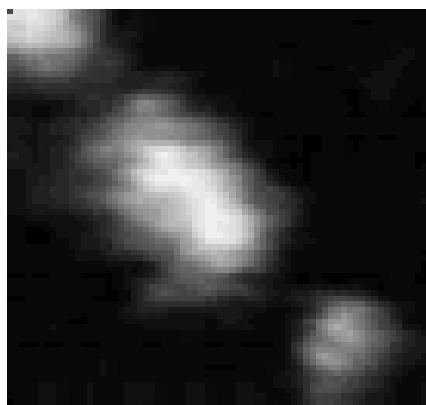
40°



movie

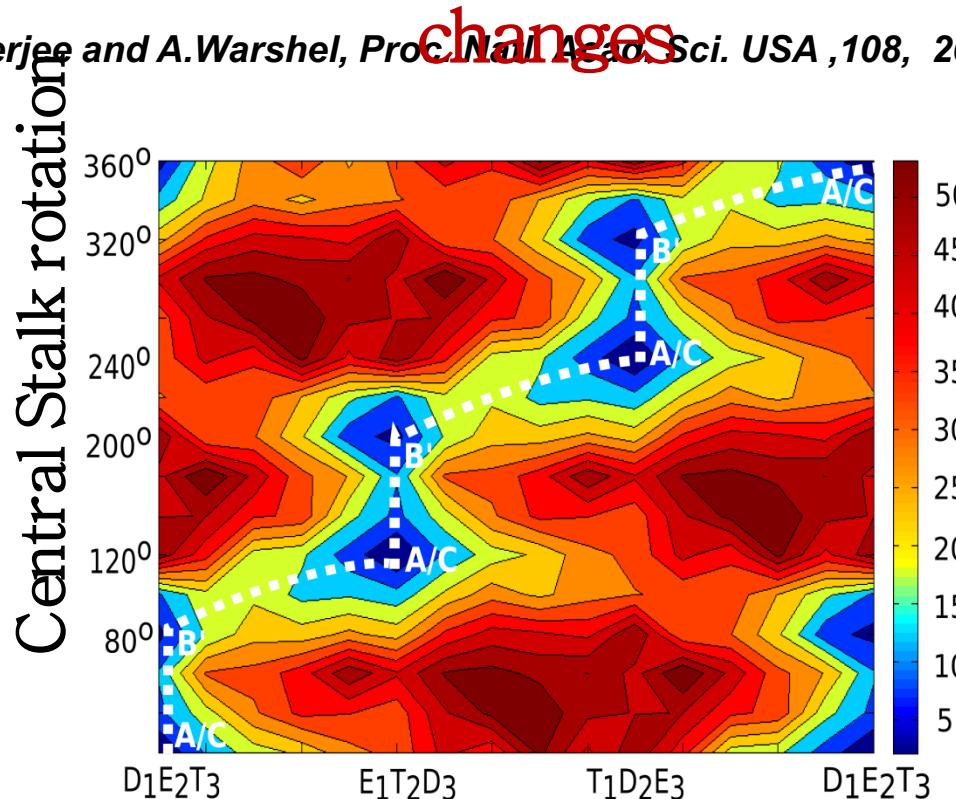


Yasuda, R. et al., Nature, 2001.



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)



Conformation change of catalytic subunits

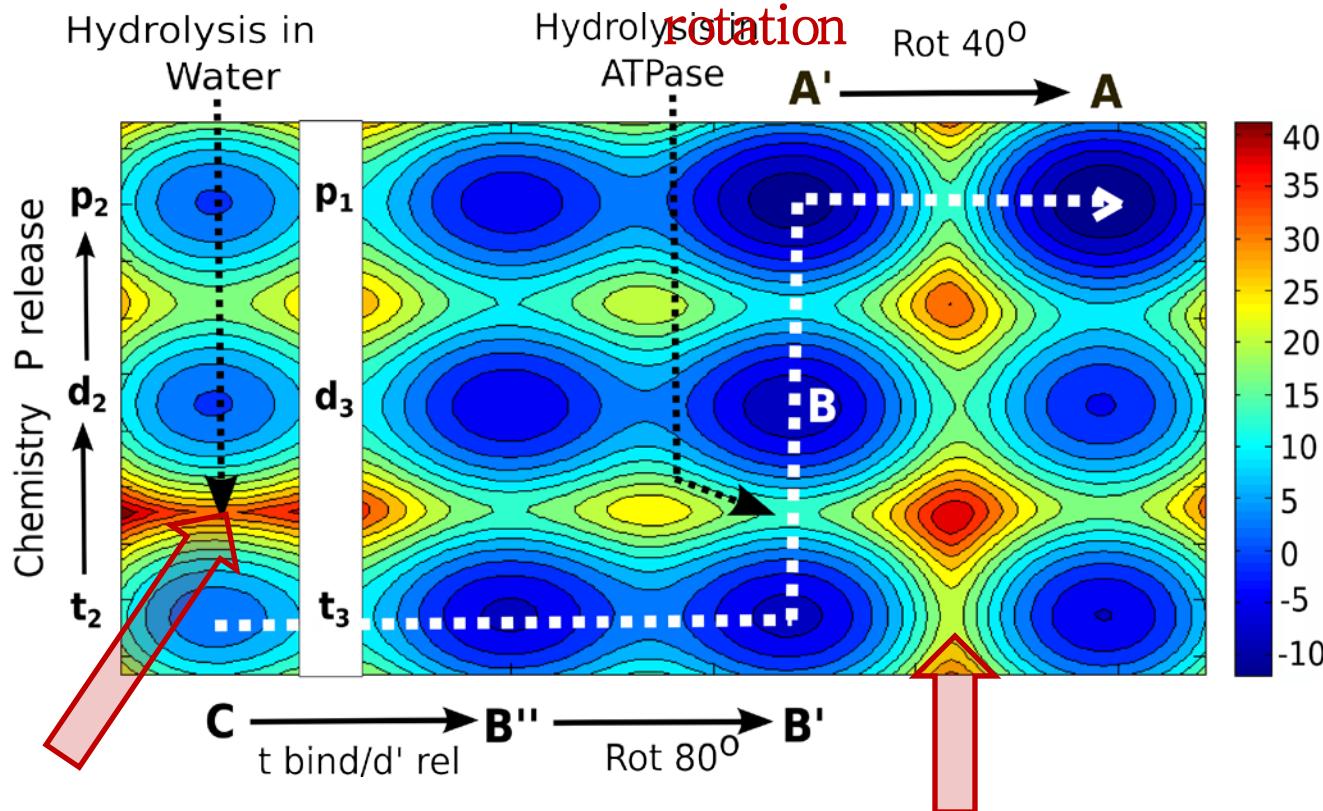
The least energy path clearly shows the 80° /40° substeps.

The 80° rotation has small electrostatic barrier.

The 40° rotation and conformation change of catalytic subunits has

Simplified surface of F₁-ATPase function shows the coupling of ATP hydrolysis with central stalk rotation

The functional surface reveals why catalysis occurs after 80°

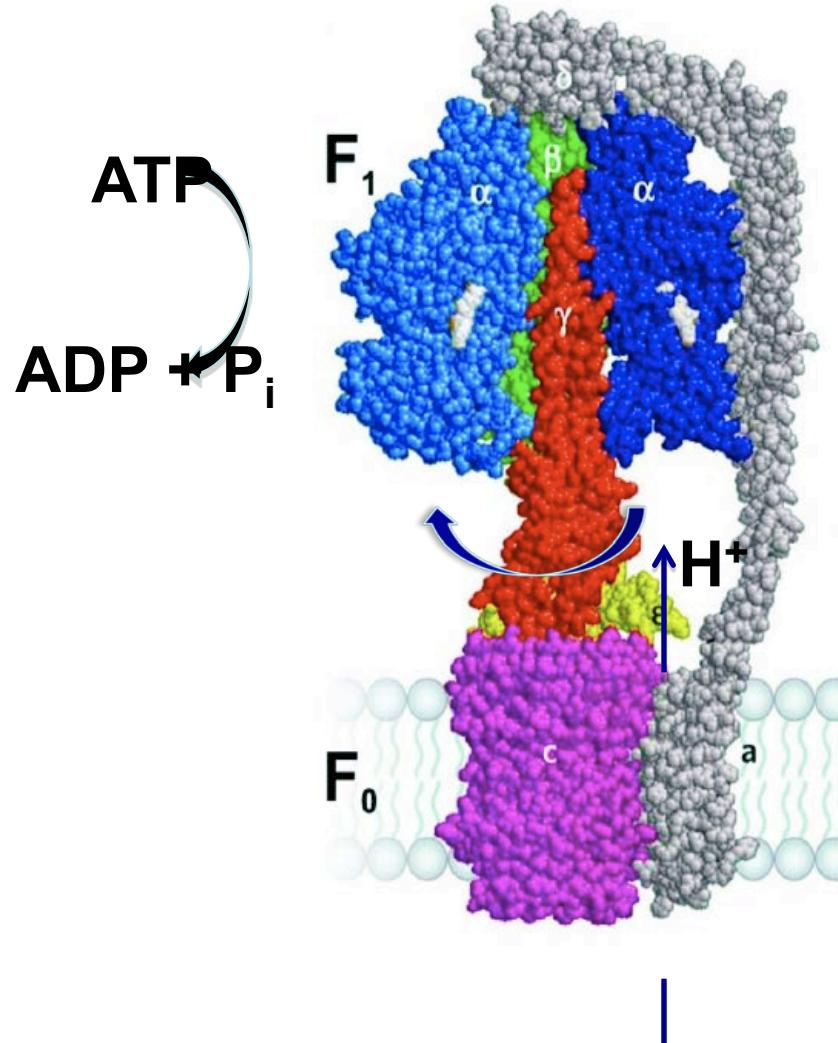


ATP hydrolysis in water has very high barrier and will need months to occur

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

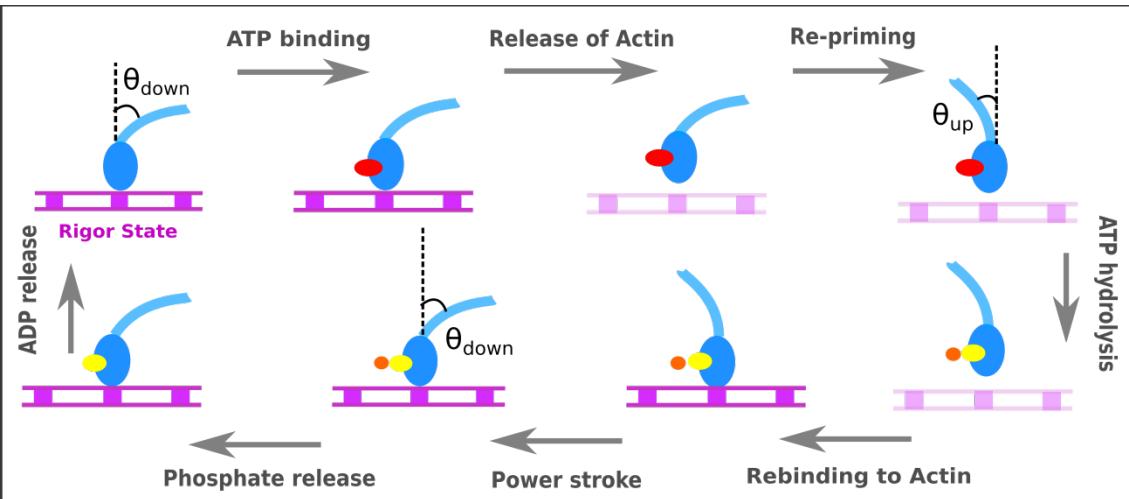


Almost no backsteps as myosin V walks over actin filament

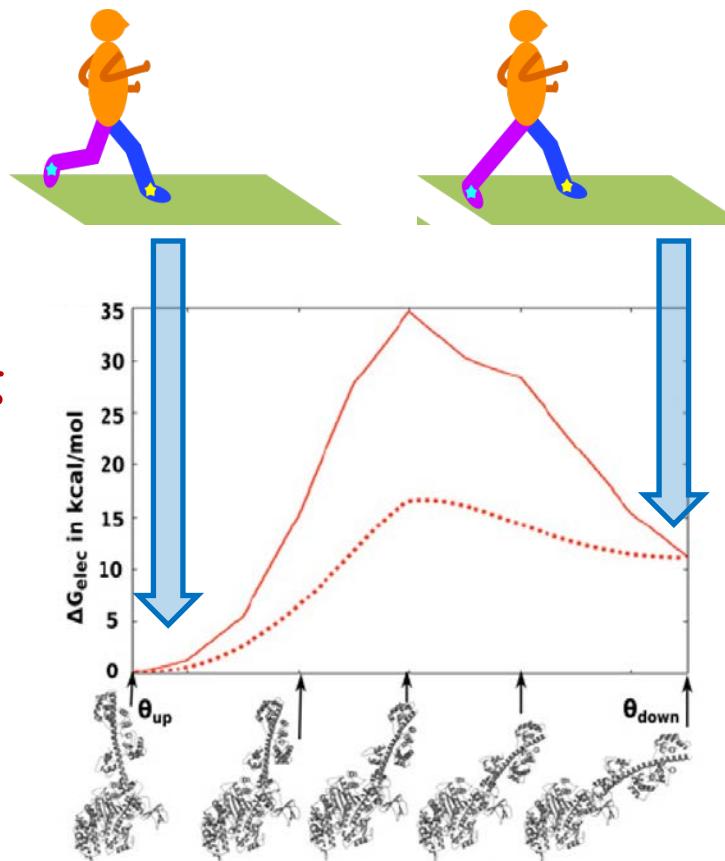
It is hard to understand unidirectional movement, even in our daily life !!!



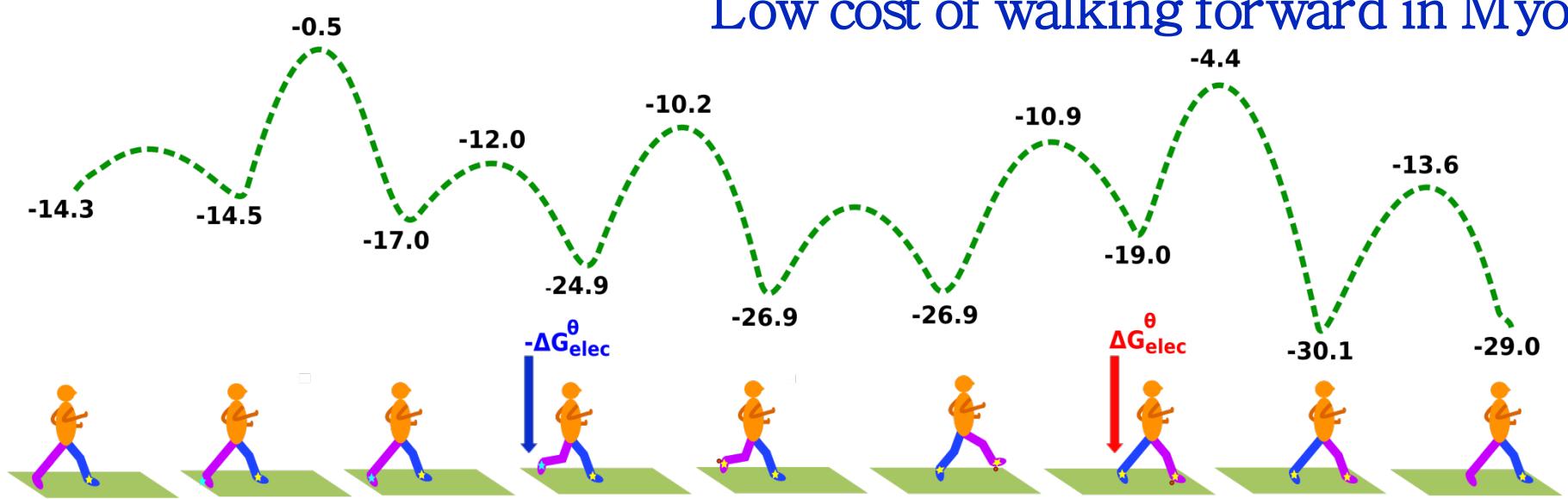
CG energetics of a single leg as it bends (changes conformation)



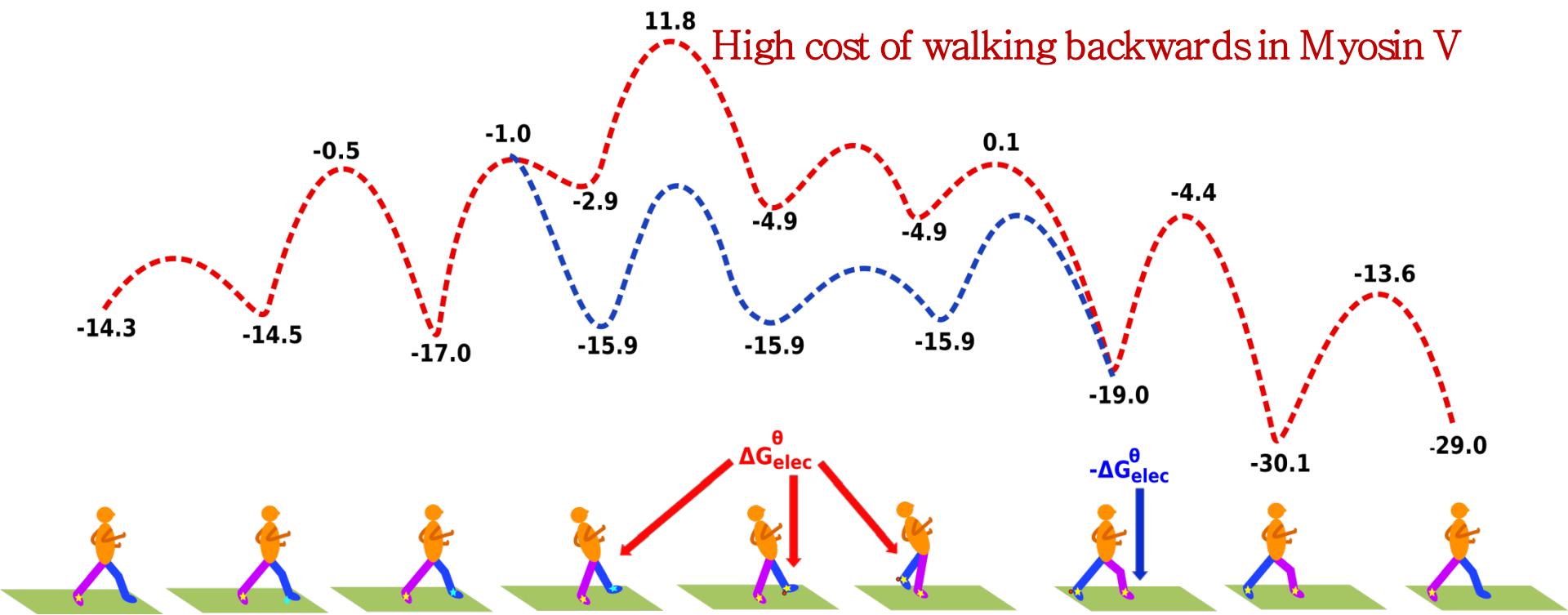
Schematic functional cycle of myosin V single leg



Low cost of walking forward in Myosin



High cost of walking backwards in Myosin V



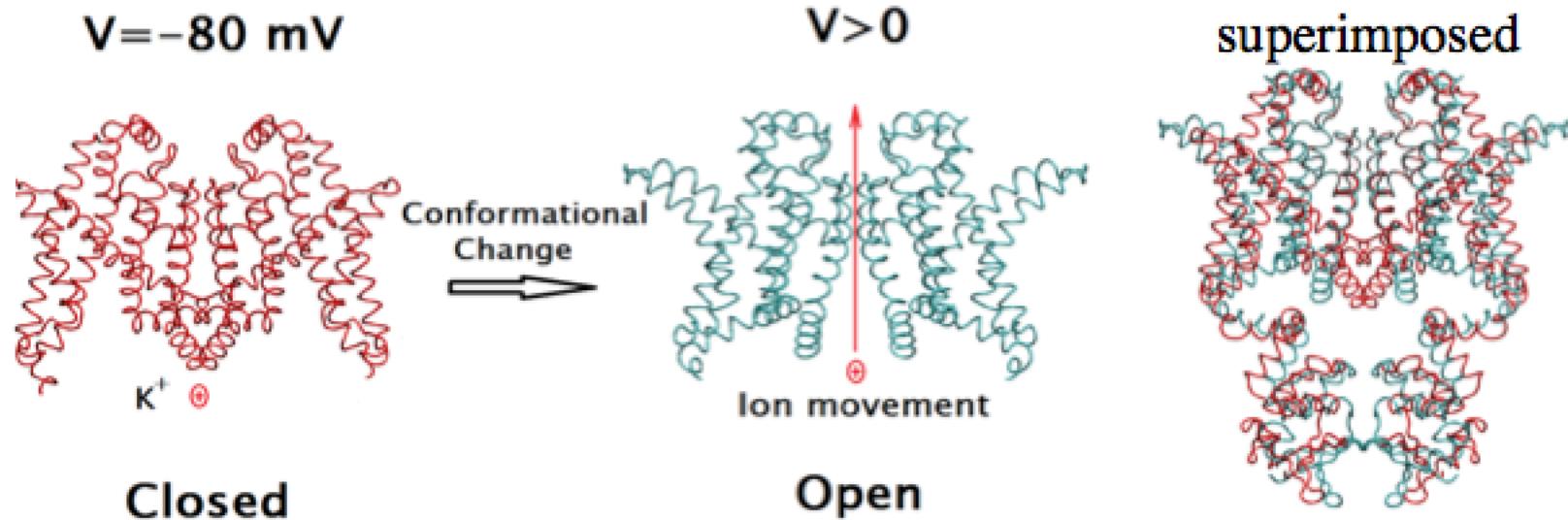
Life Transistors

Voltage-activated Ion Channel

Part of the structure

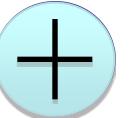
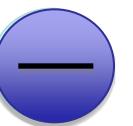
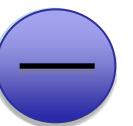
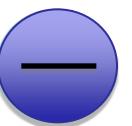
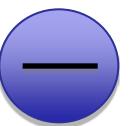
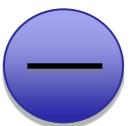
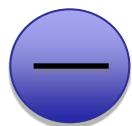


Roderick MacKinnon

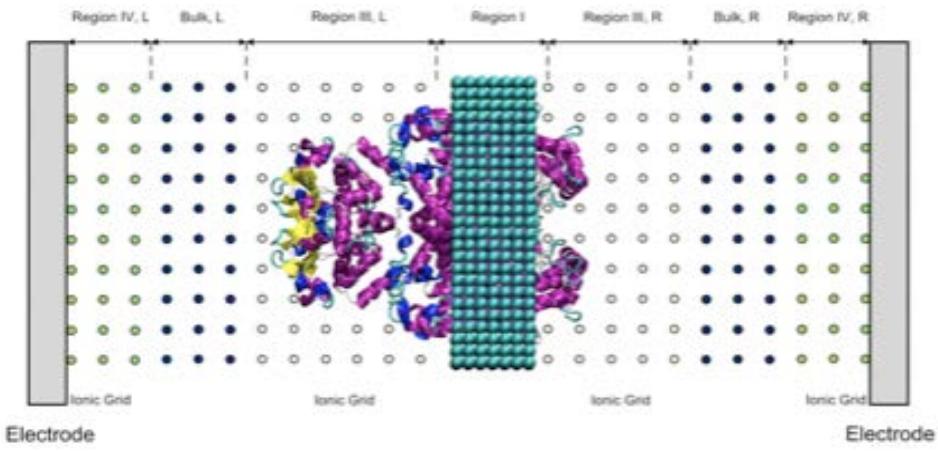


Kv1.2 channel: A delayed rectifier subtype of shaker potassium channels that is selectively inhibited by a variety of scorpion venoms





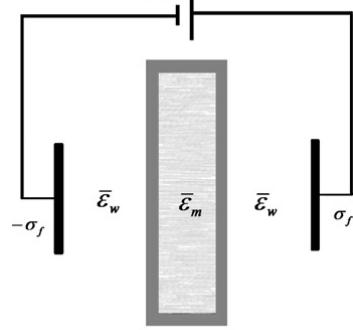
Ionic Strength Effect and External Potential



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

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

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



For protein-containing systems
Need to approximate

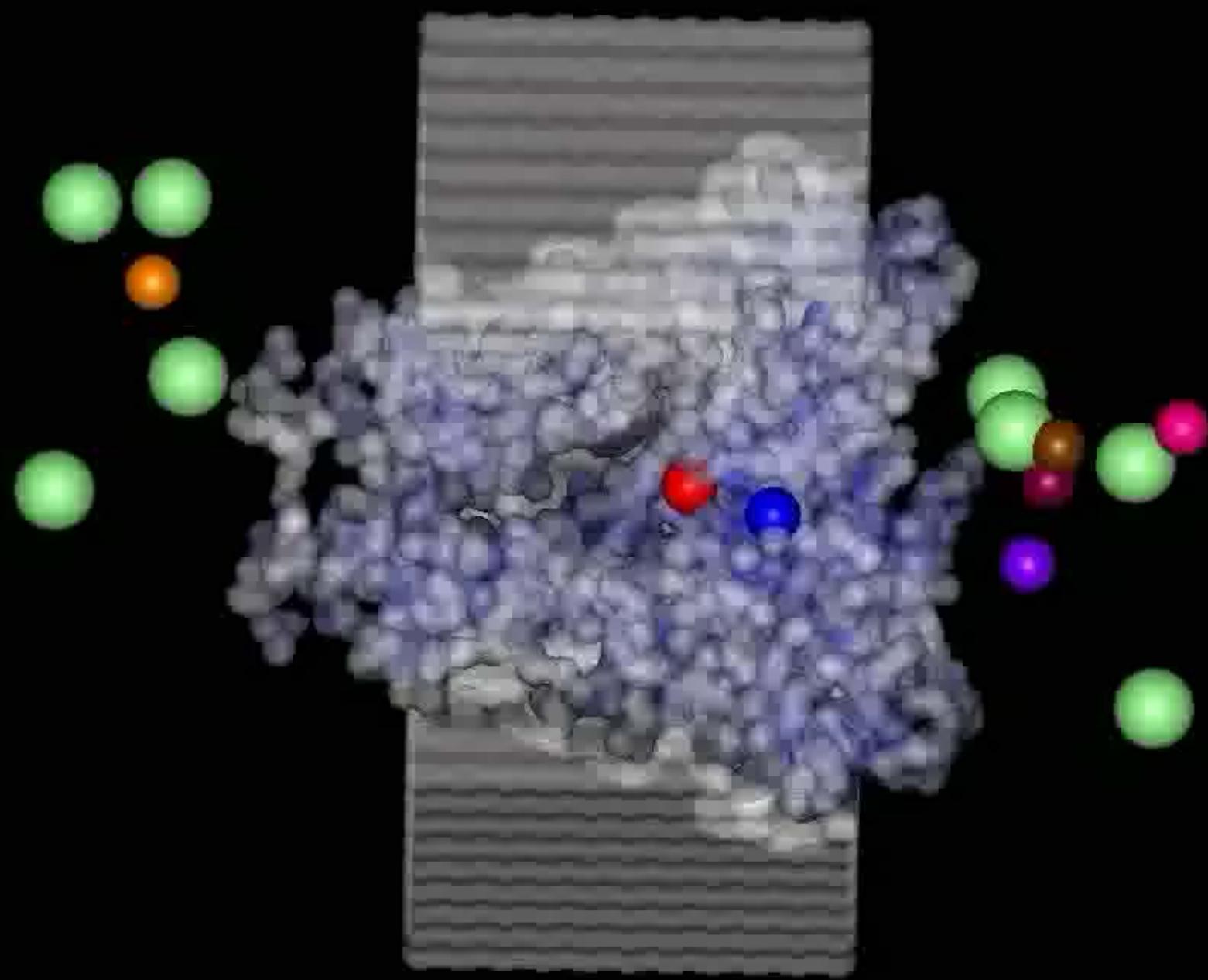




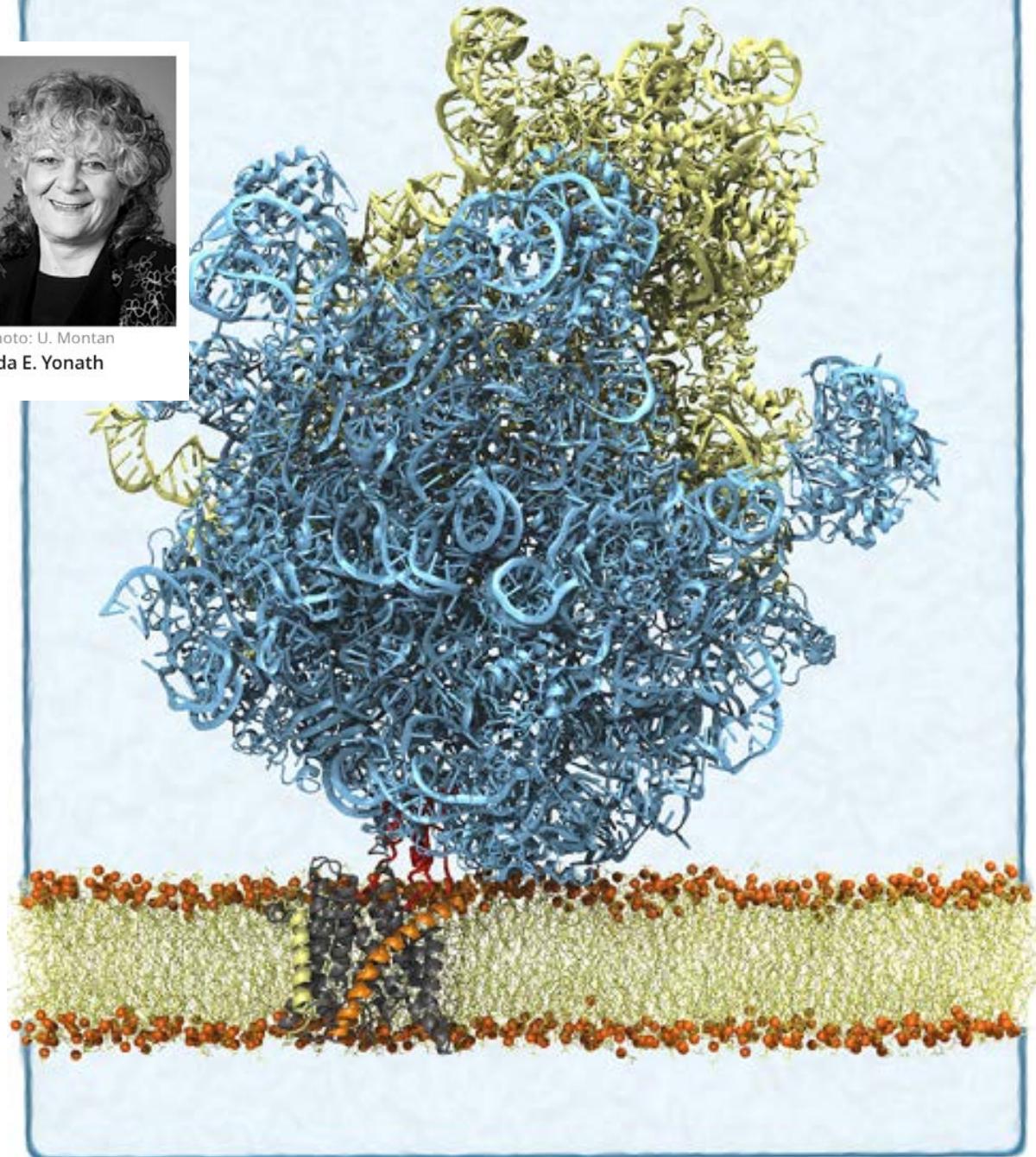
Photo: U. Montan
**Venkatraman
Ramakrishnan**



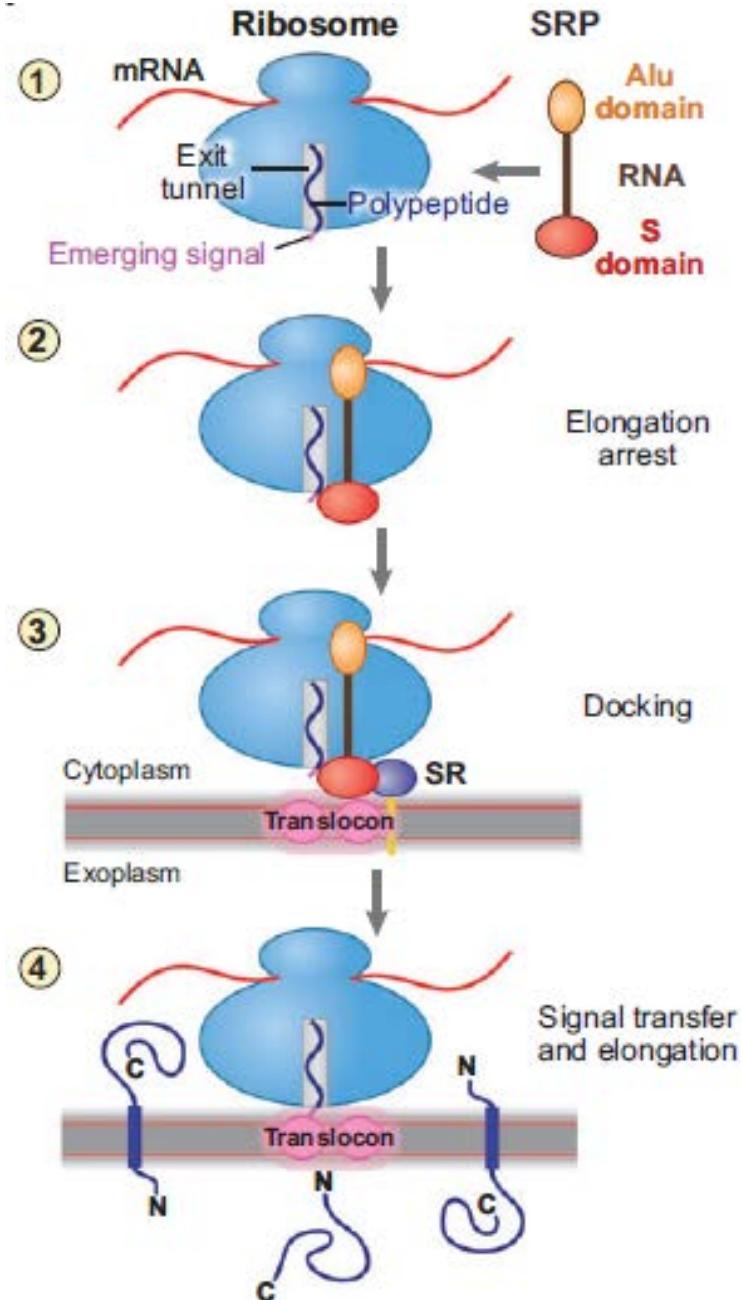
Photo: U. Montan
Thomas A. Steitz



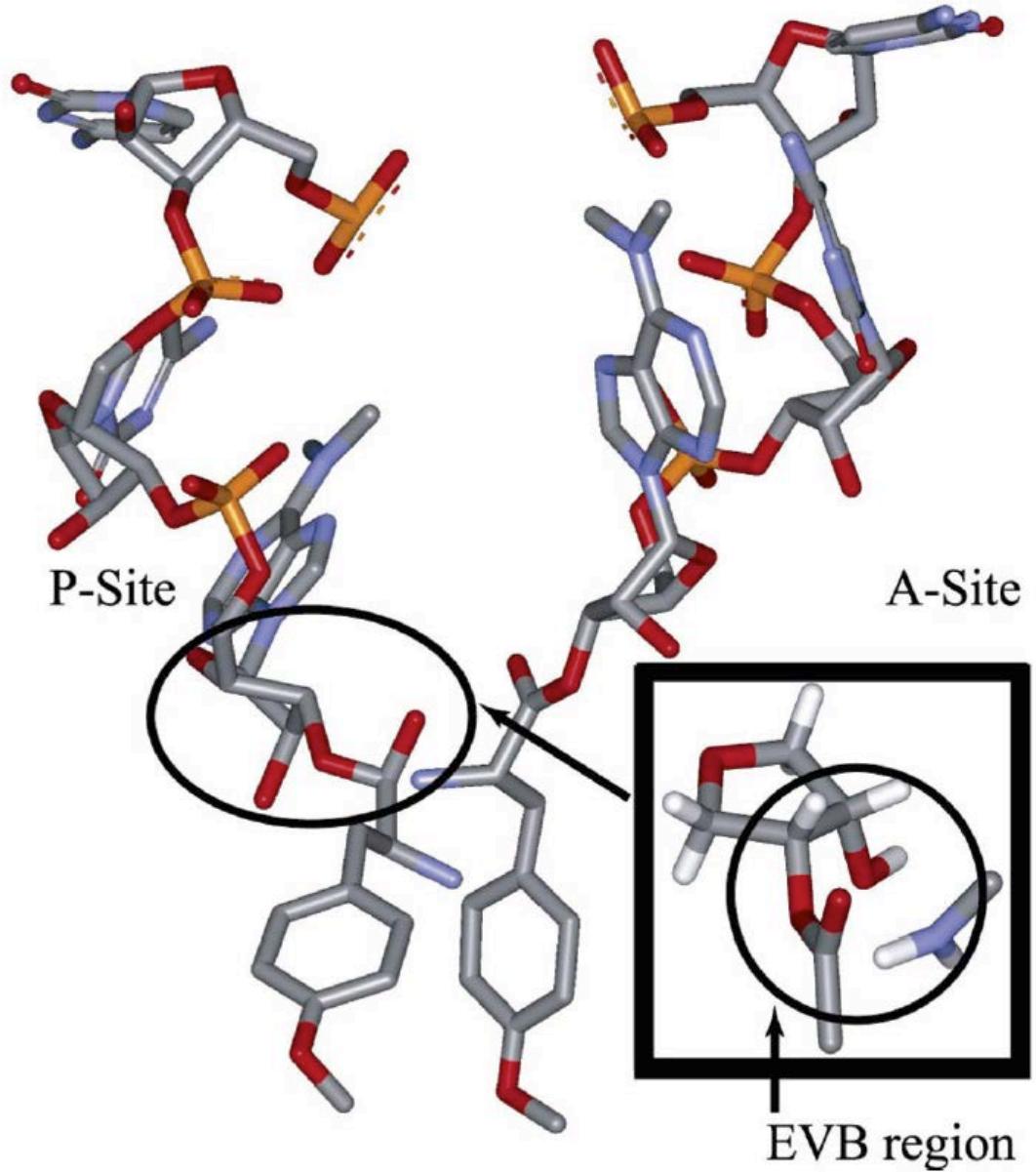
Photo: U. Montan
Ada E. Yonath

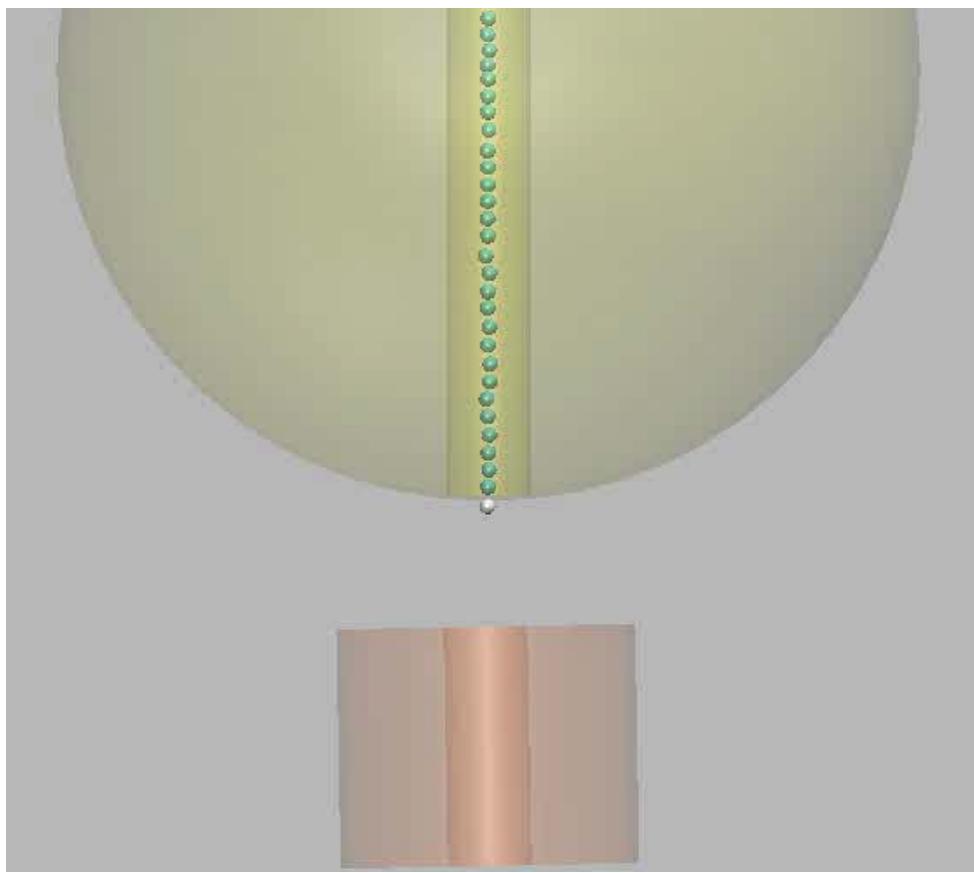


Translocon and Ribosome Coupling

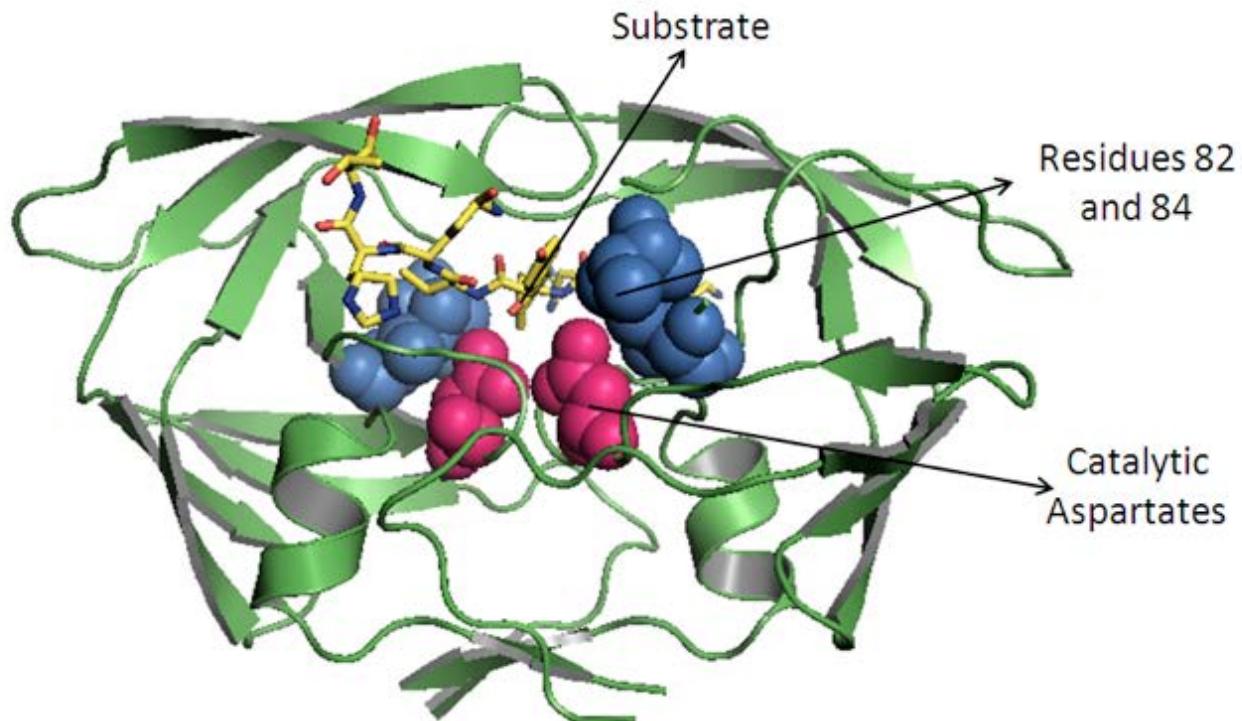


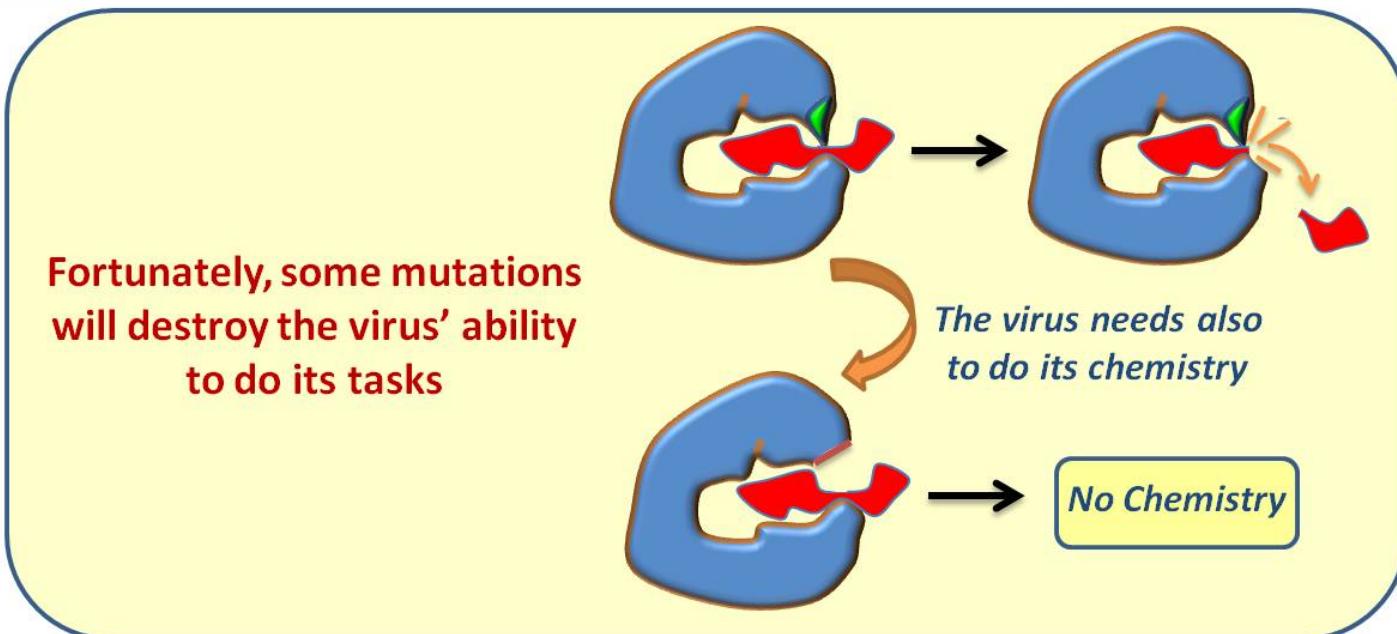
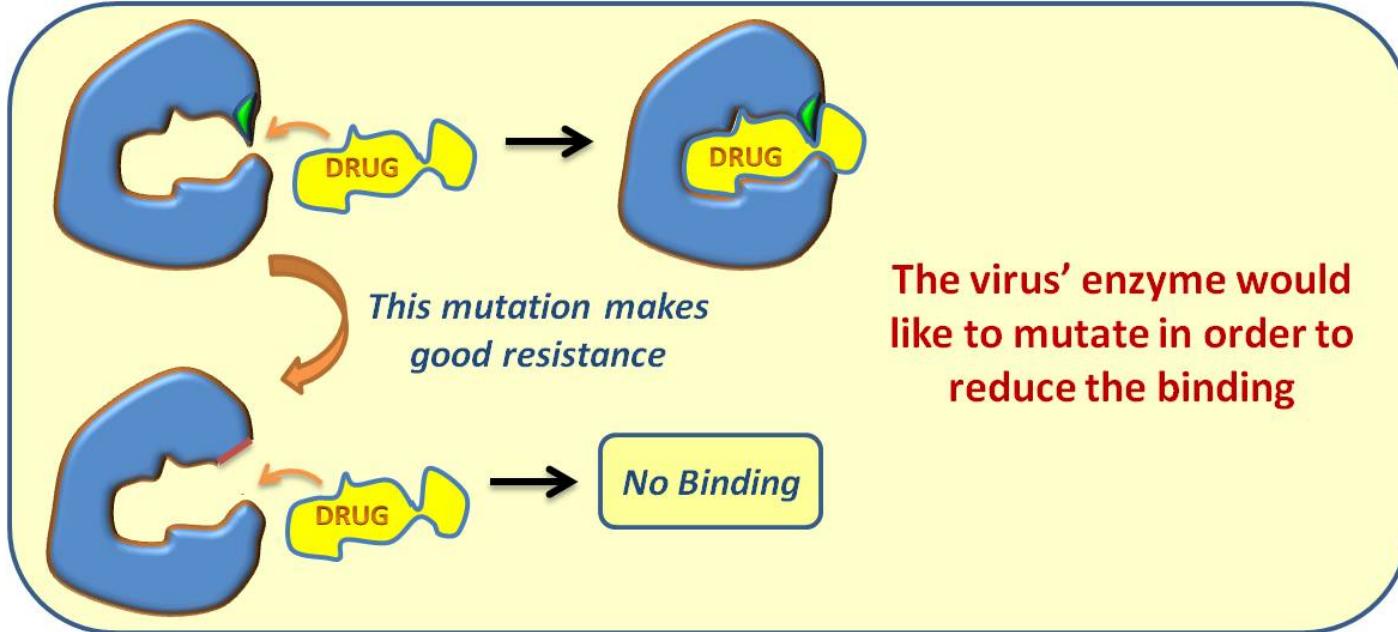
White and von Heijne,
Annu. Rev. Biophys.,
2008





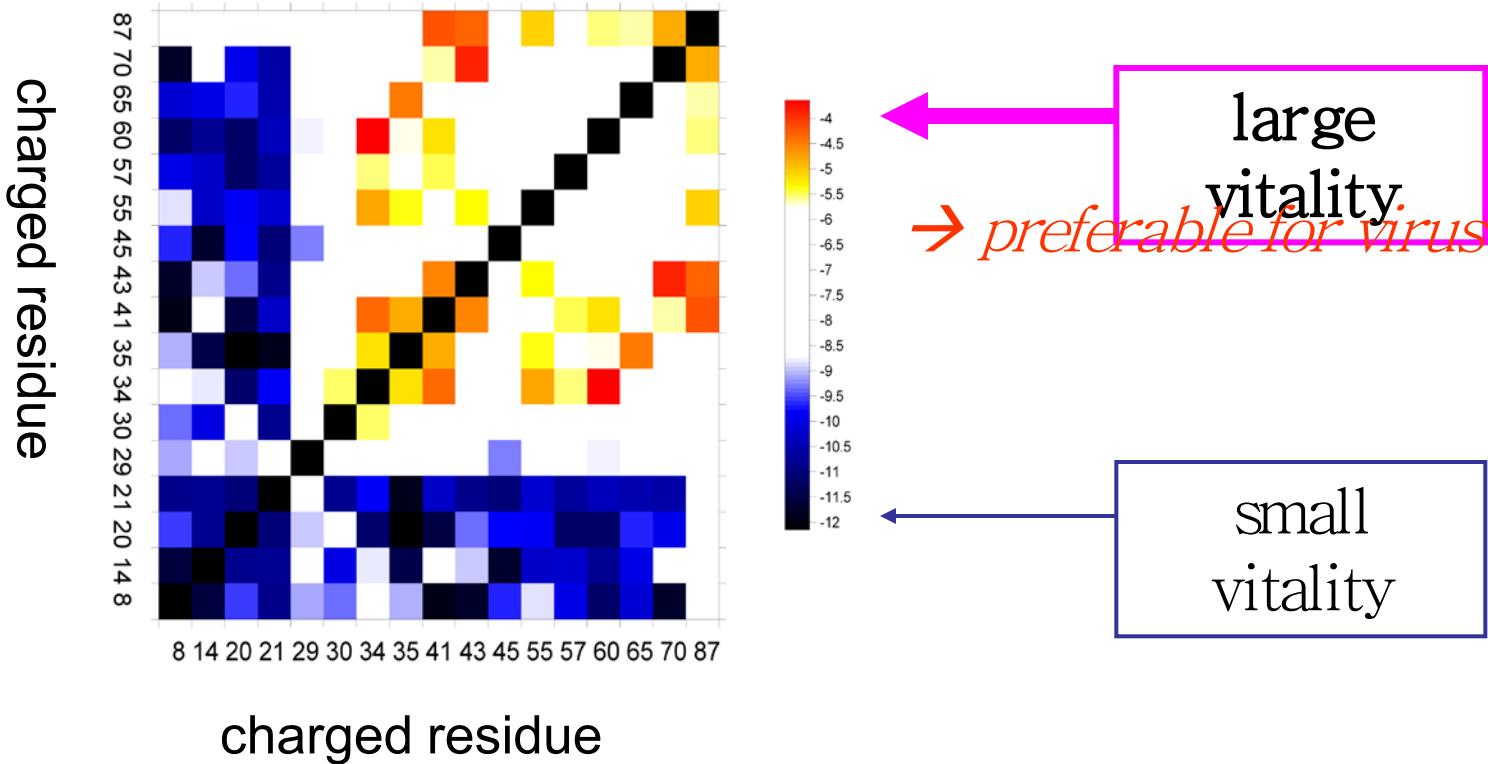
Drug Resistance





Vitality diagram for double mutant

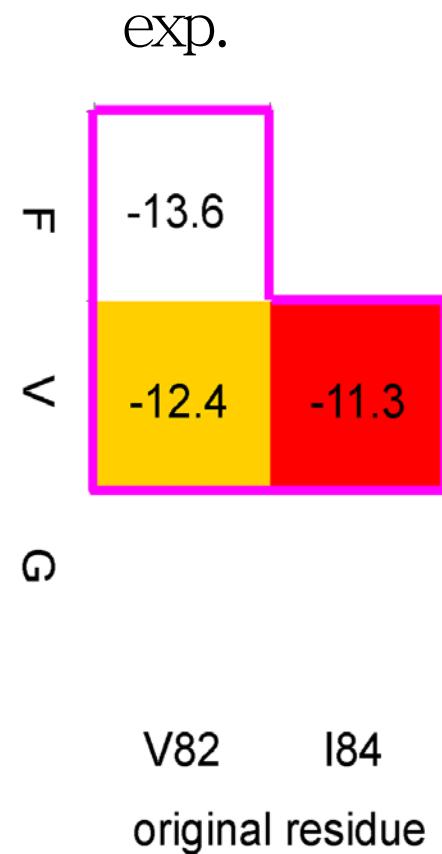
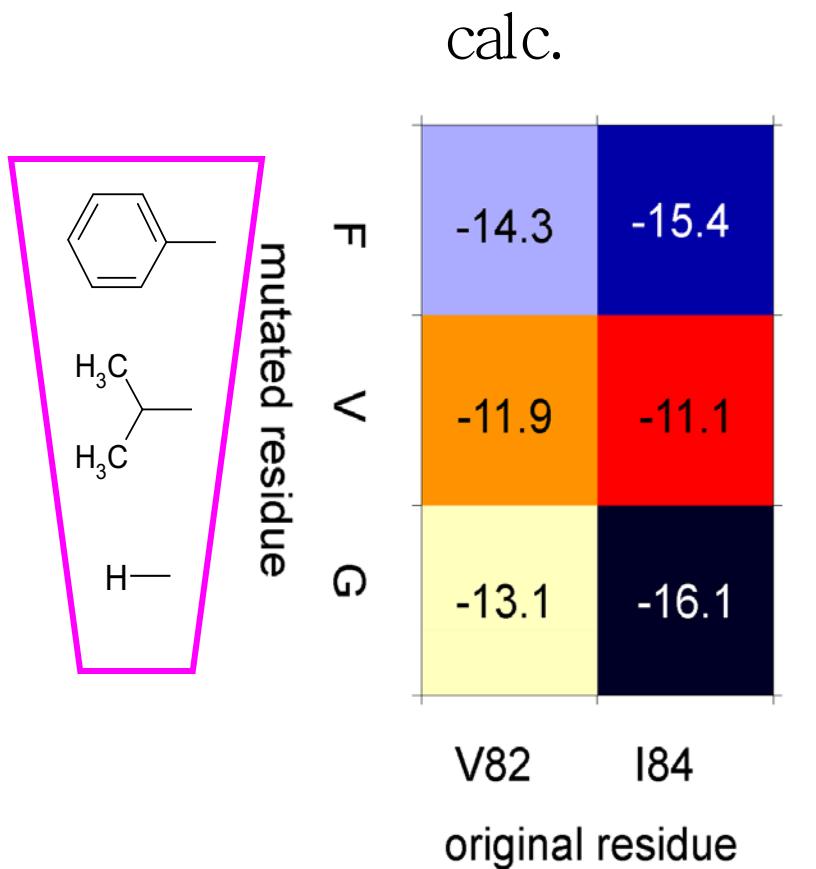
$$-\Delta\Delta G_{bind}^{N \rightarrow M}(TS) + \Delta\Delta G_{bind}^{N \rightarrow M}(drug)$$



Ishikita & Warshel (2007)

Influence of the size of nonpolar residue on vitality value

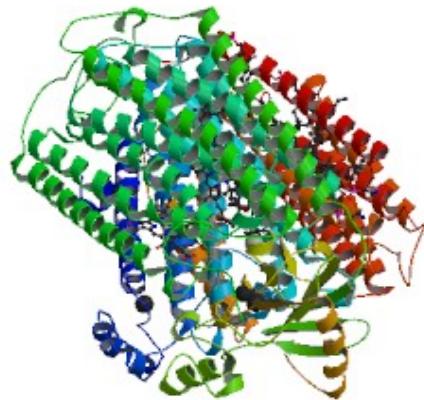
$$-\Delta\Delta G_{bind}^{N \rightarrow M}(TS) + \Delta G_{bind}(drug)$$



Ishikita & Warshel (2007)

STRUCTURE-FUNCTION RELATIONSHIP

STRUCTURE



FUNCTION



Missing link

ENERGY



SOLVATION BY PROTEIN + WATER



MAINLY ELECTROSTATIC!

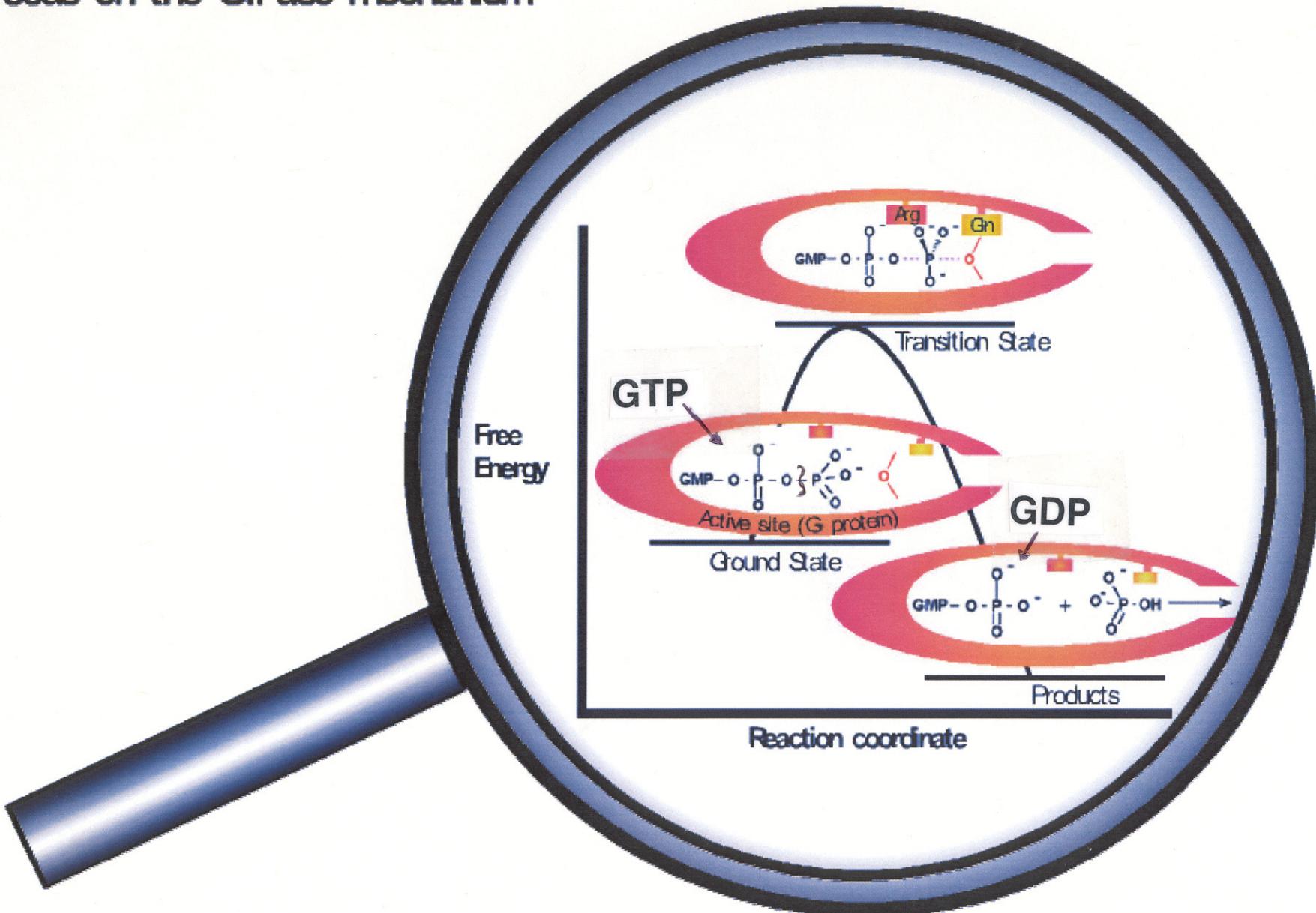


Acknowledgements

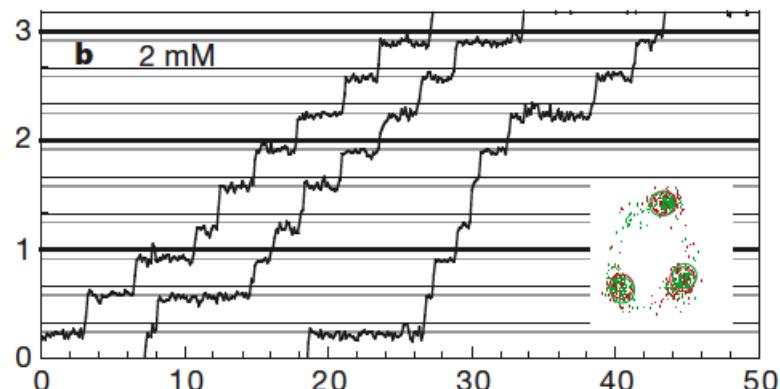
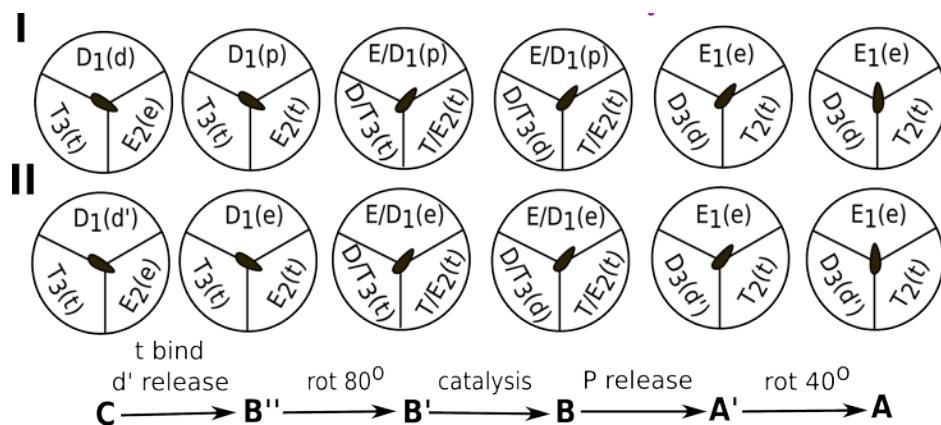
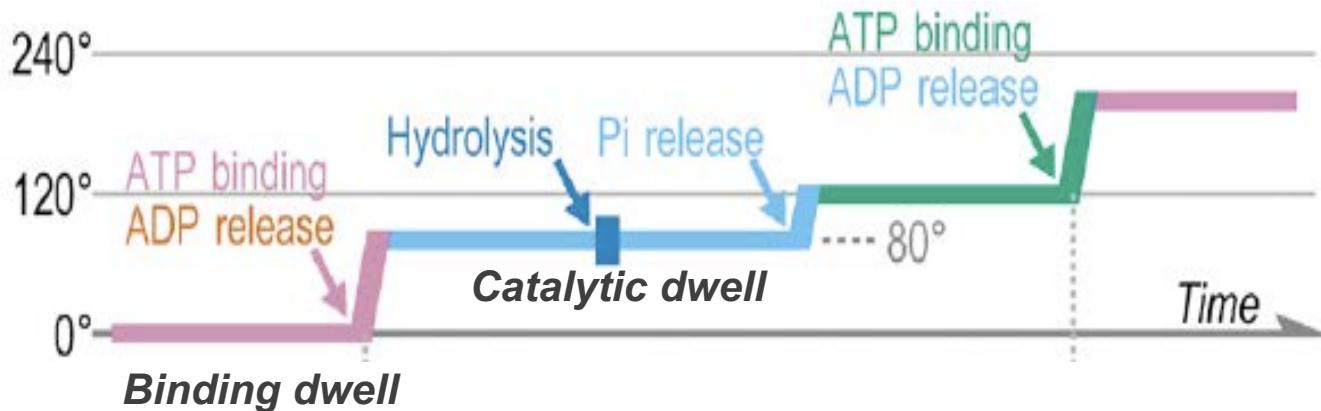
A.Adamczyk	M.Kato	A.Reymer
J.Aqvist	I.Kim	M.Roca
J.Bentzien	G.King	E.Rosta
J.Bertran	M.Klahn	R.Rucker
M.Bohac	B.Kormos	S.Russel
R.P.Bora	M.Kosloff	A.Rychkova
S.Braun-Sand	I.Kupchenko	P.Schopf
A.Burykin	S.Kuwajima	N.Schutz
J.Cao	J.Lameira	T.Schweins
S.Chakrabarty	R.Langen	Y.Sham
Z.T.Chu	F.Lee	P.Sharma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



Focus on the GTPase mechanism



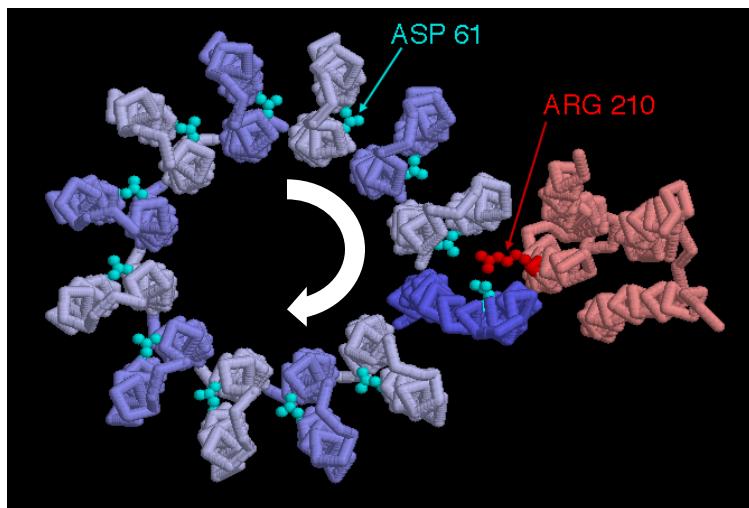
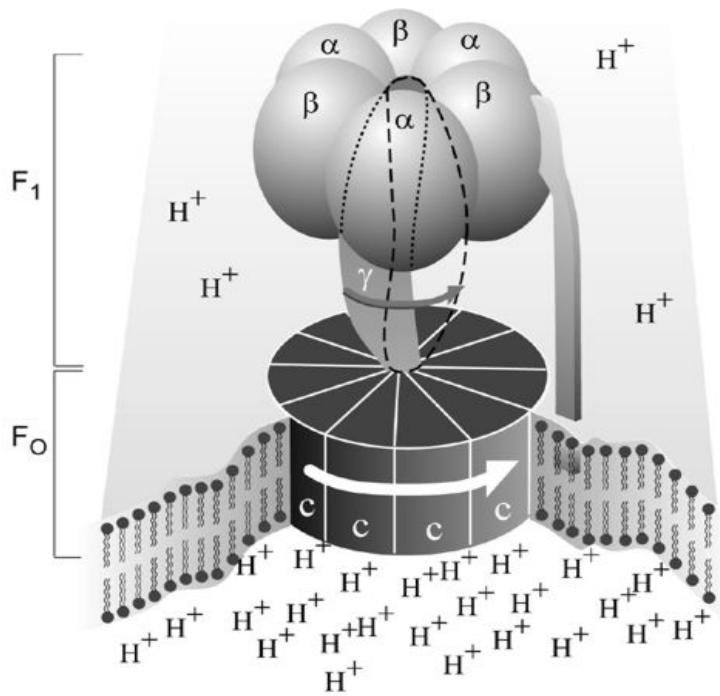
Mechano-Chemical Coupling between γ and α/β dimers : As revealed by numerous single-molecule studies



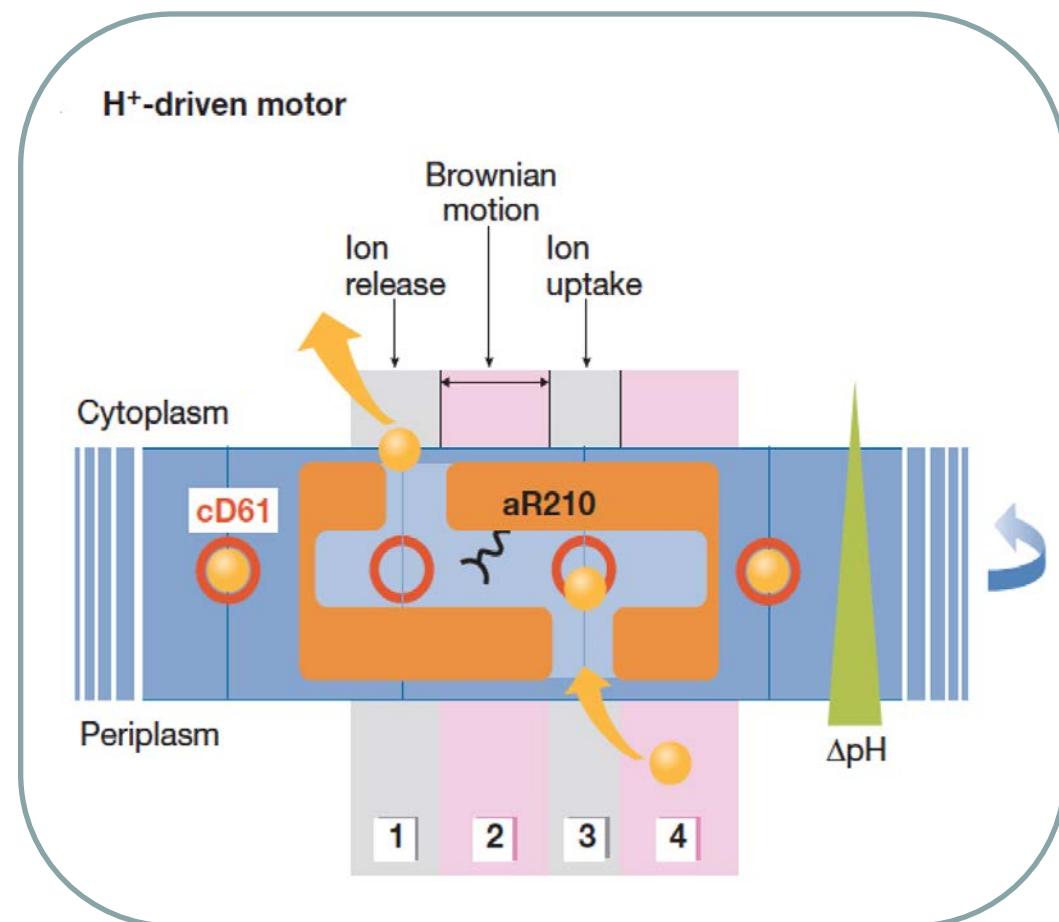
Shimo-Kon, R. et. al., Biophys. J., 2010.

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

Understanding the action of proton driven F_0 motor

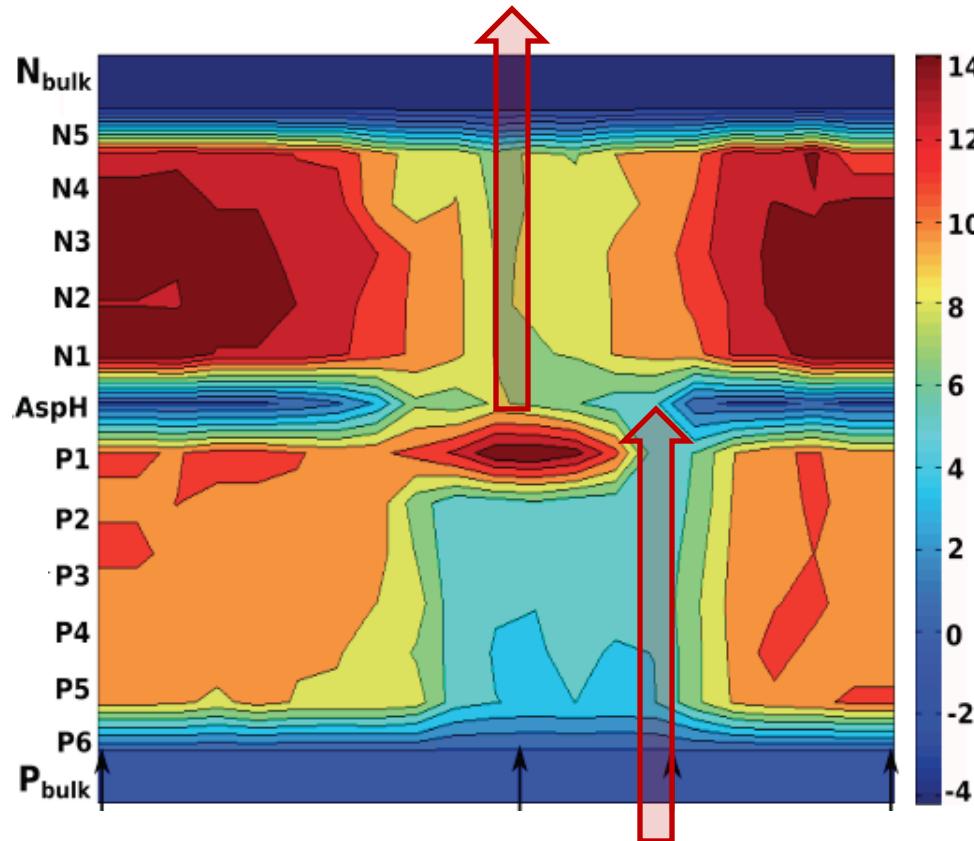


A 2D schematic model of F_0 rotation coupled to ion transport



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

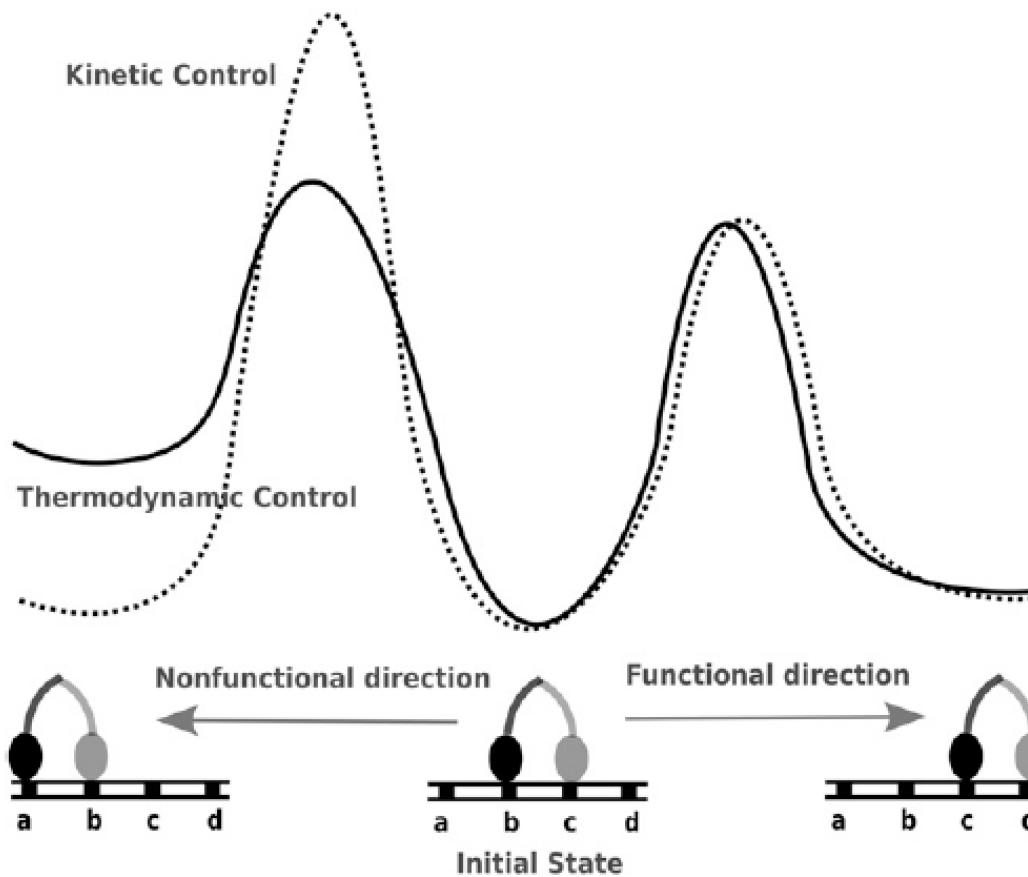
Release of protons to
the high pH side



Release of protons to
the high pH side

Uptake of protons from
the low pH side

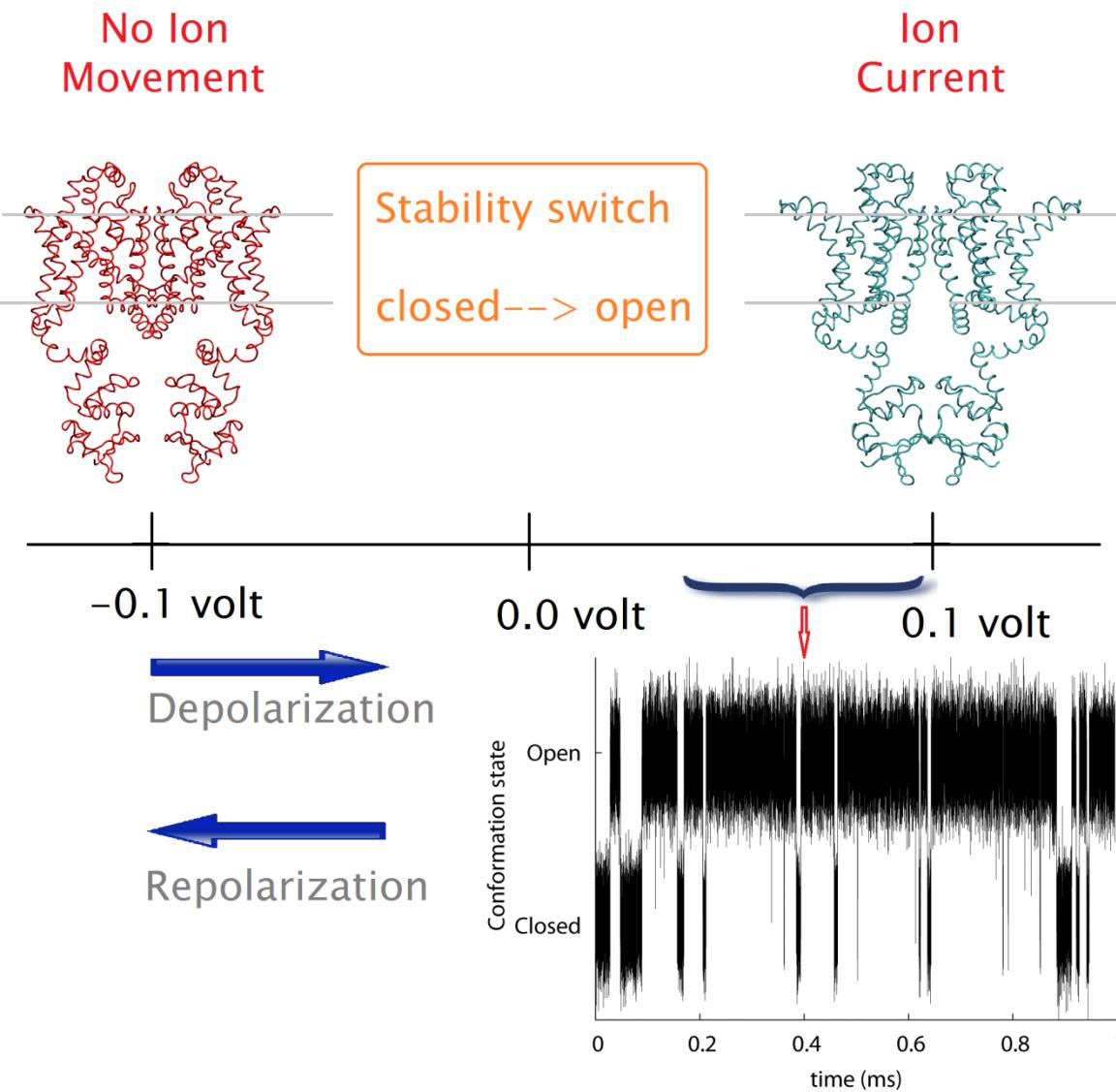
Free energy surface that can explain unidirectional walking motion



Backward steps
are obstructed
by higher barriers
or free energies

Forward steps
are preferred
by lower barriers
or free energies

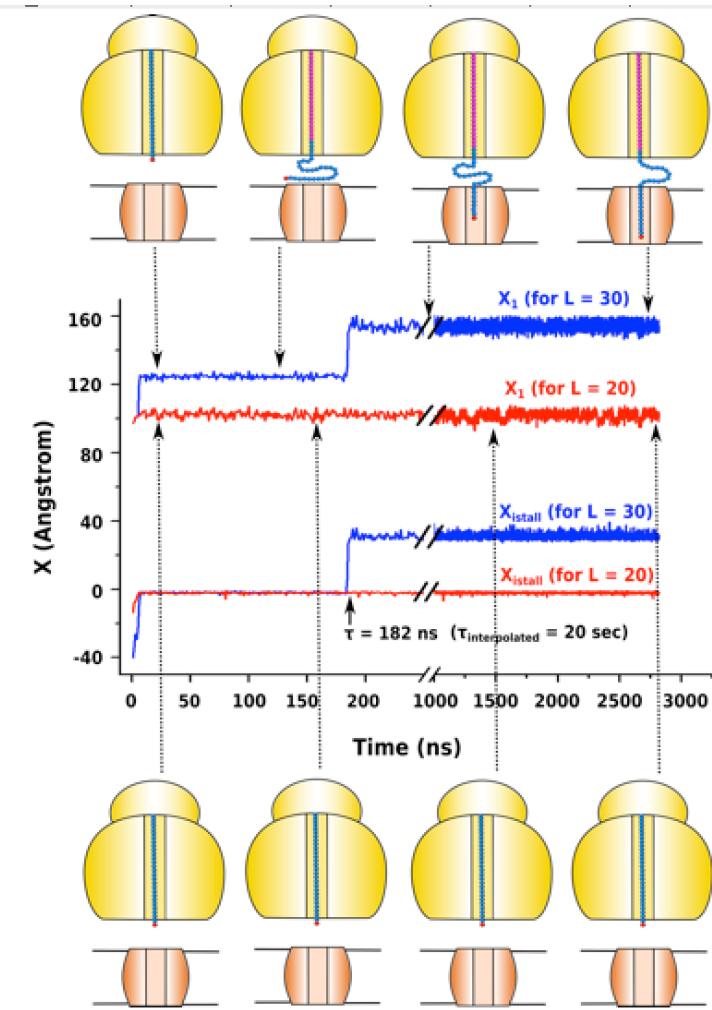
Free Energy of the System



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)



A General Cycle For Moving between Different Multiscale Leveles

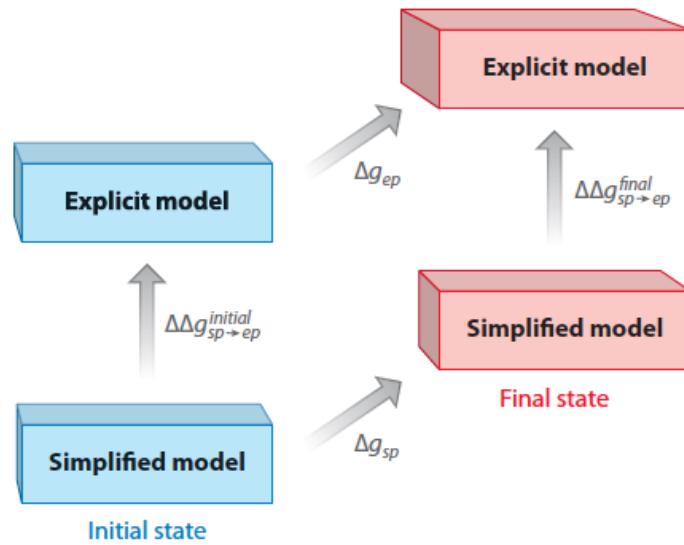
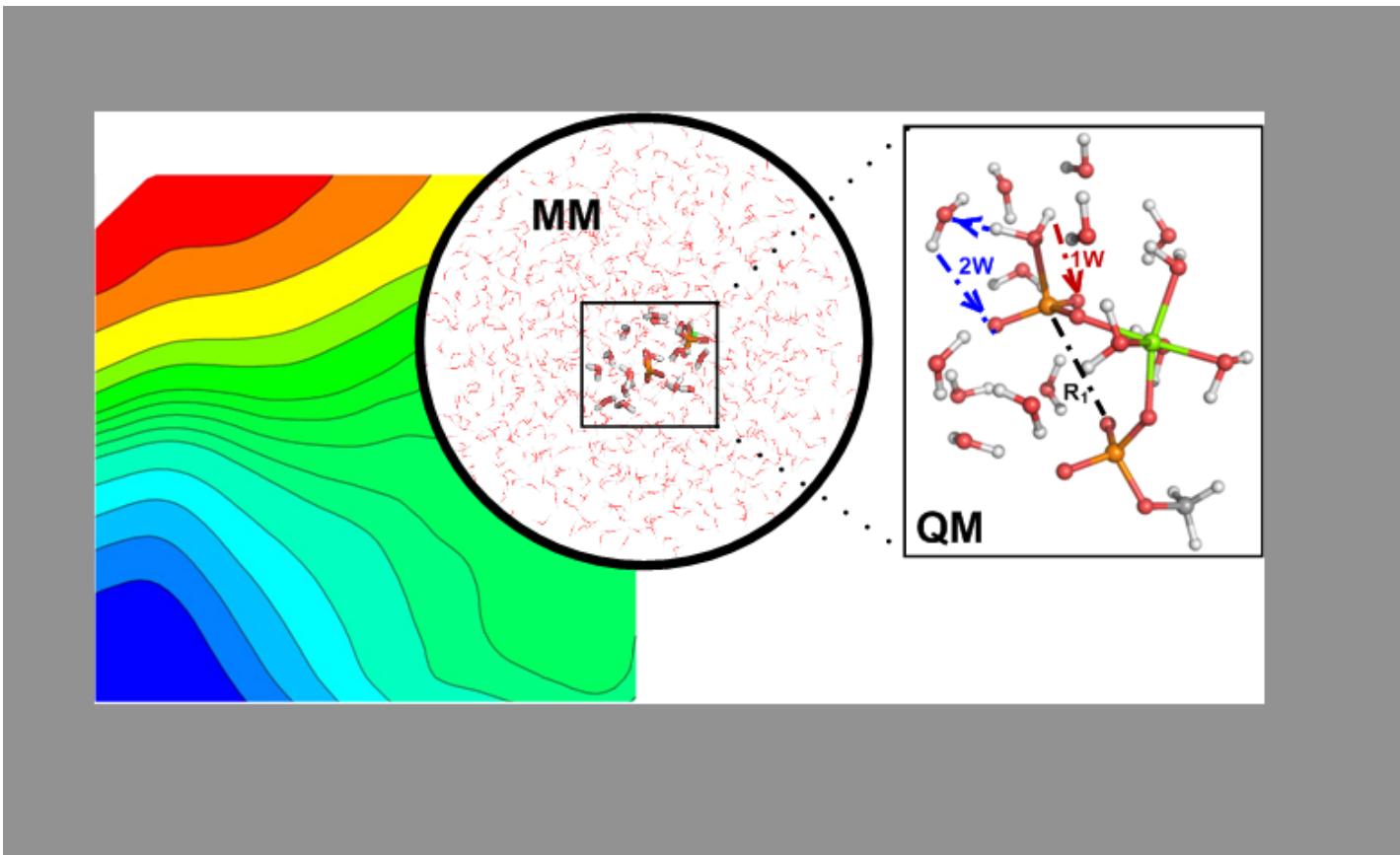


Figure 2

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 $\Delta\Delta g_{sp \rightarrow ep}$ for the initial and final states to obtain Δg_{ep} .

JPC 2013

Systematic QM(ai)/MM PMFs



γ_M / γ_N	
<i>exp</i>	<i>calc</i>
V82A 0.53	V82A 0.17
V82F 0.25	V82F 0.08
I84V 0.36	I84V 0.36

Saquinavir

γ_M / γ_N	
<i>exp</i>	<i>calc</i>
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

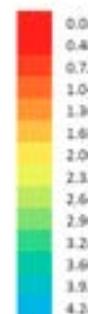
DMP323

V82A 0.74	V82A 1.00
V82F 1.69	V82F 2.00
I84V 1.50	I84V 2.62

SD146

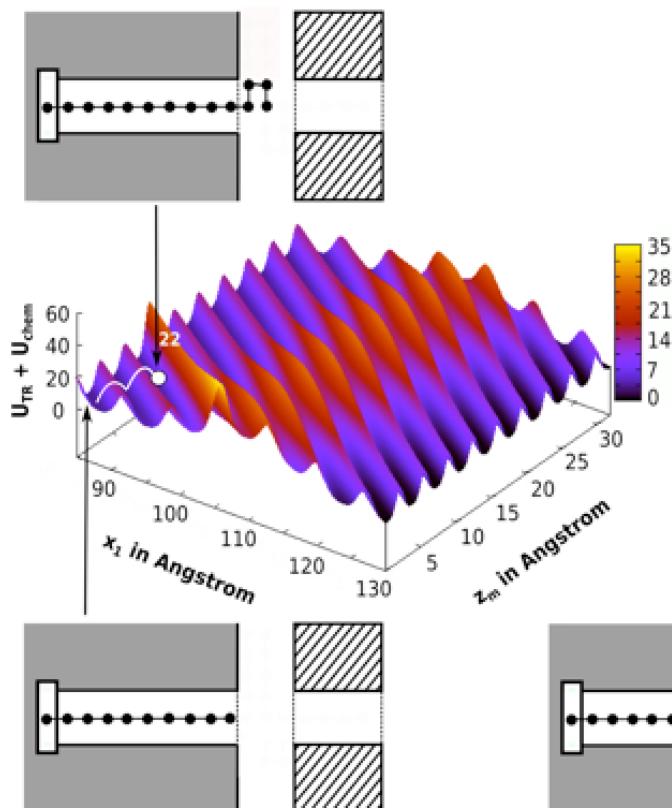
V82A 0.80	V82A 0.50
V82F 1.55	V82F 1.36
I84V 3.56	I84V 3.05

XV638

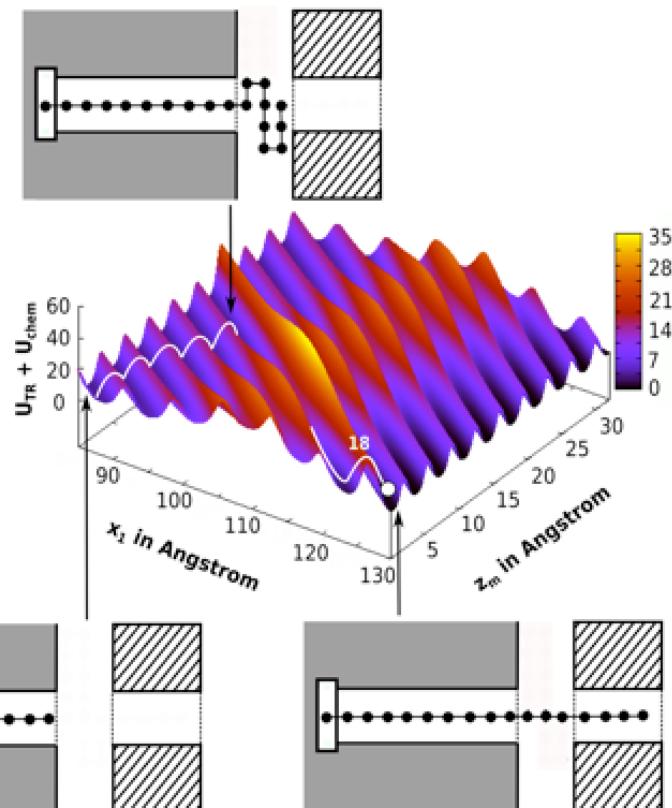


PNAS 2013

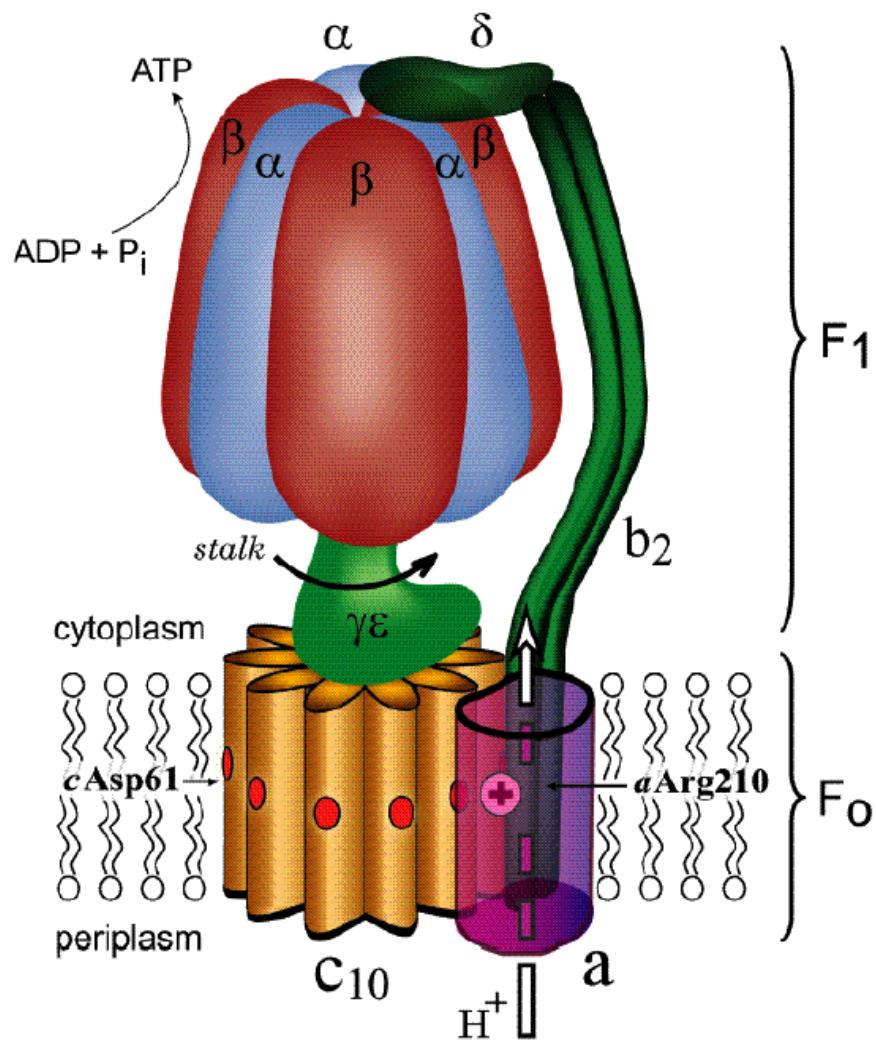
A



B



Adenosine Triphosphate (ATP) Synthase



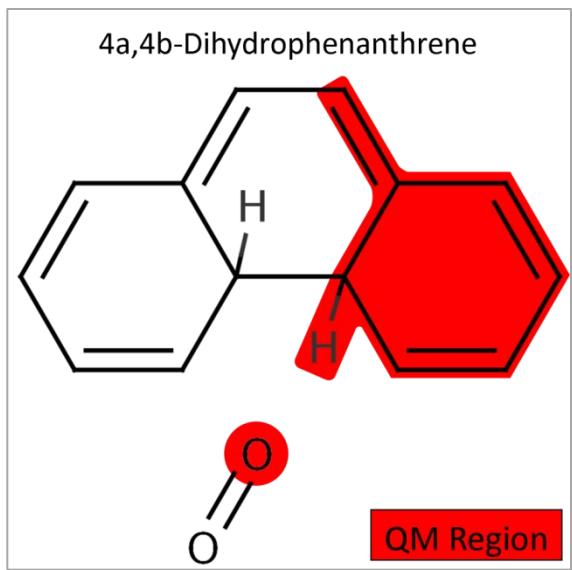
Rotary catalysis: Two protein motors coupled via common central stalk $\gamma\delta$

Solvent exposed **F₁** unit ($\alpha_3\beta_3\gamma\delta\epsilon$): central stalk rotation causes conformational changes in catalytic sites, driving ATP synthesis

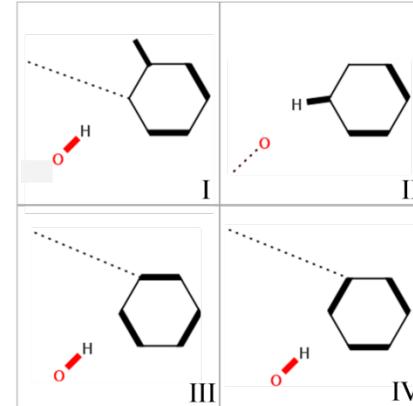
Transmembrane **F₀** unit (ab_2c_{10}): converts proton motive force into mechanical rotation of central stalk

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

QM(VB)
+MM



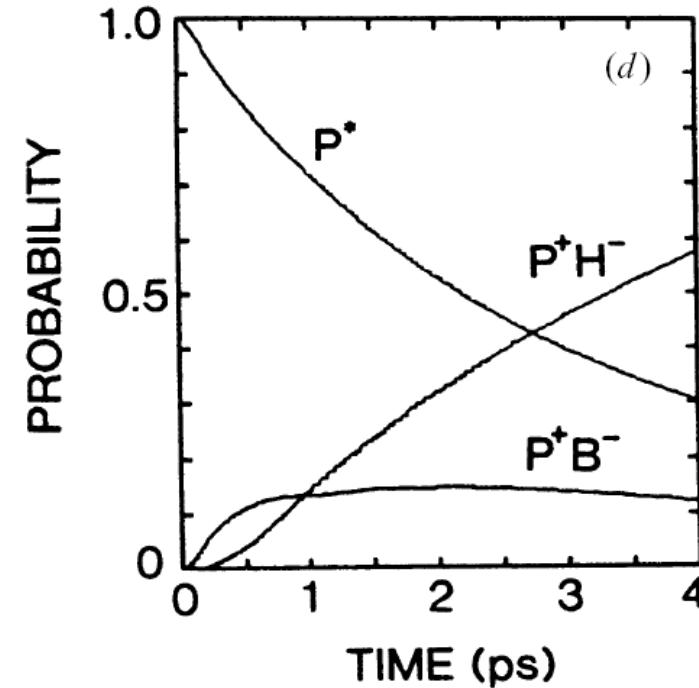
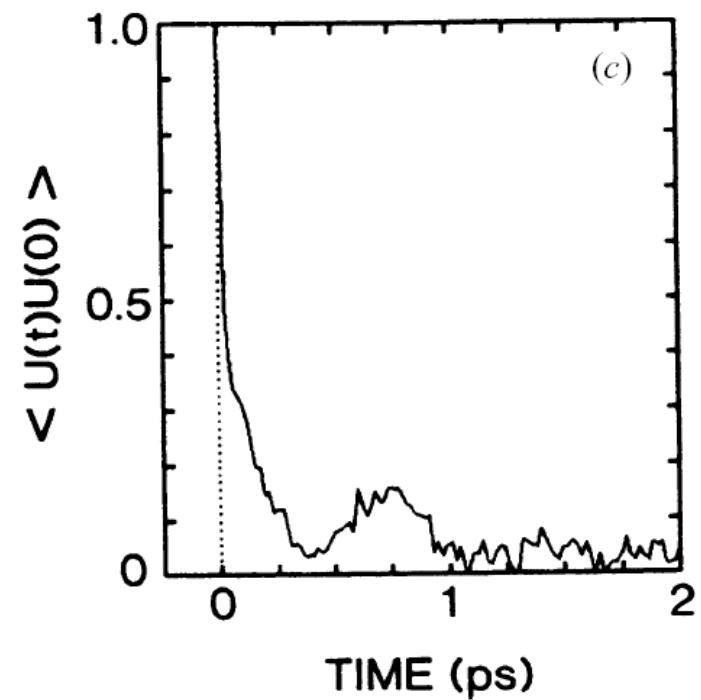
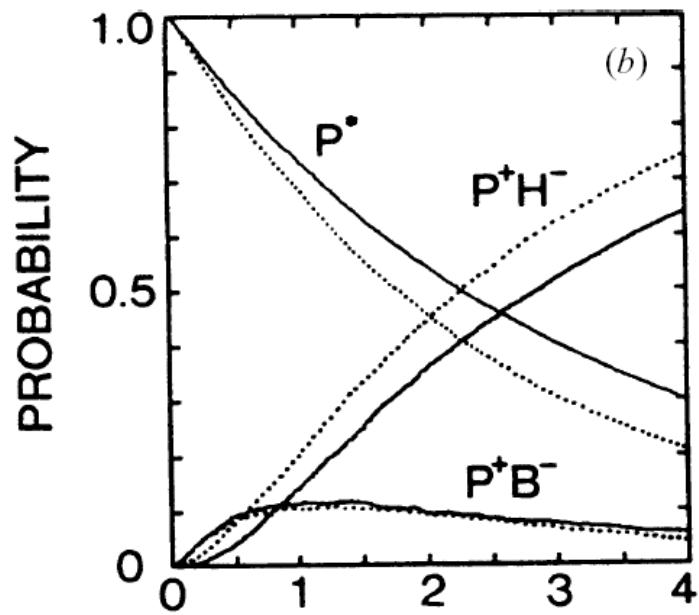
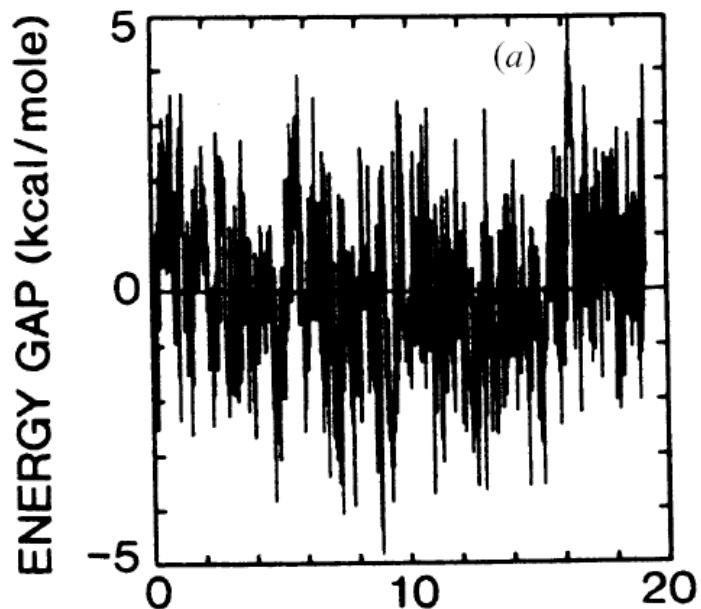
$$\mathbf{H} = \begin{pmatrix} 2^5 \left[Q + \beta + \delta_2 - \frac{1}{2}(\alpha + \gamma) \right] & -2^4 \left[Q + \alpha + \beta + \delta_2 - 2\gamma \right] & -2^2 \left[Q + \beta + \delta_2 + 6\delta_1 - \frac{1}{2}(\alpha + \gamma) \right] & -2^4 \left[Q + \beta + \delta_2 + \frac{3}{2}\delta_1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



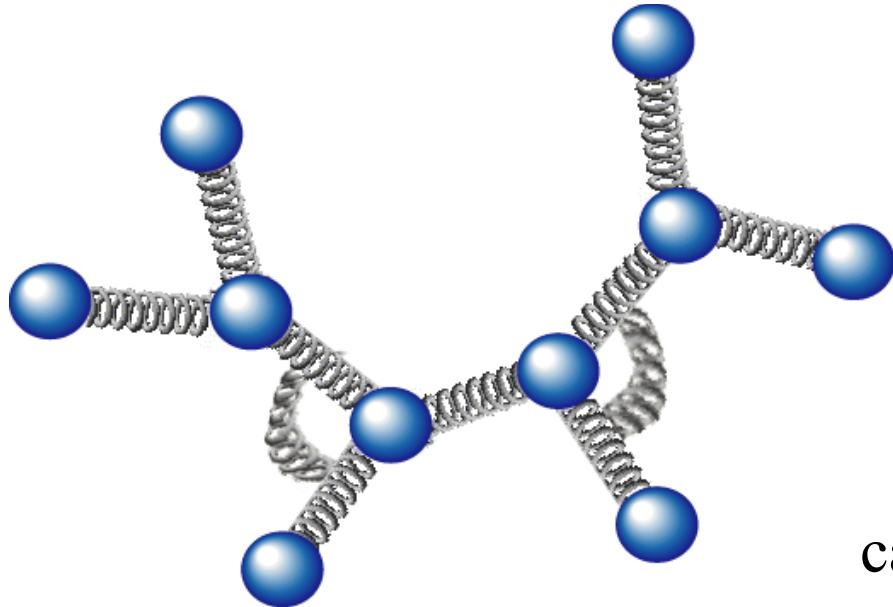
Dashed lines represent an electron removed to infinity.
Paired electrons are represented by heavy lines.

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

QM(MO)
+MM

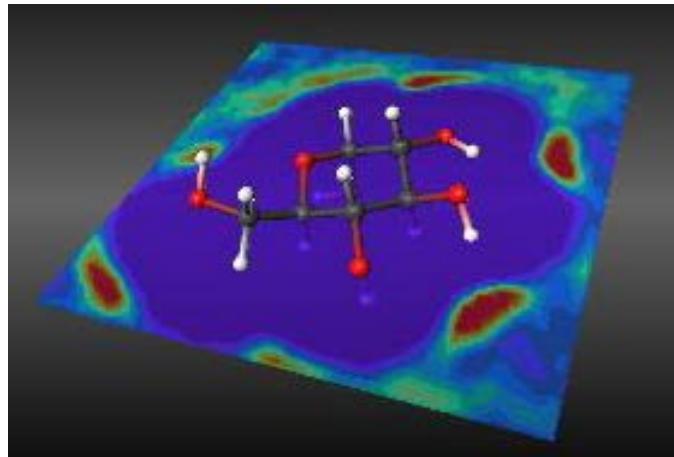


Molecular Mechanics



cannot study chemistry

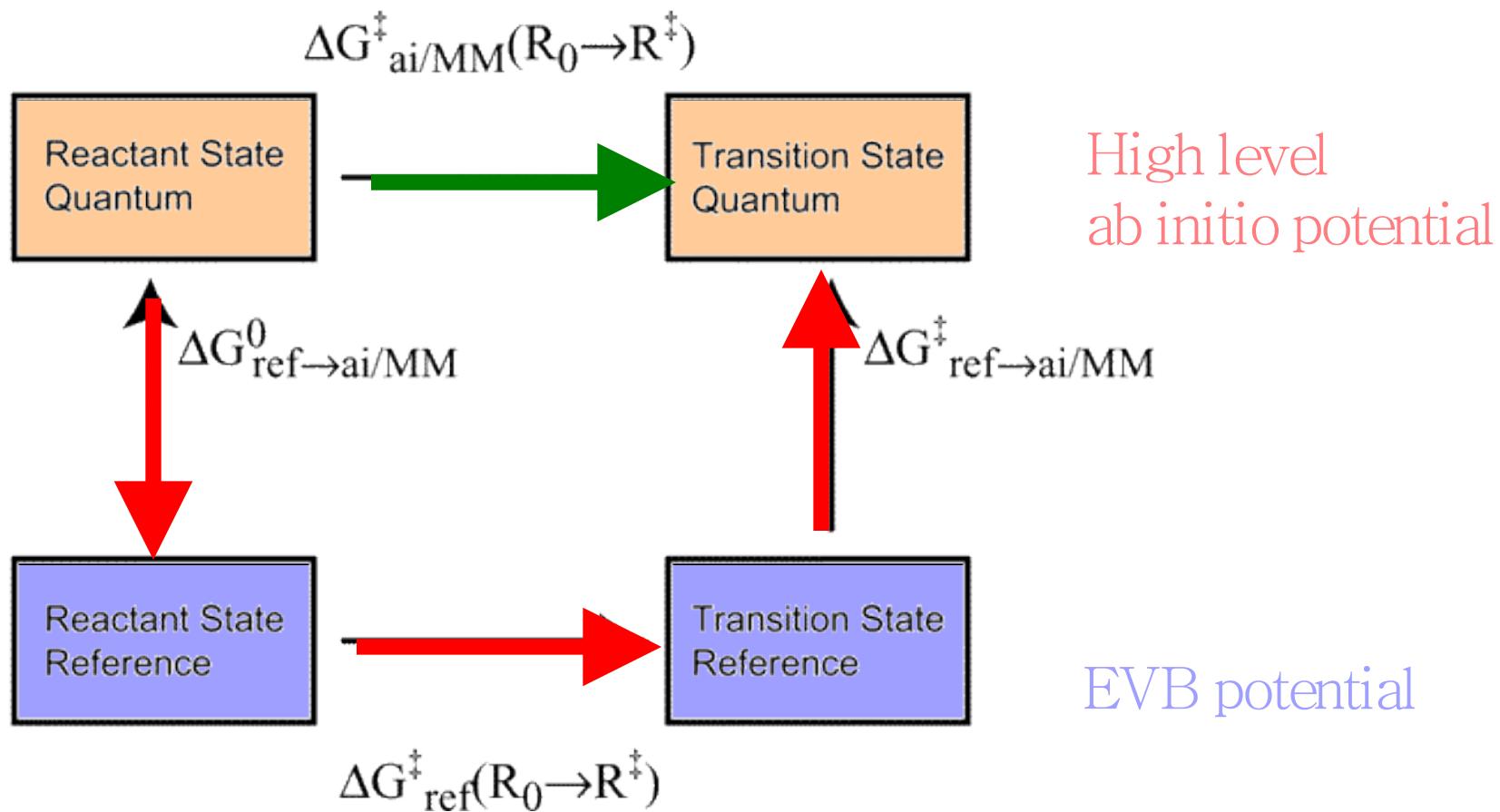
Quantum Mechanics



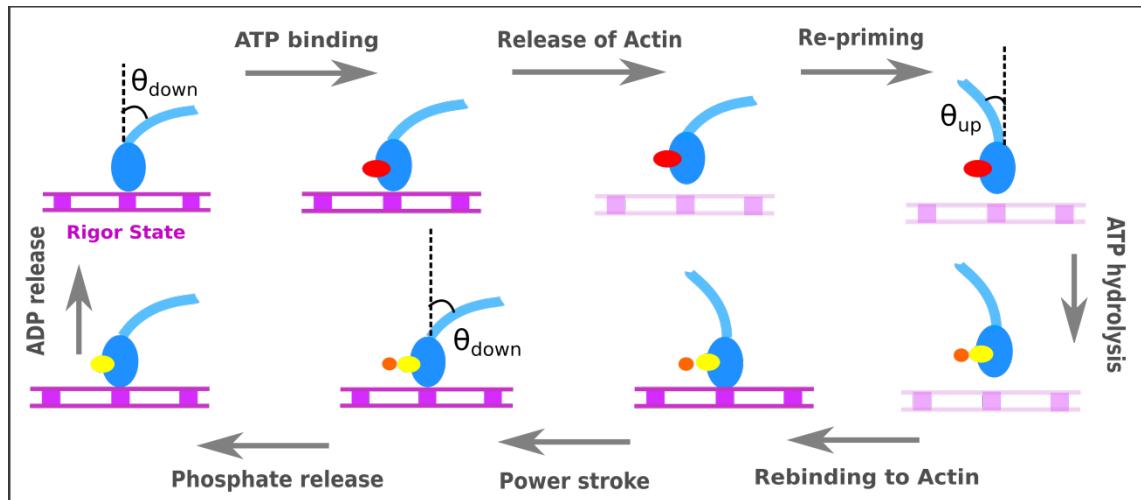
very expensive

Our Pradynamics (since 1992 with different names)

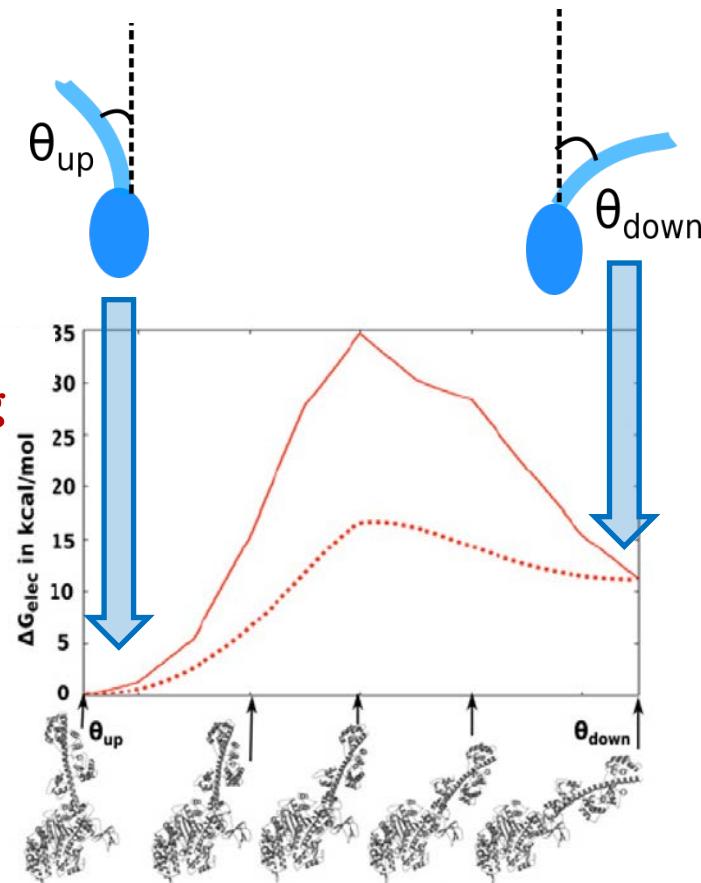
A General Thermodynamic Cycle For MultiScale Modeling

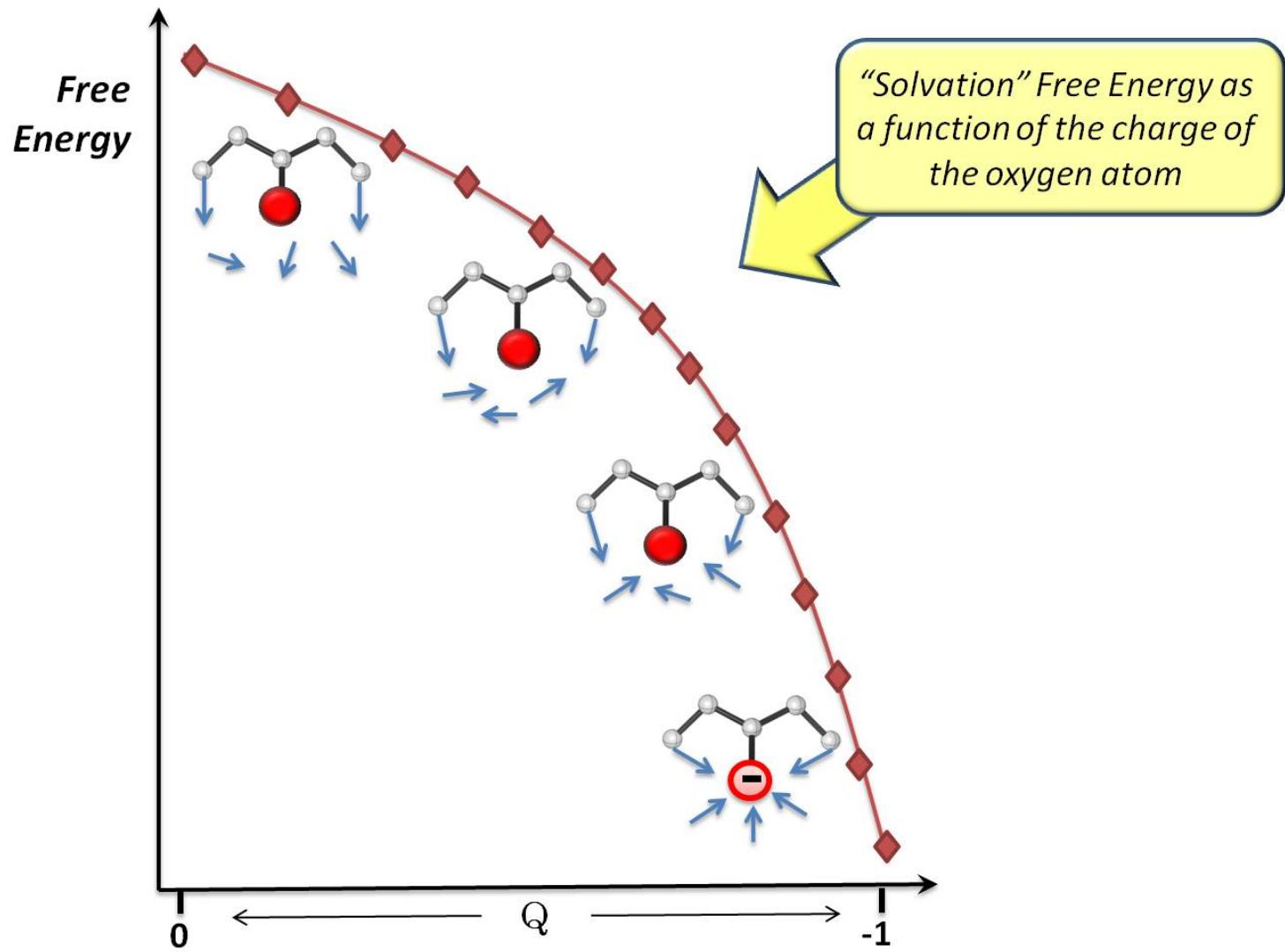


CG energetics of a single leg as it bends (changes conformation)



Schematic functional cycle of myosin V single leg





Pontifical Academy
1983

Free energy perturbation in
enzyme catalysis

SPECIFICITY IN BIOLOGICAL INTERACTIONS

77

