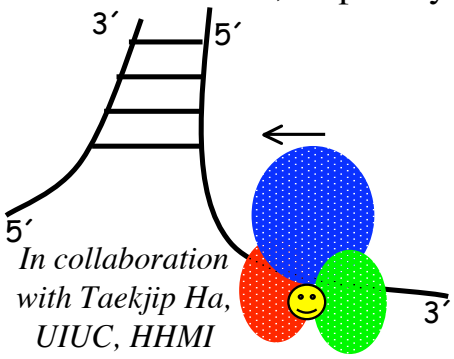
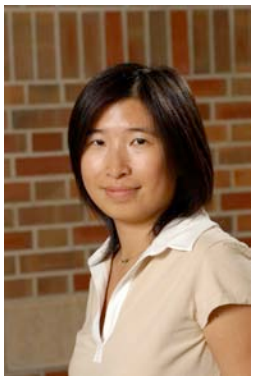
PcrA Helicase
A Molecular Motor Studied from the Electronic to the Funtional Level

Klaus Schulten, Dept. Physics and Beckman Inst., U. Illinois, Urbana

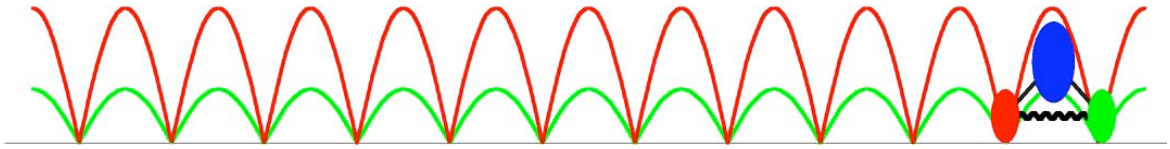


In collaboration with Taekjip Ha, UIUC, HHMI



Jin Yu

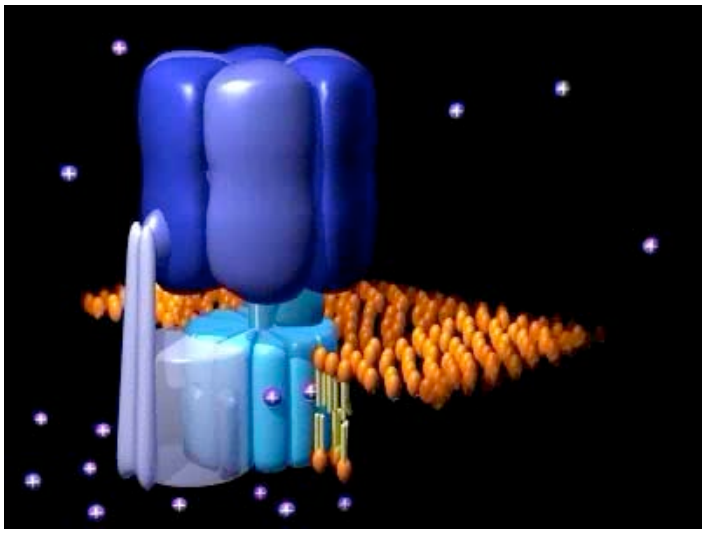
Markus Dittrich

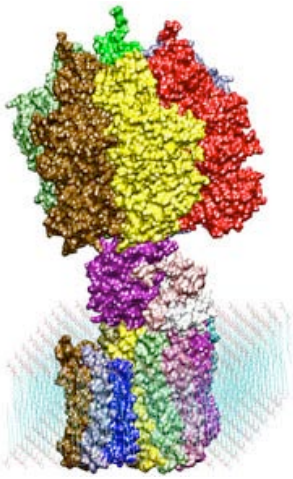


Why did we chose PcrA helicase for study? Its small!

(Of course, it is also an important system otherwise)

We started actually with ATP synthase. It's much bigger!





Movie courtesy W. Junge et al.

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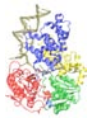

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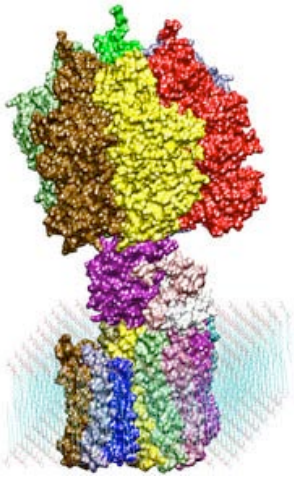
Preparing
F1-ATPase
for simulation



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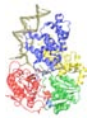

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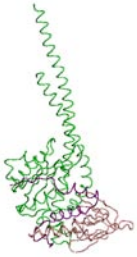

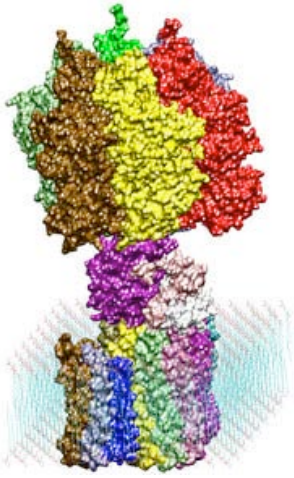

• Start with DCCD-inhibited structure, has near-complete stalk. (Gibbons 2000, PDB code 1E79)
• Total 327,000 atoms (3325 residues, 92,000 water molecules, nucleotides, and ions).
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E. Tajkhorshid et al., *Adv. Protein Chemistry* **66**, 195-247(2003)

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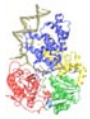

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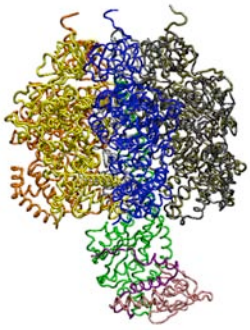

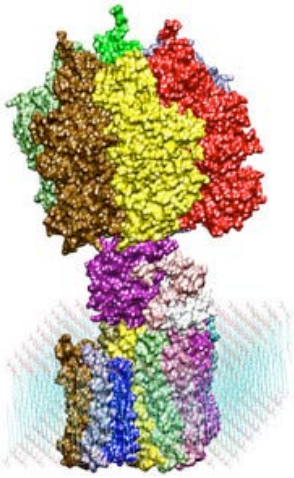

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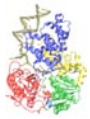

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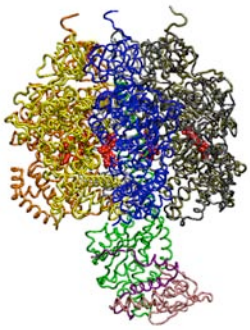

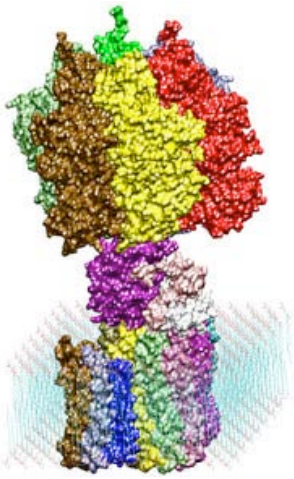

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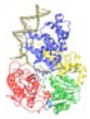

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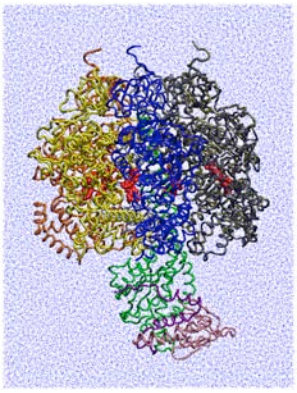

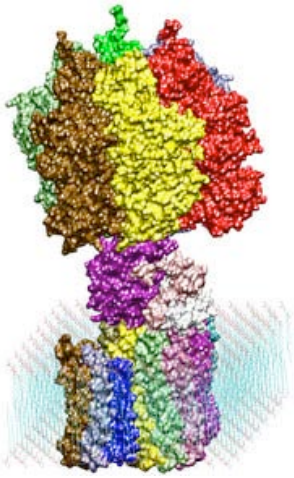

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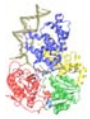

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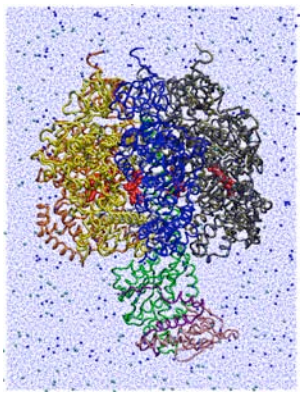

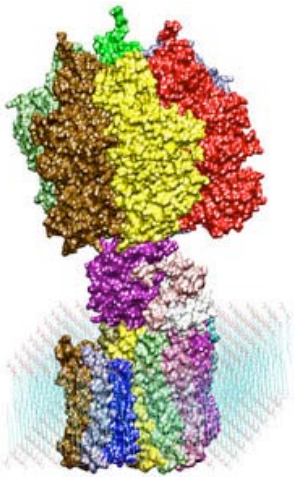

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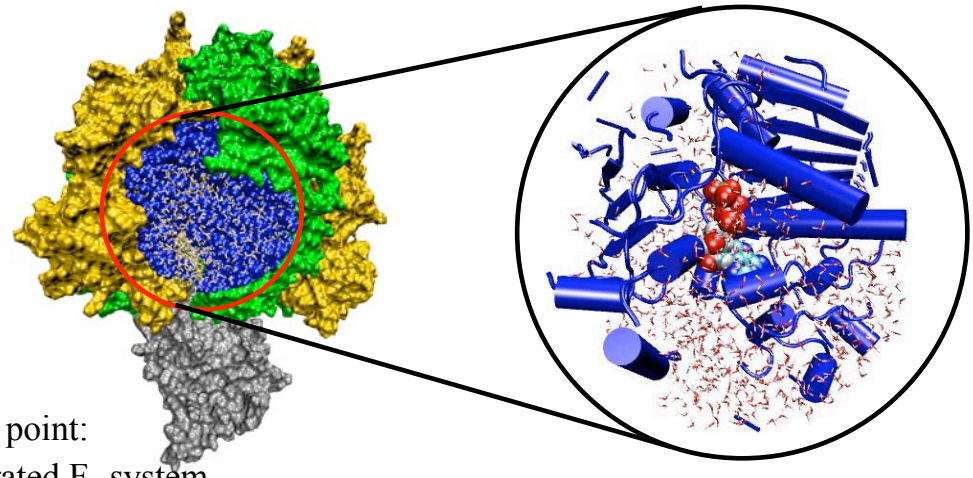
Naturally, we began with the hydrolysis reaction



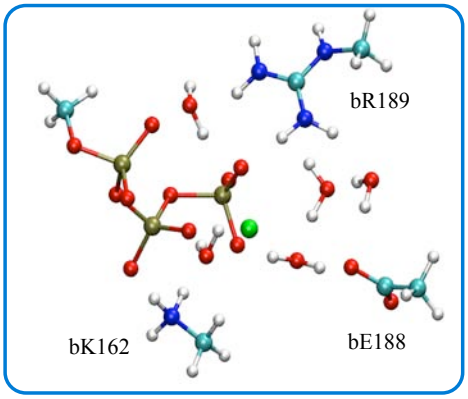
Starting point:
Equilibrated F_1 system
with ~ 327,000 atoms
(structure used
Gibbons et al.,2000,
bovine mitochondrial ATP synthase)

Reduced sub-systems were used to perform QM/MM simulations of ATP hydrolysis in b_{TP} and b_{DP}.

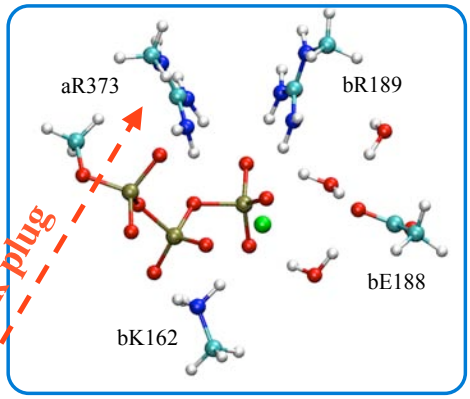
M. Dittrich, S. Hayashi, and K. Schulten, *Biophys. J.* **85**: 2253-2266 (2003); *Biophys. J.* **87**: 2954-2967 (2004)

Nucleotide Conformation

reactant state (b_{TP})



reactant state (b_{DP})

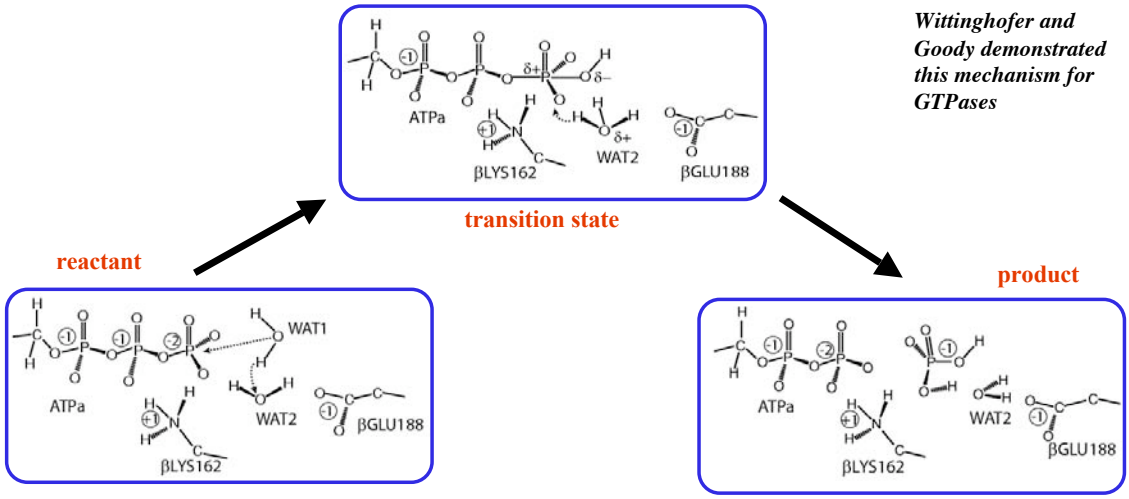


spark plug

b_{TP} and b_{DP} have a similar conformation apart from a large movement of the **"arginine finger"** residue aR373.

M. Dittrich, S. Hayashi, and K. Schulten, *Biophys. J.* **85**: 2253-2266 (2003); *Biophys. J.* **87**: 2954-2967 (2004)

Efficient ATP Hydrolysis via Proton Relay



Wittinghofer and Goody demonstrated this mechanism for GTPases

Proton transfer via a proton relay mechanism is the energetically dominant pathway in b_{TP} and b_{DP}.

M. Dittrich, S. Hayashi, and K. Schulten, *Biophys. J.* **85**: 2253-2266 (2003); *Biophys. J.* **87**: 2954-2967 (2004)

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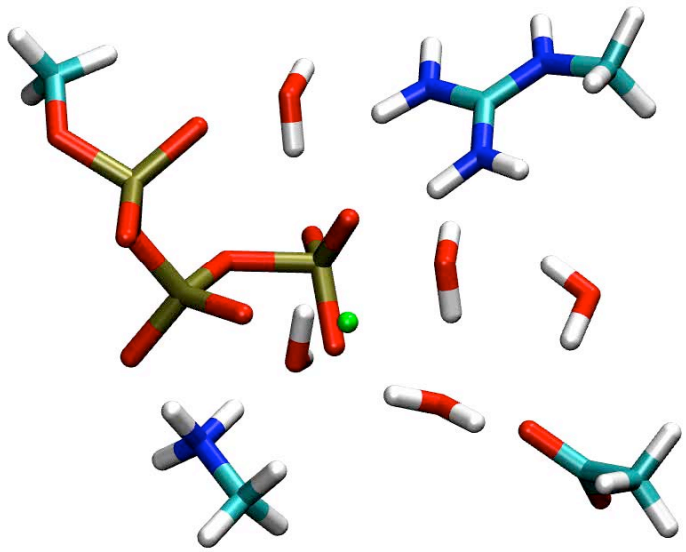
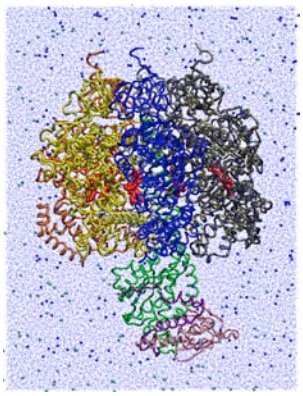


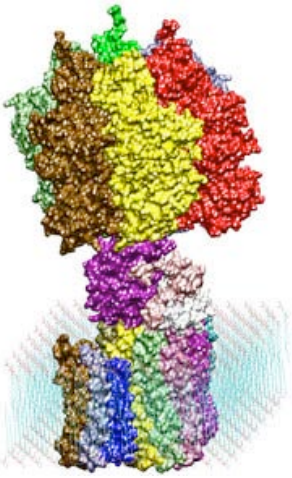
To which purpose?

M. Dittrich, S. Hayashi, and K. Schulten, *Biophys. J.* **85**: 2253-2266 (2003); *Biophys. J.* **87**: 2954-2967 (2004)

We thought to determine the causal relationship between ATP hydrolysis and stalk rotation (torque generation).

Preparing F1-ATPase for simulation





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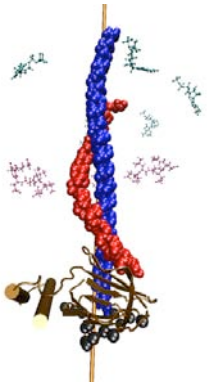
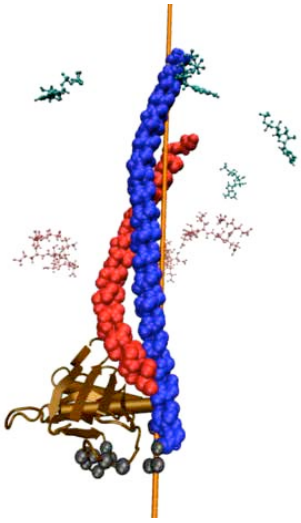
Winding of γ coiled-coil

t= 0 ns
θ= 0°



t= 3.0 ns
θ= 72°



Different coupling for the two γ helices:
γ 1—50, partially via δ subunit
γ 197—272, directly to F_o

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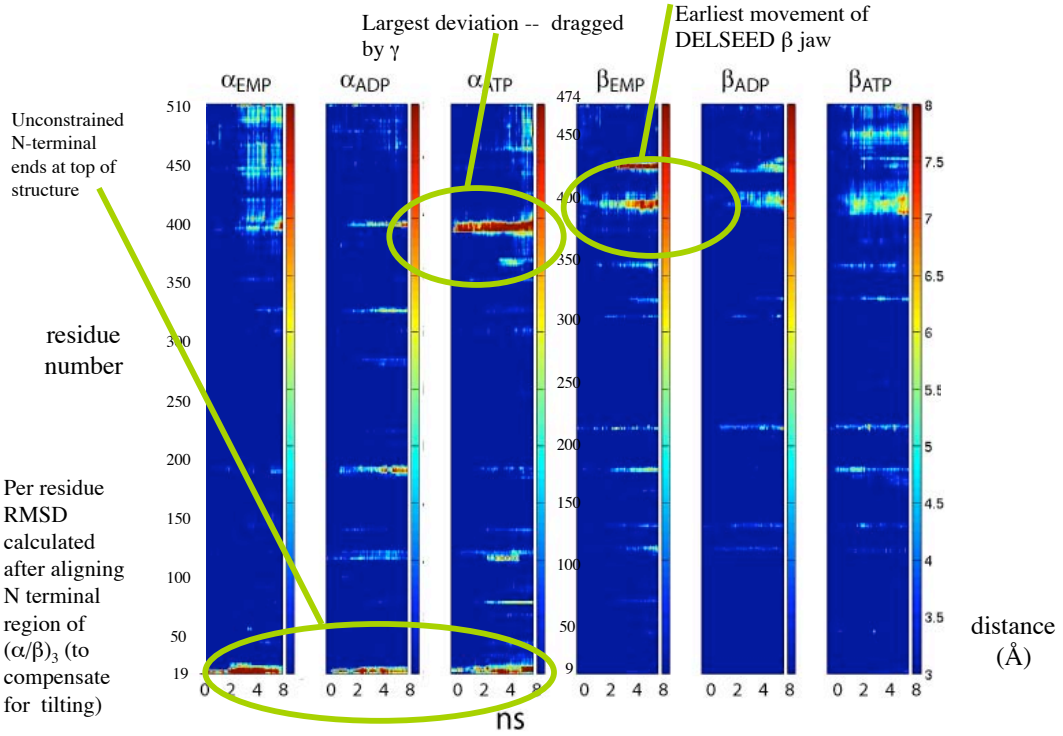
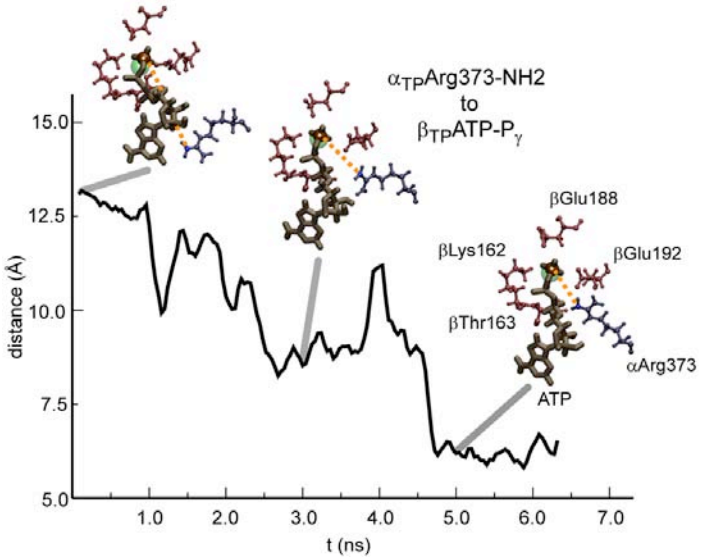
Per-residue subunit motions



α_{TP} Arg373 enters P_i pocket



Motion of α_{TP} Arg373 guanidinium group towards β_{TP} phosphate binding pocket during enforced stalk rotation. α_{TP}Arg373 is seen to play an important role in hydrolysis in QM/MM simulations (Dittrich, 2004).

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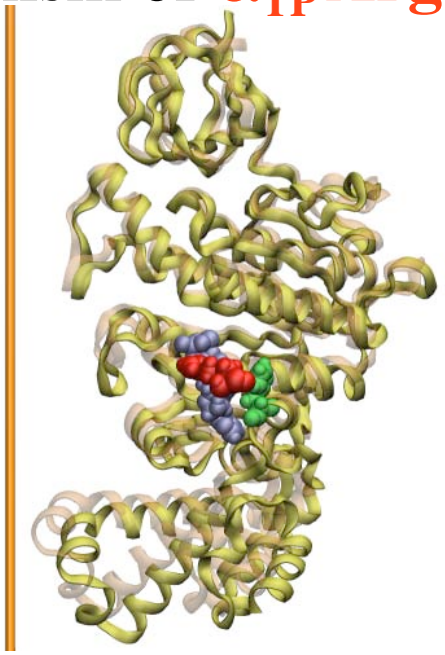
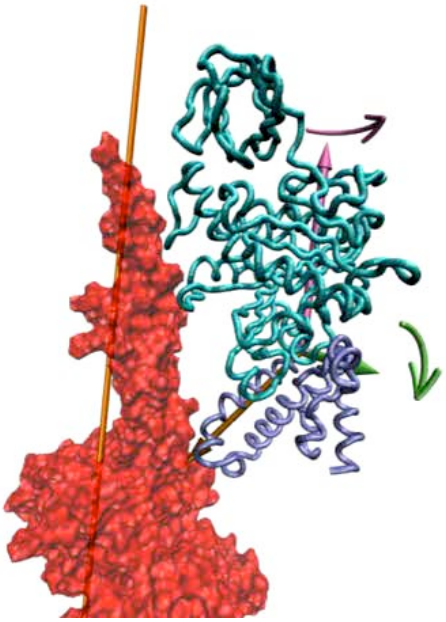
Mechanism of α_{TP} Arg373 movement



120° γ rotation

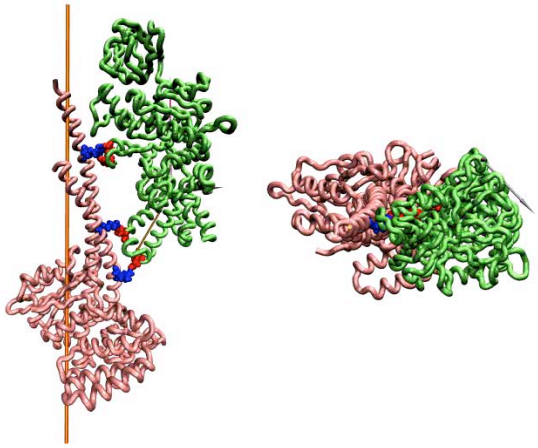
Rotation of the γ stalk pushes on β_{TP} C-terminal jaw. As β_{TP} hinges open, β_{TP}Phe474 is shifted. This frees α_{TP}Arg373 to move along ATP toward P_{γ}.

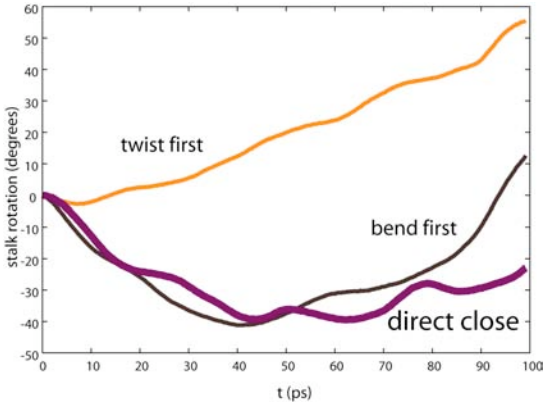
Closing β_E along different paths



force target motions

β_E closing, stalk rotation, and salt bridges



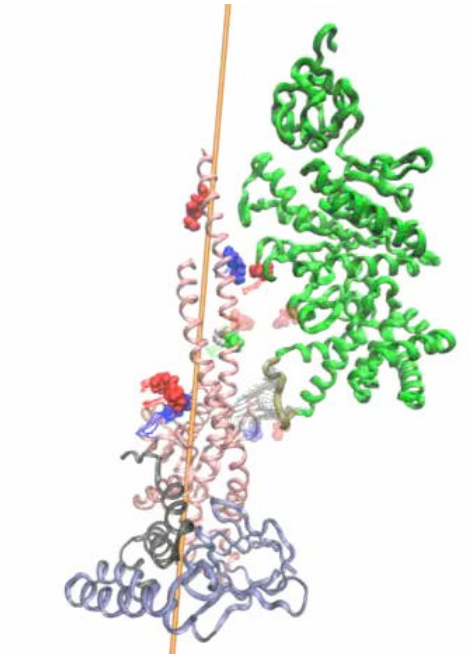


"direct close"

• Motion of stalk restricted to rotation around axis via harmonic springs during enforced β closing.
• Long-lived b-stalk salt bridges drawn as vdW

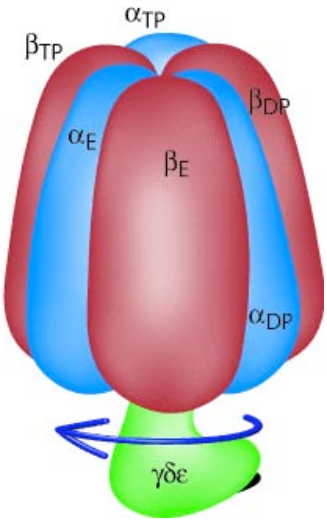
• "Direct close" path ends with largest hydrolysis-direction rotation. Collision with center of coiled-coil might be avoided if salt bridges break.

Ionic bridges pull α_{ATP} into β_{ATP}



The largest deviation in $(\alpha/\beta)_3$ observed during simulation is the clockwise shift of the α_{ATP} 402-411 "jaw" loop. Long-lived hydrogen-bonded ion pairs and several intermittent bond partners (which become available as γ subunit coiled-coil is twisted) pull α_{ATP} towards β_{ATP}. The force from shifted α_{ATP} might help β_{ATP} open to release ATP. Interactions from δ and ε subunits, and γ coiled-coil winding, might affect the availability or strength of bond partners.

But after all simulations we got no clue how the hydrolysis reaction is coupled to stalk rotation!



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And so has nobody else, experimentalists included.

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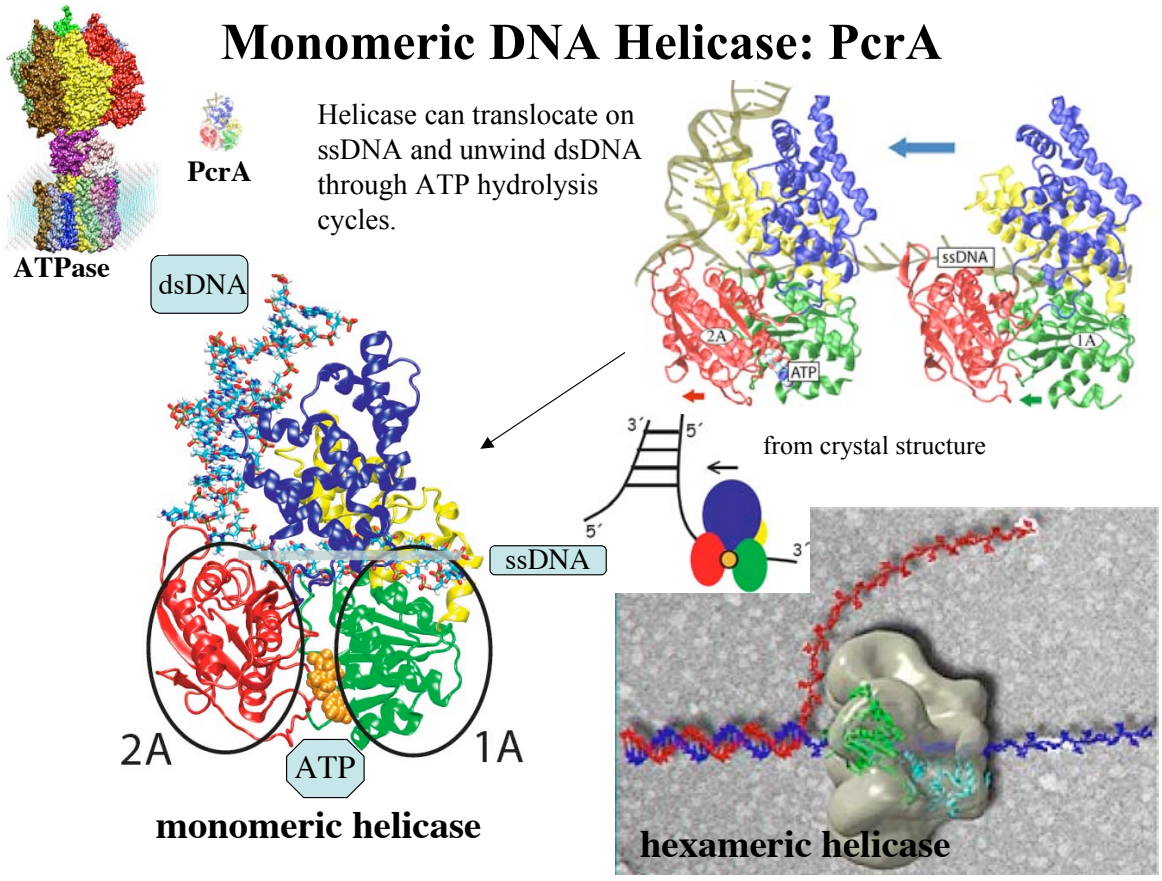
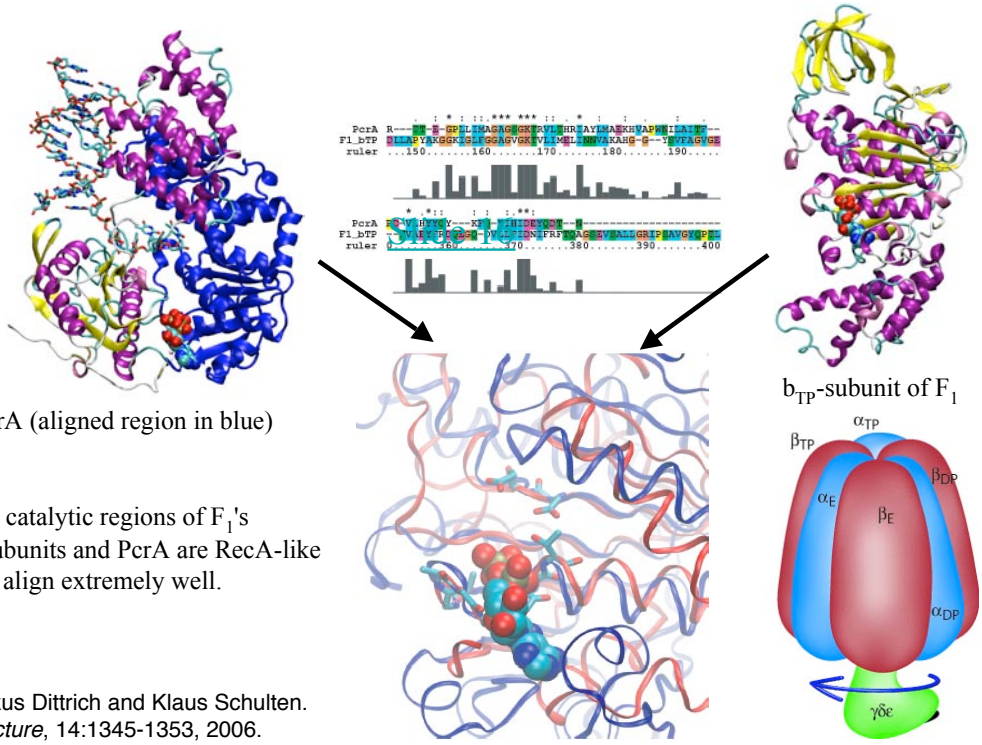
Time to switch the approach and look for a simpler system.

Markus Dittrich and Klaus Schulten. *Structure*, 14:1345-1353, 2006; Jin Yu, Taekjip Ha, and Klaus Schulten. *Biophysical J.*, 91:2097-2114, 2006; Markus Dittrich, Jin Yu, and Klaus Schulten. *Topics in Current Chem.*, 2006.



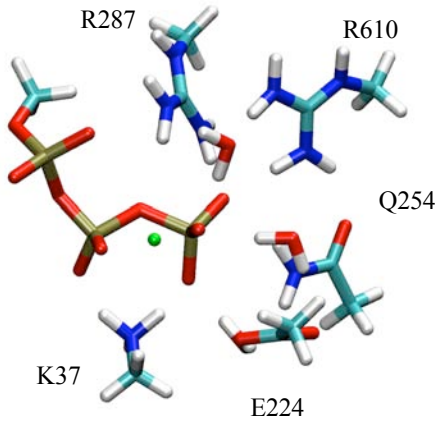
Structure and Sequence Alignment of Catalytic Regions of F_1 and PcrA-Helicase Reveals Amazing Similarity



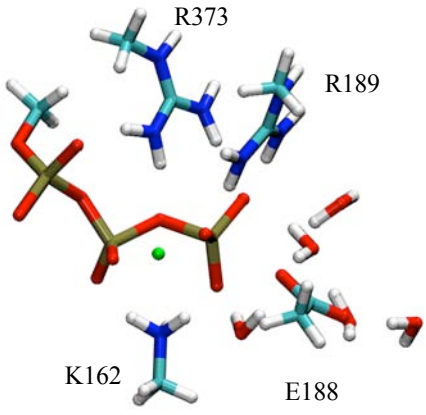
The catalytic regions of F_1's b-subunits and PcrA are RecA-like and align extremely well.

Markus Dittrich and Klaus Schulten. *Structure*, 14:1345-1353, 2006.

Structural Comparison of Catalytic Core Region of F_1 and PcrA-Helicase



catalytic core region of PcrA

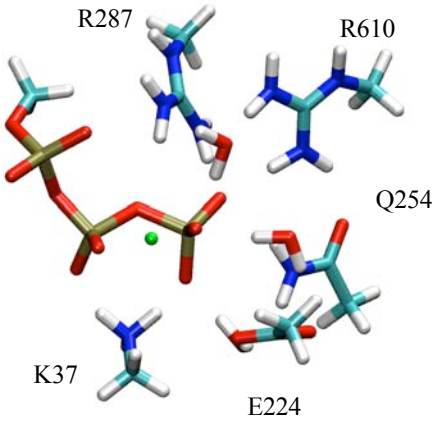


catalytic core region of F_1 b_{TP} subunit

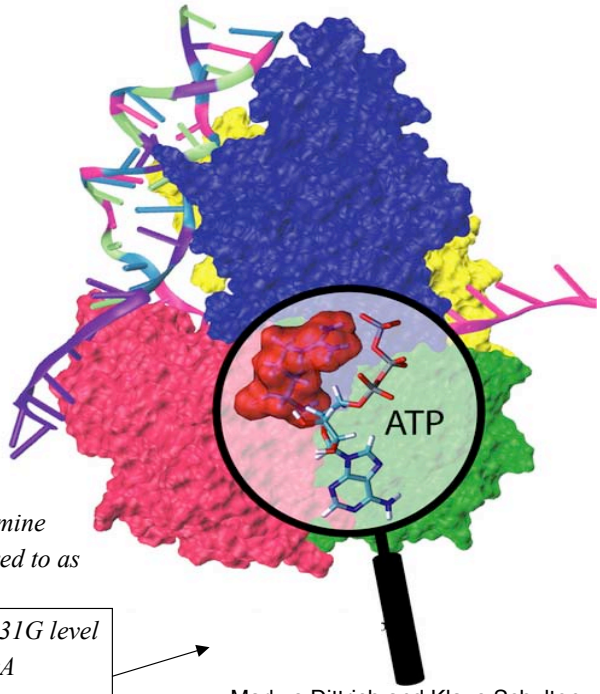
The catalytic region in PcrA has an additional glutamine residue (Q254) close to the gamma-phosphate referred to as the sensor I residue.

Markus Dittrich and Klaus Schulten. *Structure*, 14:1345-1353, 2006; Jin Yu, Taekjip Ha, and Klaus Schulten. *Biophysical J.*, 91:2097-2114, 2006; Markus Dittrich, Jin Yu, and Klaus Schulten. *Topics in Current Chem.*, 2006.

Structural Comparison of Catalytic Core Region of F_1 and PcrA-Helicase



catalytic core region of PcrA

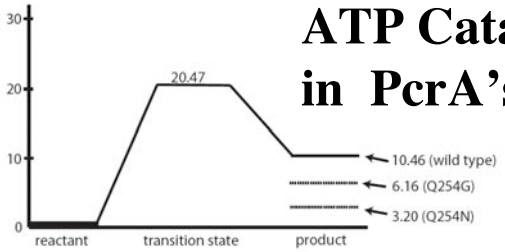


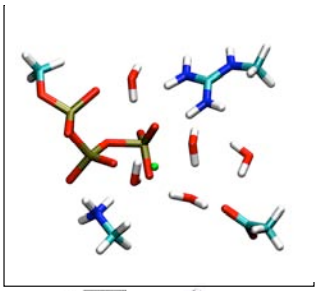
Markus Dittrich and Klaus Schulten. *Structure*, 14:1345-1353, 2006.

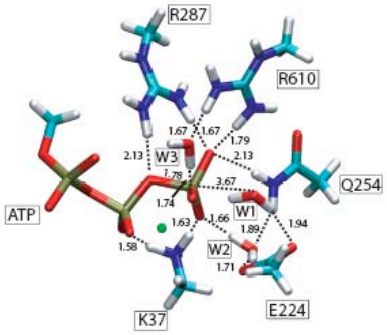
The catalytic region in PcrA has an additional glutamine residue (Q254) close to the gamma-phosphate referred to as the sensor I residue.

We have used QM/MM calculations at the B3LYP/6-31G level to investigate the chemo-mechanical coupling in PcrA (system size: ≃20,000 MM atoms, 77 QM atoms)

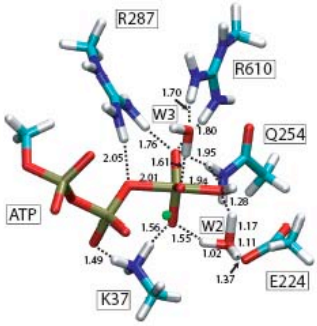
ATP Catalytic Pathway in PcrA's binding site



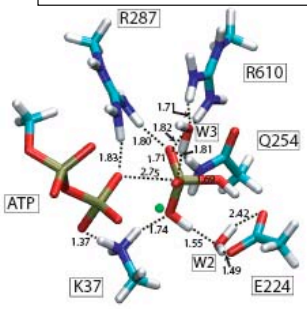




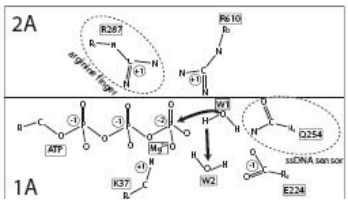
reactant state

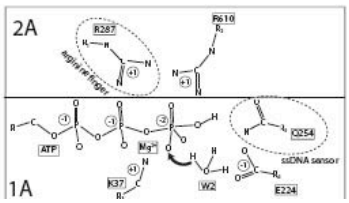


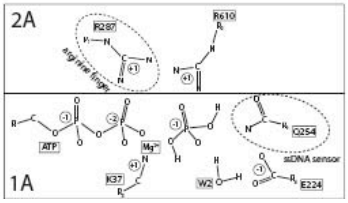
transition state



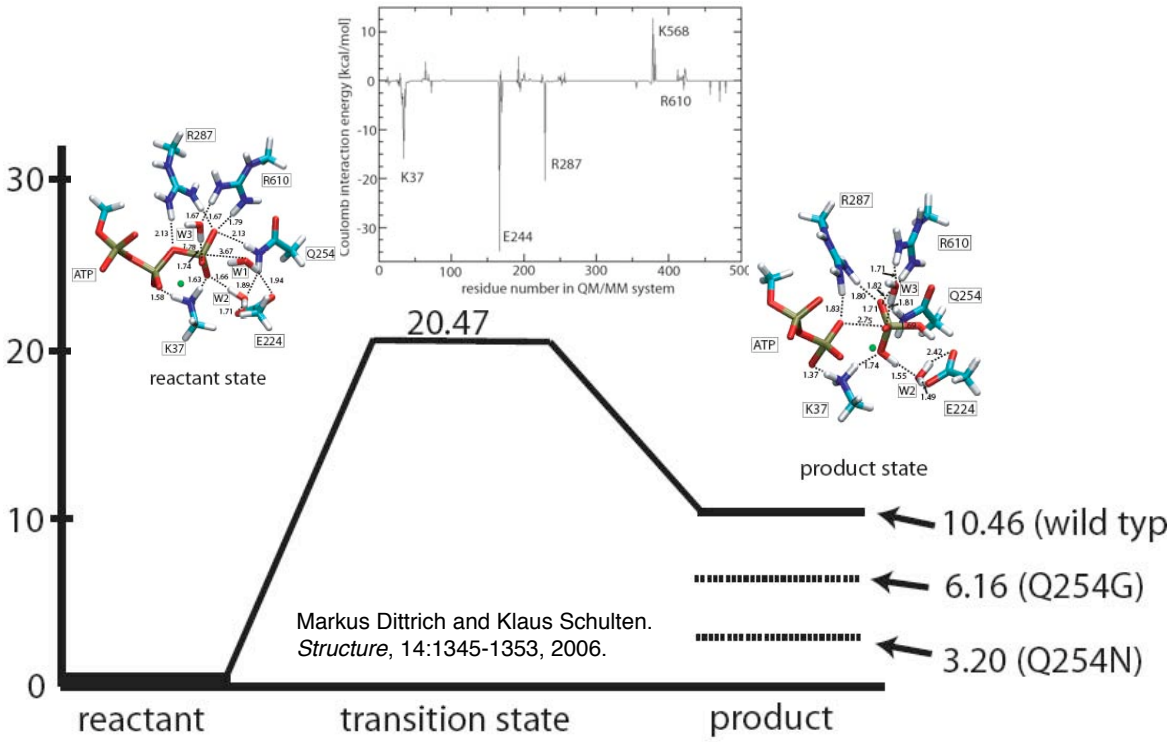
product state



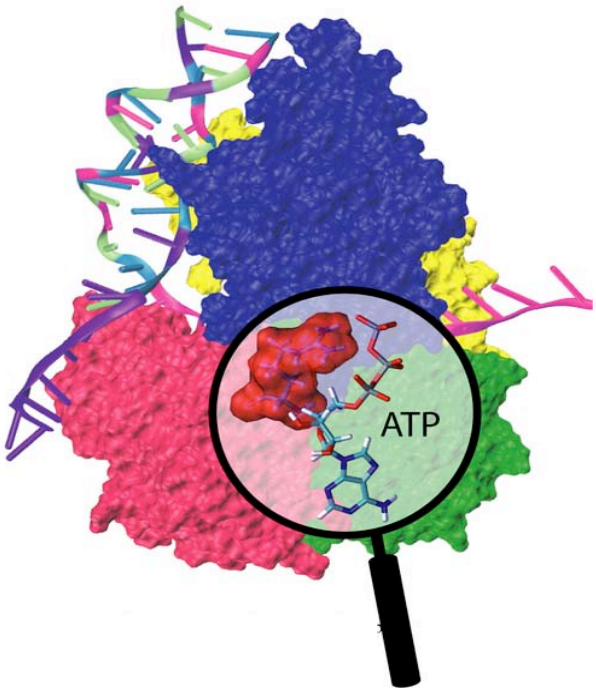




Coupling of PcrA to its Catalytic Site

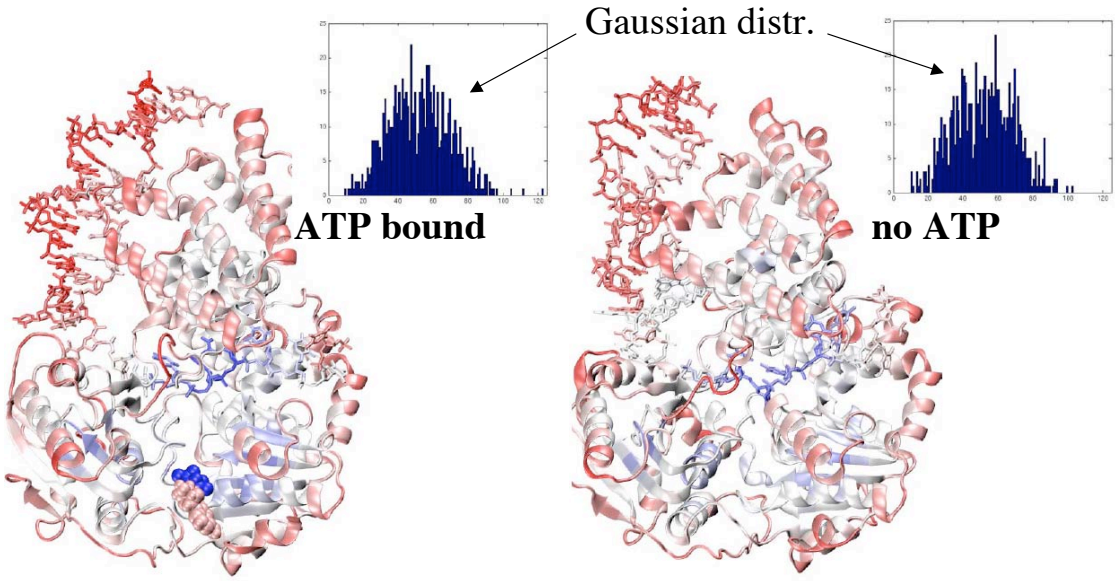


Let us look now at the entire system!



Connection Degree in Elastic Network Model of PcrA

(number of neighboring C_α or P atoms within 15 Angstroms)



Neighbor geometry similar for both states of PcrA

Markus Dittrich and Klaus Schulten. *Structure*, 14:1345-1353, 2006; Jin Yu, Taekjip Ha, and Klaus Schulten. *Biophysical J.*, 91:2097-2114, 2006; Markus Dittrich, Jin Yu, and Klaus Schulten. *Topics in Current Chem.*, 2006.

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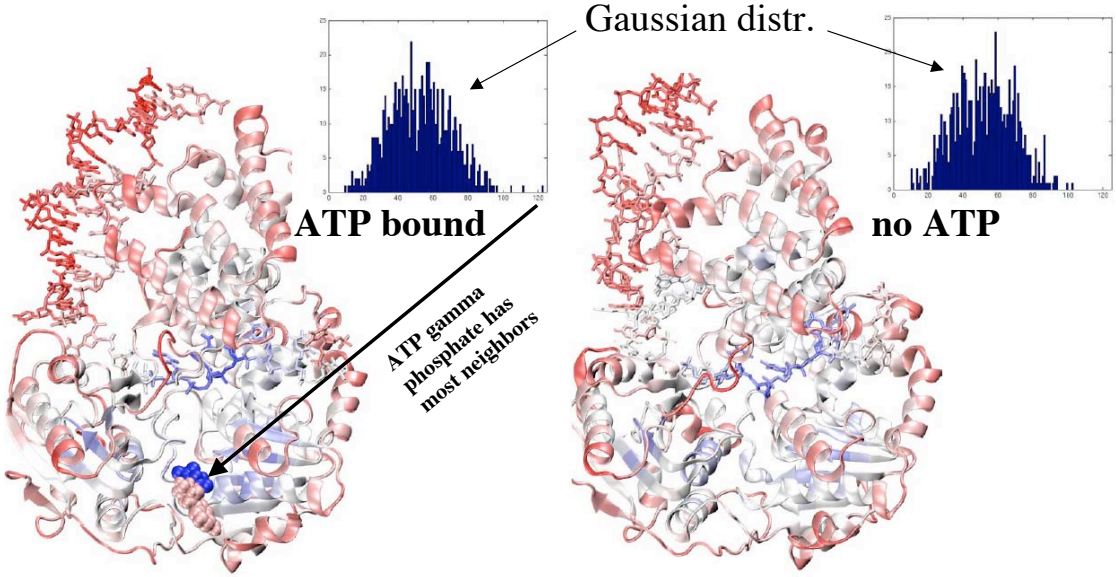

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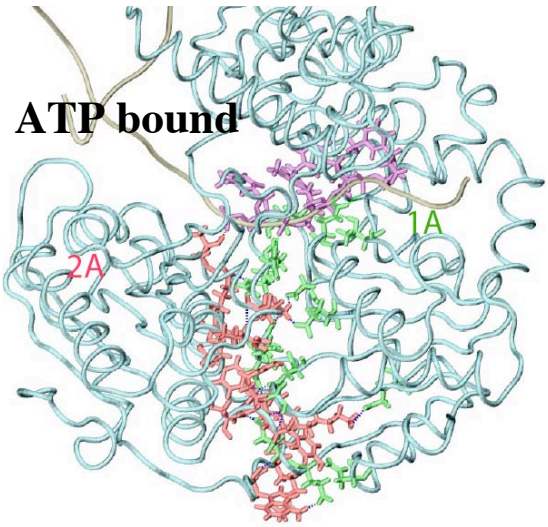
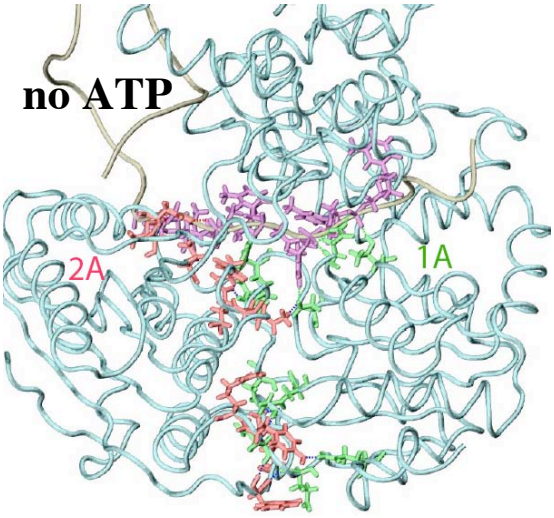
Hydrogen Bonding Associations in PcrA as Detected through MD





number of hydrogen bonds between protein and ssDNA = 8 (1A -> 6; 2A -> 1)

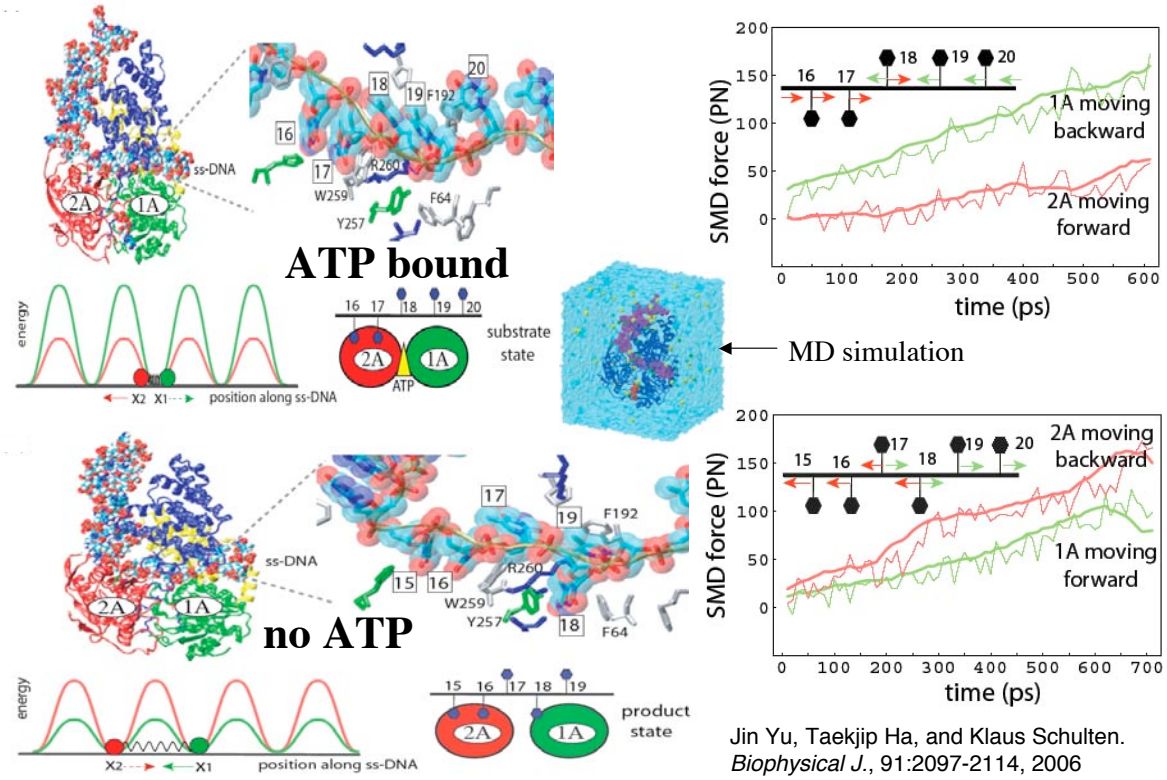
number of hydrogen bonds between 1A and 2A = 18

number of hydrogen bonds between protein and ssDNA = 14 (1A -> 5; 2A -> 5, but lower energy)

number of hydrogen bonds between 1A and 2A = 13

(energy cut-off = - 1kcal/mol; calculated using FLEXWEB)

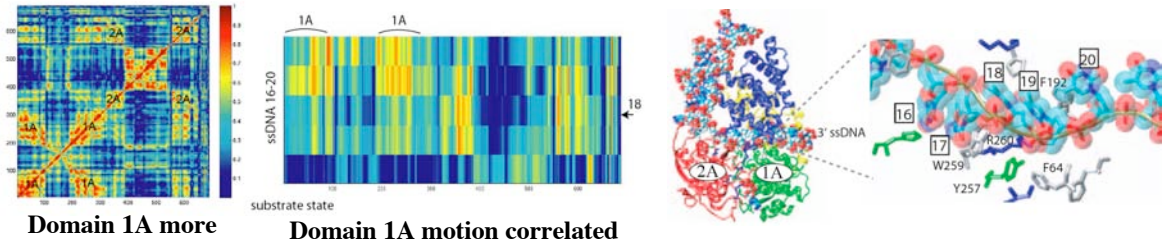
Molecular Motor Helicase



Jin Yu, Taekjip Ha, and Klaus Schulten. *Biophysical J.*, 91:2097-2114, 2006

Correlation of Motion in PcrA as Detected in MD Simulations

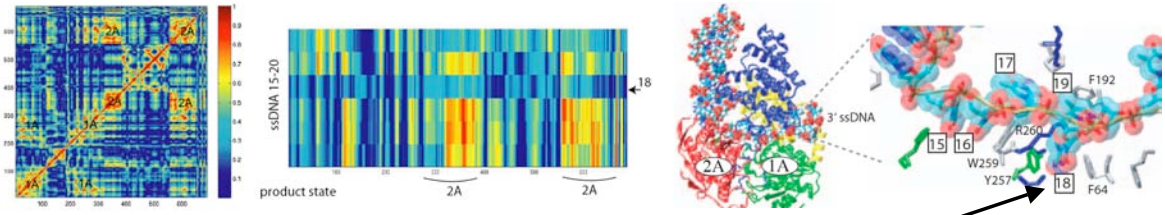
Correlation matrix **with ATP bound**



Domain 1A more collective than 2A

Domain 1A motion correlated with ssDNA 19, 20

Correlation matrix **without ATP**



Domain 2A more collective than 1A

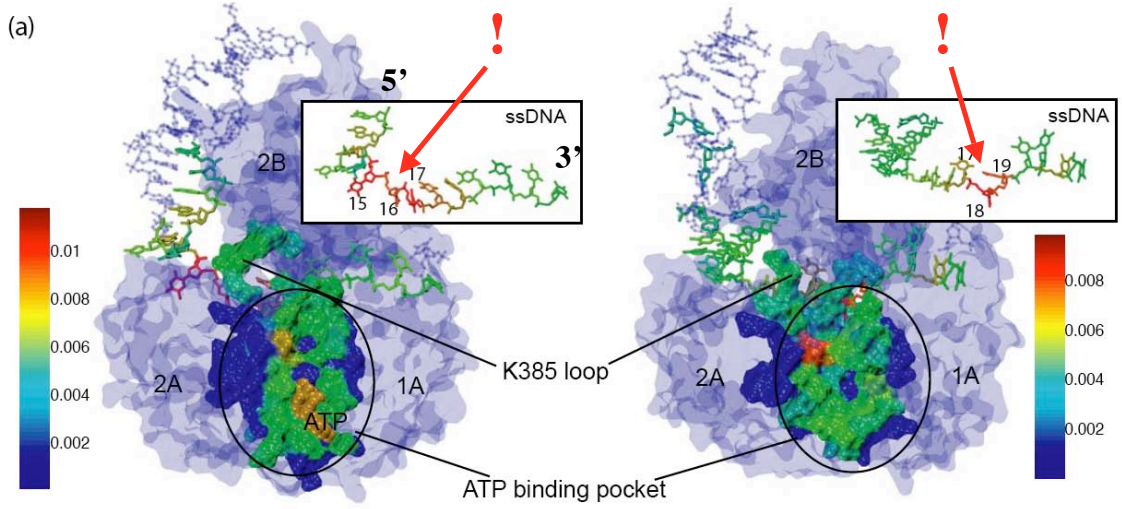
Domain 2A motion correlated with ssDNA 15-17

ssDNA 18 uncorrelated

Markus Dittrich and Klaus Schulten. *Structure*, 14:1345-1353, 2006; Jin Yu, Taekjip Ha, and Klaus Schulten. *Biophysical J.*, 91:2097-2114, 2006; Markus Dittrich, Jin Yu, and Klaus Schulten. *Topics in Current Chem.*, 2006.

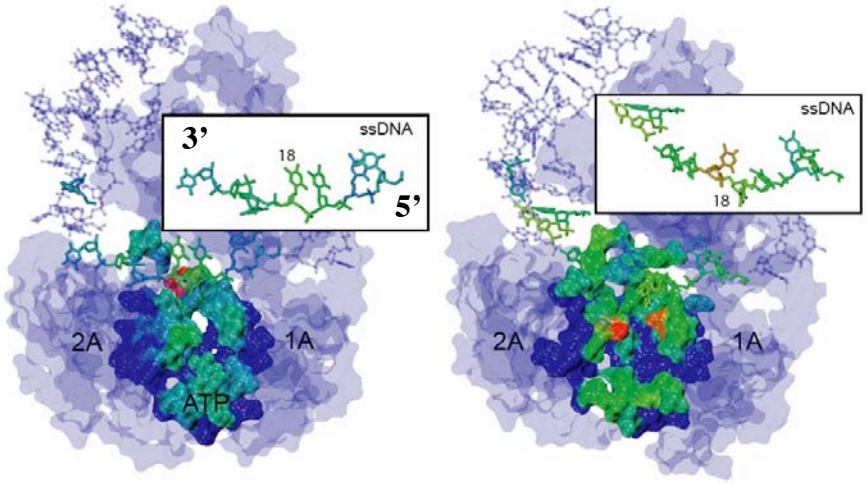
Dynamic Coupling Analysis
Based on Elastic Network Model

(poly-T DNA, 5' -> 3')



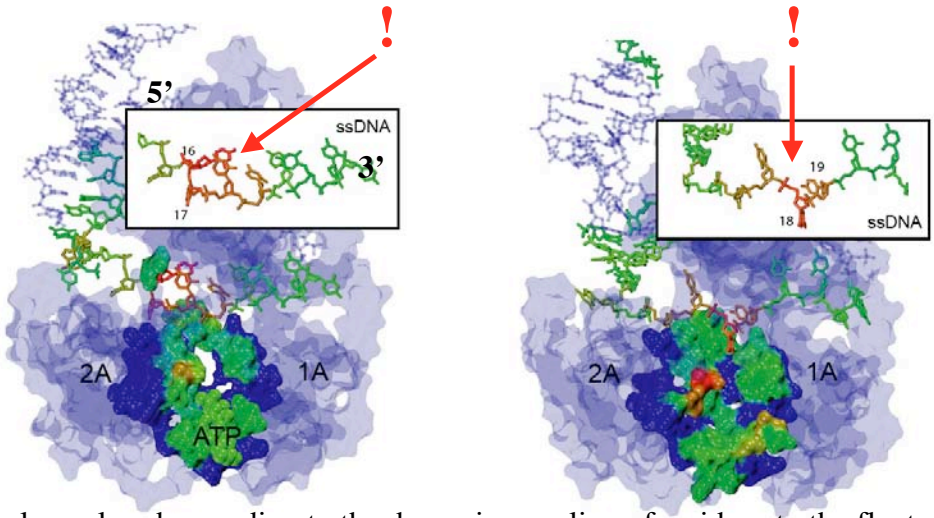
Complex colored according to the dynamic coupling of residues to the fluctuation of the ATP binding pocket; the dynamic coupling is probed through perturbation of a residue's spring constant and monitoring the ensuing effect on the vibrational fluctuation of the ATP binding pocket.

Dynamic Coupling Analysis
Based on Elastic Network Model
(poly-T DNA, 3' -> 5')



Complex colored according to the dynamic coupling of residues to the fluctuation of the ATP binding pocket; the dynamic coupling is probed through perturbation of a residue's spring constant and monitoring the ensuing effect on the vibrational fluctuation of the ATP binding pocket.

Dynamic Coupling Analysis
Based on Elastic Network Model
(poly-C DNA, 5' -> 3')



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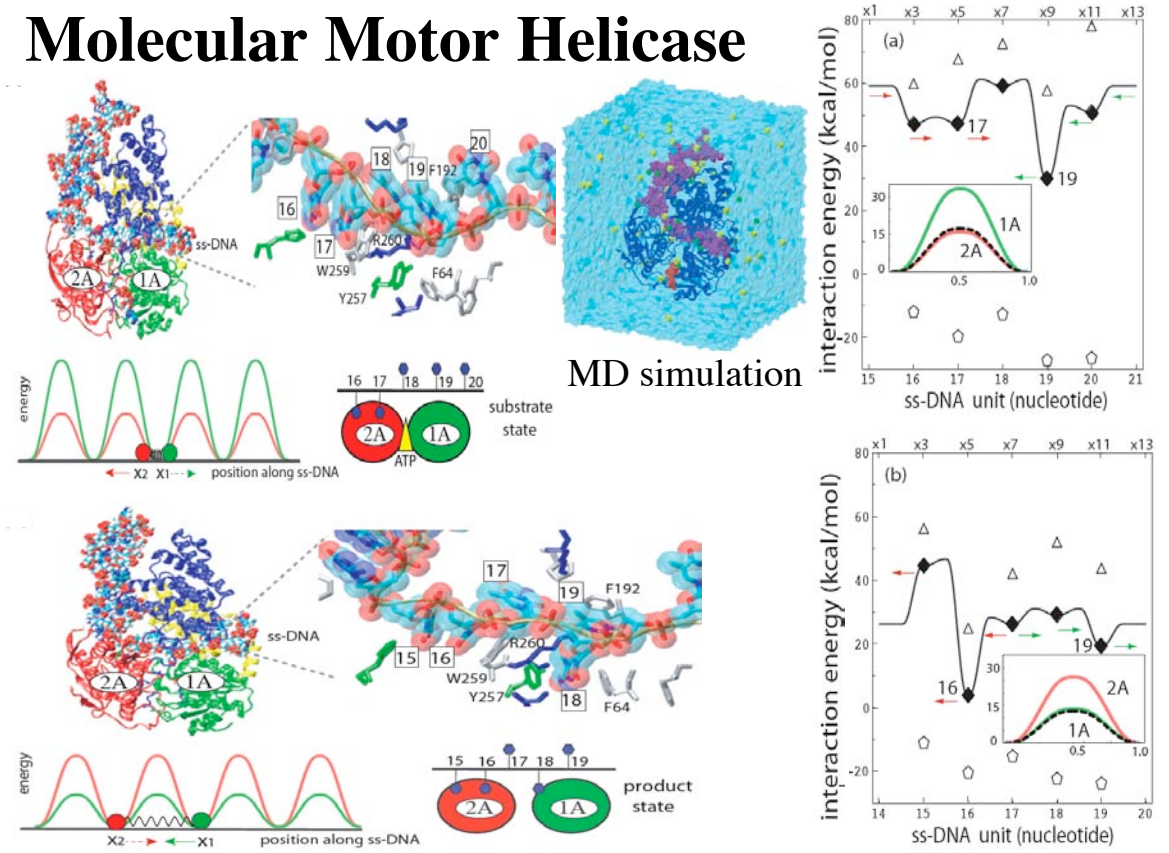
Molecular Motor Helicase



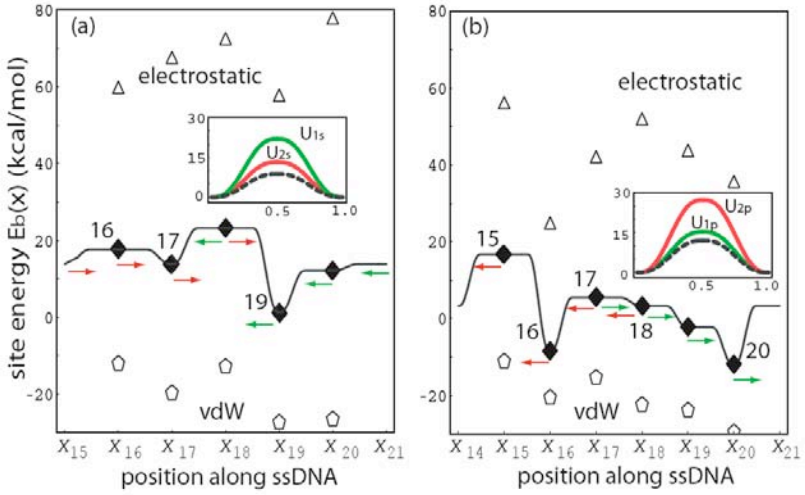
Determination of Effective Potential for ssDNA Motion

$$A_{2p} = E_b(x_{17}) - E_b(x_{18}) + E_b(x_{15}) - E_b(x_{16}) + 4\delta$$

$$A_{1p} = E_b(x_{18}) - E_b(x_{20}) + 4\delta$$

$$A_{2s} = E_b(x_{18}) - E_b(x_{17}) + E_b(x_{16}) - E_b(x_{17}) + 4\delta$$

$$A_{1s} = E_b(x_{18}) - E_b(x_{19}) + 4\delta$$



Stochastic Model

$$m\,\ddot{x} = -\gamma\dot{x} + \tilde{f}(t)$$

$$\langle \tilde{f}(t) \rangle = 0 \qquad \langle \tilde{f}(t)\,\tilde{f}(t') \rangle = 2\gamma k_B T \delta(t-t')$$

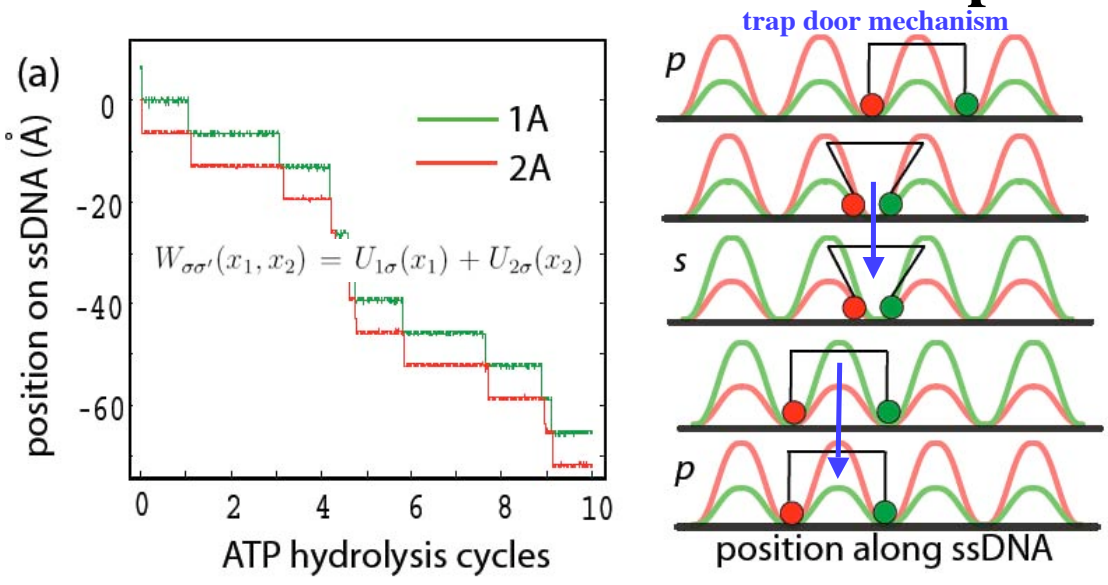
$$x_i(t+\Delta t) = x_i(t) - \frac{1}{\gamma}\frac{\partial W_{\sigma\sigma'}(x_1,x_2)}{\partial x_i}\Delta t + \sqrt{2D\,\Delta t}Z$$

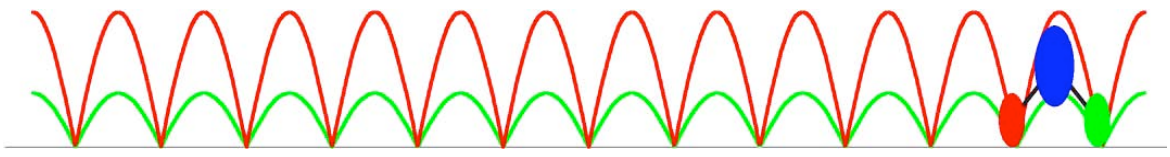
$$W_{\sigma\sigma'}(x_1,x_2) = U_{1\sigma}(x_1) + U_{2\sigma}(x_2) + V_{\sigma'}(x_1,x_2)$$

$$U_{2s}(\Delta x) = \sum_{i=15}^{18} E_b(x_i+\Delta x) - E_b(x_i) \quad \Delta x \in [0, 0.5]$$

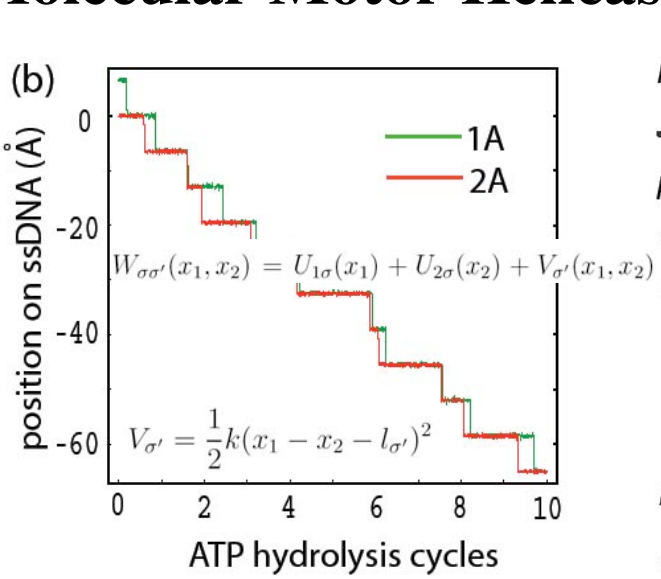
Markus Dittrich and Klaus Schulten. *Structure*, 14:1345-1353, 2006; Jin Yu, Taekjip Ha, and Klaus Schulten. *Biophysical J.*, 91:2097-2114, 2006; Markus Dittrich, Jin Yu, and Klaus Schulten. *Topics in Current Chem.*, 2006.

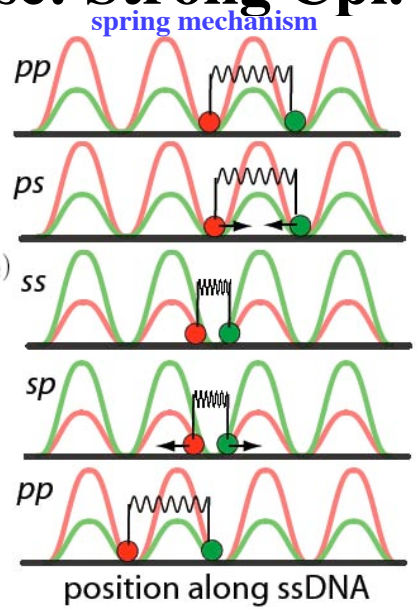
Molecular Motor Helicase: Weak Cpl.

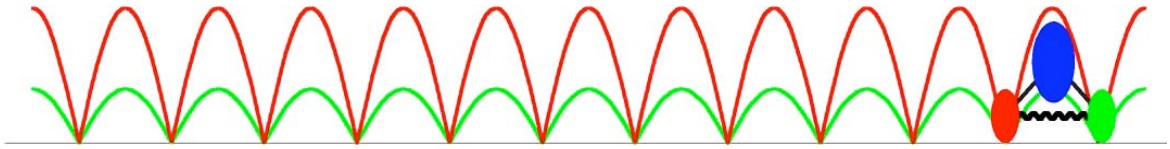




Molecular Motor Helicase: Strong Cpl.

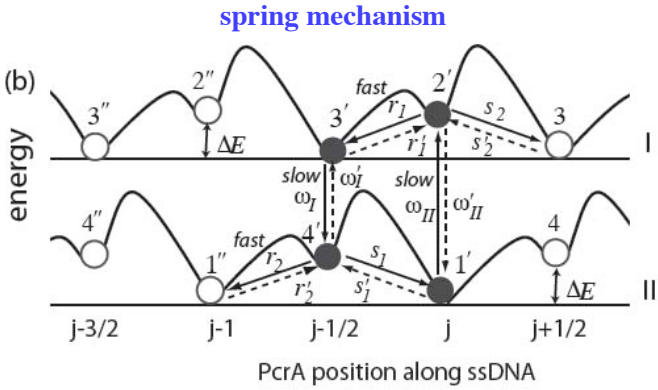


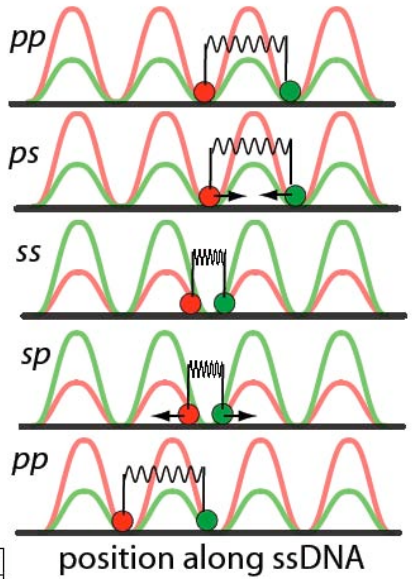




Kinetic Model for Strong Coupling

I = ATP bound II = free



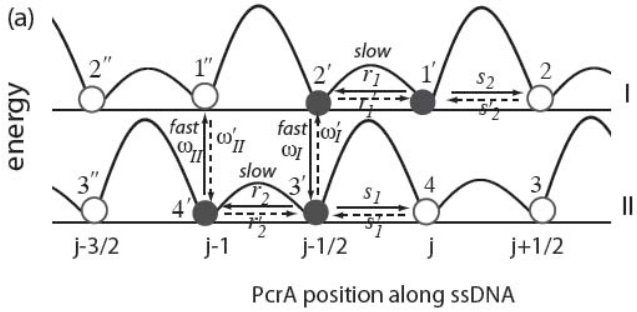


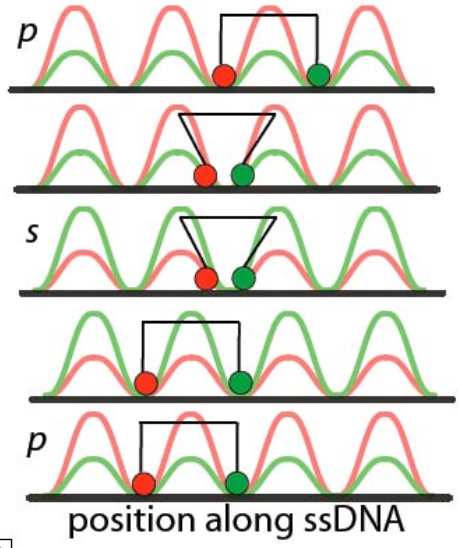
time	physical correspondence	weak coupling	strong coupling
$1/r_1$	1A moving forward	19 ms	0.3 ms
$1/s_2$	2A moving backward	3×10^6 s	4×10^4 s
$1/r_2$	2A moving forward	0.8 ms	0.03 ms
$1/s_1$	1A moving backward	6×10^2 s	14 ms
$1/\omega_I$	no domain movement	100 ns (assumed)	15.4 ms
$1/\omega_{II}$	no domain movement	100 ns (assumed)	4.6 ms

Kinetic Model for Weak Coupling

I = ATP bound II = free

trap door mechanism





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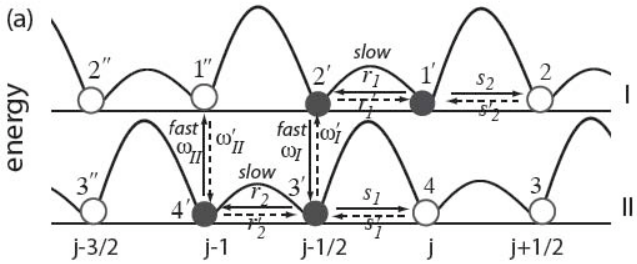
Kinetic Model

$$
\begin{aligned}
\dot{P}_{1'} &= J_{1'4'} + J_{1'4} + J_{1'2'} \\
\dot{P}_{2'} &= J_{2'3'} + J_{2'3} + J_{2'1'} \\
\dot{P}_{3'} &= J_{3'2'} + J_{3'2''} + J_{3'4'} \\
\dot{P}_{4'} &= J_{4'1'} + J_{4'1''} + J_{4'3'}
\end{aligned}
$$



$$J_{\mu\nu} = k(\mu \leftarrow \nu)\, P_\nu - k(\nu \leftarrow \mu)\, P_\mu$$

Under non-stationary conditions one need to solve

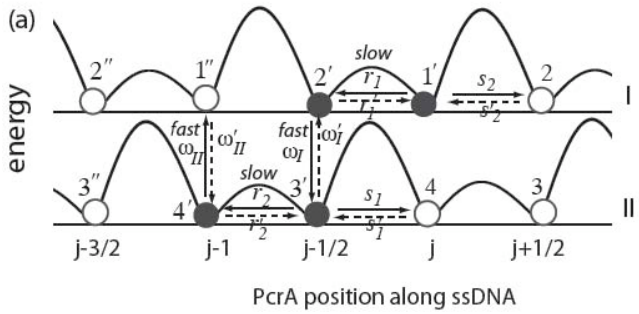
$$\dot{\mathbf{P}} = \mathbf{K}\,\mathbf{P}$$

$$\mathbf{P}^T = (\ldots P_1\, P_2\, P_3\, P_4\, P_{1'}, P_{2'}, P_{3'}, P_{4'}, \ldots)$$

Kinetic Model

Under stationary conditions
One solves:

$$\mathbf{M}\tilde{\mathbf{P}} = 0$$



$$\mathbf{M} = \begin{pmatrix} -r_2 - s_1 - \omega'_{II} & \omega_{II} & r'_2 + s'_1 & 0 \\ \omega'_{II} & -r'_1 - s'_2 - \omega_{II} & 0 & r_1 + s_2 \\ r_2 + s_1 & 0 & -r'_2 - s'_1 - \omega_I & \omega'_I \\ 0 & r'_1 + s'_2 & \omega_I & -r_1 - s_2 - \omega'_I \end{pmatrix}$$

Flux / Translocation Speed

$$J_{2'3} = J_{32} \ldots = J^I \qquad J_{4''1''} = J_{1''4'} = J_{4'1'} = J_{1'4} = J_{41} \ldots = J^{II}$$

$$v = -(r_1 r_2 - s_1 s_2) C \qquad A_{2p} > A_{1p} \text{ and } A_{2s} < A_{1s}$$

Flux / Translocation Speed

$$v = -(r_1 r_2 - s_1 s_2) C \qquad C \text{ is positive}$$

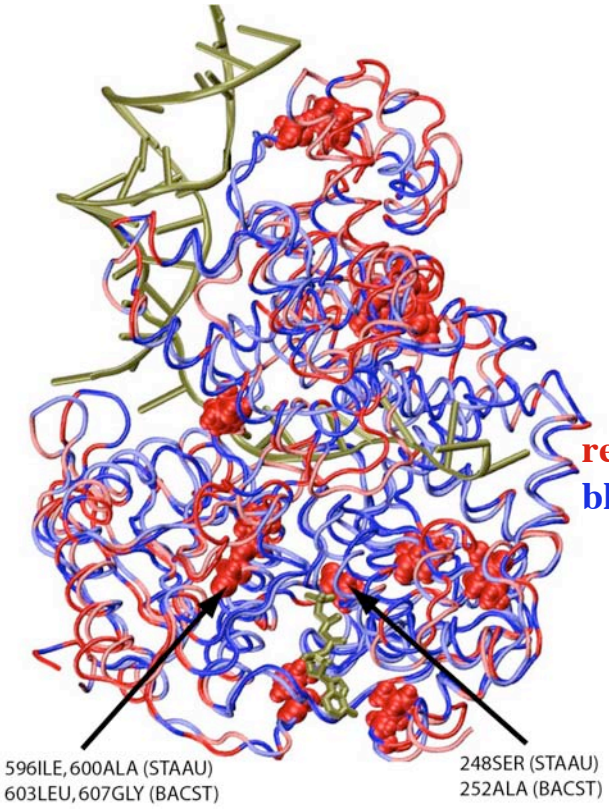
$$A_{2p} > A_{1p} \text{ and } A_{2s} < A_{1s}$$

$$A_{2p} = E_b(x_{17}) - E_b(x_{18}) + E_b(x_{15}) - E_b(x_{16}) + 4\delta$$
$$A_{1p} = E_b(x_{18}) - E_b(x_{20}) + 4\delta$$

$$A_{2s} = E_b(x_{18}) - E_b(x_{17}) + E_b(x_{16}) - E_b(x_{17}) + 4\delta$$
$$A_{1s} = E_b(x_{18}) - E_b(x_{19}) + 4\delta$$

Translates into molecular mechanism

Identification of Amino Acids Essential for PcrA Directionality

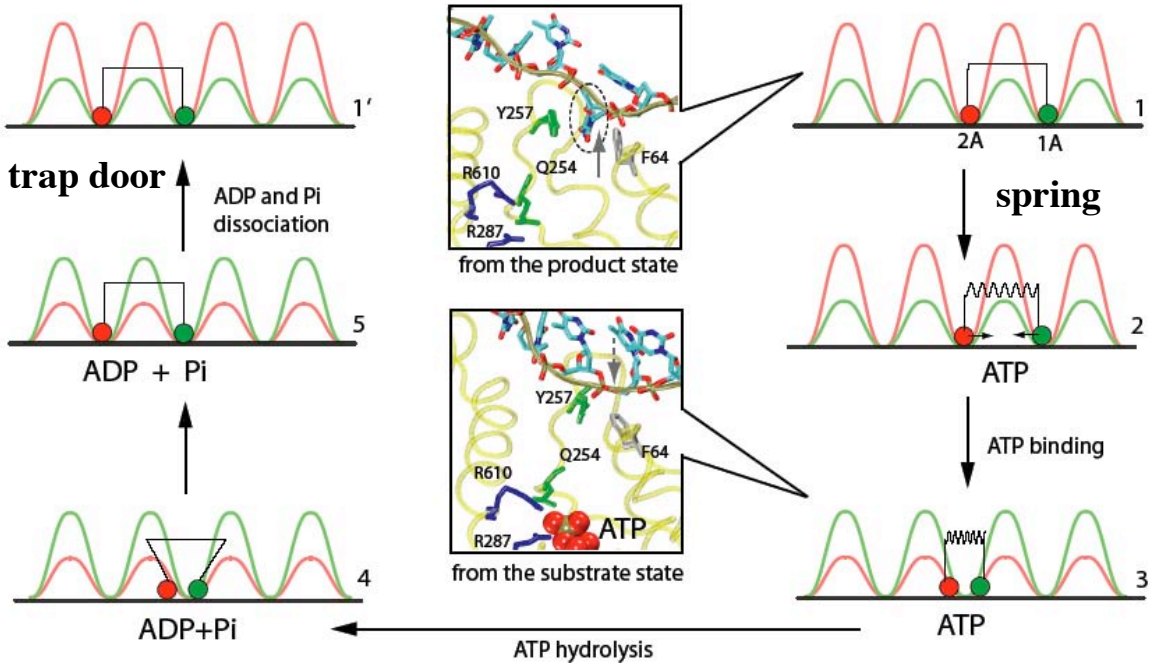


Comparision of
PcrA-STAAU (bipolar)
PcrA-BACST (unipolar)

red - light red : variant AAs
blue - light blue : conserved AAs

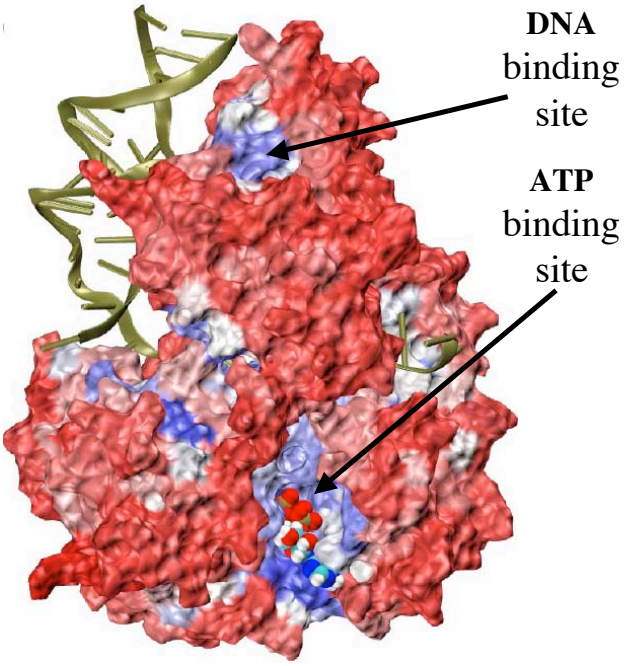
Mutations of these AAs might reverse polarity of PcrA!

Combined Model: spring + trap door

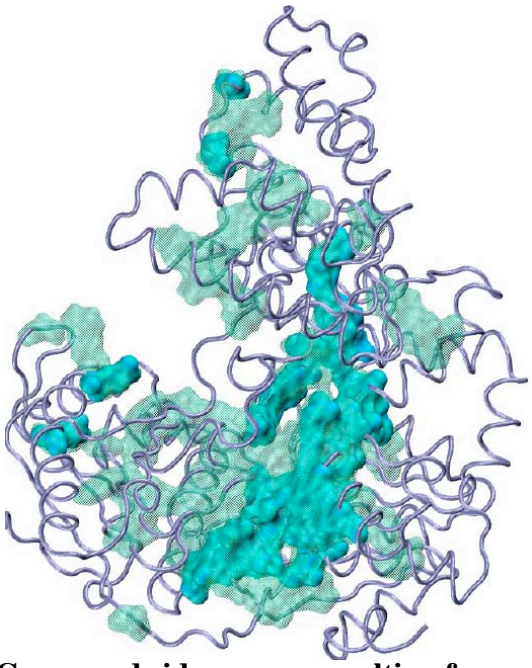


Markus Dittrich and Klaus Schulten. *Structure*, 14:1345-1353, 2006; Jin Yu, Taekjip Ha, and Klaus Schulten. *Biophysical J.*, 91:2097-2114, 2006; Markus Dittrich, Jin Yu, and Klaus Schulten. *Topics in Current Chem.*, 2006.

Sequence Analysis of PcrA to Identify Conserved Side Groups

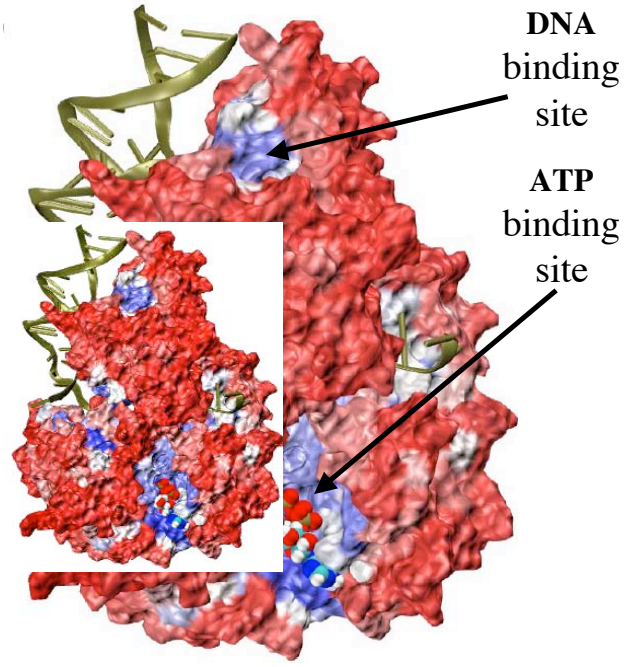


Conserved (blue) side groups resulting from comparison of PcrA+Rep+UvrD helicases

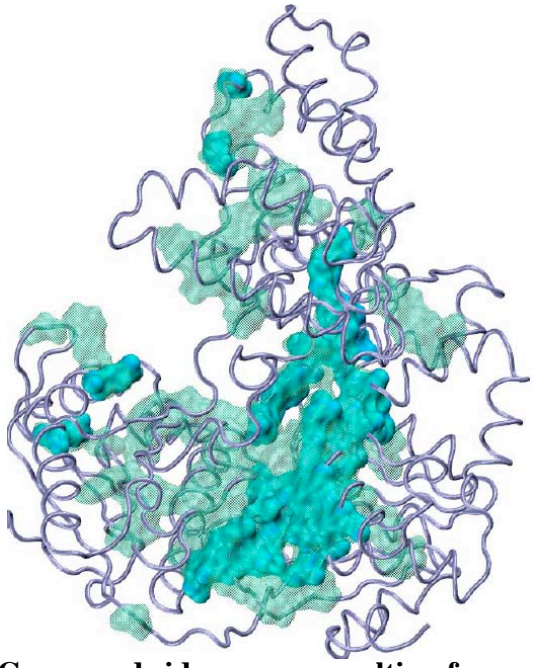


Conserved side groups resulting from comparison of PcrA+Rep+UvrD (dark) and PcrA (light) helicases

Sequence Analysis of PcrA to Identify Conserved Side Groups



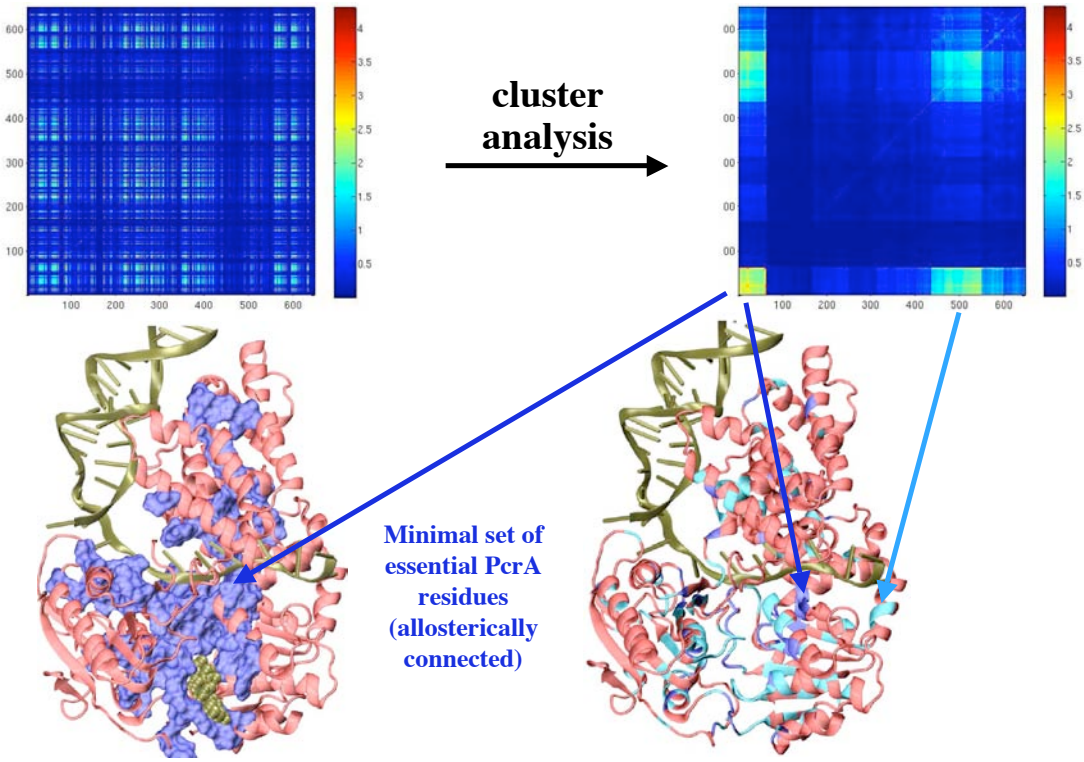
Conserved (blue) side groups resulting from comparison of PcrA+Rep+UvrD helicases



Conserved side groups resulting from comparison of PcrA+Rep+UvrD (dark) and PcrA (light) helicases

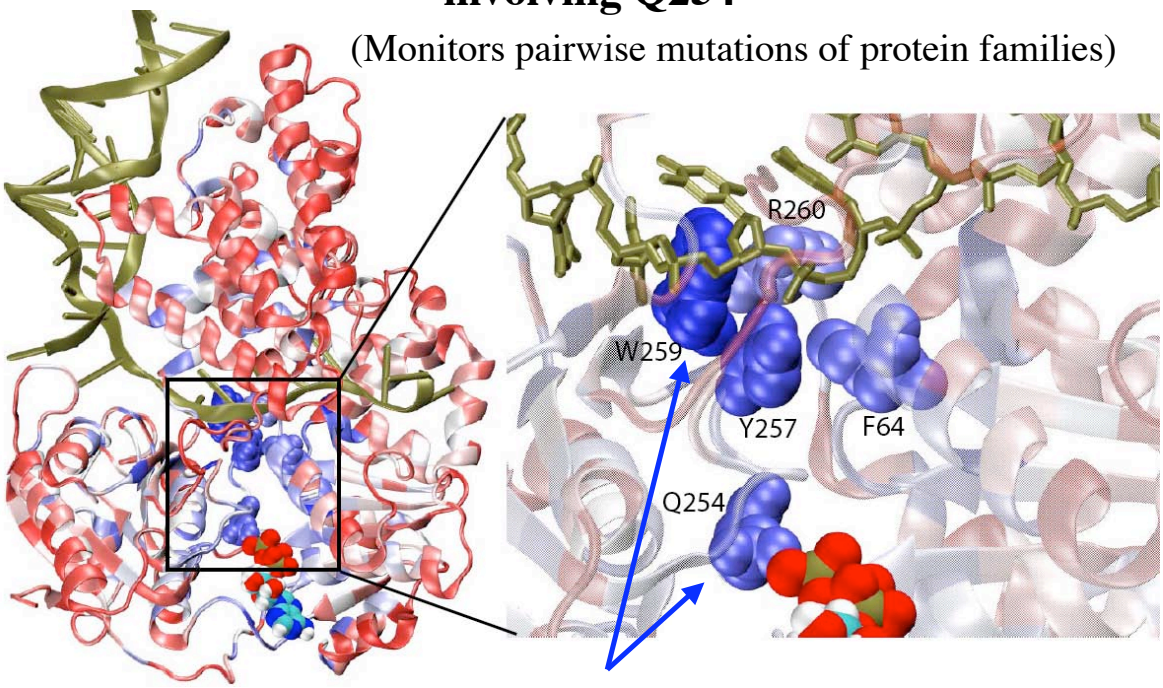
Co-evolution Statistical Coupling Analysis

(Monitors pairwise mutations of protein families)



Co-evolution Statistical Coupling Analysis involving Q254

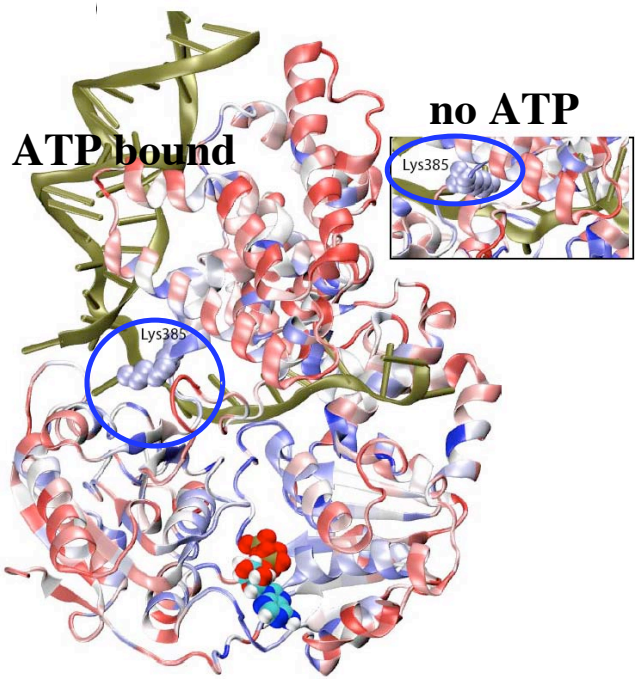
(Monitors pairwise mutations of protein families)



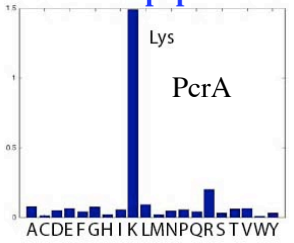
Co-Evolution partners of Q254 are clustered near ssDNA

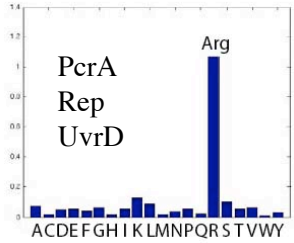
Co-evolution Statistical Coupling Analysis involving Lys385

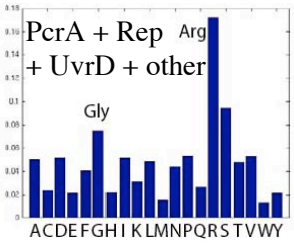
(Monitors pairwise mutations of protein families)



population of Lys385 site





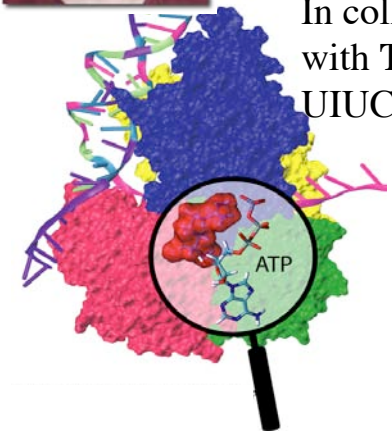


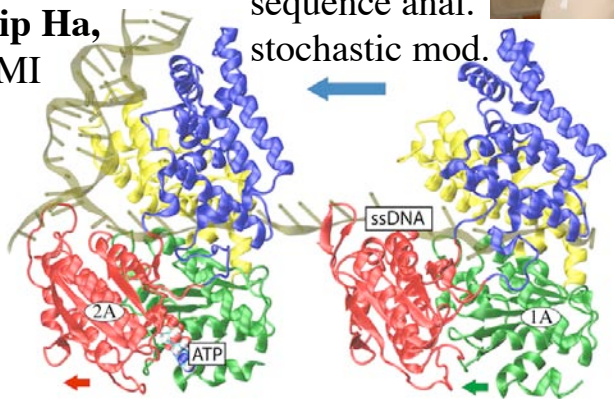
Acknowledgments

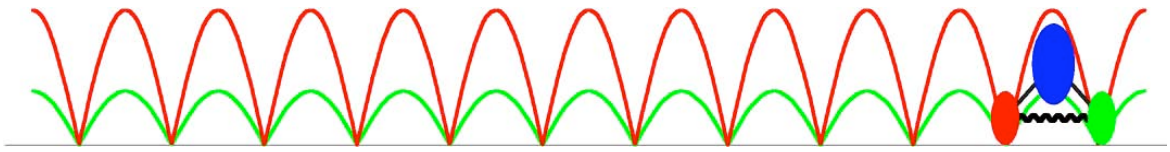
Markus Dittrich
QM/MM calc.

Jin Yu
Molecular dynamics sim.
sequence anal.
stochastic mod.

In collaboration with **Taekjip Ha,** UIUC, HHMI







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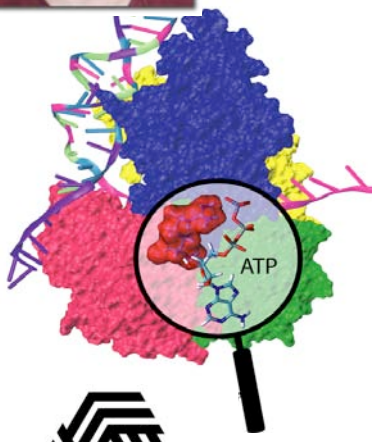

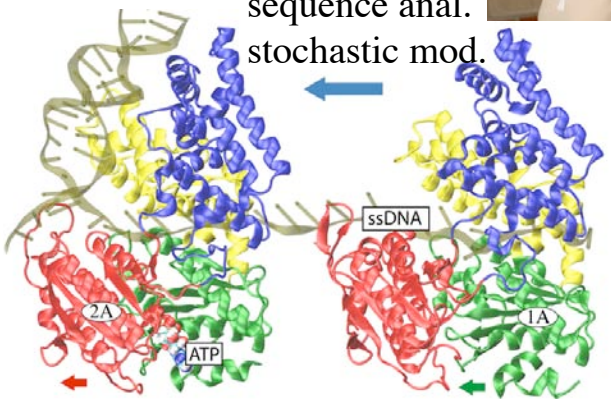

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Markus Dittrich and Klaus Schulten. *Structure*, 14:1345-1353, 2006; Jin Yu, Taekjip Ha, and Klaus Schulten. *Biophysical J.*, 91:2097-2114, 2006; Markus Dittrich, Jin Yu, and Klaus Schulten. *Topics in Current Chem.*, 2006.