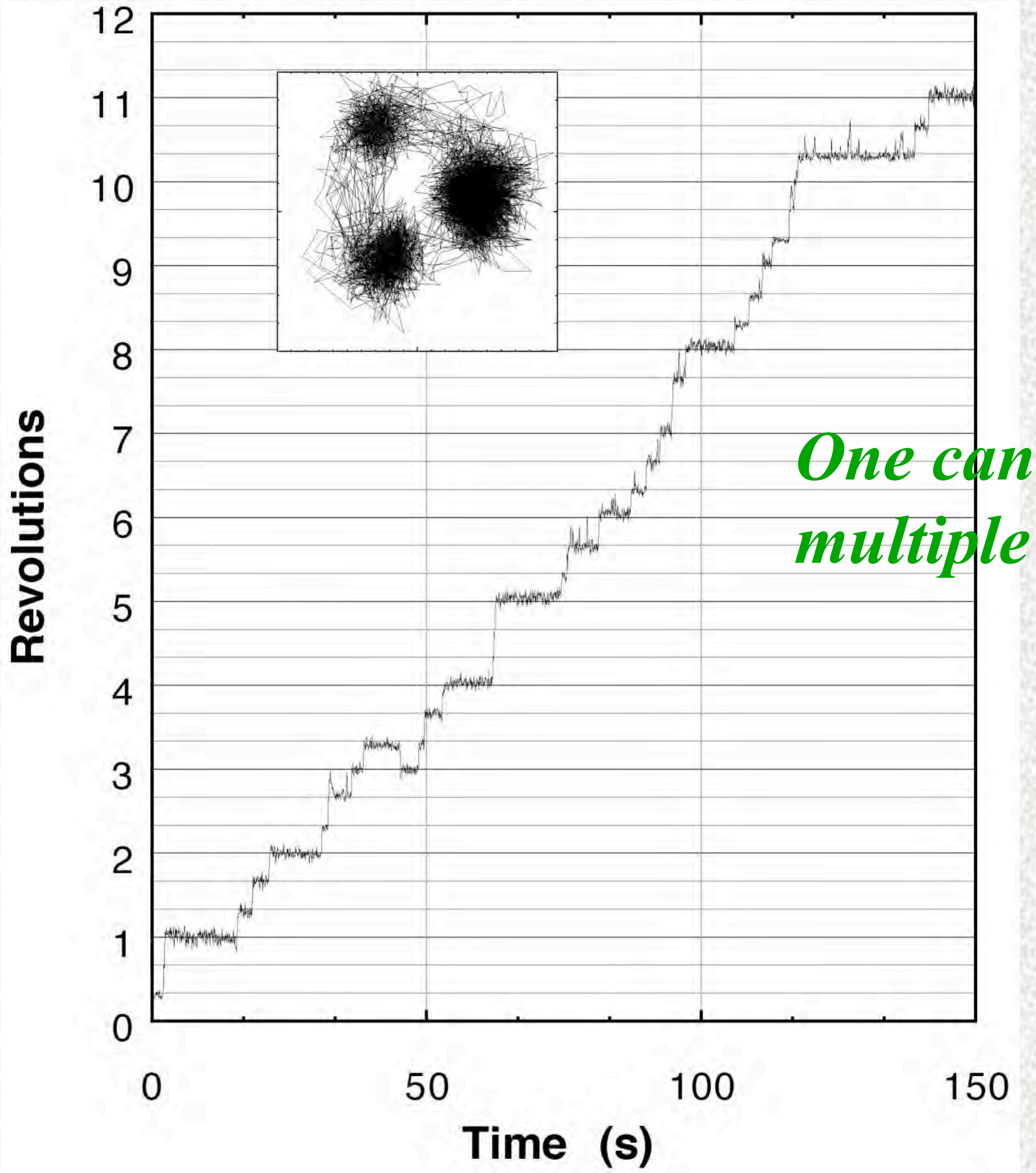
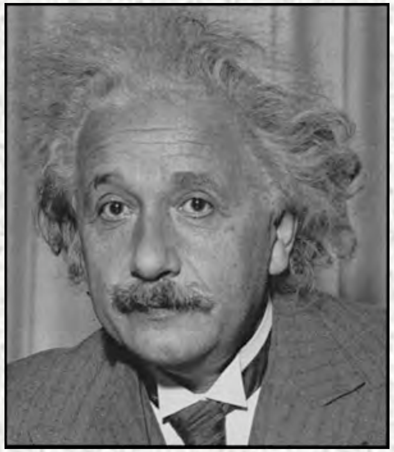


Stepwise Rotation at 20 nM ATP

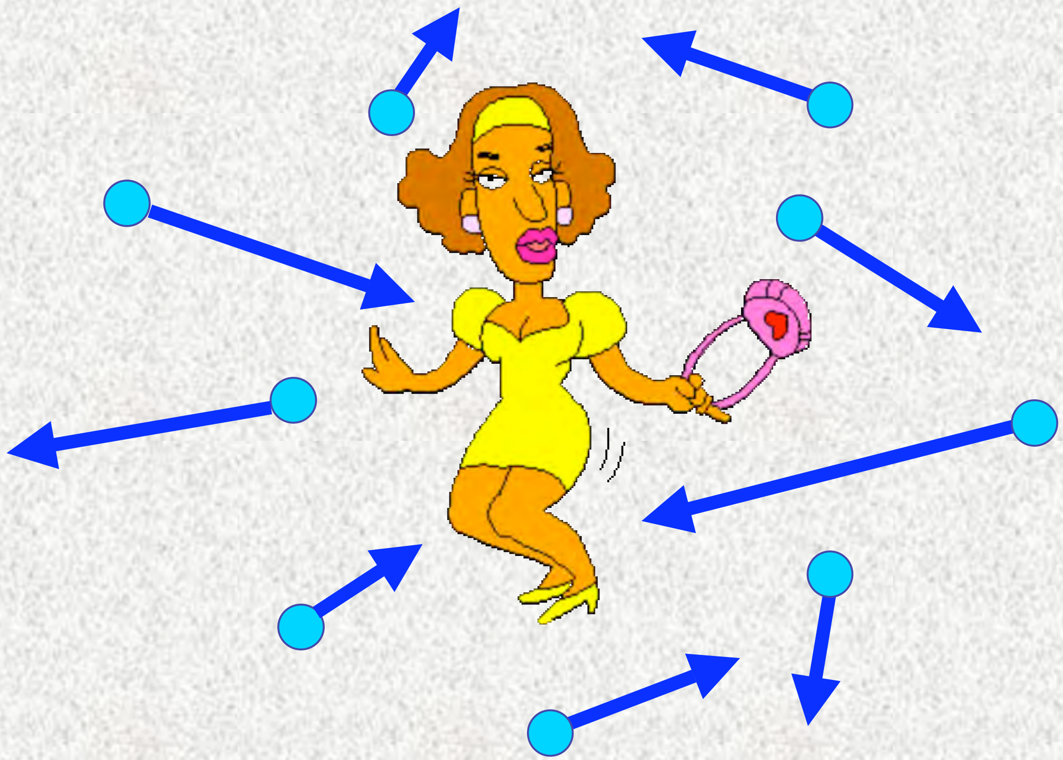
One can never synchronize multiple molecular machines.



A. Einstein

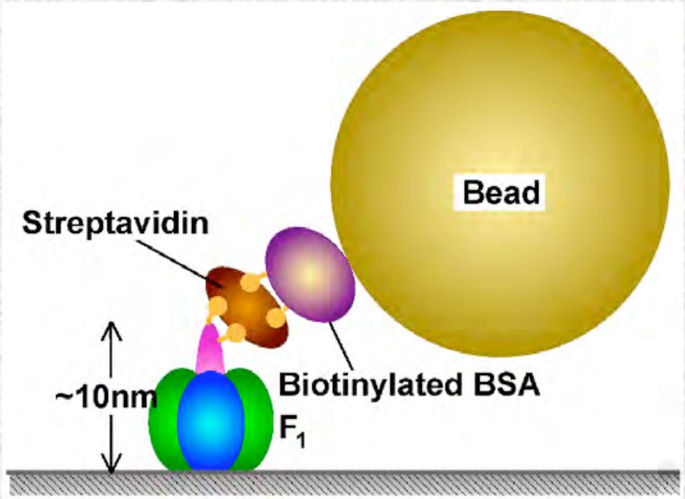
R. Yasuda & H. Noji

Water Molecules Are Bullets



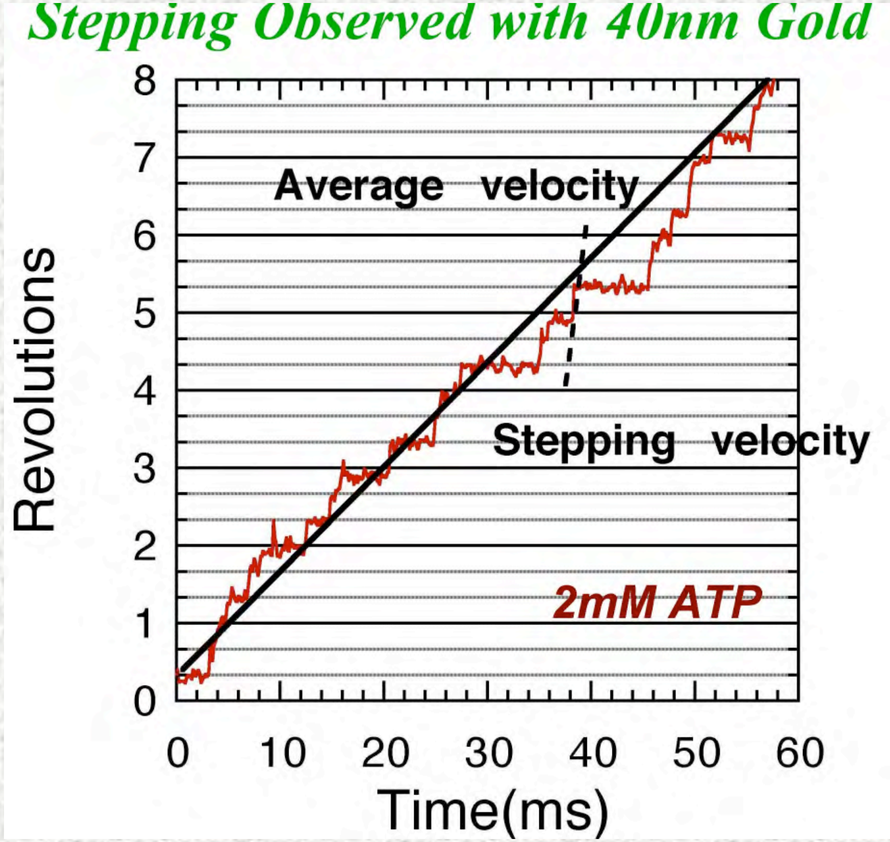
If you are a protein molecule,
water molecules surrounding you are
gun bullets in size and weight.
There average speed is 1000 km/h !
The speed of a jumbo jet, or of a real bullet from a gun.

Imaging Fast Rotation with a Nanobead(s)



40 nm gold particle

[ATP] = 2 mM
Slow replay (1/267)

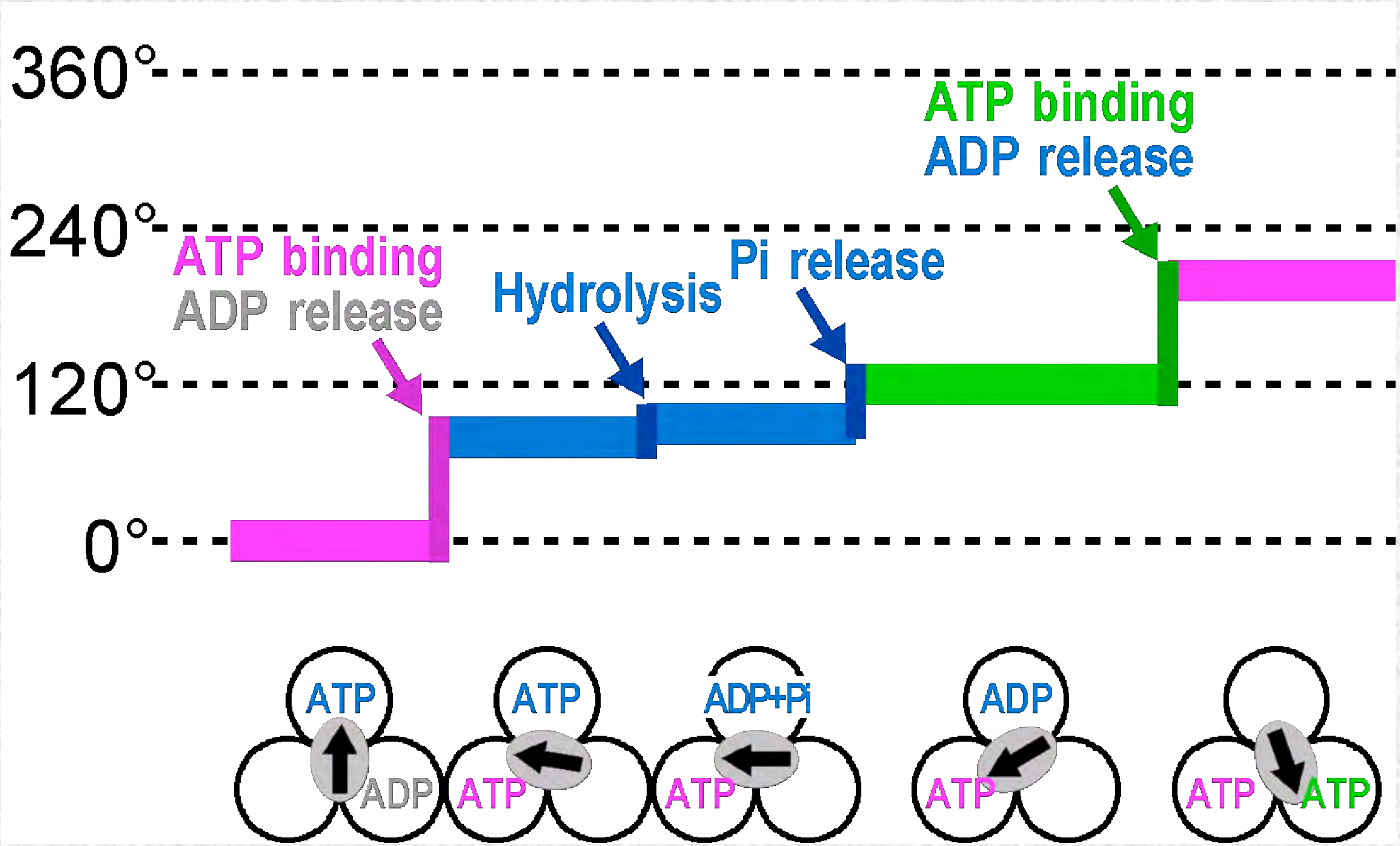


Average velocity:
130 rps = 8,000 rpm
Stepping velocity > 100,000 rpm

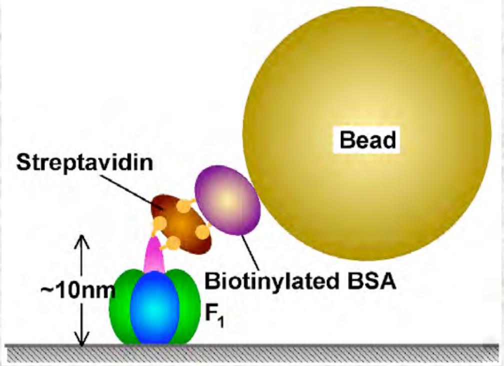
R. Yasuda

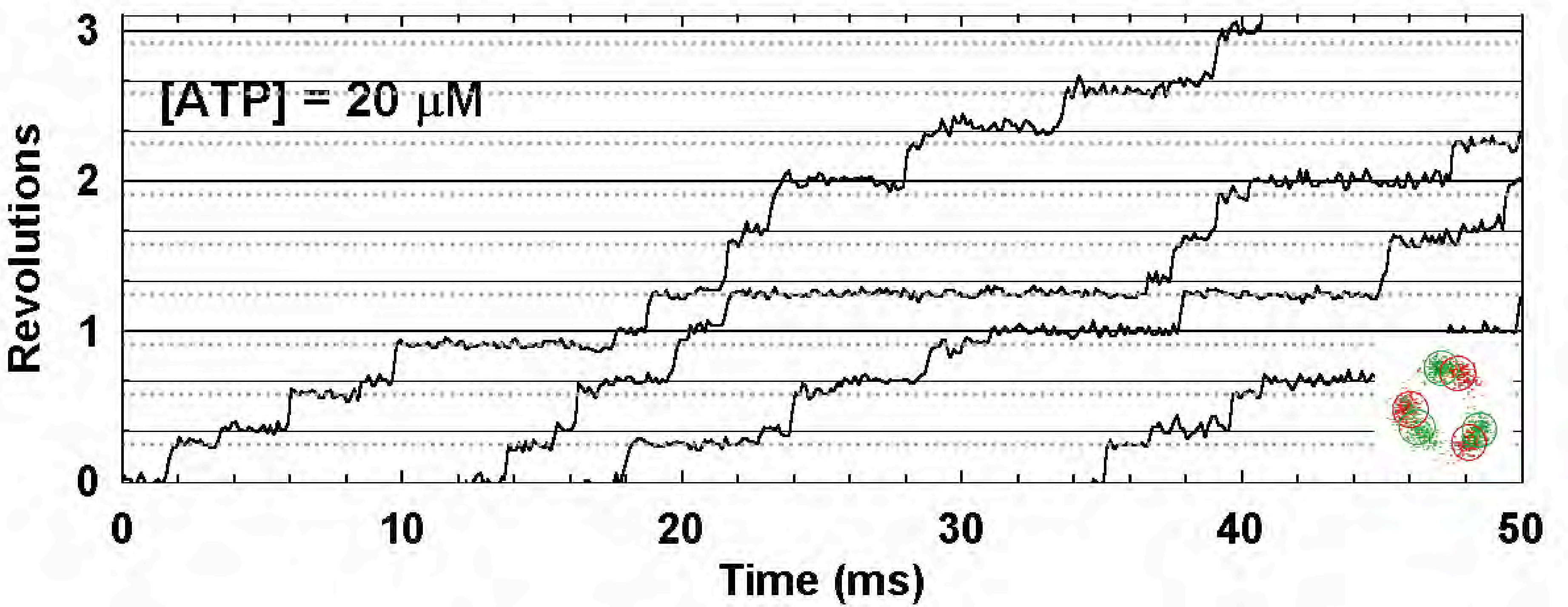
How Is Rotation (□Angle) Coupled to Chemical Reaction in the Three Catalytic Sites?

Working hypothesis for the hydrolysis sequence



Substeps Resolved



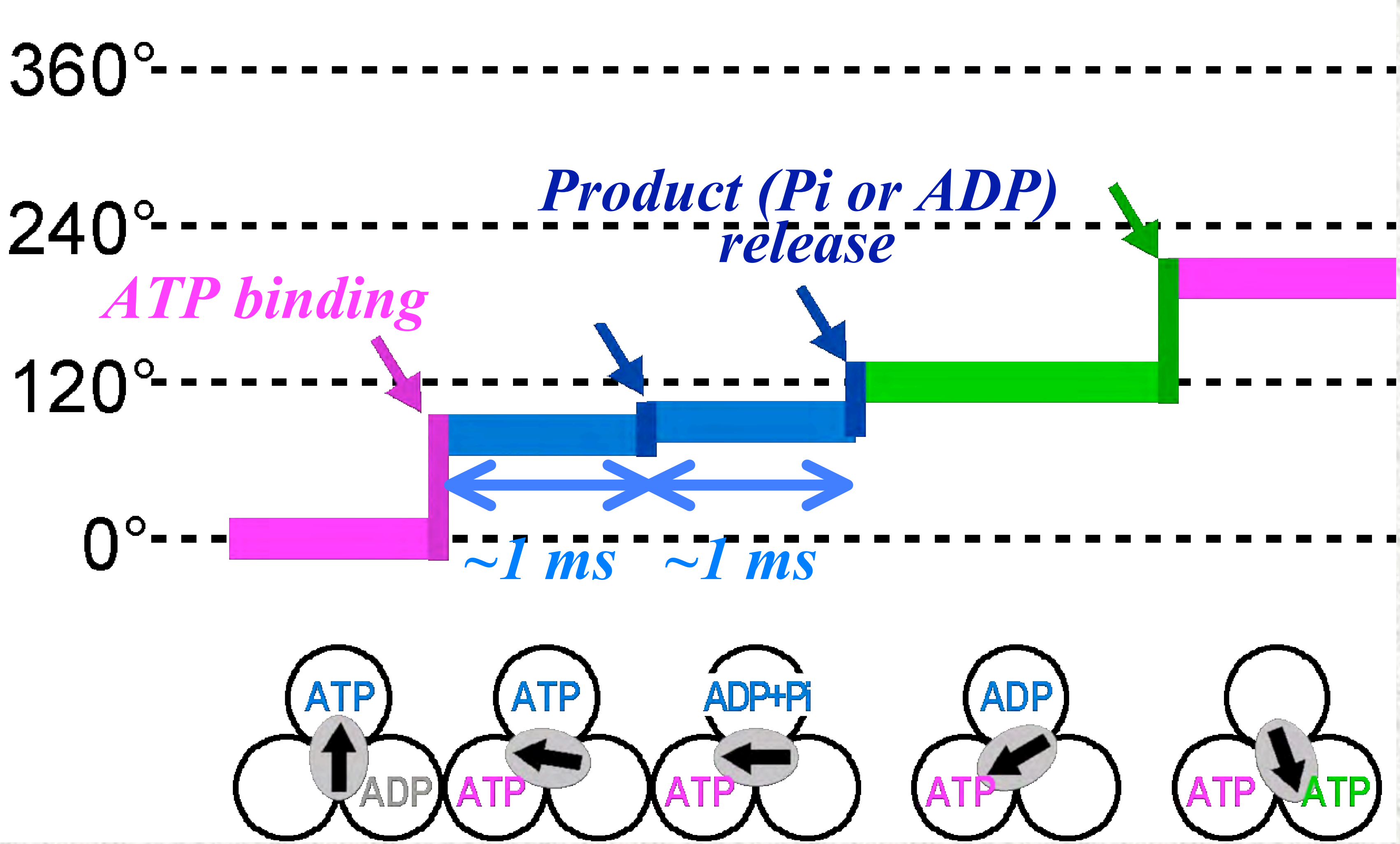


80-90° substep is driven by ATP binding.

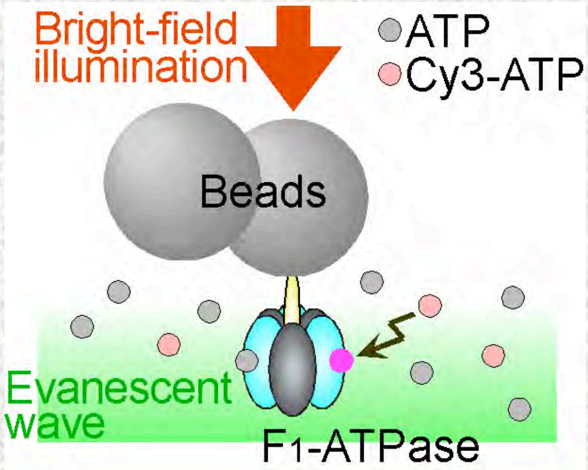
40-30° substep is driven by release of a hydrolysis product.

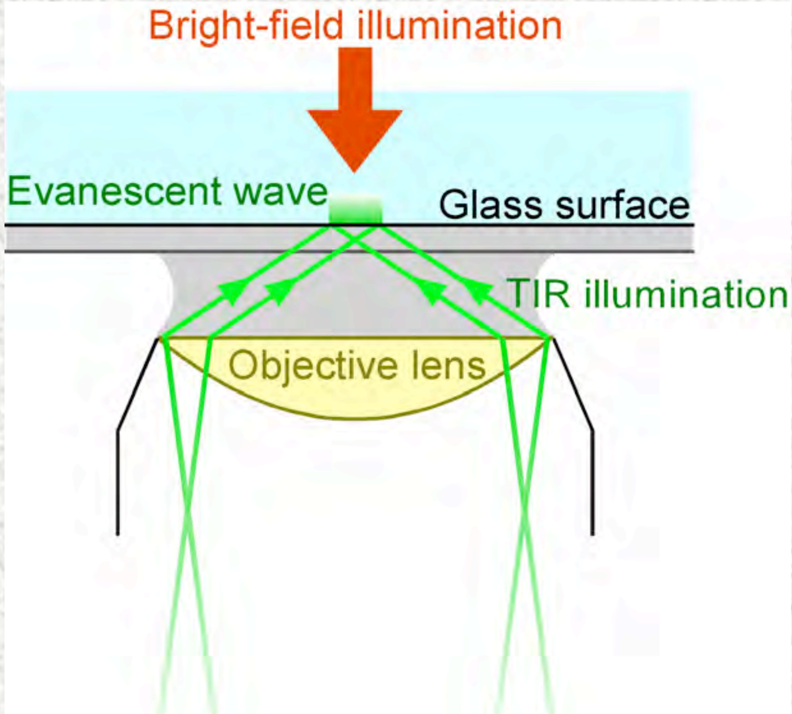
R. Yasuda

Binding and Release Drive Most of the 120º Rotation

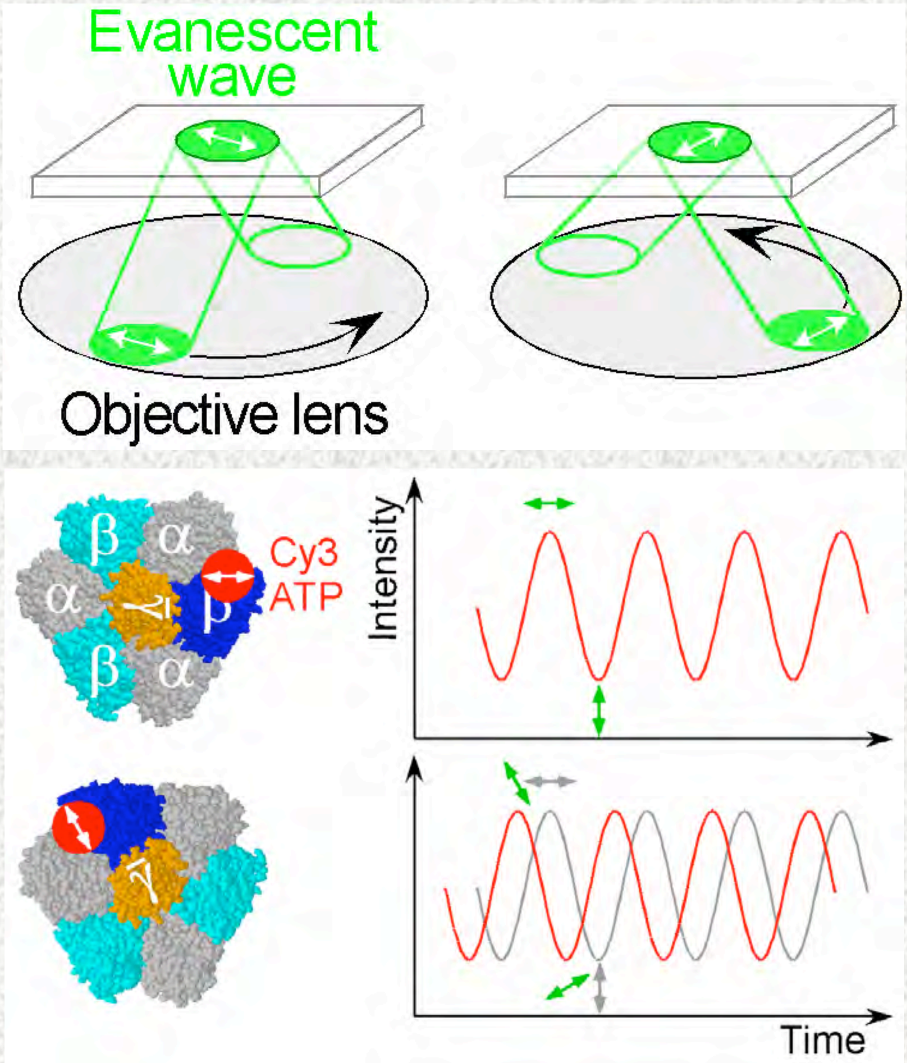


Simultaneous Observation of Rotation and ATP Binding





Detection of the orientation of Cy3-ATP



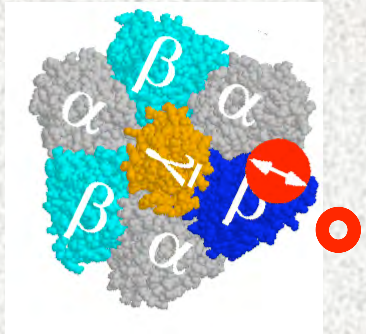
M. Tokunaga et al., 1997

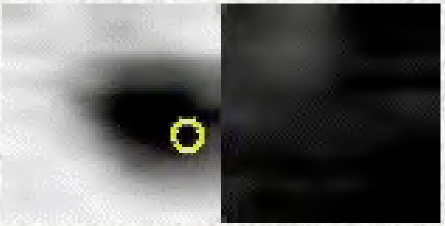
T. Nishizaka, K. Oiwa, E. Muneyuki & D. R. Trentham

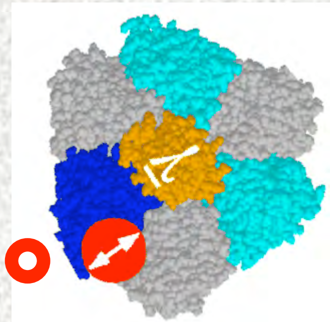
Simultaneous Observation of Rotation and ATP Binding

50nM Cy3-ATP 550nM ATP **3□slow playback**

Beads Cy3-ATP



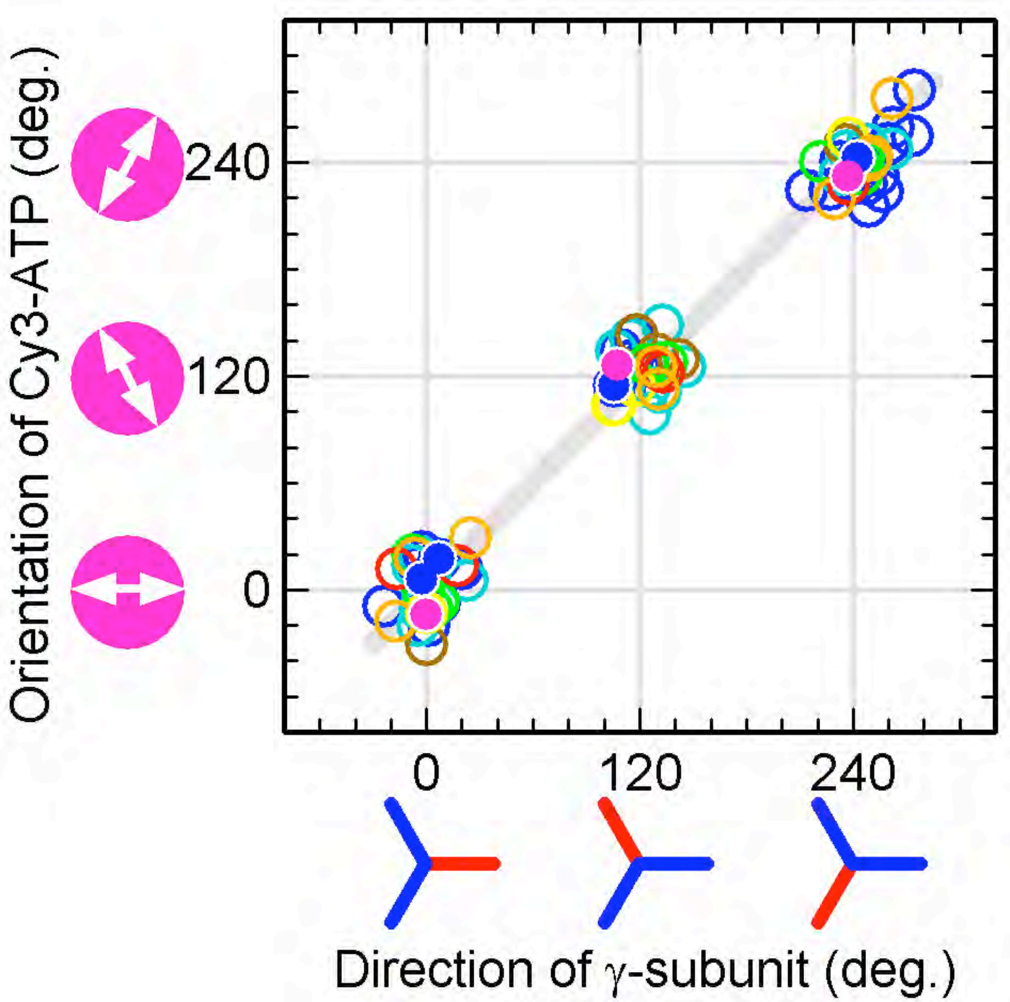




Orientations of Cy3-ATP and of the beads are correlated:
The □that has just bound Cy3-ATP
dictates the orientation of □.
Conversely, □dictates which □is to bind the next ATP.

T. Nishizaka

Cy3-ATP and Bead Orientations Are Correlated

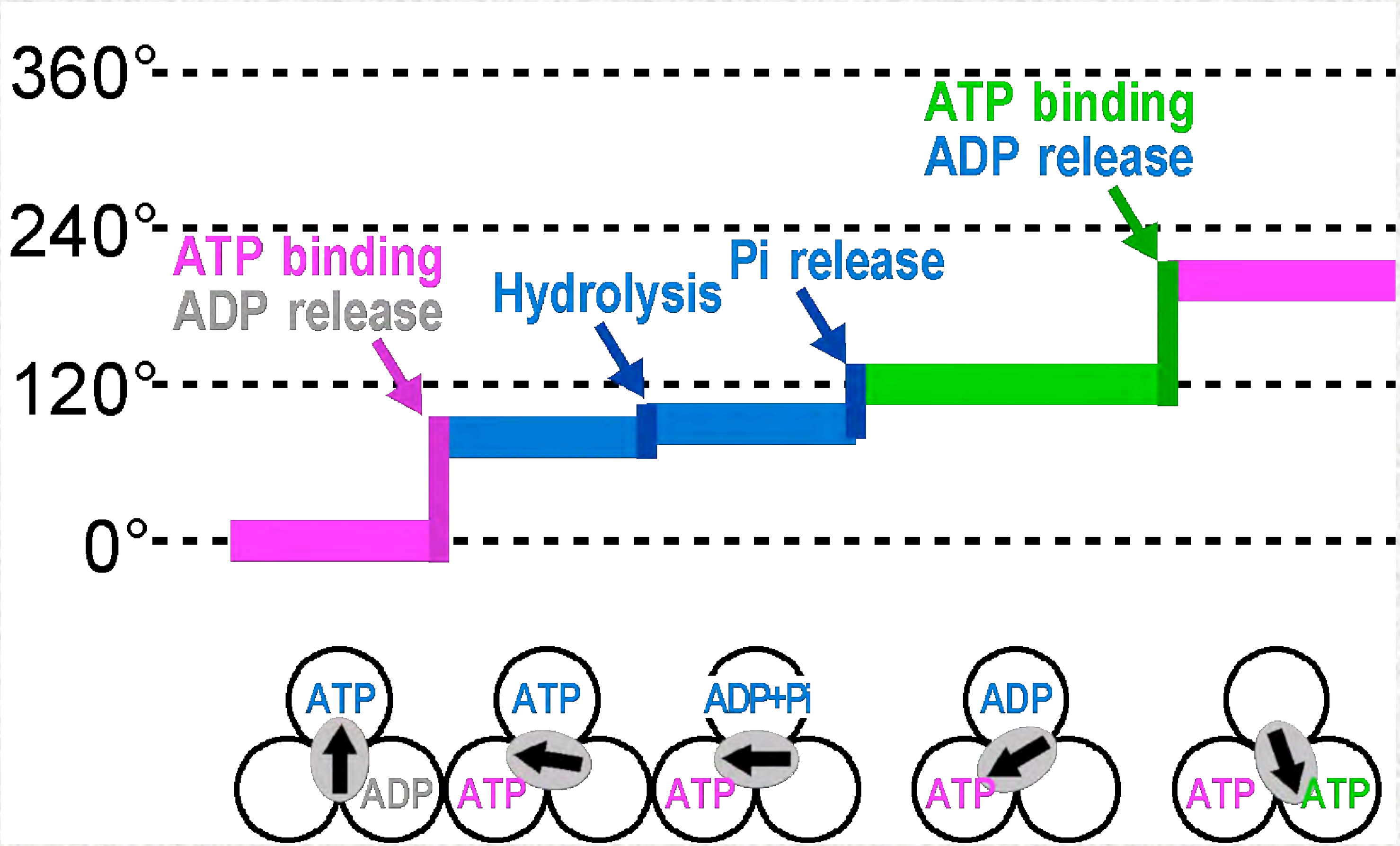


Selected data in which Cy3-ATP visited all three sites.

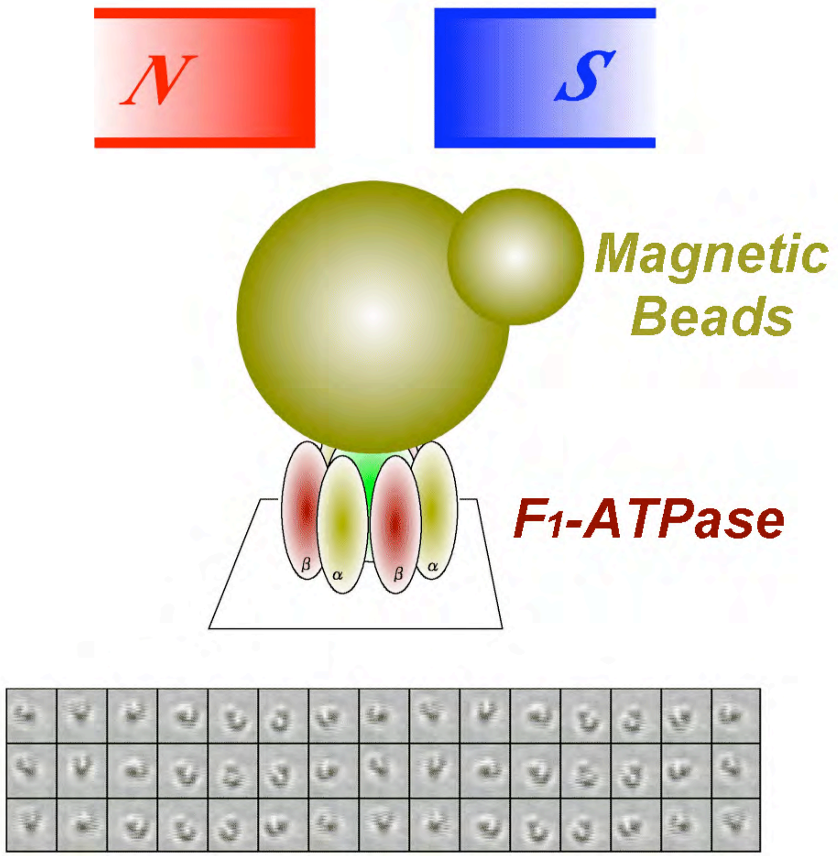
T. Nishizaka

How the Angle Dictates Chemical Reaction in the Three Catalytic Sites

Working hypothesis for the hydrolysis sequence



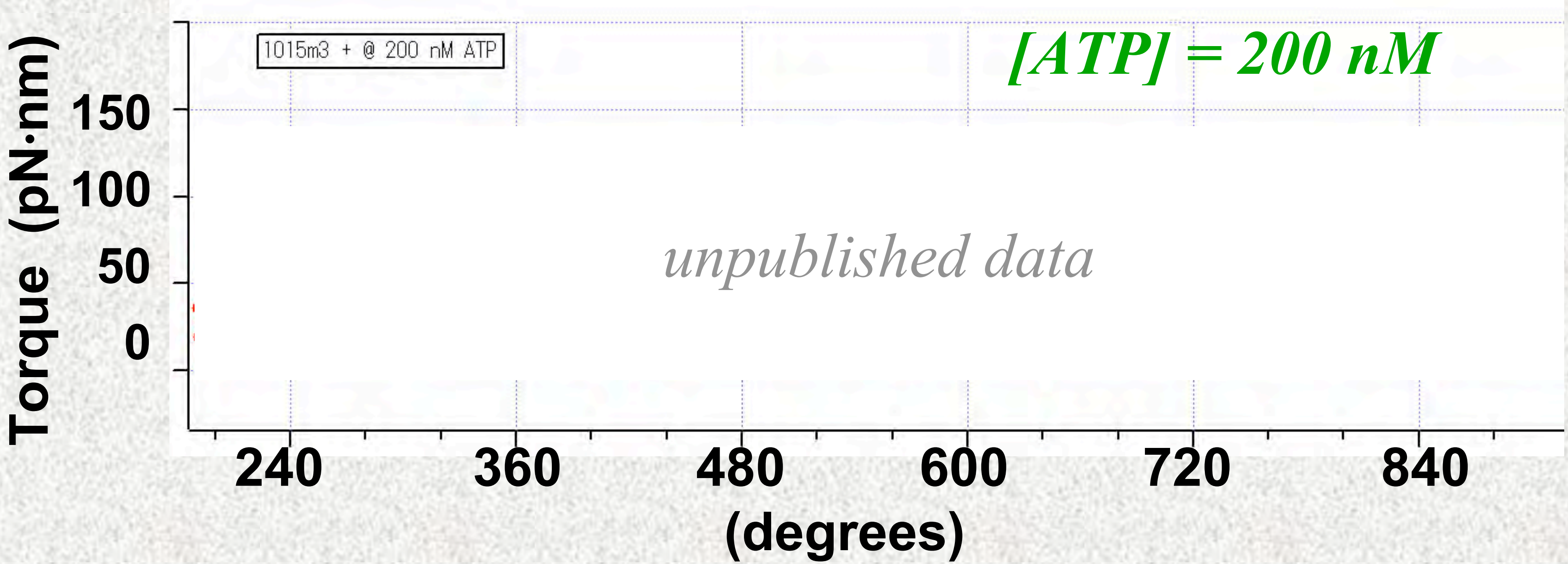
Measuring the Stall Torque of F_1 Motor with Magnetic Tweezers



[ATP] = 200 nM

H. Noji & H. Itoh

Stall Torque of F_1 Measured with Magnetic Tweezers



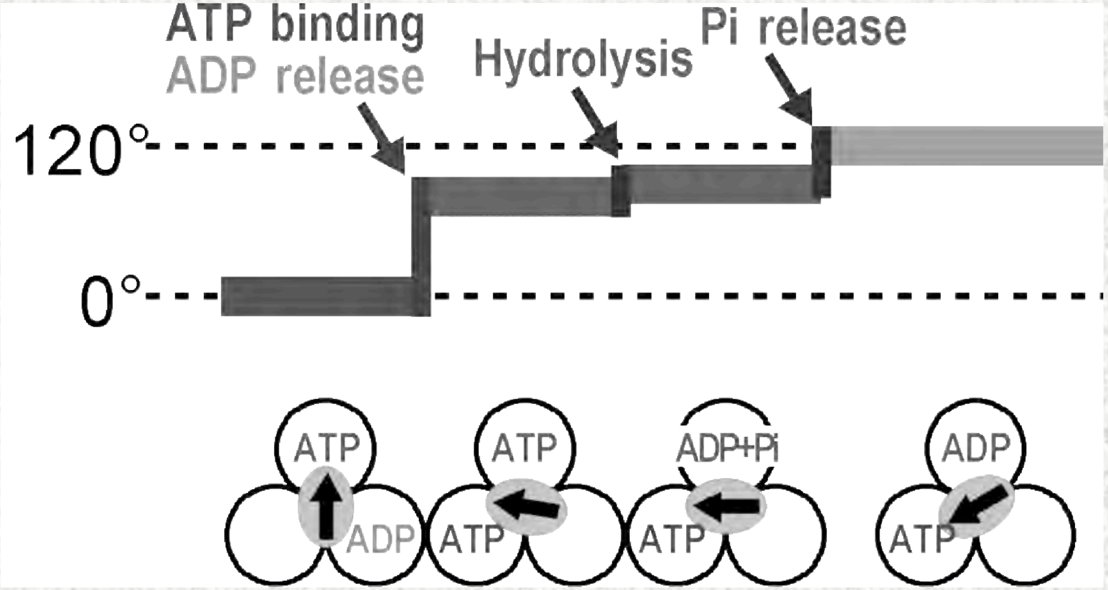
Work per step = Torque × 120°

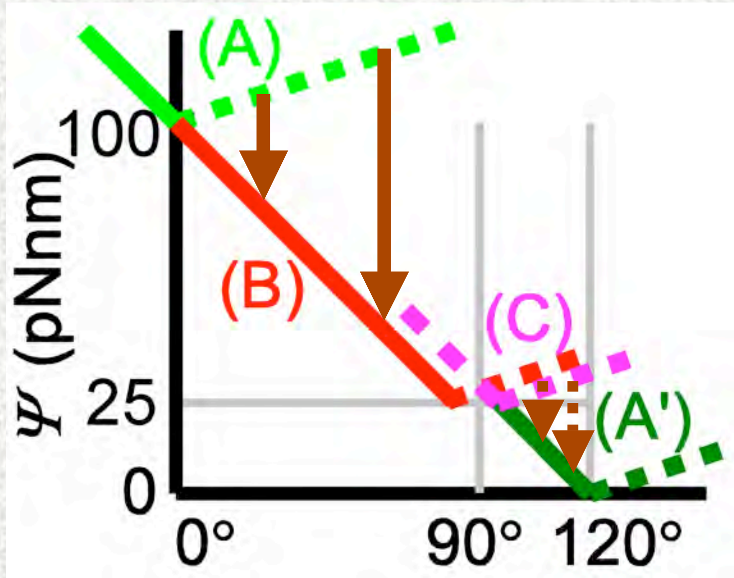
~ [50 pN·nm]×[2π/3 radian] ~ 100 pN·nm ~ ΔG_{ATP}

⇒ *Output ~ Input*

H. Noji

Kinetics and Dynamics of F_1 Rotation





(A) **(B)** **(C)** **(A')**

ATP-waiting

Post ATP binding

Post hydrolysis

During a ~80º substep, the affinity for ATP increases by

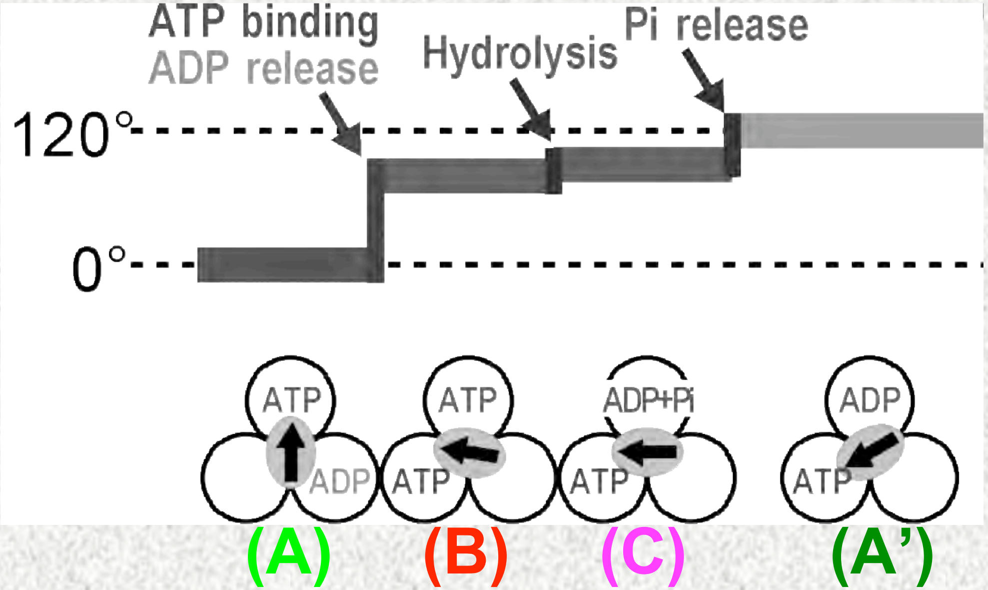
$$\exp[(\Psi_A-\Psi_B)/k_BT] > \exp(75\ \mathrm{pN{\bullet}nm}/k_BT) \sim 9\_10^7$$

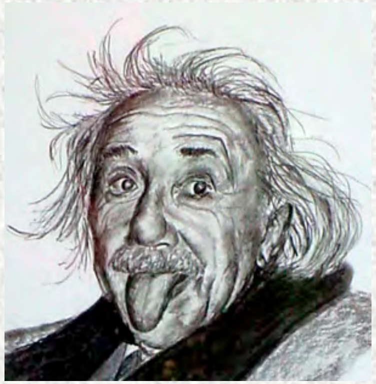
Likewise, during a ~30º substep, affinity for Pi decreases by

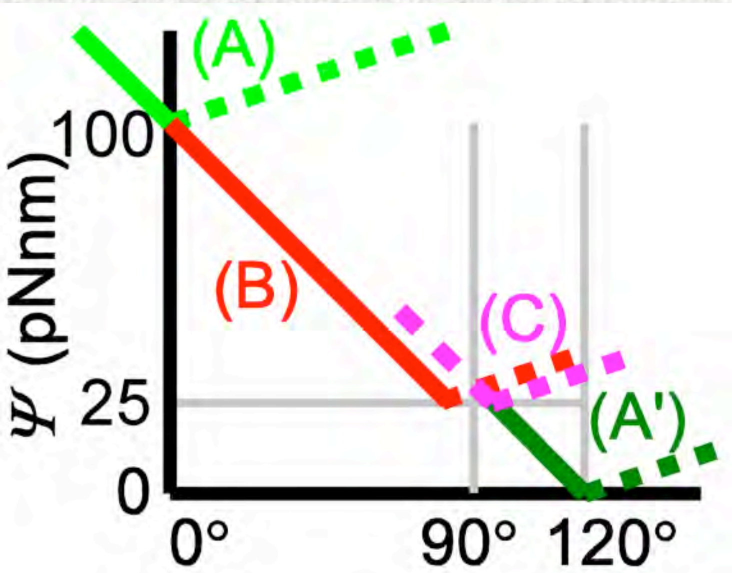
$$\exp[(\Psi_C-\Psi_{A'})/k_BT] > \exp(25\ \mathrm{pN{\bullet}nm}/k_BT) \sim 4\times10^2$$

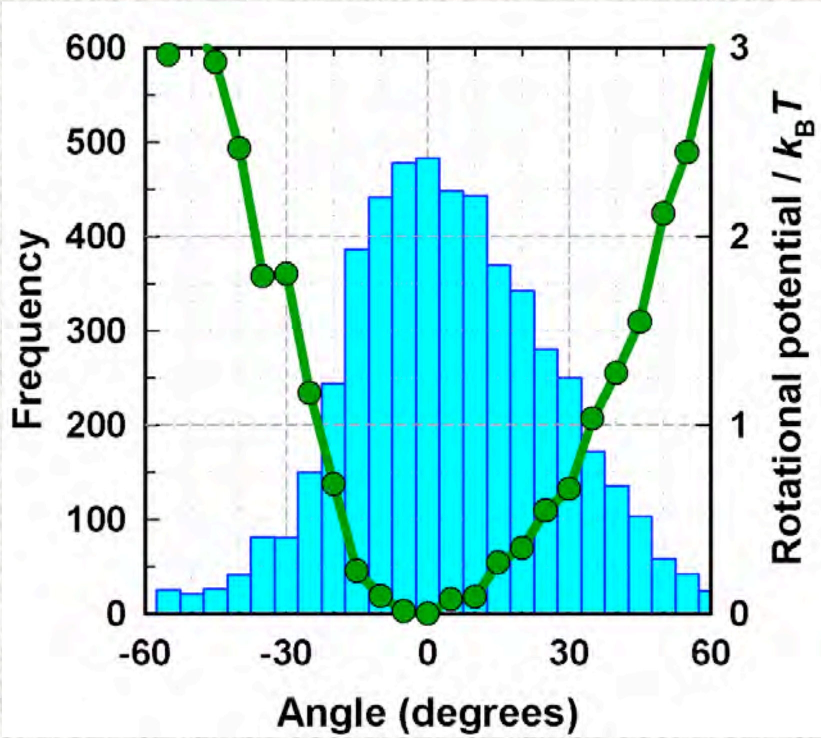
R. Yasuda

Potential Energy in the ATP-Waiting State





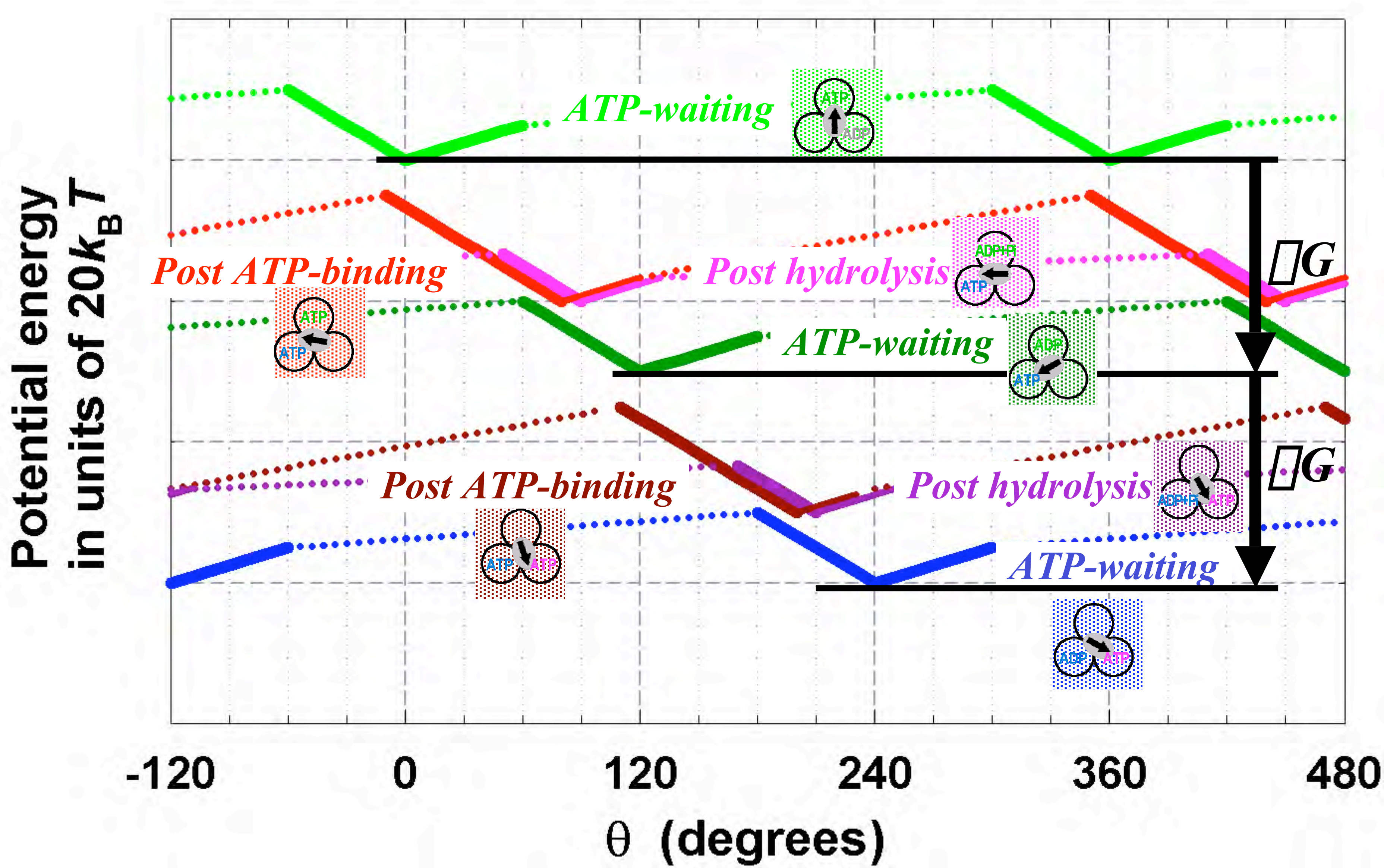




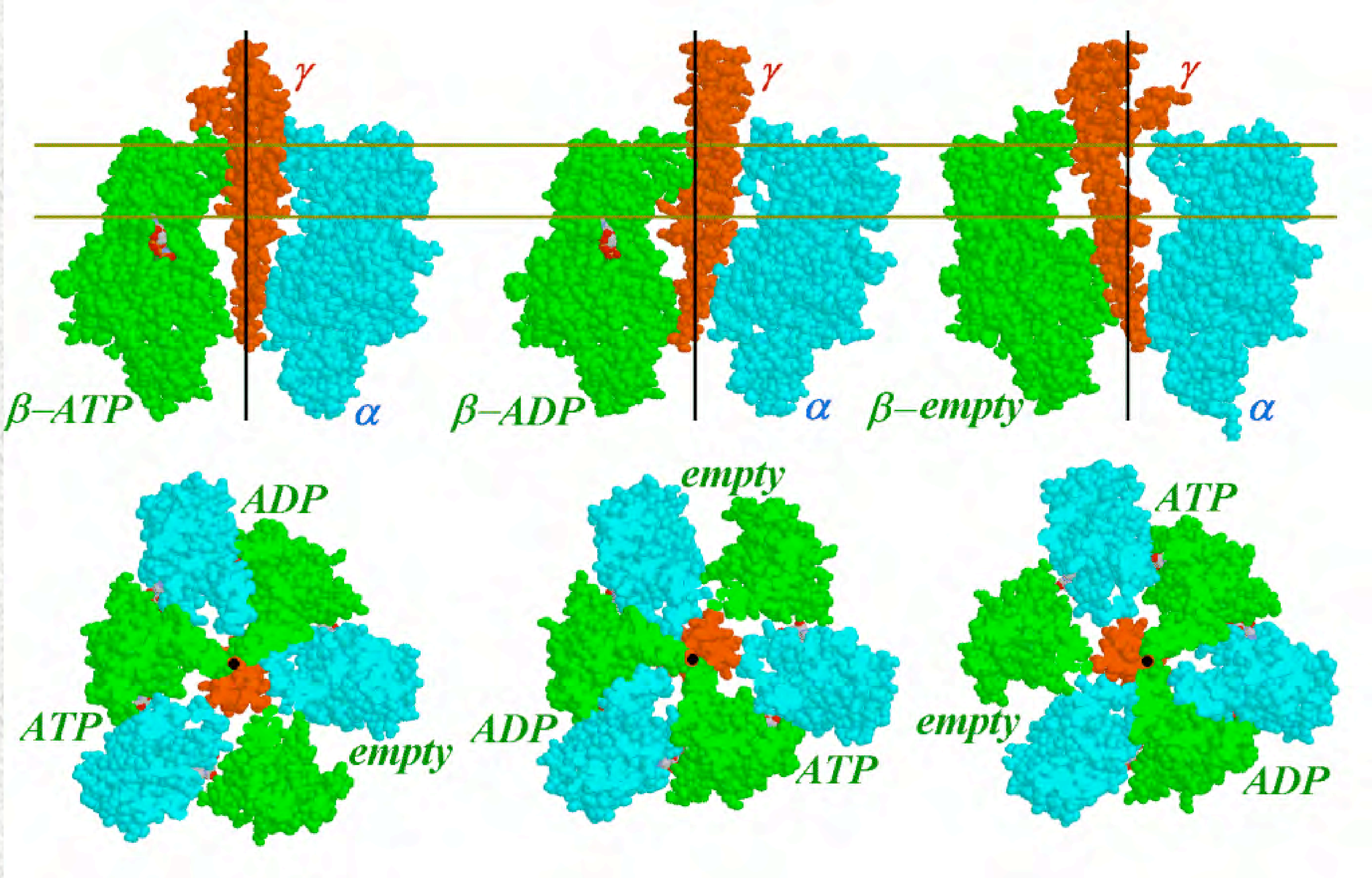
A. Einstein

R. Yasuda

Experimental Free-Energy Diagram



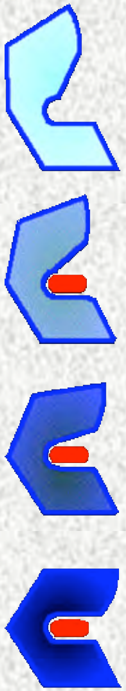
ATP Binding and ADP Release Induce Conformational Changes of



J. P. Abrahams et al., 1994

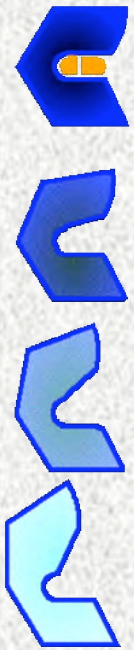
H. Wang & G. Oster, 1998

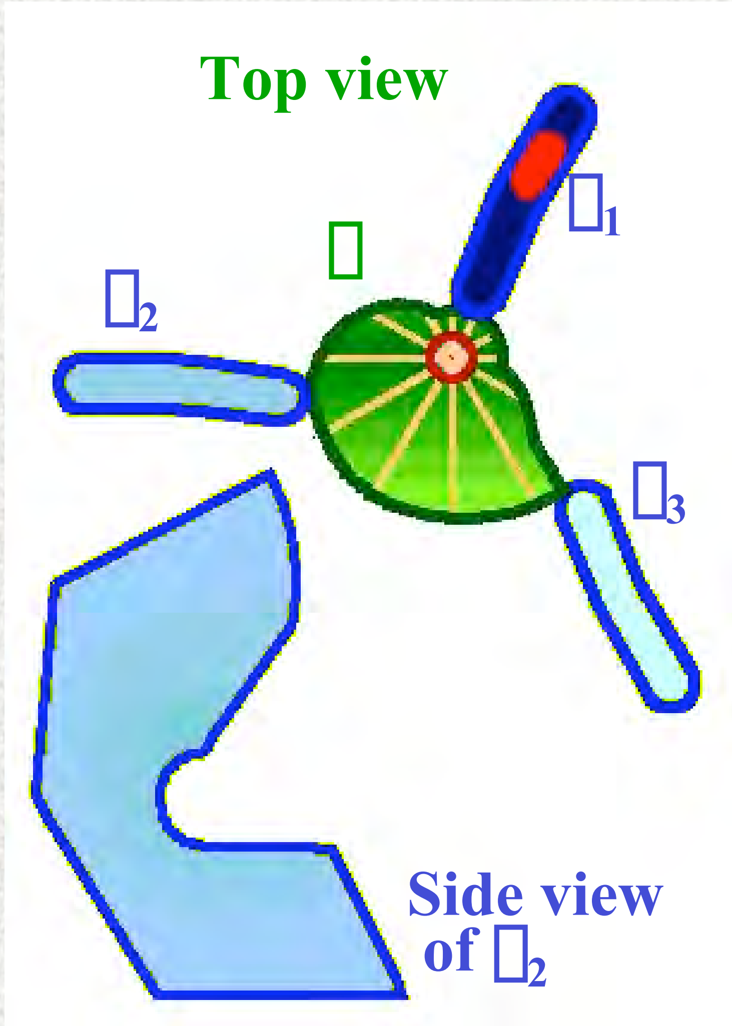
A Purely Mechanical Model for the F_1-Motor



Binding of ATP leads to bending of β.

Unbinding of Pi (and ADP) leads to unbending of β.





Nucleotide-powered pushing/pulling (through bending) drives rotation.

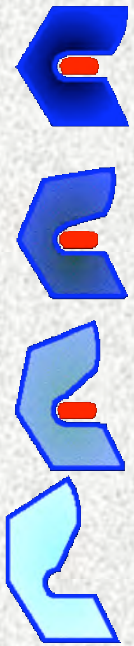
F. Oosawa & S. Hayashi, 1986

H. Wang & G. Oster, 1998

K. Kinosita, Jr. et al.
Annu. Rev. Biophys. Biomol. Struct., 33: 245 (2004)

ATP Synthesis by Forced CW Rotation

Bending of □ increases affinity for Pi (and ADP) by >4□10^{2}.



Unbending of □ decreases affinity for ATP by >9_10^{7}.

Dancing F_1