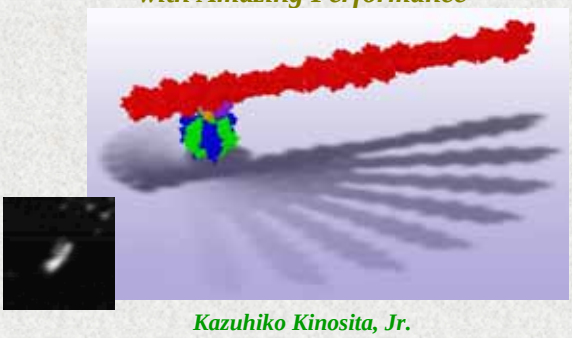
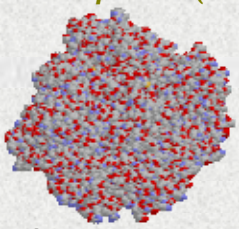
A Rotary Molecular Motor with Amazing Performance



Kazuhiko Kinosita, Jr.
Department of Physics, Faculty of Science and Engineering
Waseda University
http://www.k2.phys.waseda.ac.jp

A Protein Molecule : A Cluster of Atoms (Balls)



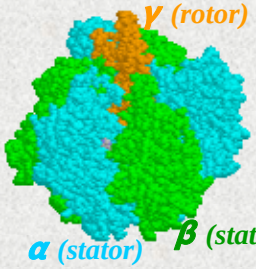
10 nm = 10×10^{-9} m
(~30 atoms wide)

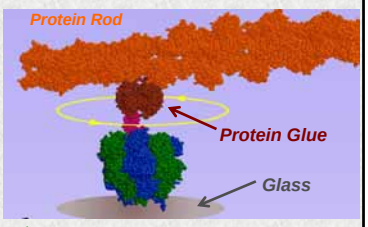
Total: 10^4-10^5 atoms

- Carbon atom (C)
- Oxygen atom (O)
- Nitrogen atom (N)
- Sulfur atom (S)
- Hydrogen atom (H)

A single protein molecule performs a marvelous function : A Molecular Machine

F_1-ATPase: A Rotary Motor Made of a Single Molecule





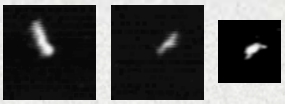
More than 10^{19} such motors in your body!

Major Players

Literature quotation

H. Noji, R. Yasuda & M. Yoshida, 1997

Observation of F_1 Rotation through a Protein Rod Attached to γ



Propeller rotation

Proof that
γ slides against $\alpha_3\beta_3$
and that
a single molecule supports rotation.

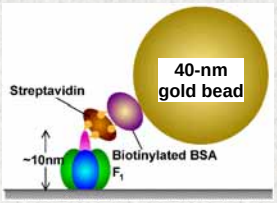
Long and short rods

Rotation is slower with a longer rod.

High and constant torque motor
Efficiency approaching ~100%

H. Noji & R. Yasuda, 1997, 1998

Molecular F_1 Runs at 120,000 rpm at 64°C



[ATP] = 2 mM
(*saturating*)

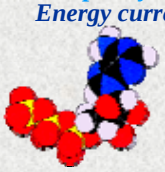
1600✕ slow

2,000 revolutions/s
= 120,000 rpm

S. Furuike, 2008

The Fuel (Energy Source) for the F_1 Motor

ATP Adenosine Triphosphate
Chemical reaction → Mechanical work (direct)
Completely reversible; Unlimited reuse
Energy currency in our body!!



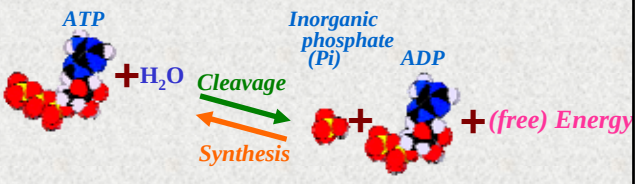
- Carbon atom (C)
- Oxygen atom (O)
- Nitrogen atom (N)
- Phosphorus atom (P)
- Hydrogen atom (H)

Uranium Physical decay → Heat → Work
Not reusable; products harmful

Gasoline (oil) Chemical reaction → Heat → Work
Not reusable; products not healthy

Electricity Direct → Various works
Hydraulic: Reversible (reusable); Clean
Energy currency of civil life

Cleavage (Hydrolysis) of ATP Produces Energy



Most of biological activities are powered by ATP: running, thinking, digesting, ····· .

How to supply ATP? (We don't eat ATP.)
By re-synthesis!!

Synthesis of ATP from ADP and Pi is the physiological role of the F_1 motor.

Physiological Function of F_1 Is to Synthesize ATP through (Forced) Clockwise Rotation

Isolated F_1 :
CCW rotation powered by ATP cleavage in the three β subunits

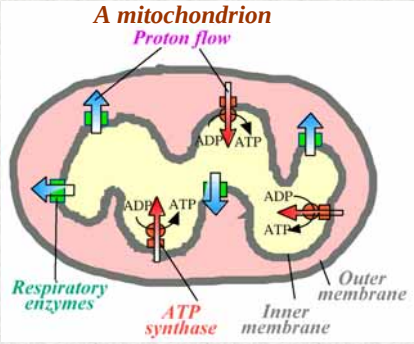
F_1 in living organisms:
CW rotation driven by proton flow through the F_o motor
↓
ATP synthesis in the three β subunits

β: driving subunit

Why do We Eat, and Why do We Breathe?

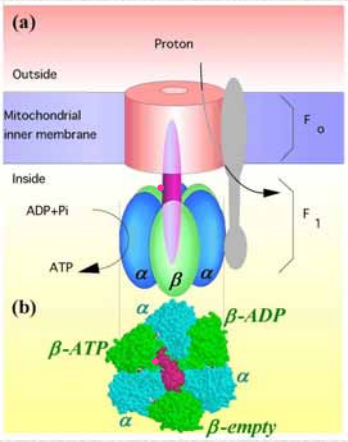
To obtain energy (= to synthesize ATP) by slowly burning the food we ingest with oxygen we inhale.

A mitochondrion



P. D. Mitchell

ATP Synthase



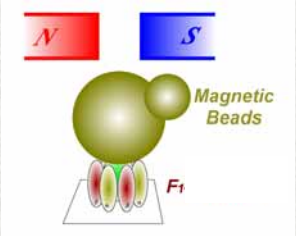
Proton-driven and ATP-driven motors connected by a common shaft (γ).

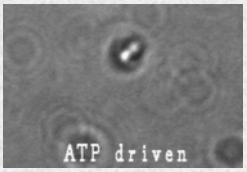
ATP synthesis/hydrolysis on three βs.

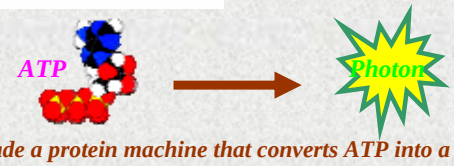
Isolated F_1 only hydrolyzes ATP; hence it is called F_1-ATPase

P. D. Boyer | F. Oosawa & S. Hayashi | J. E. Walker & colleagues

Forcing F_1 Rotation with Magnets



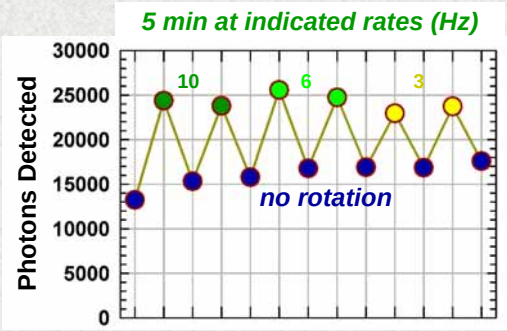




Include a protein machine that converts ATP into a photon

H. Itoh, 2004

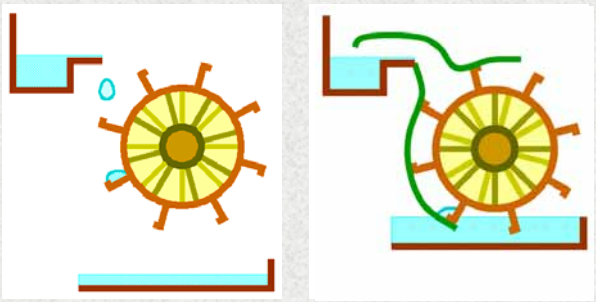
Reverse Rotation Led to ATP Synthesis!!



First chemical synthesis driven by force

Hiro Itoh @Hamamatsu Photonics Co., Ltd., 2004

Reversibility Is Not Always Guaranteed

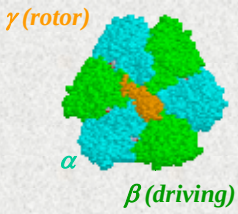


You've got to design it properly!

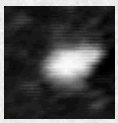
A Force (Torque) Applied to One Point Can Reverse the Operation of F_1 !!

γ - Dictator Mechanism:
The γ angle dictates how ch ions proceed in β's.
In hydrolysis, three β's co the γ angle.

Stepwise Rotation of F_1



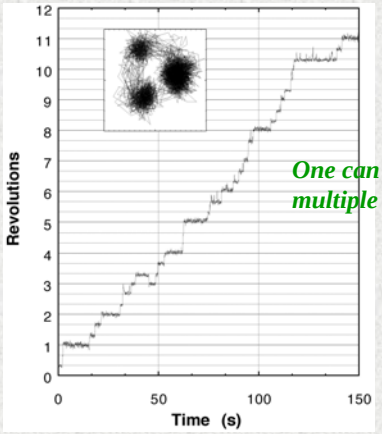
Watch!!



[ATP] = 200 nM

R. Yasuda & H. Noji, 1998

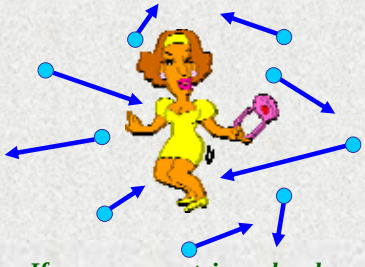
Stepwise Rotation at 20 nM ATP



One can never synchronize multiple molecular machines.

R. Yasuda & H. Noji, 1998

Water Molecules Are Bullets



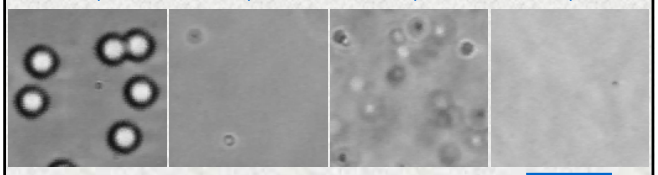
If you are a protein molecule,
water molecules surrounding you are
gun bullets in size and weight.
There average speed is 1000 km/h !
The speed of a jumbo jet, or of a real bullet from a gun.

Brownian (Thermal) Motion

Small beads in water

20 × the size of a protein molecule

$2a$ = 3.1 μm	1.0 μm	0.45 μm	0.21 μm



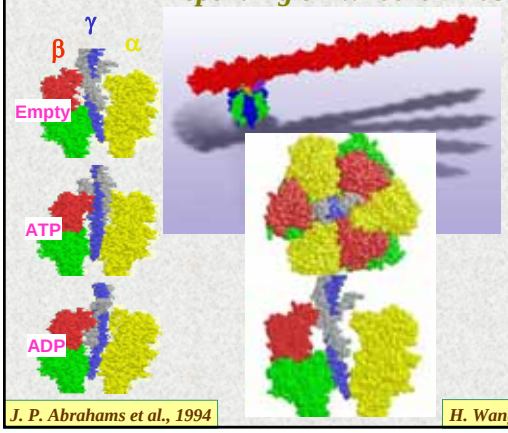
10 μm

D = 0.14 μm²/s	0.44 μm²/s	0.97 μm²/s	2.1 μm²/s
$a^2/2D$ = 9 s	0.3 s	0.03 s	0.003 s

Average time needed for movement over its own size

T. Okamoto

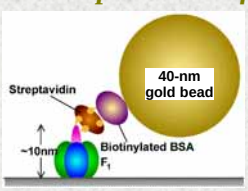
A Protein Machine Changes Its Conformation Depending on What It Binds

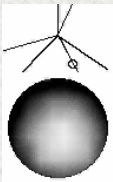


J. P. Abrahams et al., 1994

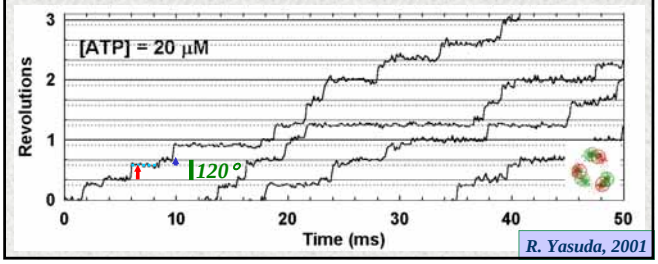
H. Wang & G. Oster, 1998

A 120° Step Consists of ~80° and ~40° Substeps



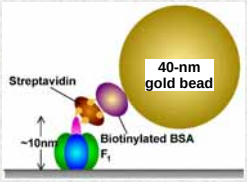


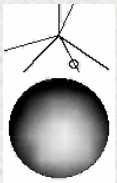
20 μM ATP
1000× slow

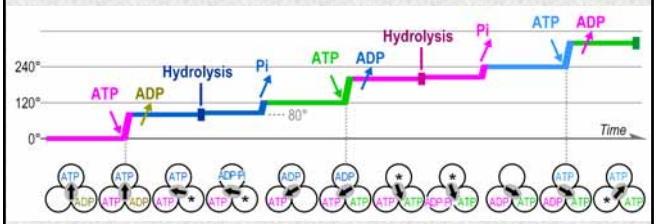


R. Yasuda, 2001

Binding and Release Drive Rotation



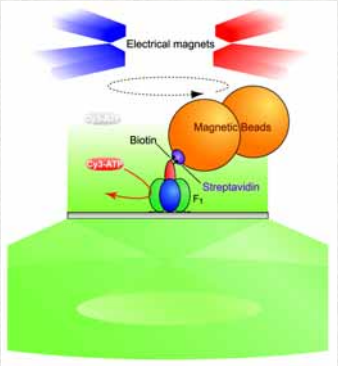


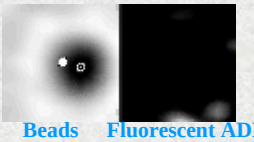


K. Adachi, K. Oiwa, 2007; R. Shimo-Kon, 2010

R. Yasuda, 2001

Nucleotide Binding/Release during Forced Rotation





Beads Fluorescent ADP

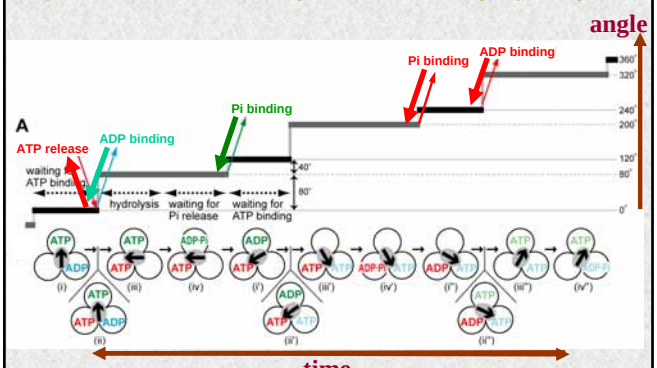
Binding → Unbinding

50 nM Cy3ADP, 10 mM Pi

Fluorescent ADP analog

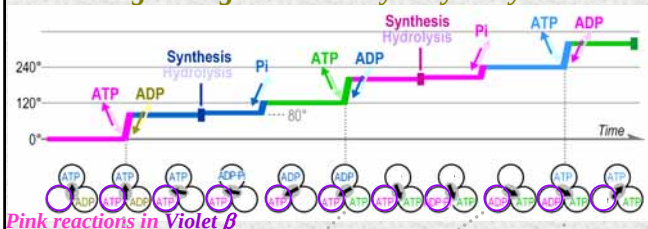
K. Adachi, K. Oiwa

Synthesis Is Essentially the Reverse of Hydrolysis



K. Adachi, K. Oiwa

Binding Changes in ATP Hydrolysis/Synthesis



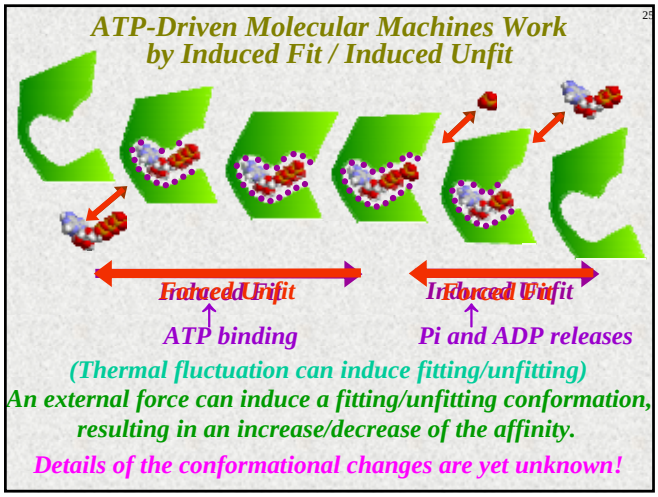
Pink reactions in Violet β

$\log K_a$ (ATP|ADP) $\log K$ (syn|hyd) $\log K_a$ (Pi) $\log K_a$ (ATP|ADP)

$\log k_{on}$ (ATP|ADP) $\log k_{on}$ (ATP|ADP)

K_a: association constant (binding affinity)

k_{on}: rate of binding


25
ATP-Driven Molecular Machines Work
by Induced Fit / Induced Unfit
Induced Fit
Induced Unfit
Induced Fit
Induced Unfit
ATP binding
Pi and ADP releases
(Thermal fluctuation can induce fitting/unfitting)
An external force can induce a fitting/unfitting conformation,
resulting in an increase/decrease of the affinity.
Details of the conformational changes are yet unknown!