

What is life? 2007

Function of Life and Fluctuation

*Single molecule nanobiology for elucidating the mechanism
involved in utilizing fluctuations by bio-system*

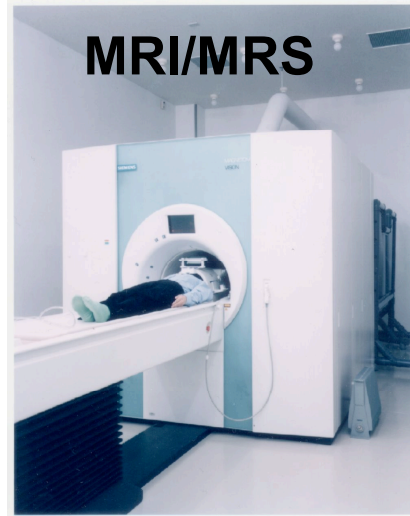


Toshio Yanagida

**(Graduate Schools of Frontier BioScience
and Medical school, Osaka Univ.)**

Brain uses only < 1 watt for thinking. Most of energy is used for spontaneous fluctuations?

20Wt
+
>1Wt?



Measurement of
temperature of brain

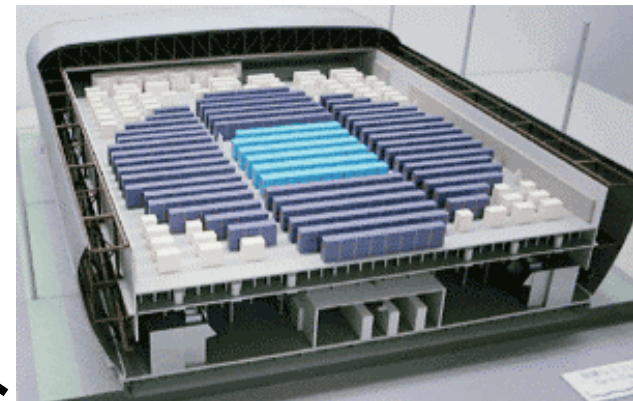
Yoshioka, Seiyama et al.

50,000Wt



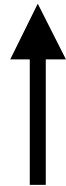
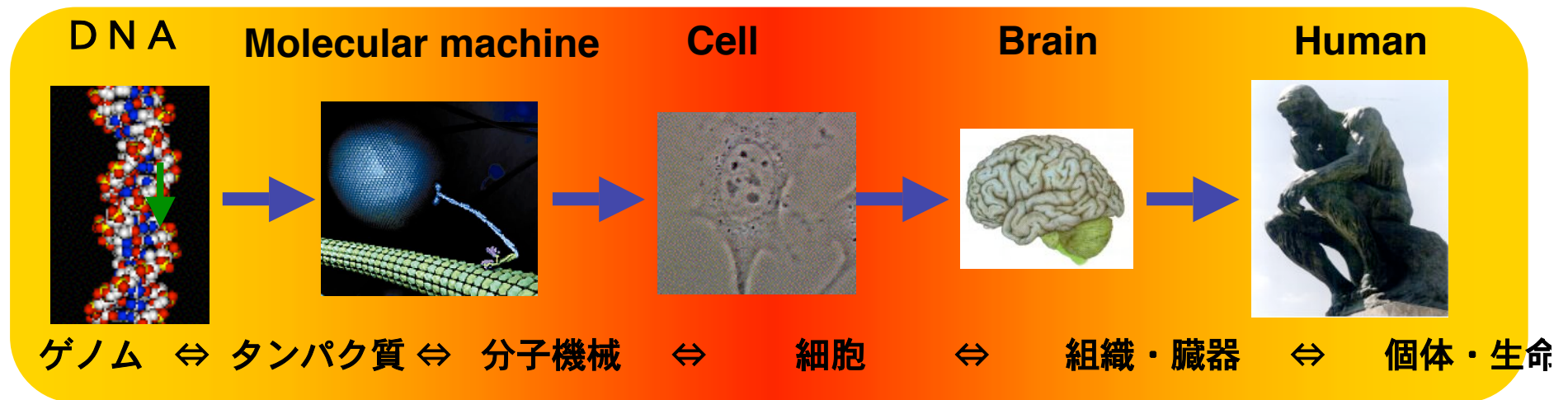
Garry Kasparov
World champion of
chess game

Chess
game

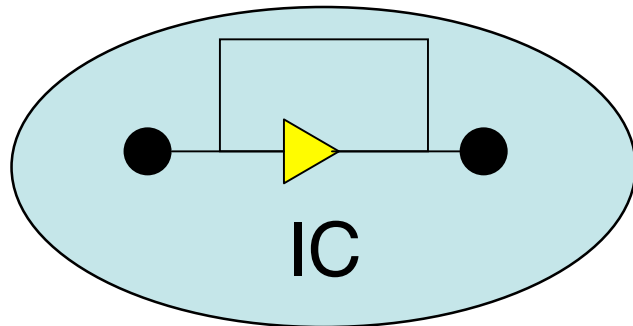


Super computer
Blue Gene (IBM)
4 T FLOPS x 2

Biological system is composed of molecular machines

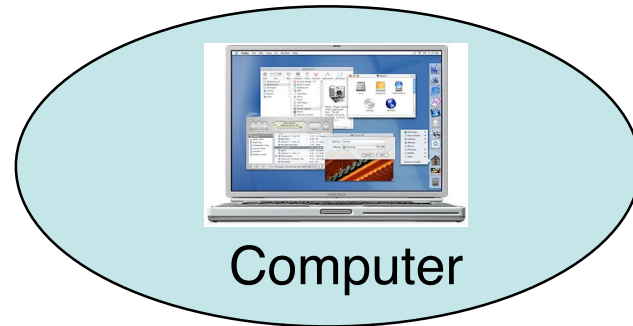


Input energy level of biological molecular machines is not far from thermal noise



IC

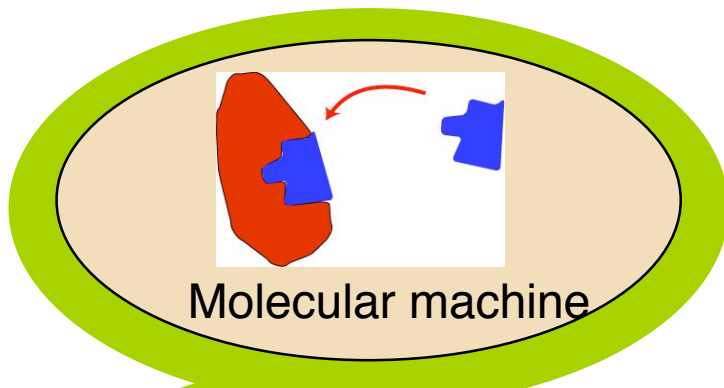
Accurate
Fast
Deterministic



Computer

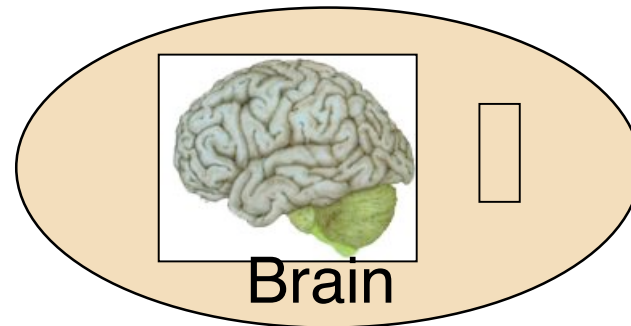
Hard
[structure
function

High level
input energy
($\sim 500k_B T$)



Molecular machine

Fuzzy
Slow
Stochastic

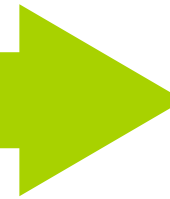


Brain

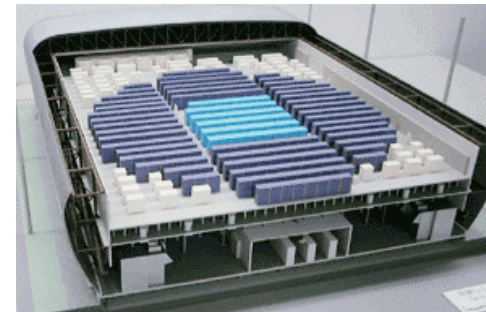
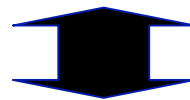
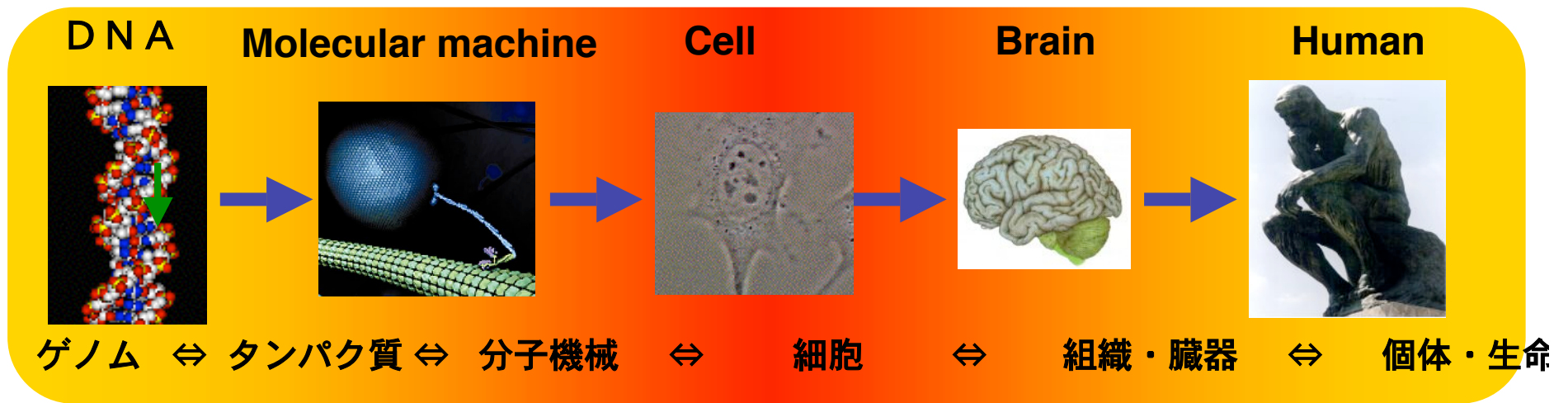
Soft
[structure
function

Low level
input energy

(\sim
 $10k_B T$)



The apparent performance of functions of biological machines is much poorer than that of manmade machines.



Comparison. 1) Operation rate and accuracy

	Man-made	Bio	(Ratio)
Operation time	~nsec (10^{-9})	~msec (10^{-3})	$1/10^6$

Accuracy (S/N)	10^{80}	10^4	$1/10^{76}$
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Rapid
Accurate

Slow
Erroneous

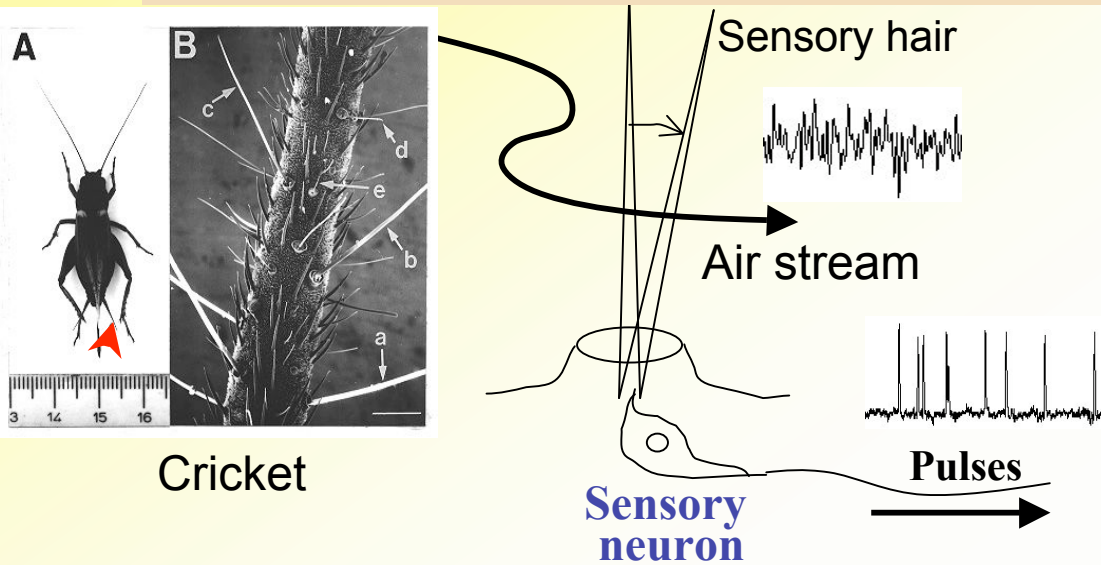
Brain



Computer

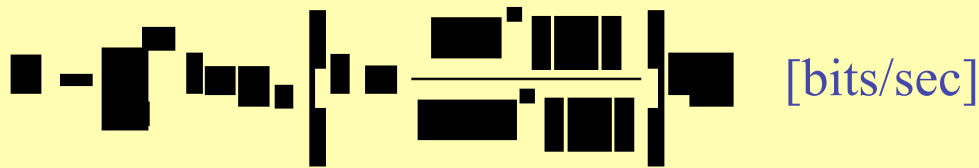
The apparent performance of biological machines is much poorer from viewpoint of manmade machine, but

Comparison. 2) Rate of data transfer



Cricket

Data carrying rate by Shannon

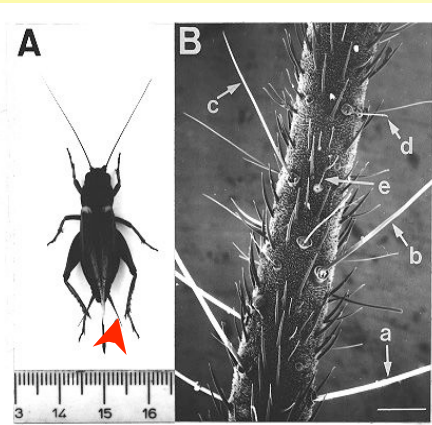


From T. Shimozawa

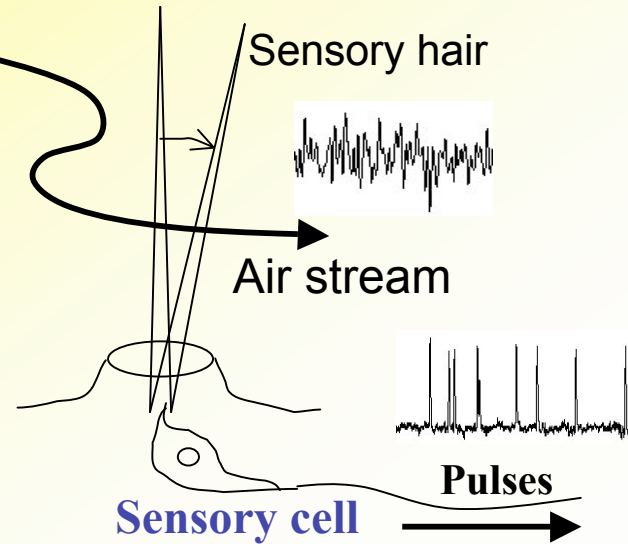
Rate of data transfer

400 bits/sec :

Performance of nerve and optical guide



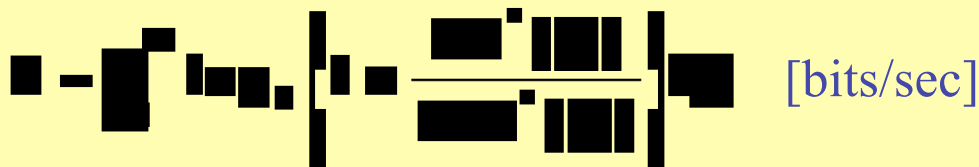
Cricket



Optical guide operated by electronic devices



Data carrying rate by Shannon



From T. Shimozawa

Rate of carrying data

400 bits/sec

:

10^9 bits/sec

Millions times slower !

Comparison. 3) Memory capacity



Kim Peak
(Model of a famous
movie” Rain Man”)

He learns
7,600 books
by rote.

映画レインマン(Rain Man)のモデルにもなった発達障害をもつキム=ピークは7600冊の本を丸暗記し、米国内すべての市外局番、高速道路、郵便番号、その都市をカバーする全テレビ局や電話会社名などを記憶している。カレンダー計算能力も持ち合わせていて、誕生日を言えば定年を迎える65歳の誕生日が何曜日になるかをたちどころに教えてくれる。

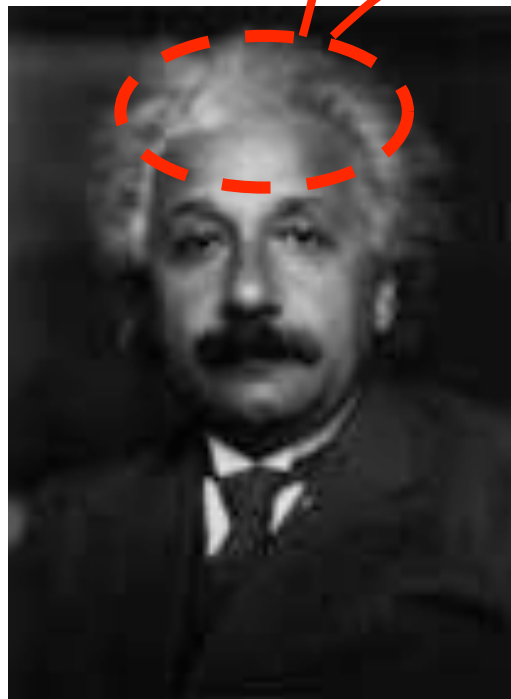
Amazing !!!!

The maximum memory
capacity of a human
brain.

Comparison. 3) Memory capacity



\$3??



Kim Peak

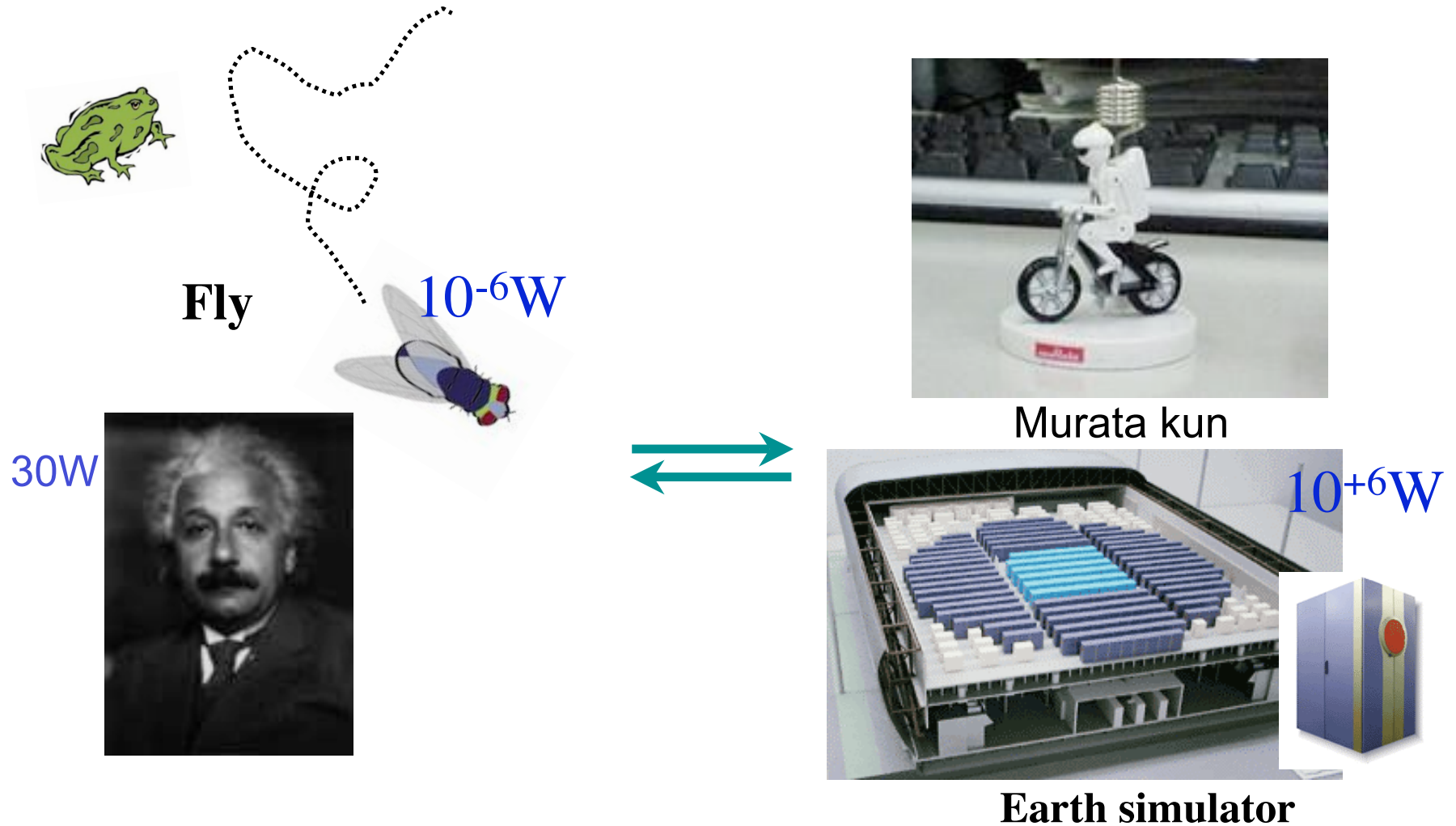
He learns
7,600 books by rote.

1 book=1GB
7,600 books=7.6GB

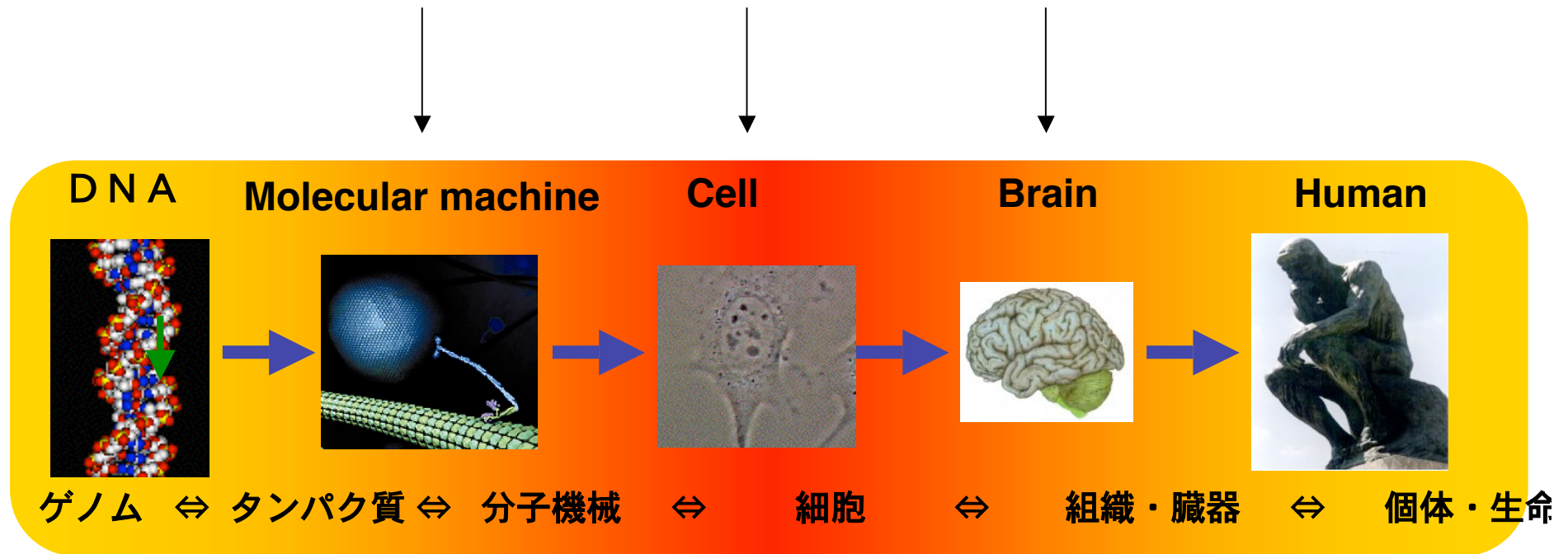
Human brain memory

|||
DVD disc= 4.7GB
200yen x 2
= 400yen (\$3)

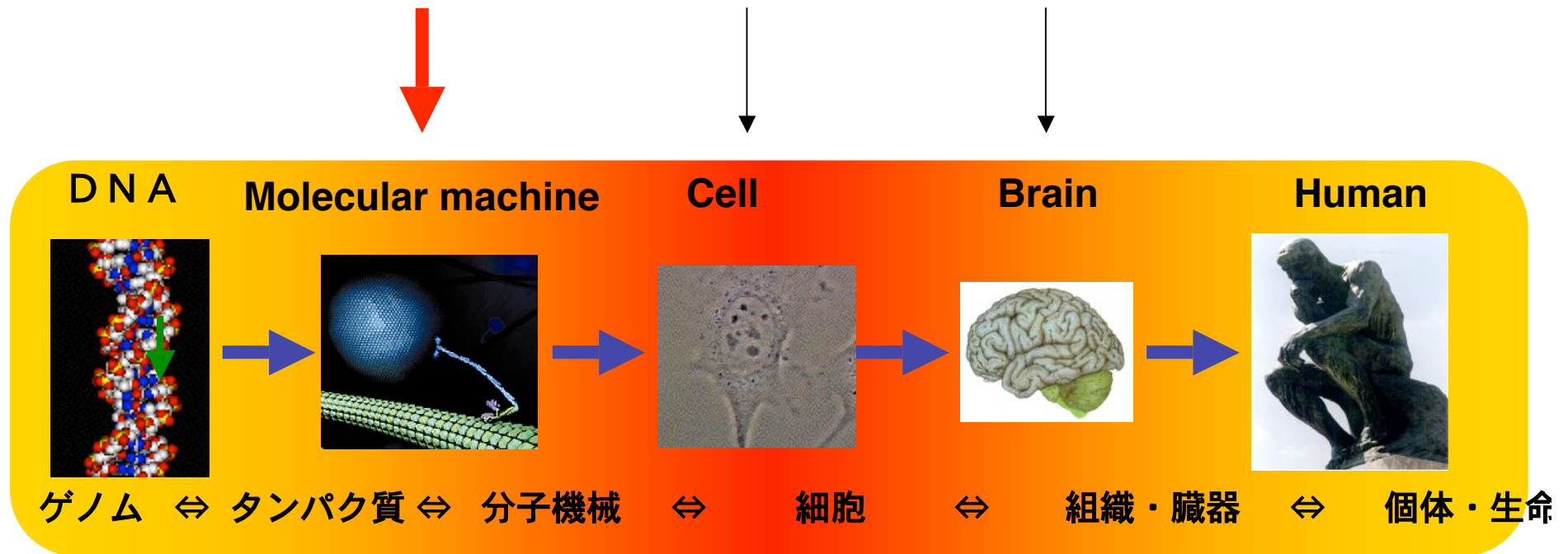
The underlying principle of biological machines should be different from that of man-made machines.



What is difference between man-made and biological machines?



What is difference between man-made and biological machines?

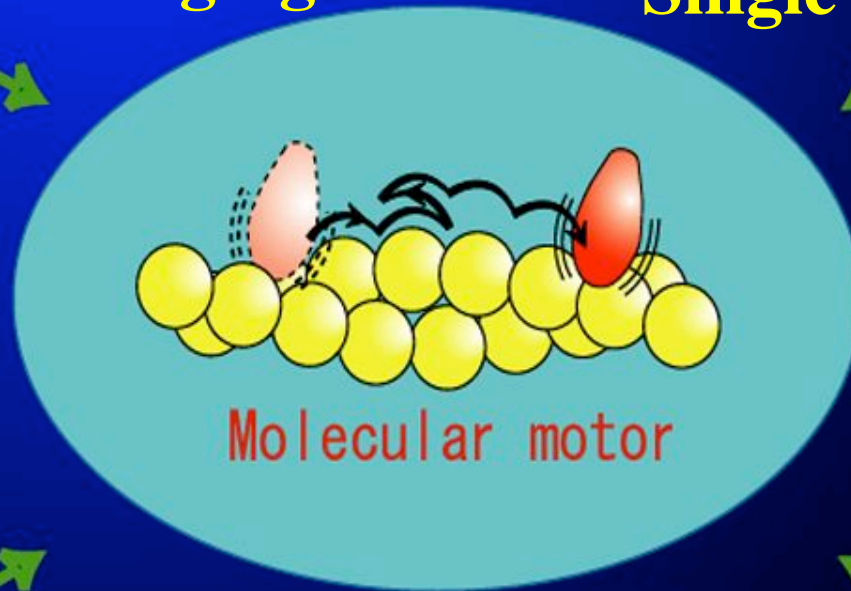


Approach

Single molecule nano-technology

Single molecule imaging

Single molecule trapping



Imaging of single
chemical reactions

Nano-manipulation
of single molecules

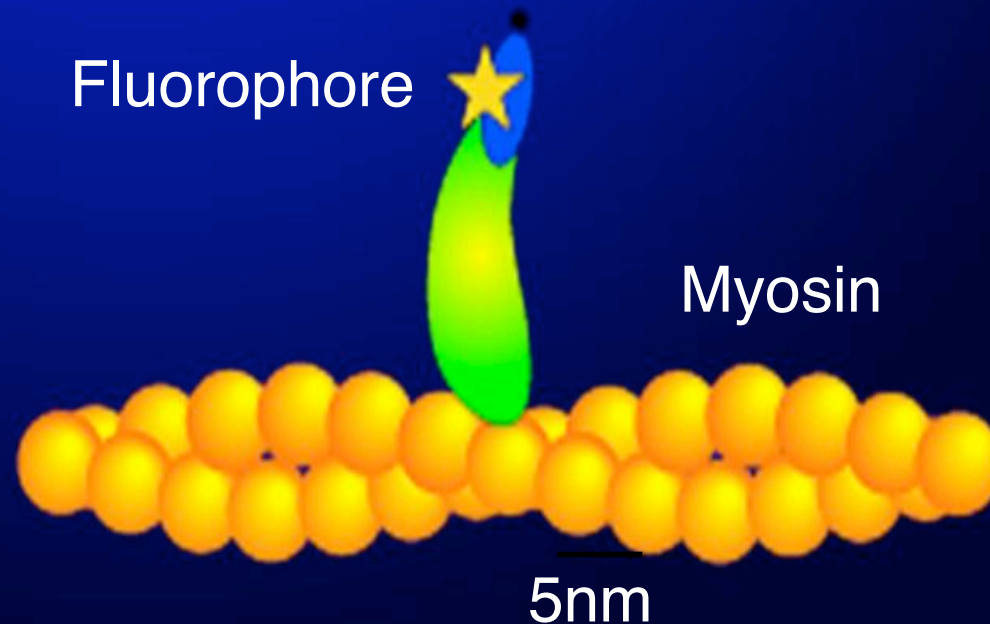
Aim of our research is to approach the engineering principle of flexible bio-machines by uncovering unique operation of biological devices.

Single molecule nano-detection

1) Single molecule imaging



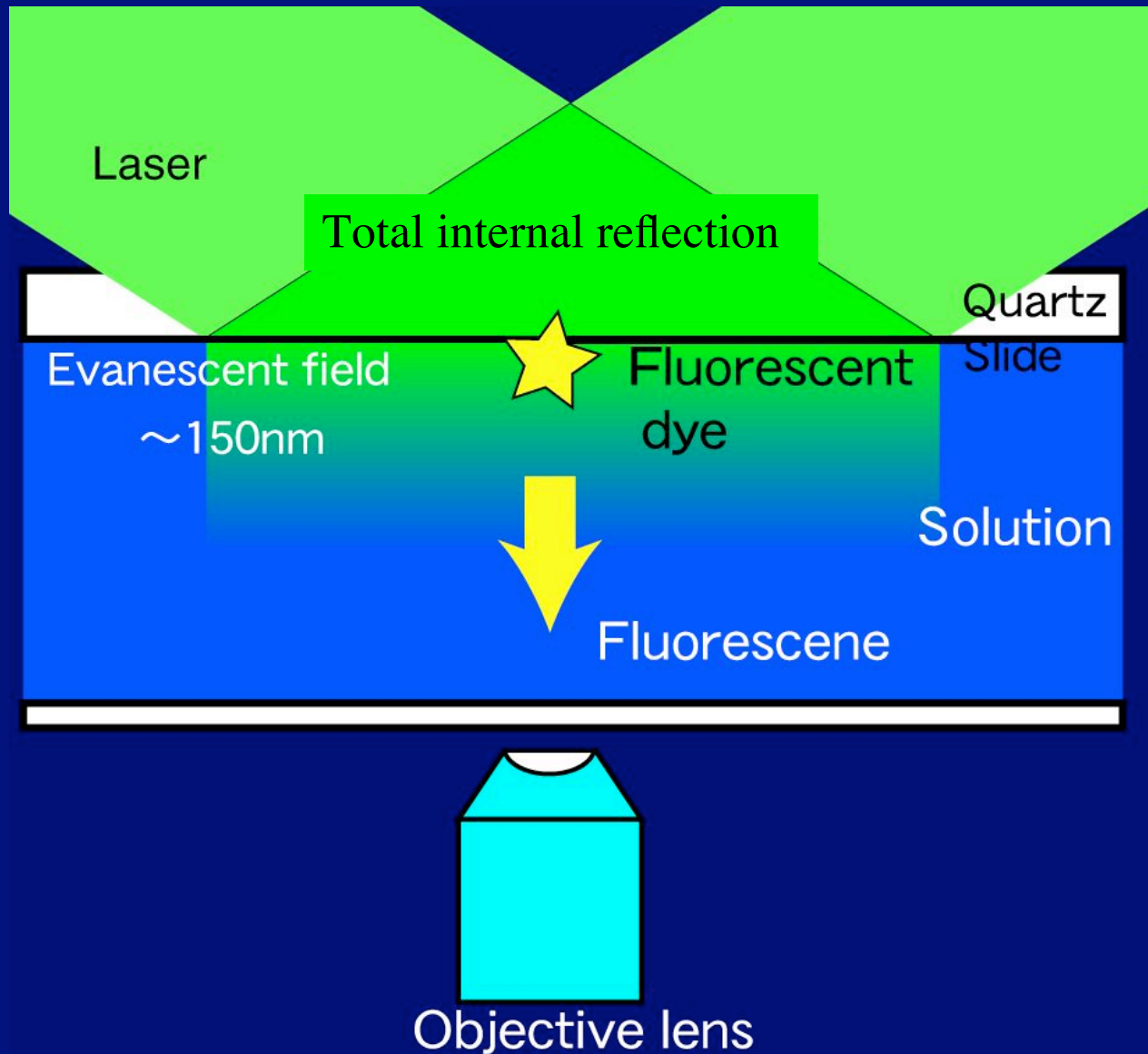
2) Single molecule nano manipulation



Stars at a great distance can be seen in the dark.



Imaging of single fluorescent dyes



TIRF

Near field

**Background noise:
2000-fold smaller
than conventional
fluorescence
microscope**

Fluorescence images of single fluorophores

Cy3-HMM



200 nm

Main players

Funatsu, Harada, Saito, Tokunaga, et al. *Nature* 374, '95

Applications

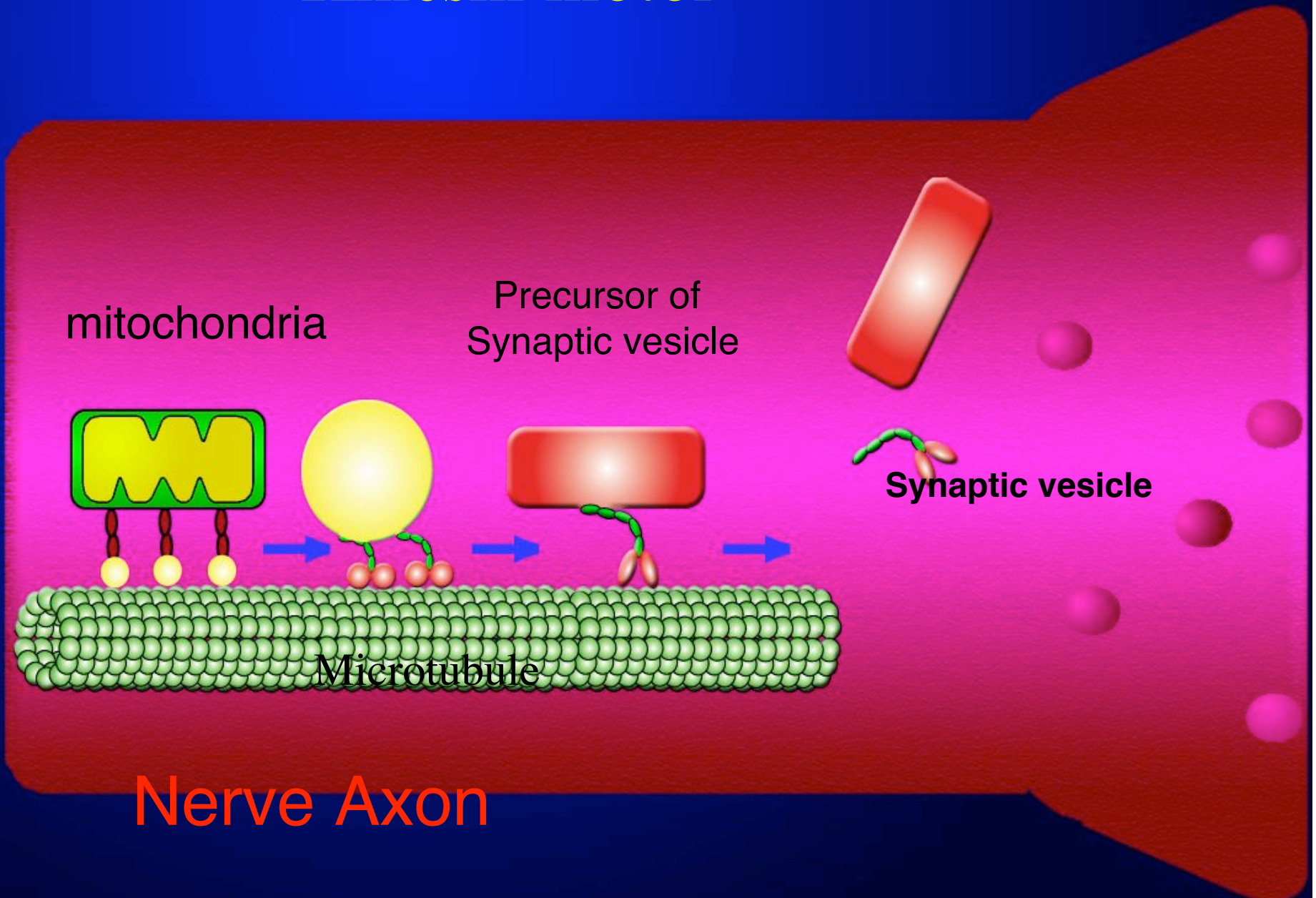
1) Kinsein

2) Myosin

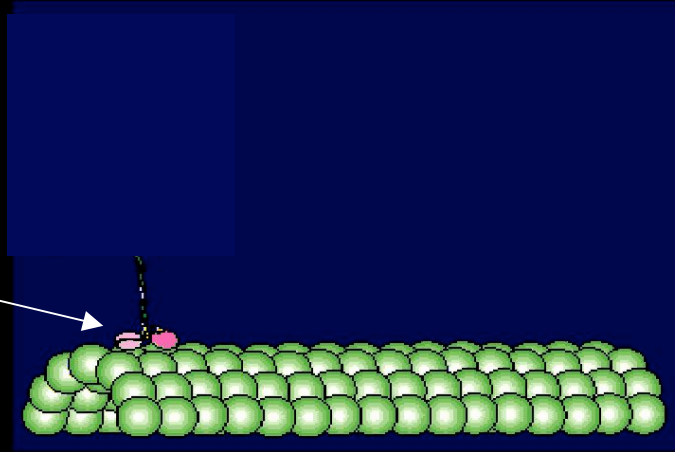
3) ATPase reaction

(enzyme reaction)

Kinesin motor

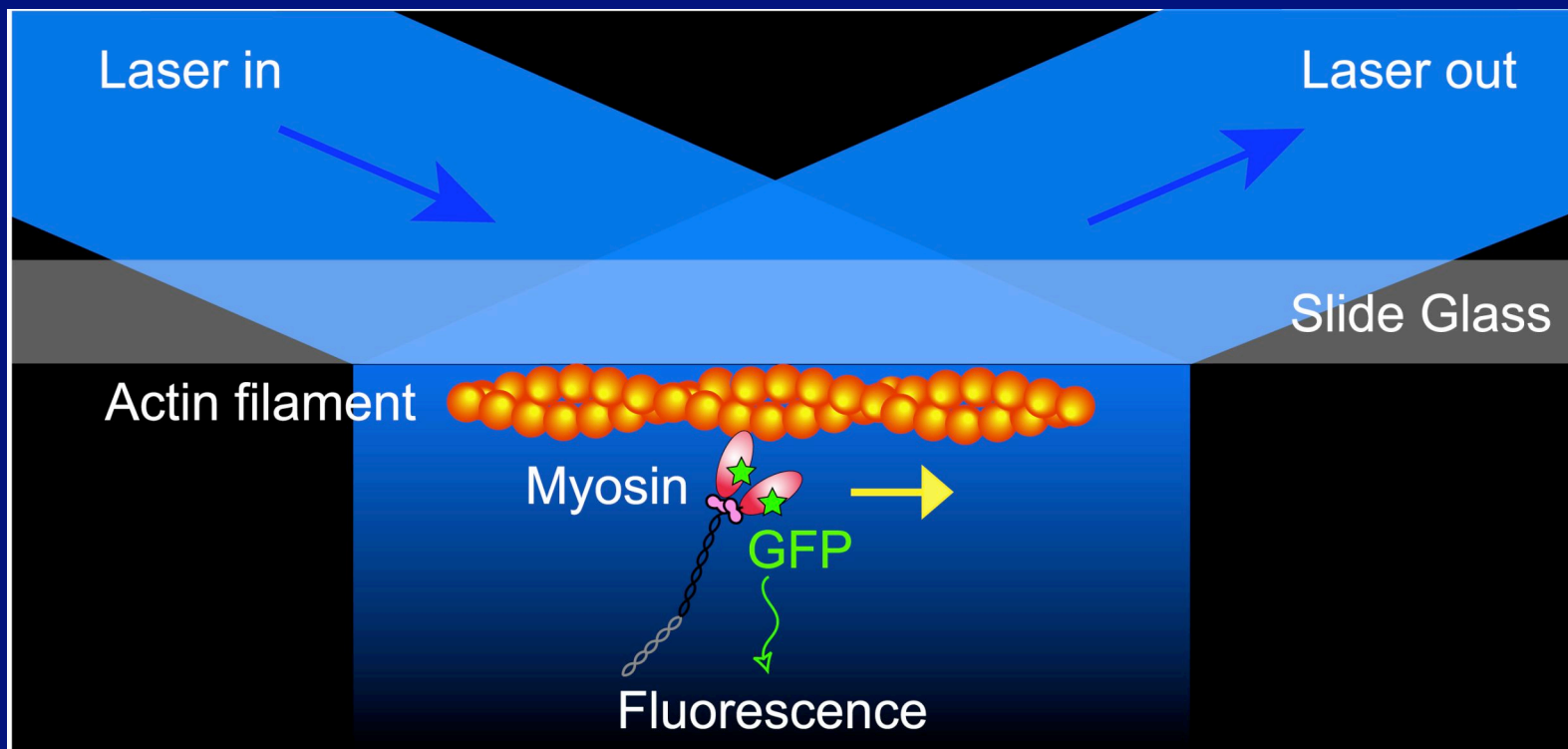
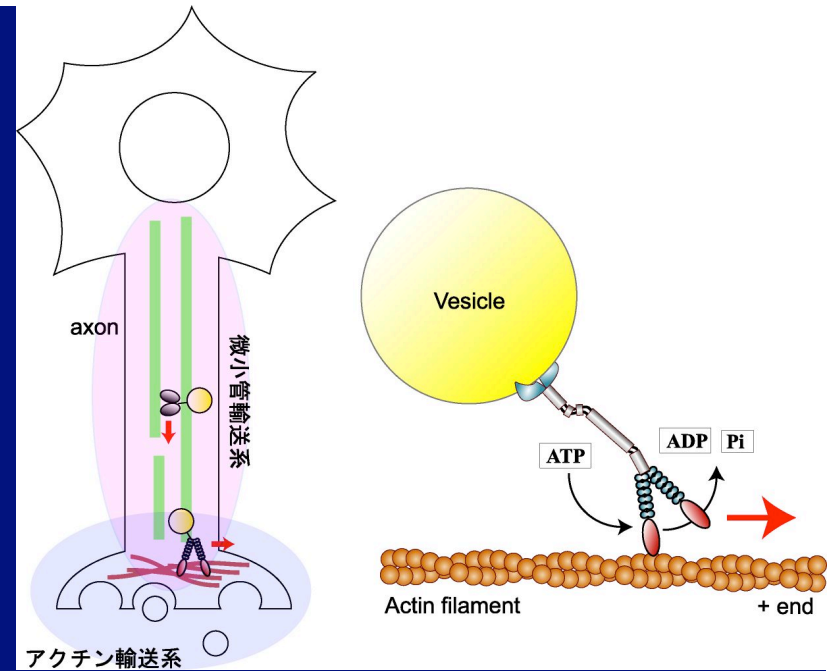


Kinesin is
nanometers
in size

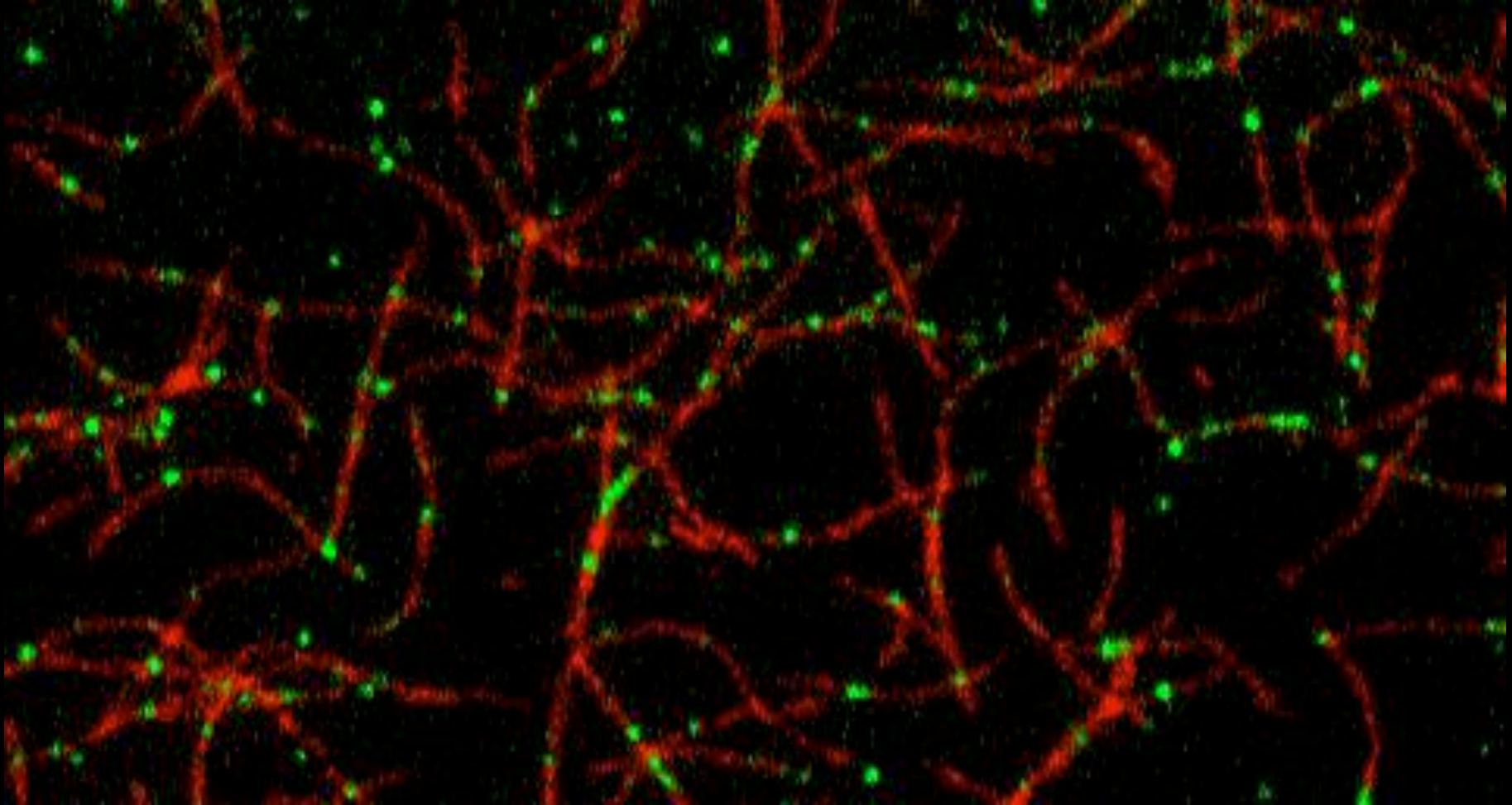


1nm (ナノメートル)
= 1 千万分の 1 cm

Unconventional myosin (V) functions as a transporter in cells.

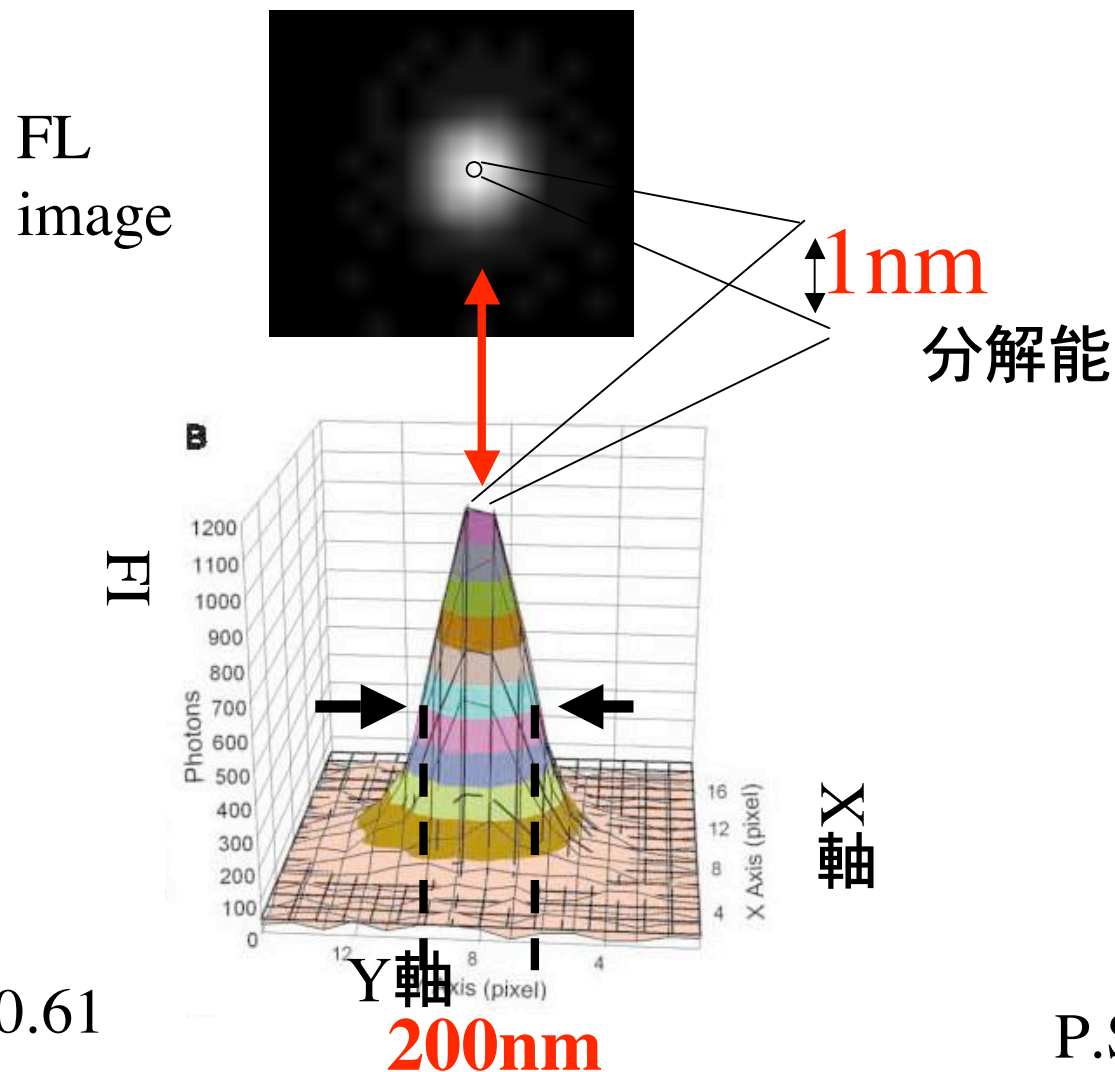


Class V myosin



Nishikawa, Iwane, et al.

Real time fluorescence nano-imaging by computer image analysis (FIONA)



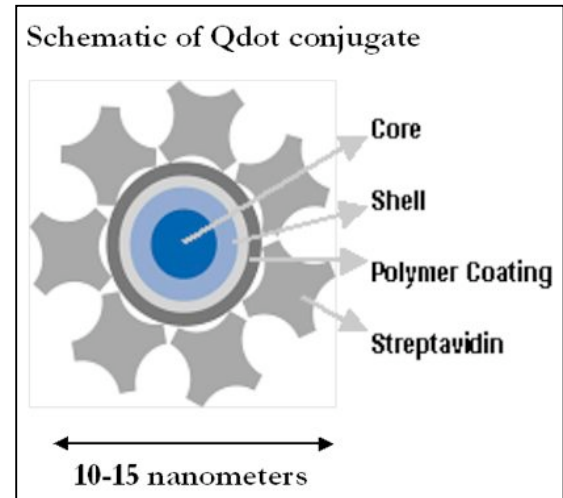
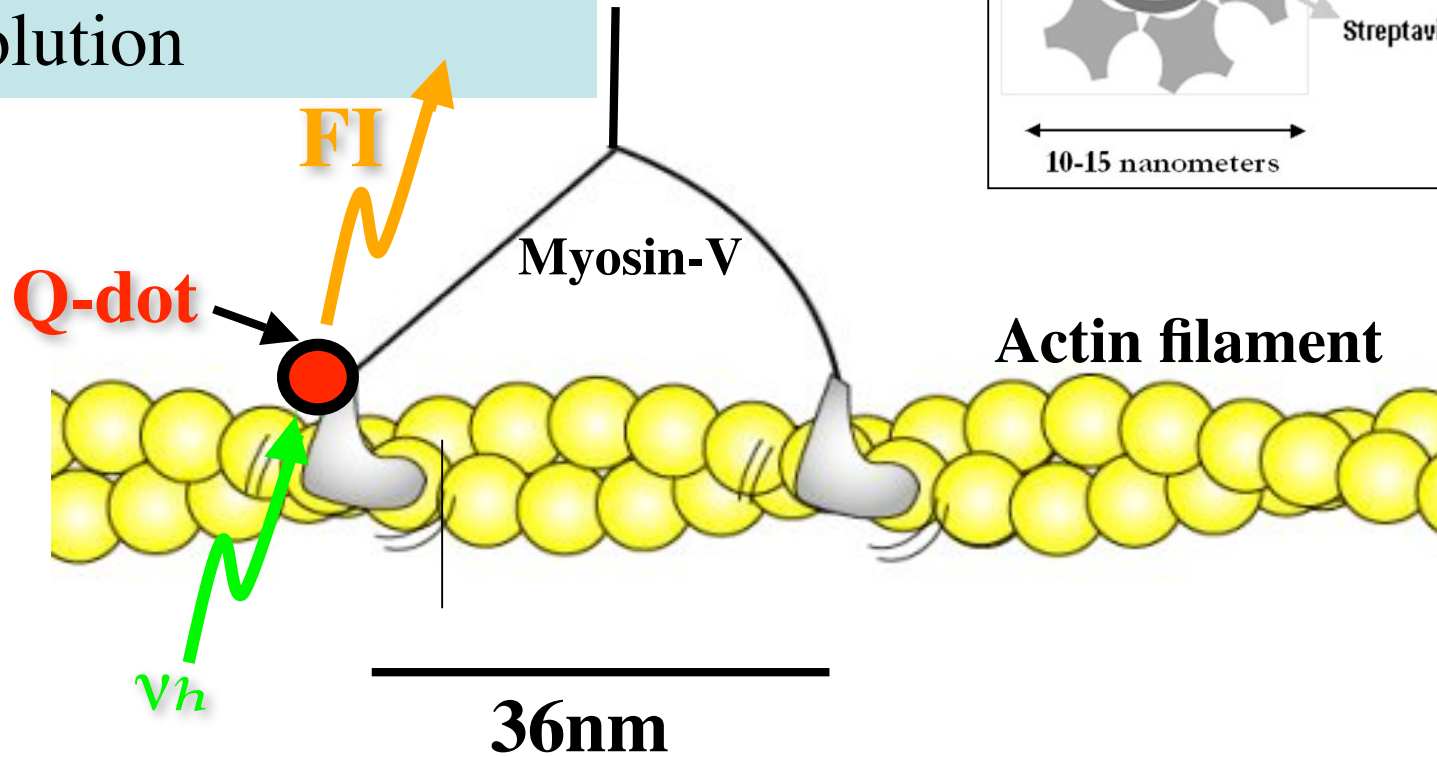
$$D = (\lambda / NA) \cdot 0.61$$


P.Selvin et al

How does myosin-V move?

nm, ms

spatiotemporal
resolution



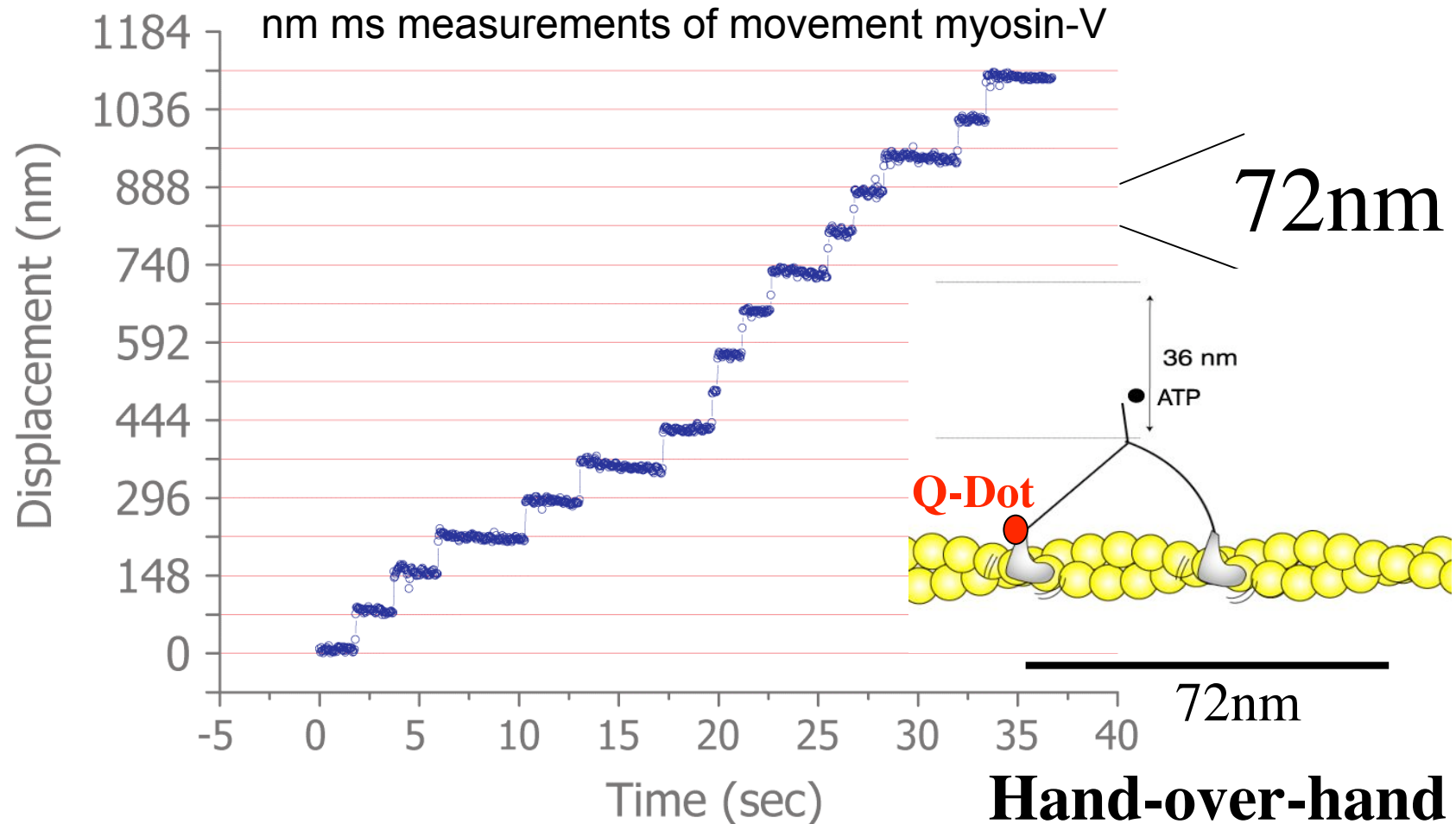
A cluster of stars, possibly a star-forming region, is visible in the center of the image. The stars are of varying brightness and are concentrated in a roughly circular area. The background is black, making the stars stand out.

10uMx120camx10倍速

FIONA powered by Qdot

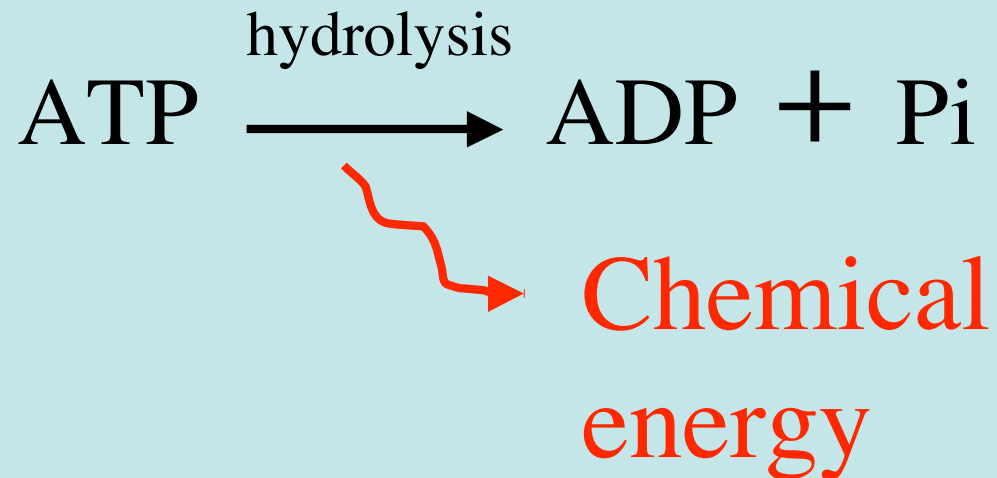
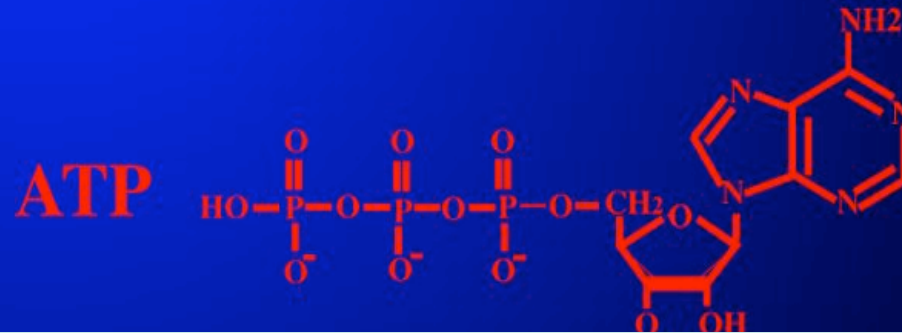


Sugawa, nishikawa et al in preparation

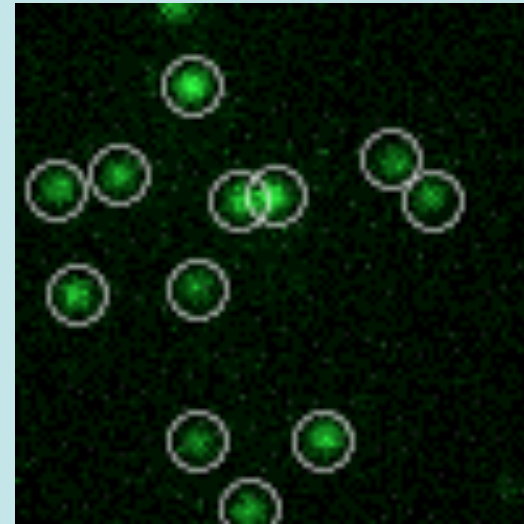
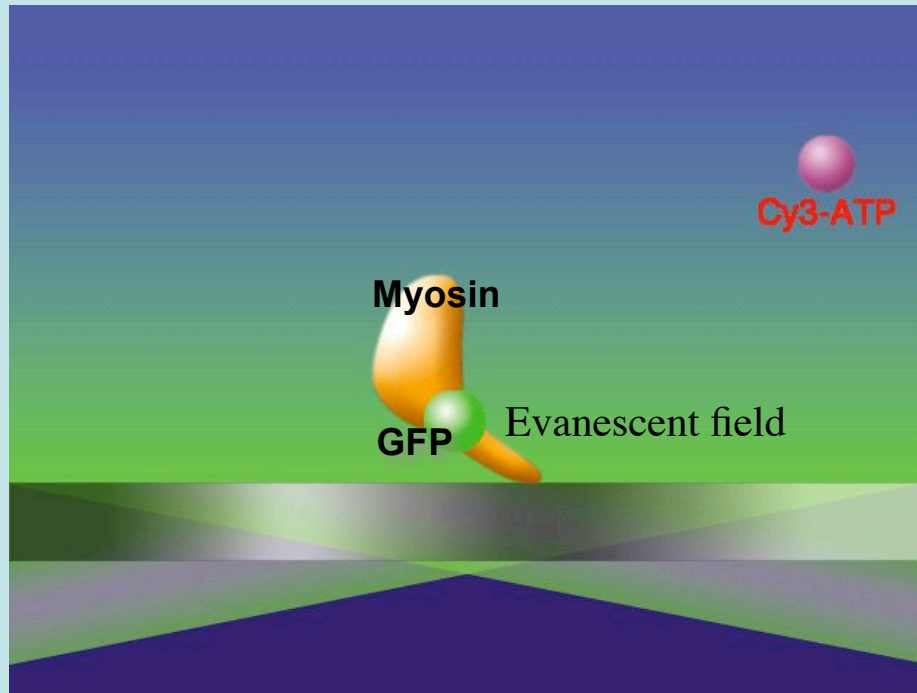


Direct observation of individual ATPase cycles

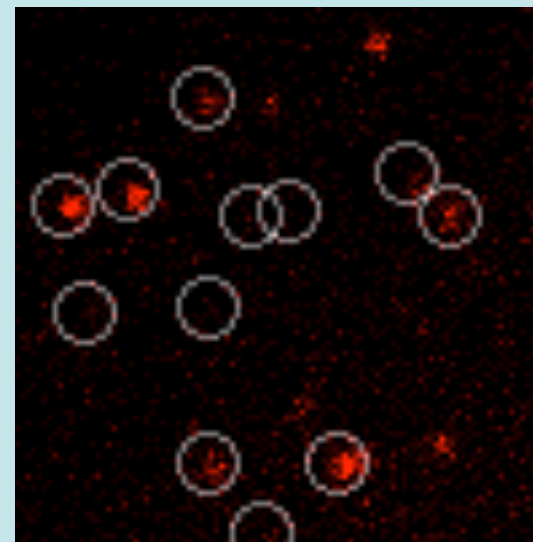
Molecular machines are fueled by chemical energy from ATP (Adenosine triphosphate) hydrolysis.



Single molecule imaging of ATP hydrolysis cycles by myosin



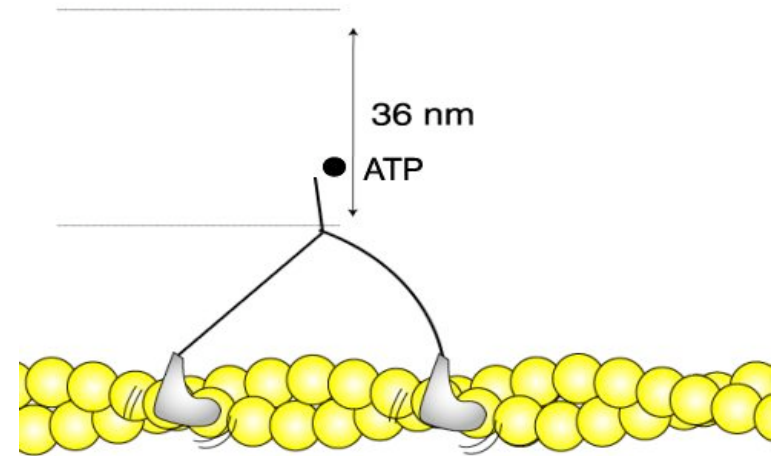
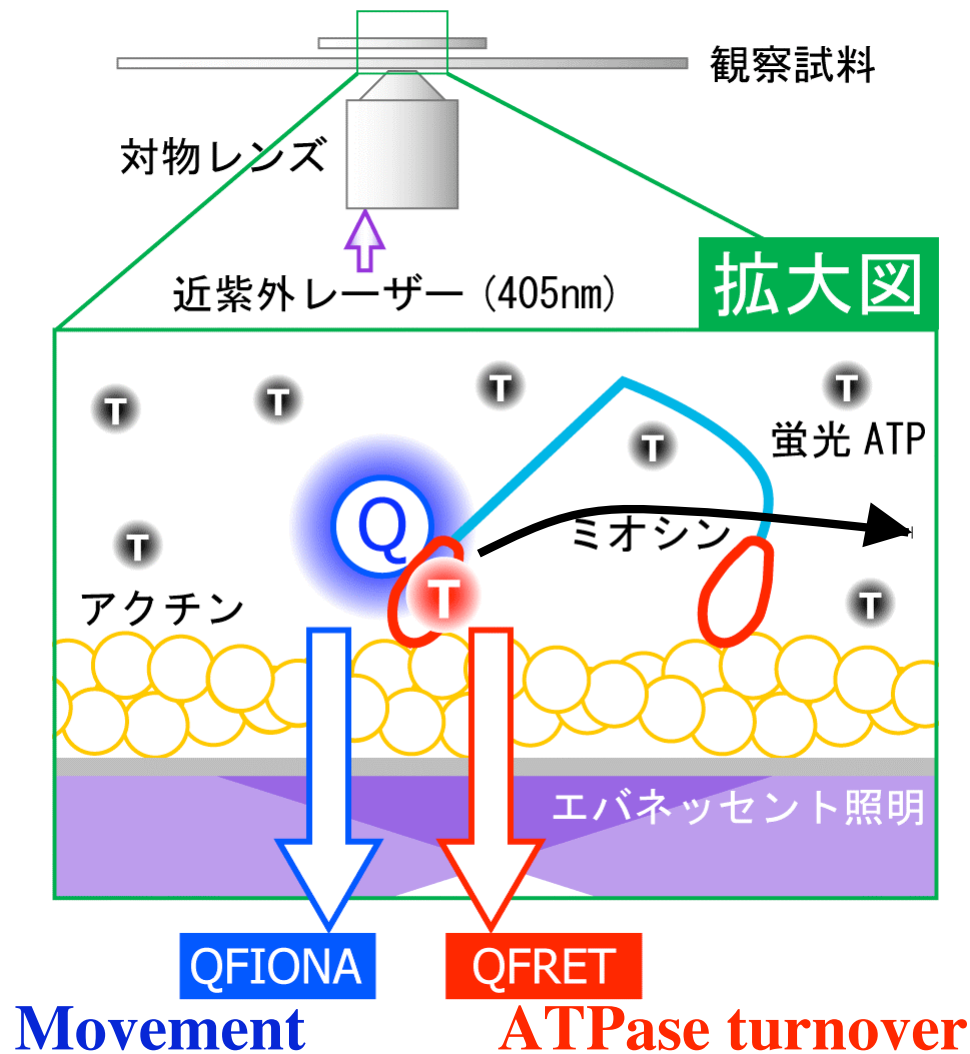
**GFP-
myosin**



**Cy3-
ATP**

Funatsu, Harada, Saito, Tokunaga, et al. *Nature* 374,'95
Komori, Nishikawa et.al. in preparation in preparation

Simultaneous observation of ATPase turnover and movement by QFRET & QFIONA



Okada et al. BBRC 2007
Komori, Nishikawa et al.
Poster

Single molecule detection technology

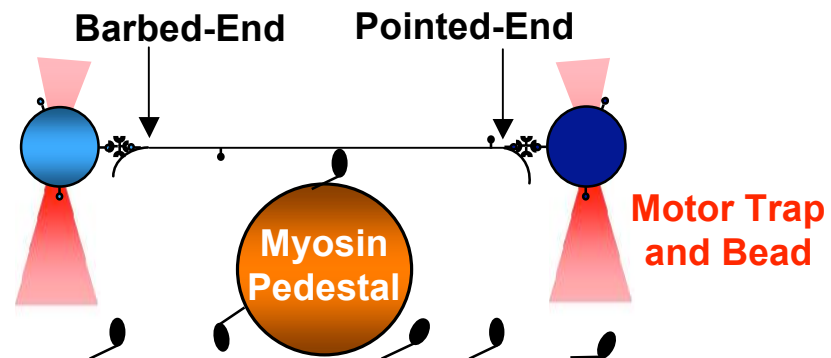
1) Single molecule imaging

2) Single molecule nano-manipulation



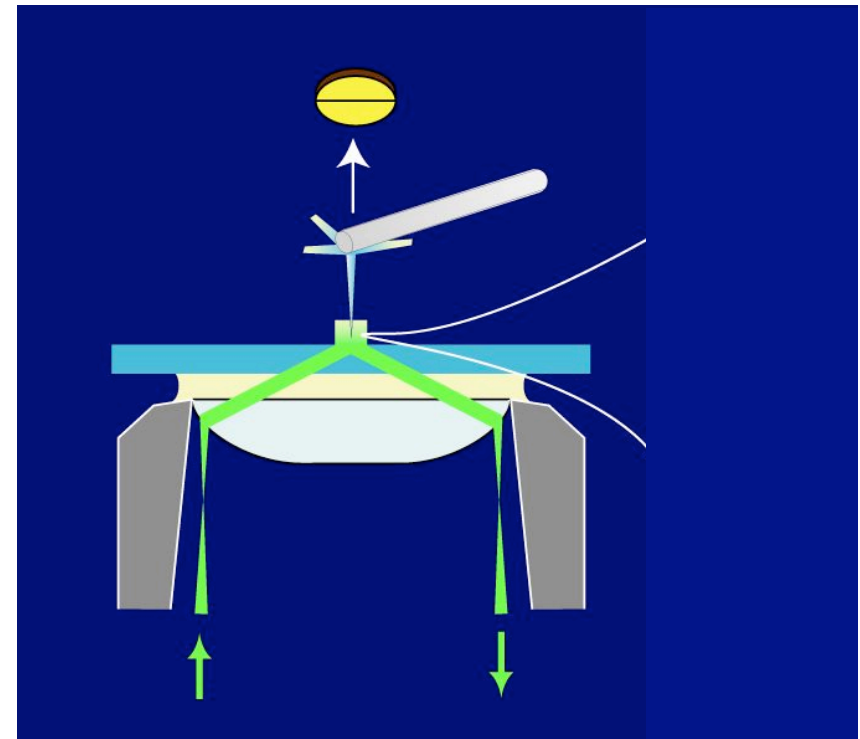
Single molecule nano-manipulation

Optical trapping nanometry



Finer,..Spudich, Nature 368 1994

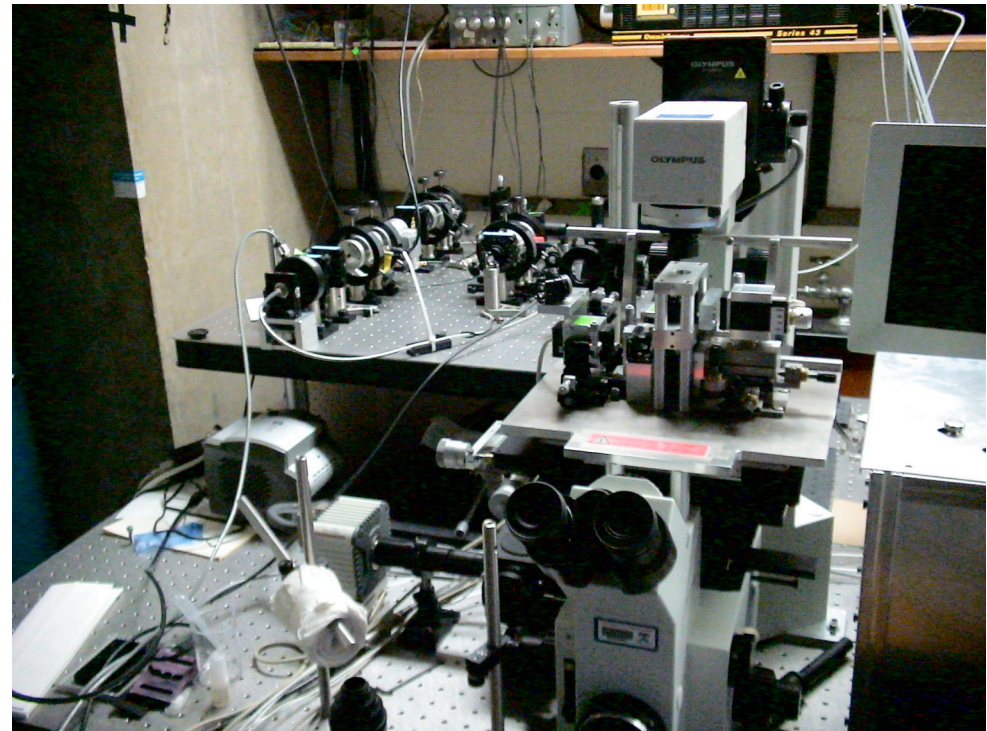
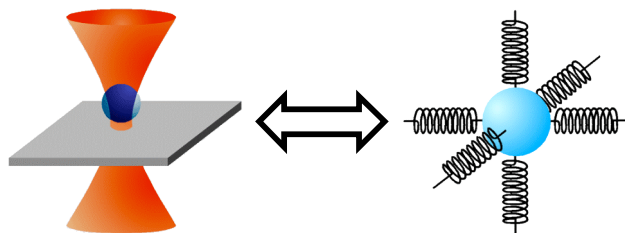
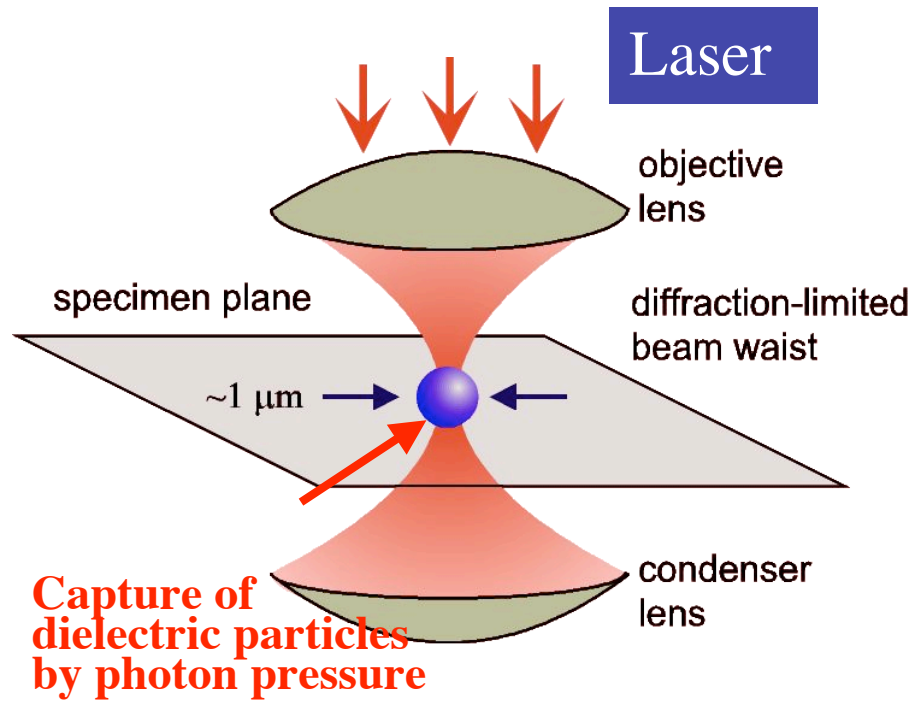
Scanning probe nanometry



Ishijima...ty, Nature, 1991,
BBRC 1994

Kitamura, Tokunaga...ty,
Nature397,1999

Single molecule nano-manipulation by optical tweezers



A. Ashkin *et al.*, 1986
Opt. Lett. 11:288-290

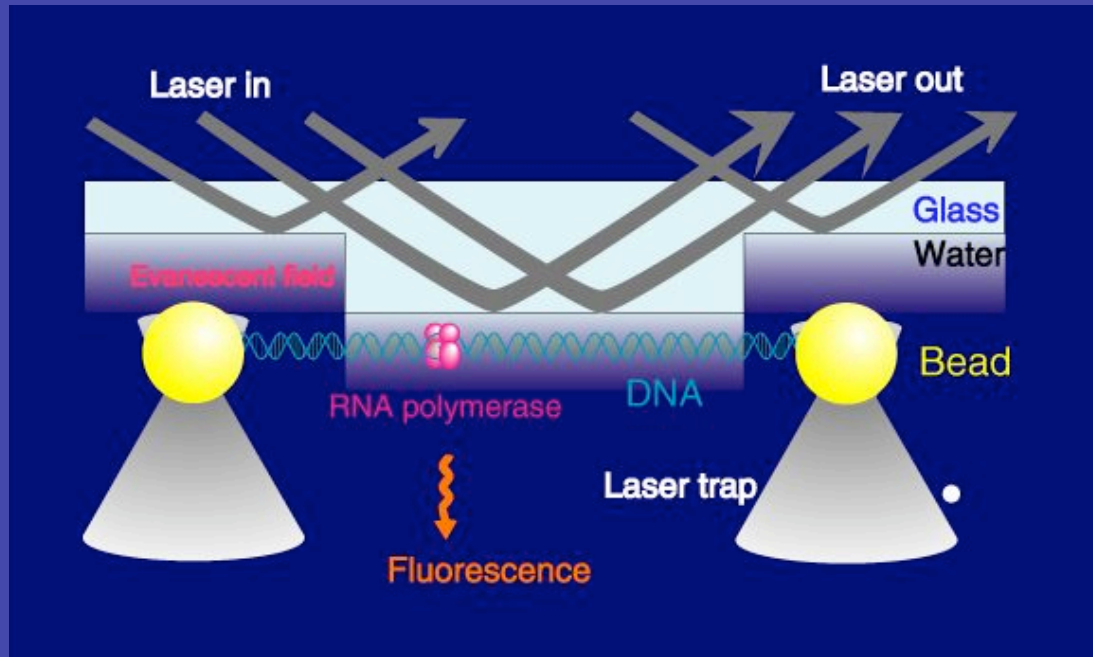
遺伝情報読みとり装置

光ピンセットによるDNAの1分子操作

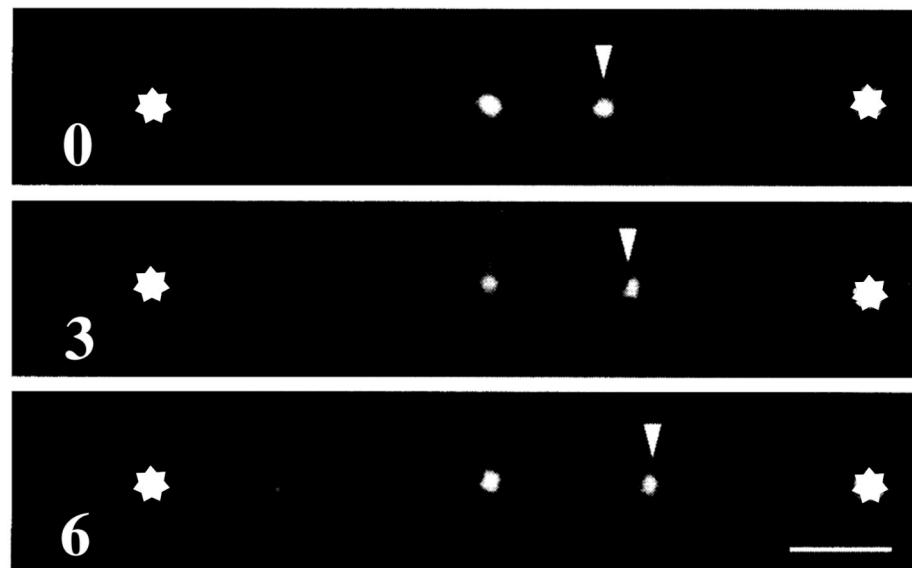


Harada et al.

RNAPによるプロモーターサーチの分子イメージング

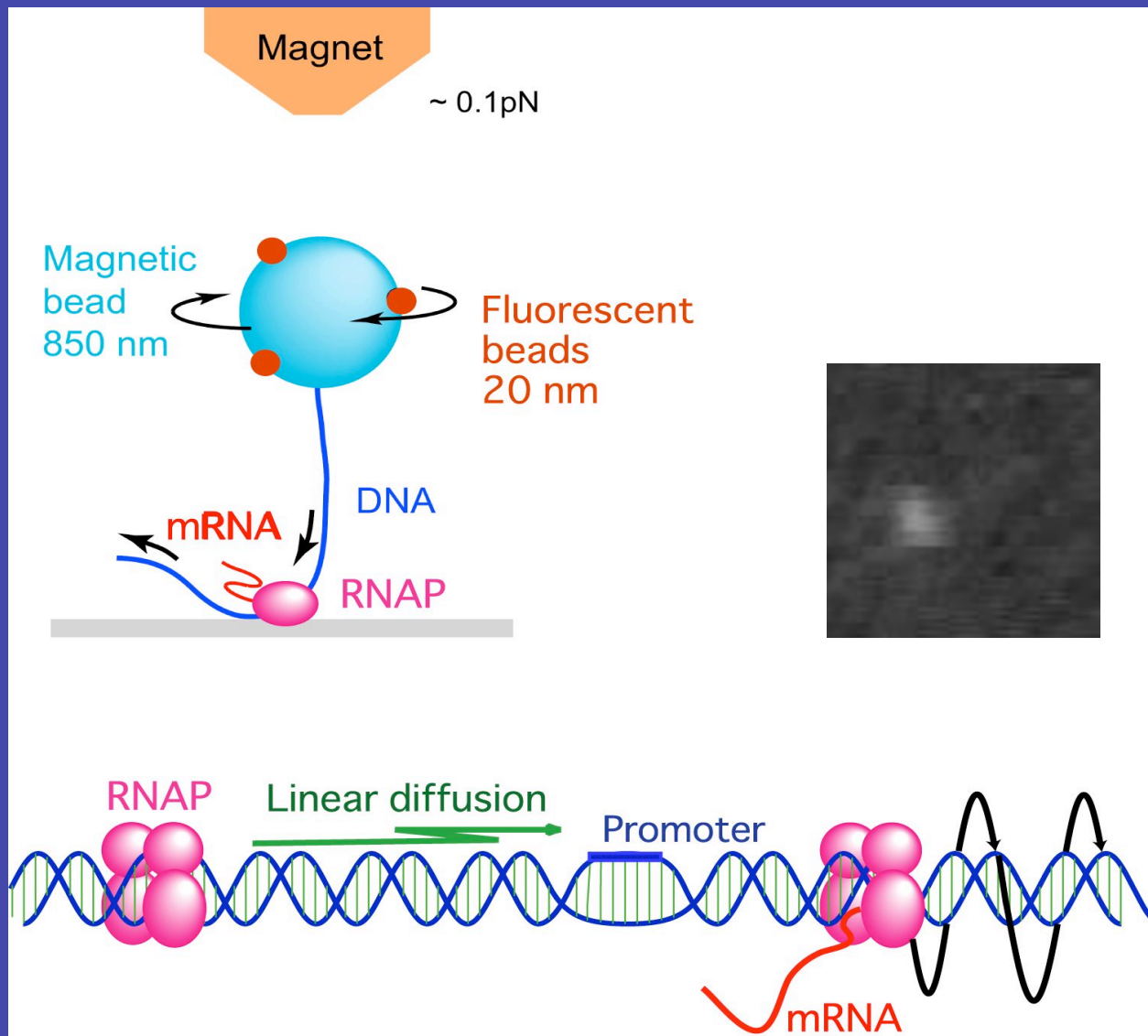


RNAポリメラーゼはDNAに沿って滑走して、プロモーターをさがす。



Harada, Funatsu, et al
Biophys. J. (1999)

遺伝情報発現過程の1分子イメージングと計測 (DNA 1分子のシーケンス)



転写中
DNAは
回転する

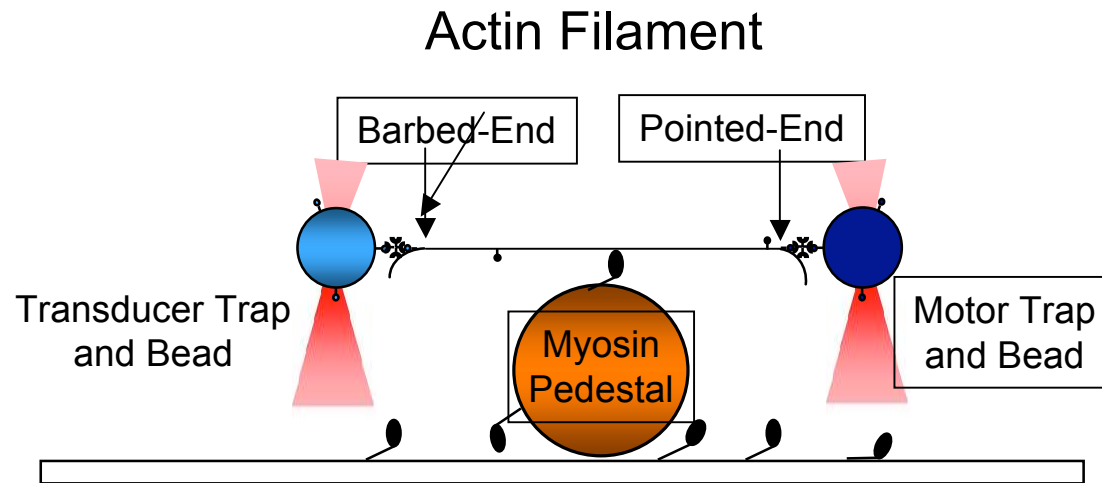
Manipulation of a single actin filament by an optical tweezers



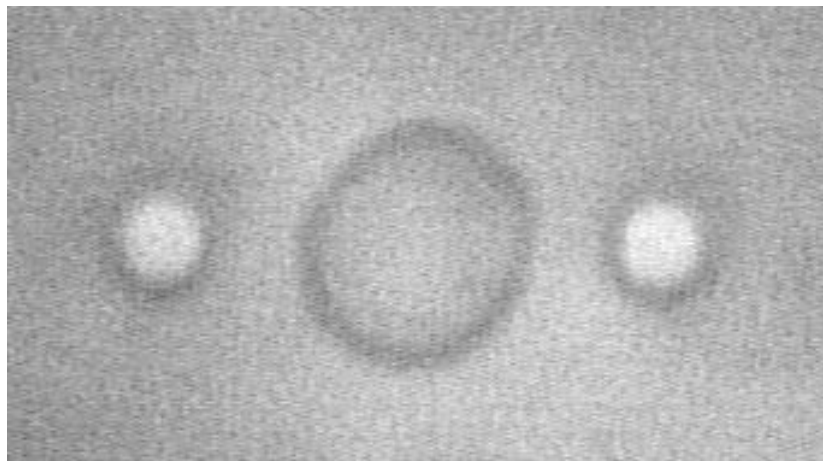
Movie

Single molecule mechanics of myosin motor

A



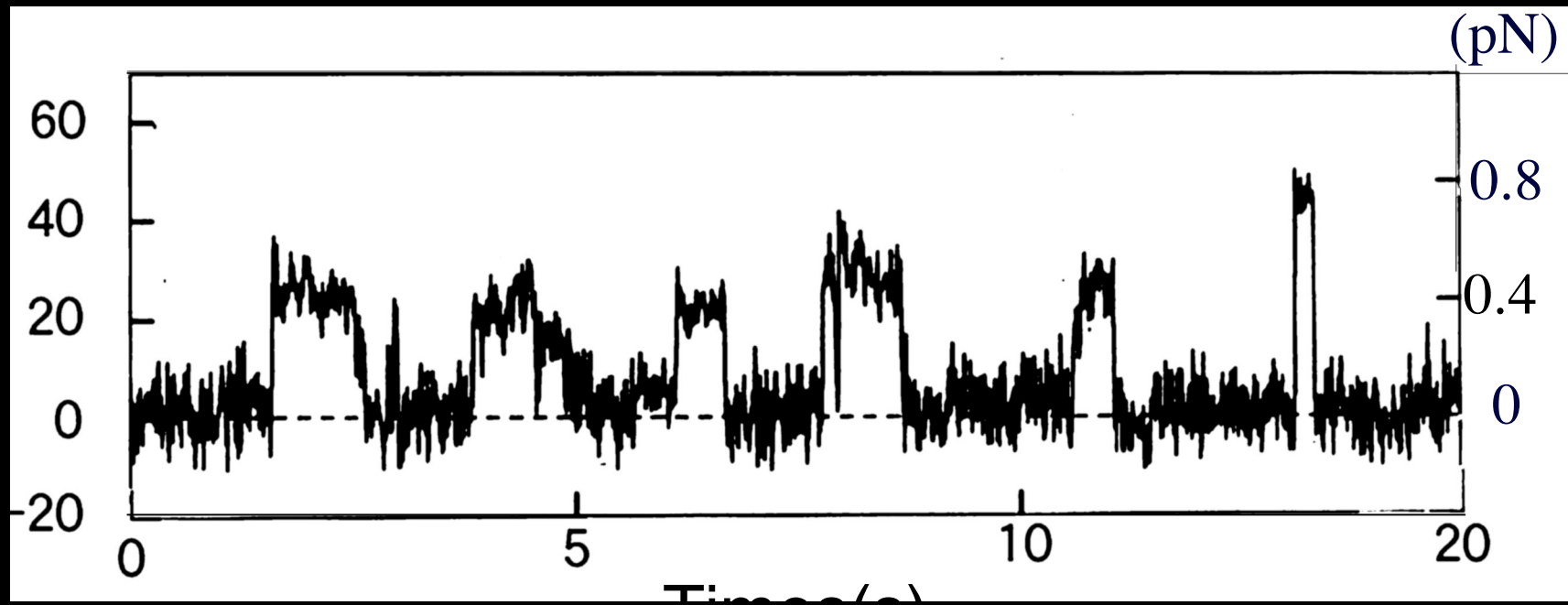
B



Force = 10 picoNewtons
= 10×10^{-12} N
 $\approx 22 \times 10^{-12}$ lbs
= 0.000,000,000,022 lbs

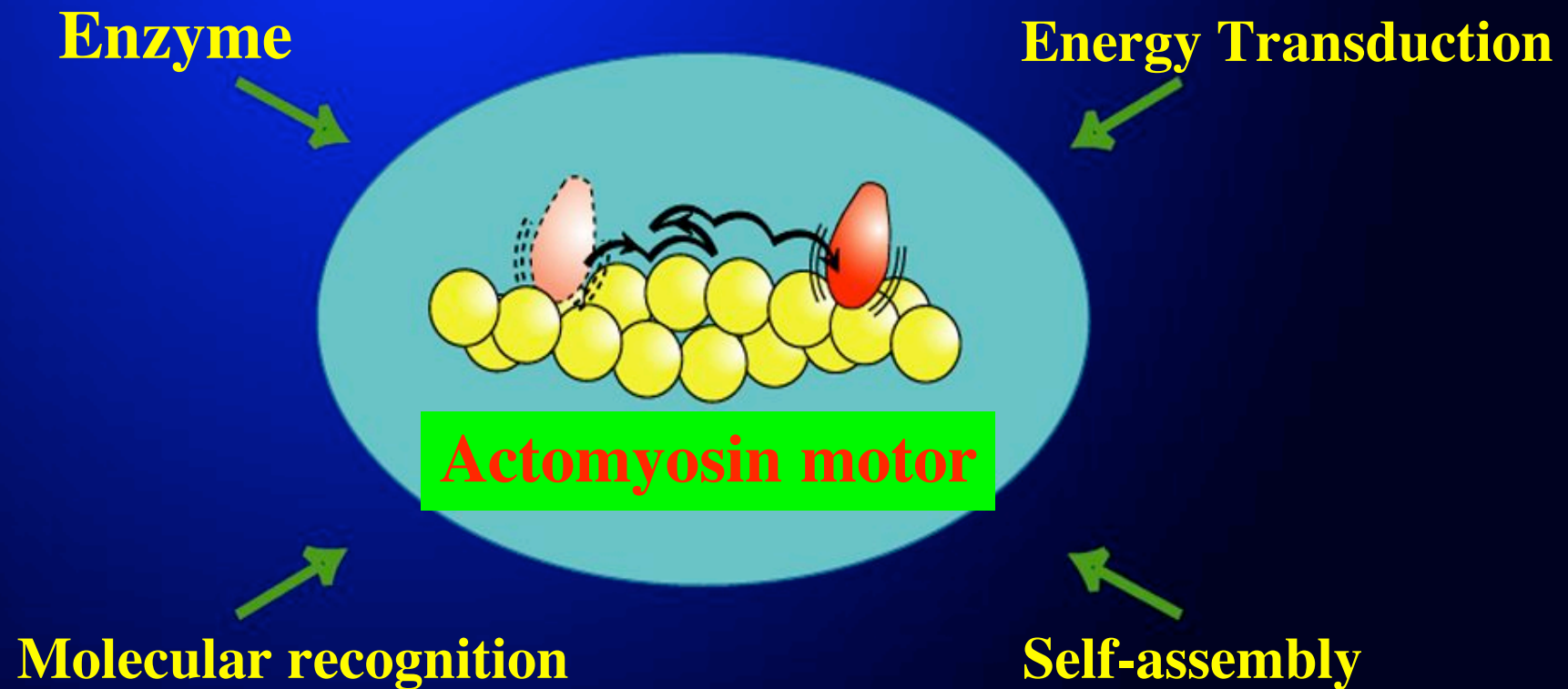
Displacement
= 10 nanometers

Displacements caused by a single myosin



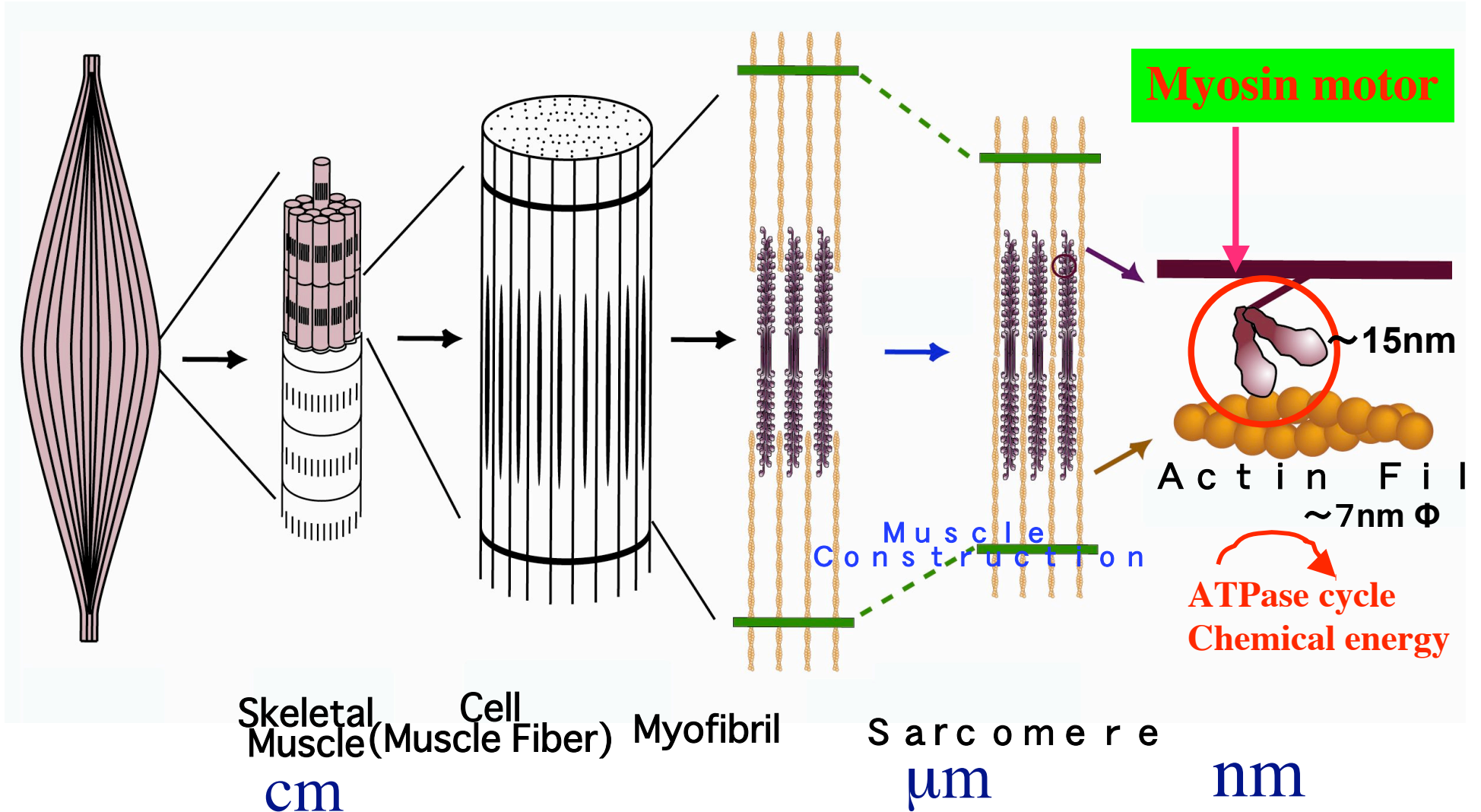
Tanaka H, et al. Biophys.J.75'98

How do biological molecular machines work?

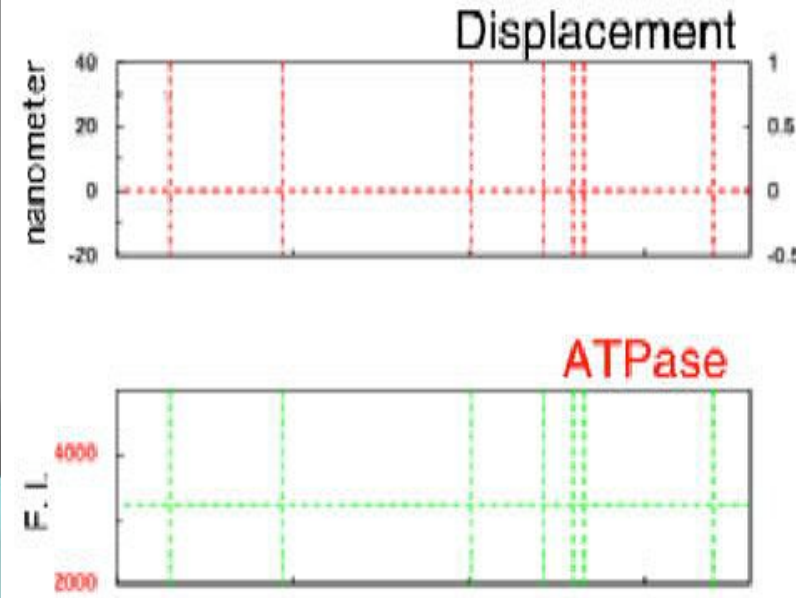
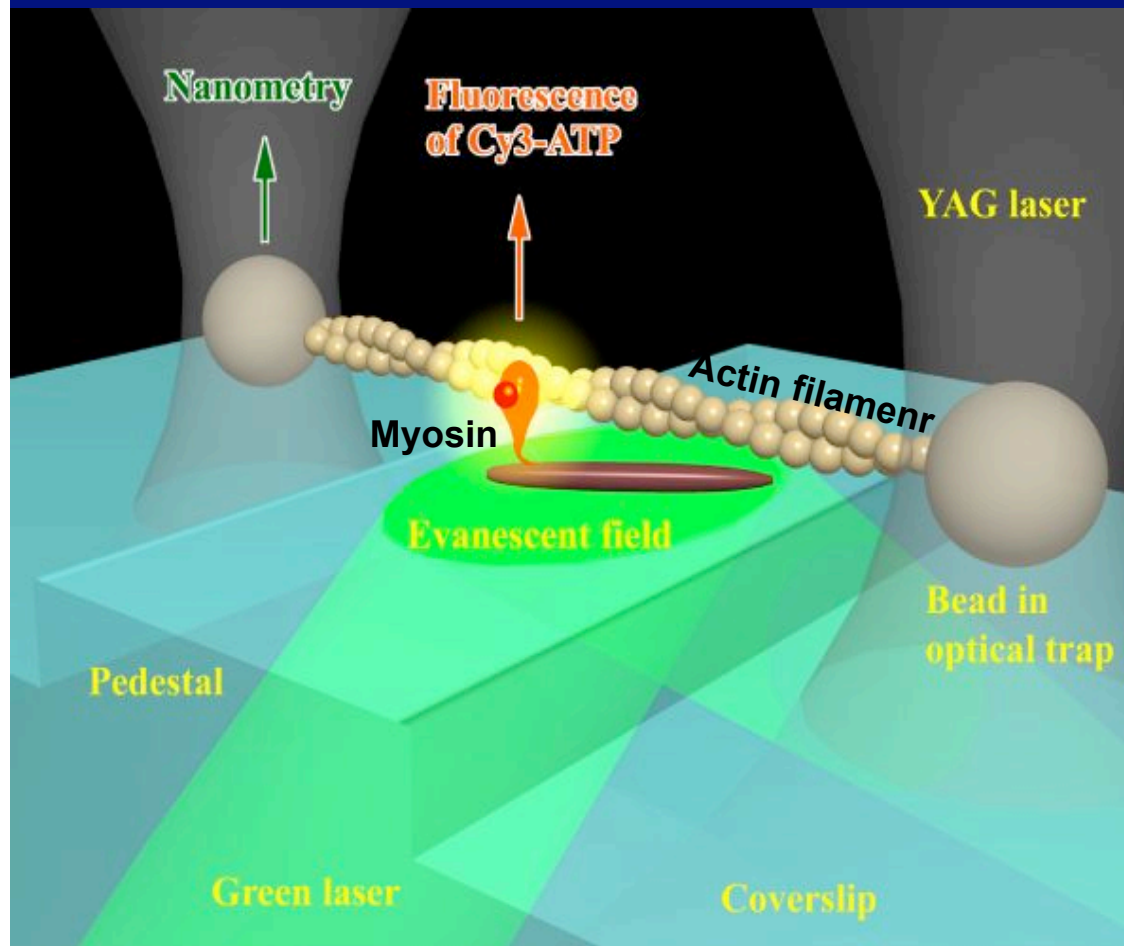


Important functions of proteins are integrated in the molecular motor.

Myosin, molecular motor in muscle



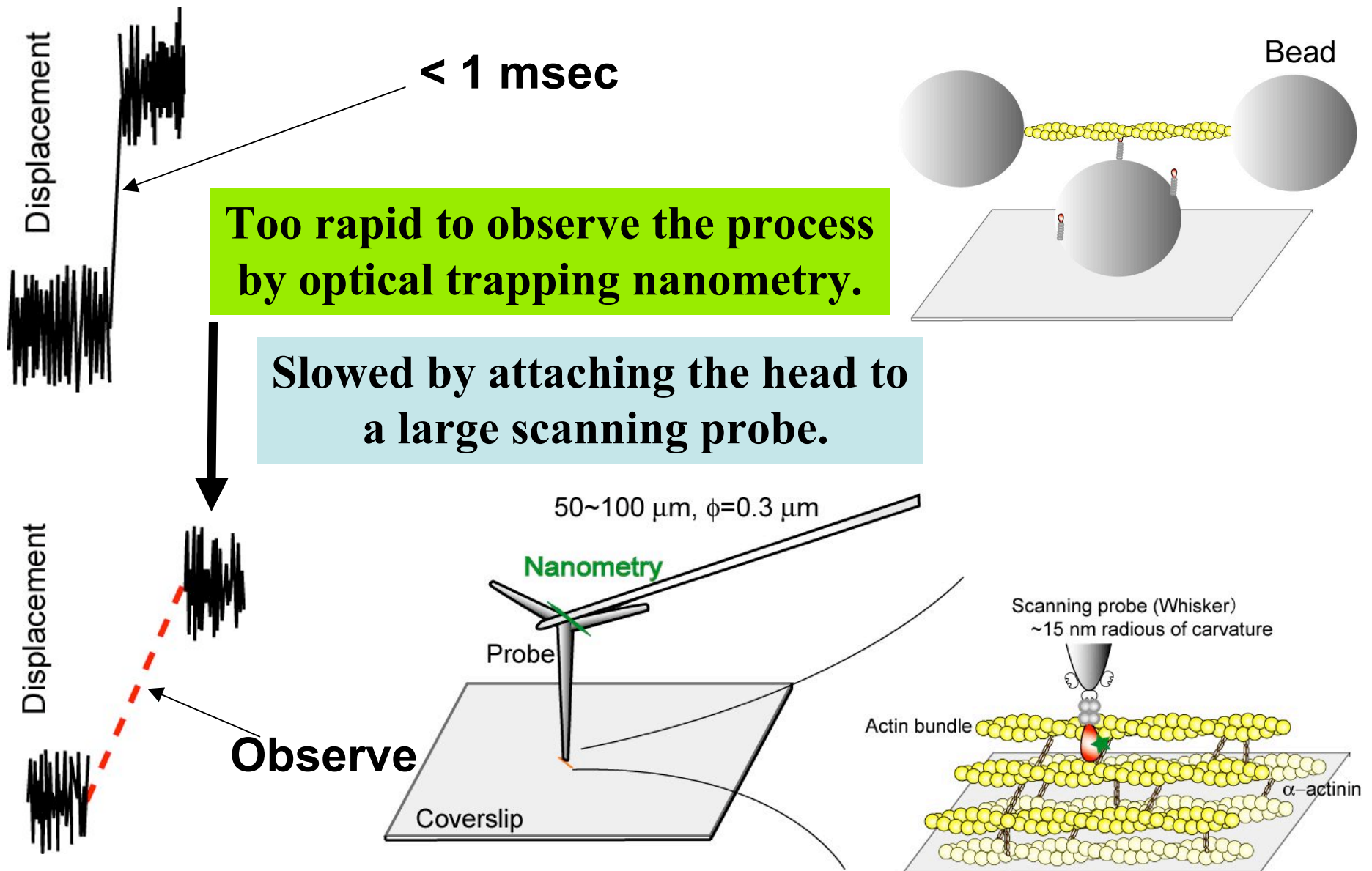
Simultaneous Measurement of Individual Chemical and Mechanical Reactions



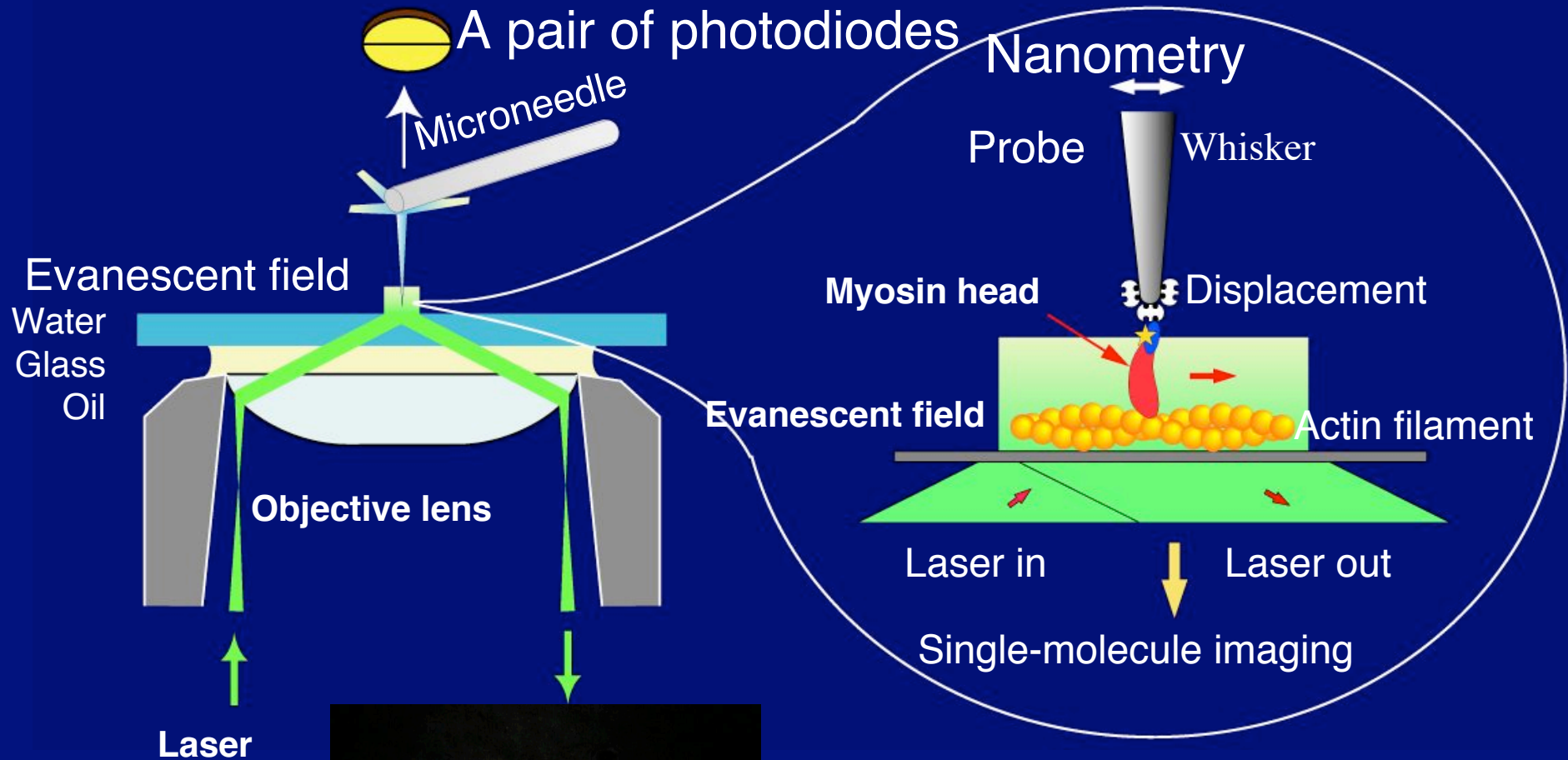
Optical microscope

Ishijima, Kojima,ty., Cell 92 '98
Komori, Nishikawa et al. 2007

Resolving the process of displacements by a myosin head with scanning probe nanometry



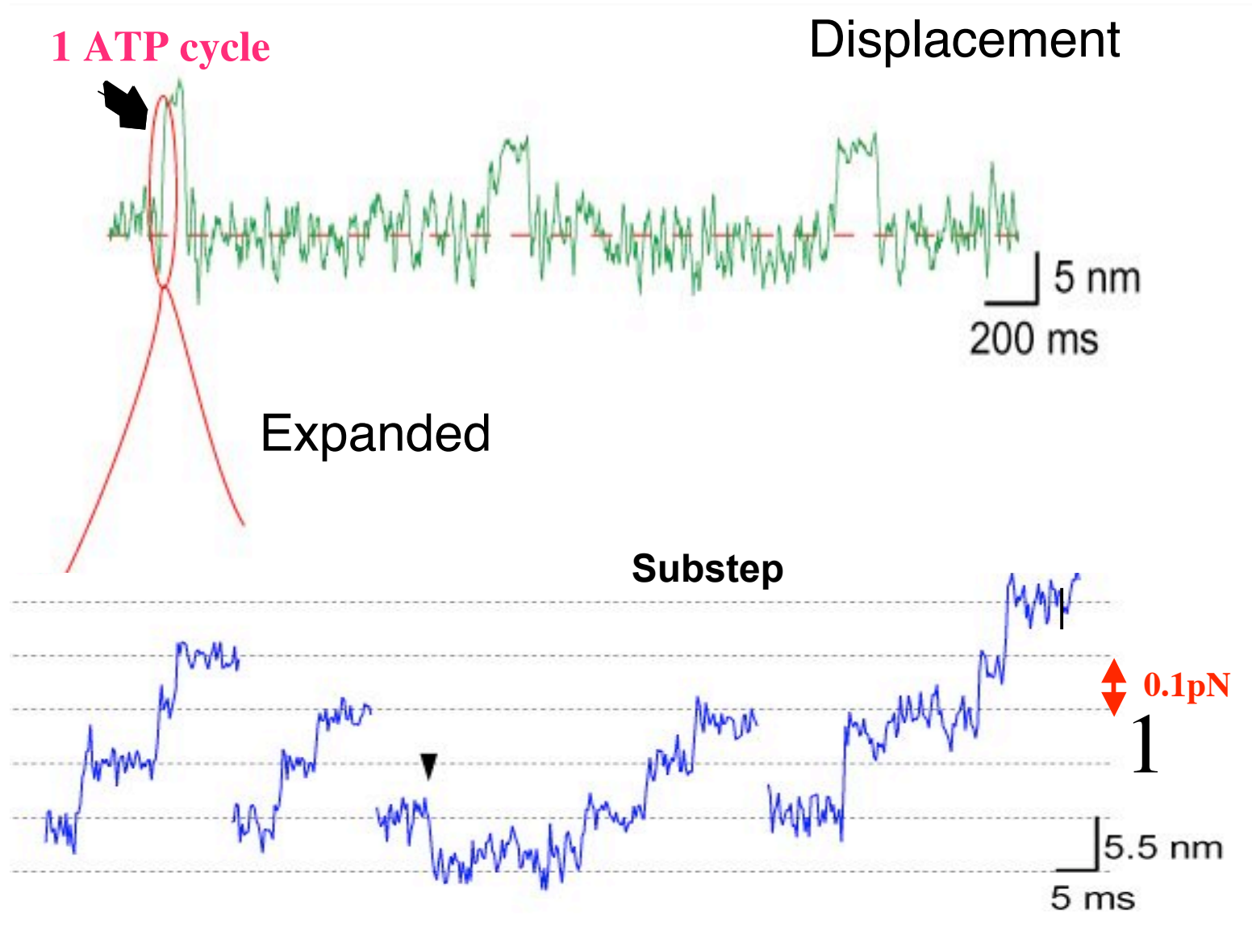
Spying on the process of a displacement



Images of single myosin heads

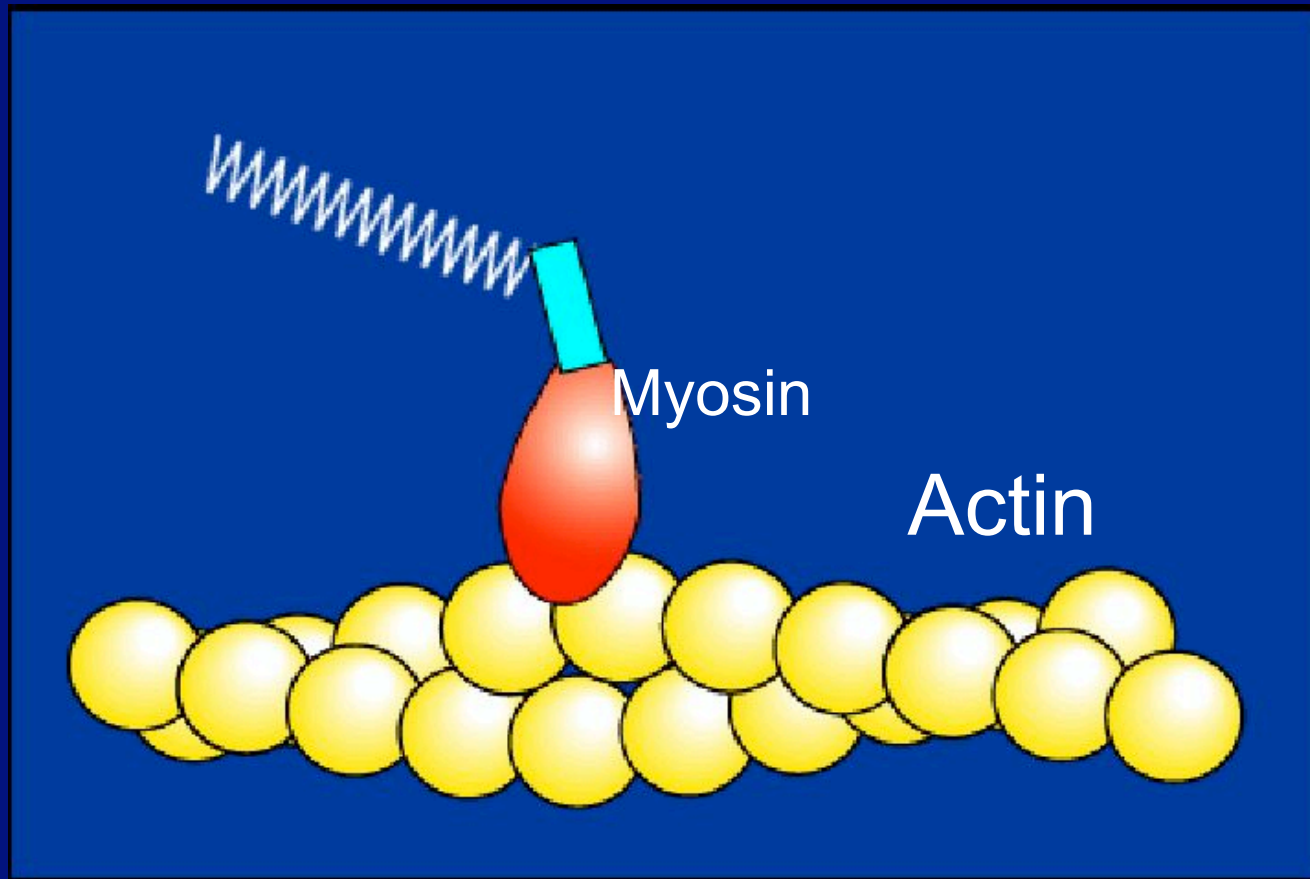
Kitamura, Tokunaga, Iwane,...TY
Nature 397 '99, Biophyscs, 1, 2006
Okada et al. BBRC, 2007

Process of displacements



Result

A myosin head walks on actin monomers
by Brownian motion



Movie

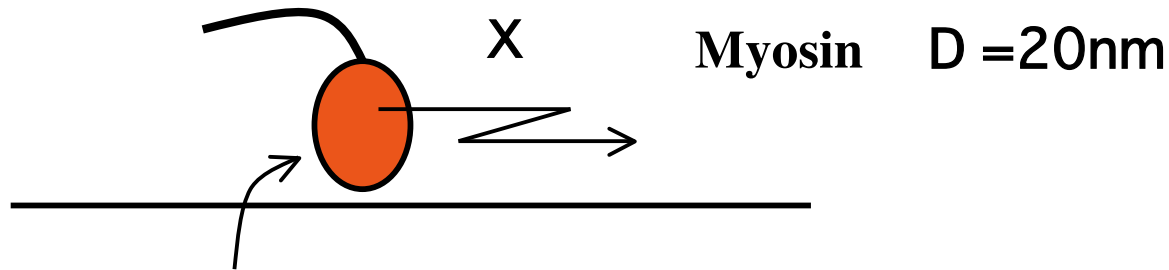
5.5 nm

Brownian motion is sufficient to produce the movement and force of myosin?

Brownian motion is sufficient to generate muscle contraction?

Diffusion equation $\langle x^2 \rangle = 2Dt$

Diffusion coefficient



$$D = \frac{k_B T}{6 \pi a} = \frac{4 \text{ nm} \cdot \text{pN}}{6 \times \pi \times 10^{-2} \text{ g/ms} \cdot 10 \text{ nm}} = 2 \times 10^{-9} \text{ m}^2/\text{s}$$

If travel distance $D = \langle x^2 \rangle^{1/2} = 20 \text{ nm}$ の場合

$$t = \frac{\langle x^2 \rangle}{2D} = \frac{(20 \text{ nm})^2}{(2 \times 2 \times 10^{-9} \text{ m}^2/\text{s})} \sim 10^{-7} \text{ s}$$

$$\text{Velocity} = \frac{20 \text{ nm}}{t} = 20 \text{ cm/s}$$



Cf. velocity of myosin in muscle = $20 \mu\text{m/s}$

Brownian motion is much faster than the motion of myosin

To produce 2pN force by Brownian motion, it takes ?????? s

If travel distance $D = \langle x^2 \rangle^{1/2} = 20 \text{ nm}$ の場合

$$t = \frac{\langle x^2 \rangle}{2D} = \frac{(20 \text{ nm})^2}{(2 \times 2 \times 10^{-9} \text{ m}^2/\text{s})} \sim 10^{-7} \text{ s}$$

$$\text{Velocity} = \frac{20 \text{ nm}}{t} = 20 \text{ cm/s}$$



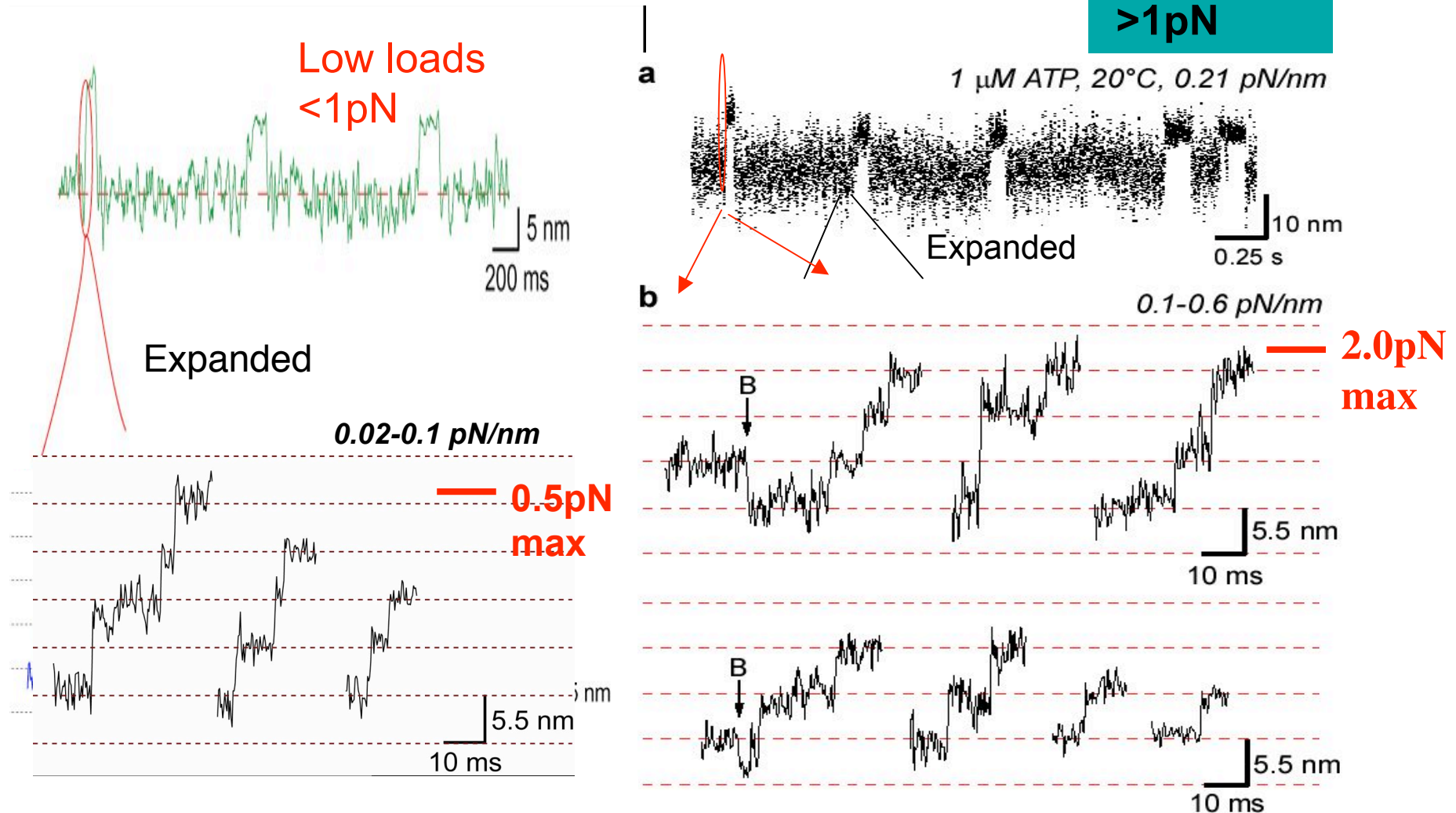
Cf. velocity of myosin in muscle = $20 \mu\text{m/s}$

Brownian motion is much faster than the motion of myosin

To produce 2pN force by Brownian motion,

it takes $t = 2 \times 10^{-7} \text{ s} \exp(2\text{pN} \times 20\text{nm}/kT) = \sim 2\text{ms}$

Load dependence of substeps

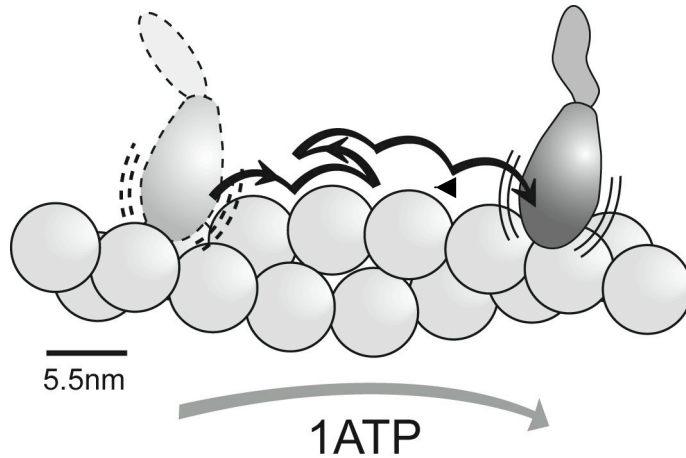


The stepsize (5.5nm) is unchanged but the dwell time increases and the number of backward steps increases .

Kitamura, Tokunaga,...TY, Biophysics 1 (2005)

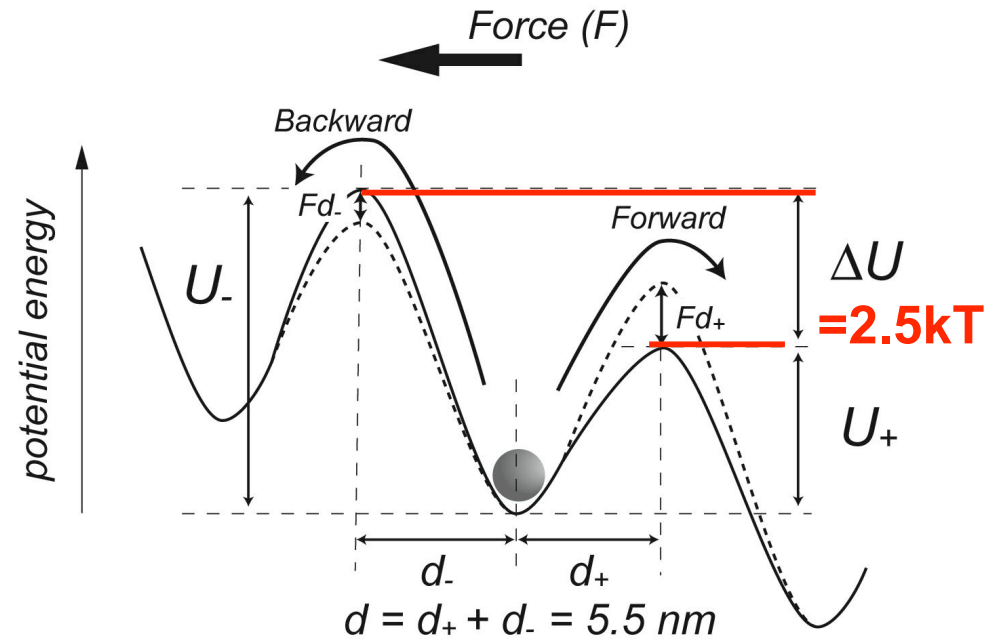
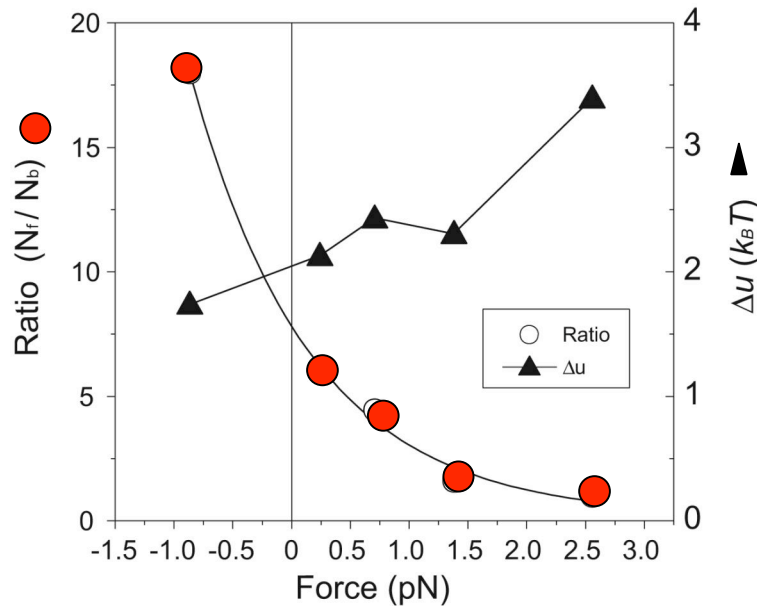
Bias potential energy

*Kitamura et al. Biophysics 1 (2005),
Okada et al. BBRC(2007)*



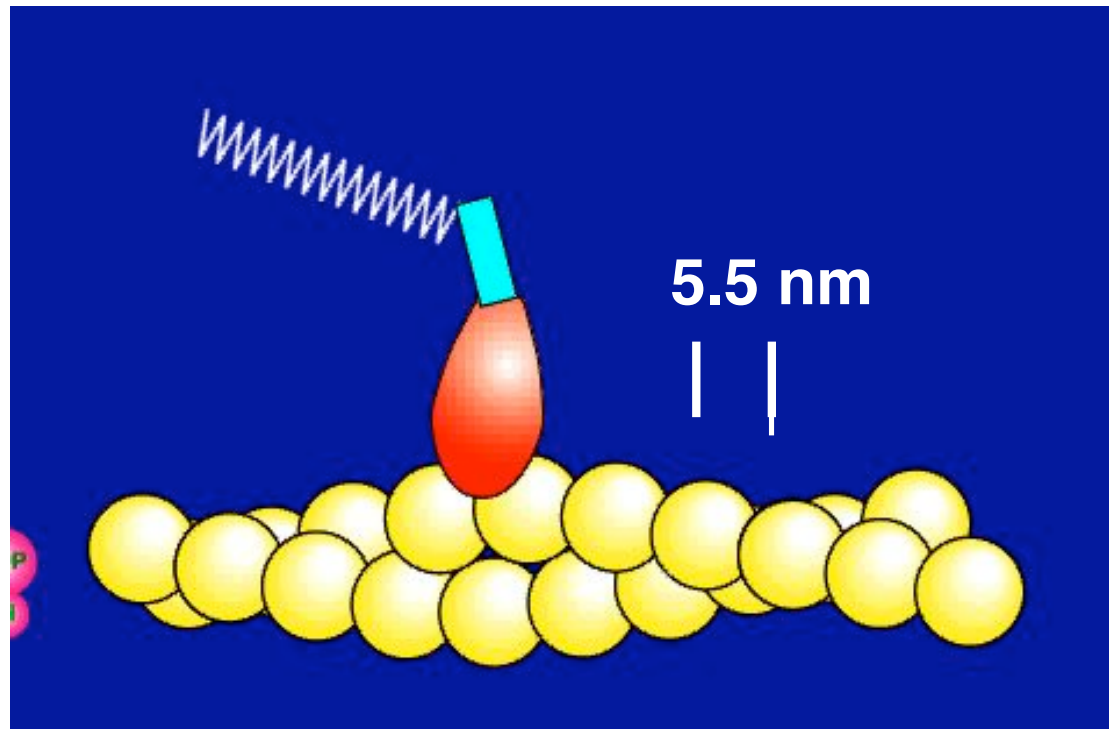
**Forward step/Backward step
= $\exp[(\Delta u - Fd)/kT]$**

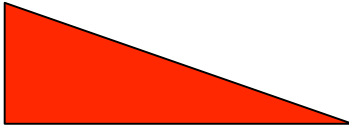
Δu = Bias potential energy



Bias potential energy = 2.5 kT/5.5nm substep or 10kT /displacement
 ||
 (50% of ΔG_{ATP})

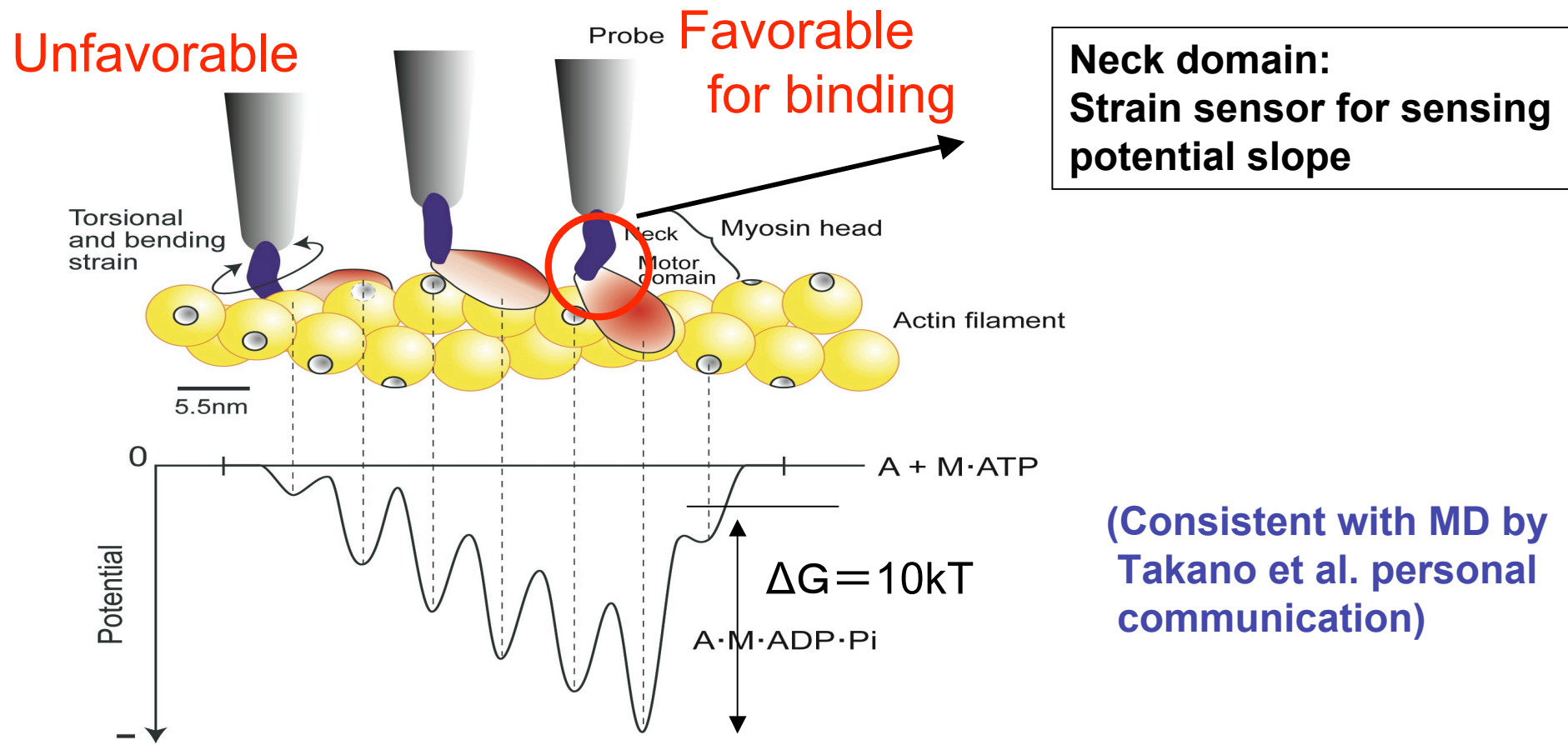
Myosin head walks on actin monomers according to a potential slope along actin helix by Brownian motion.



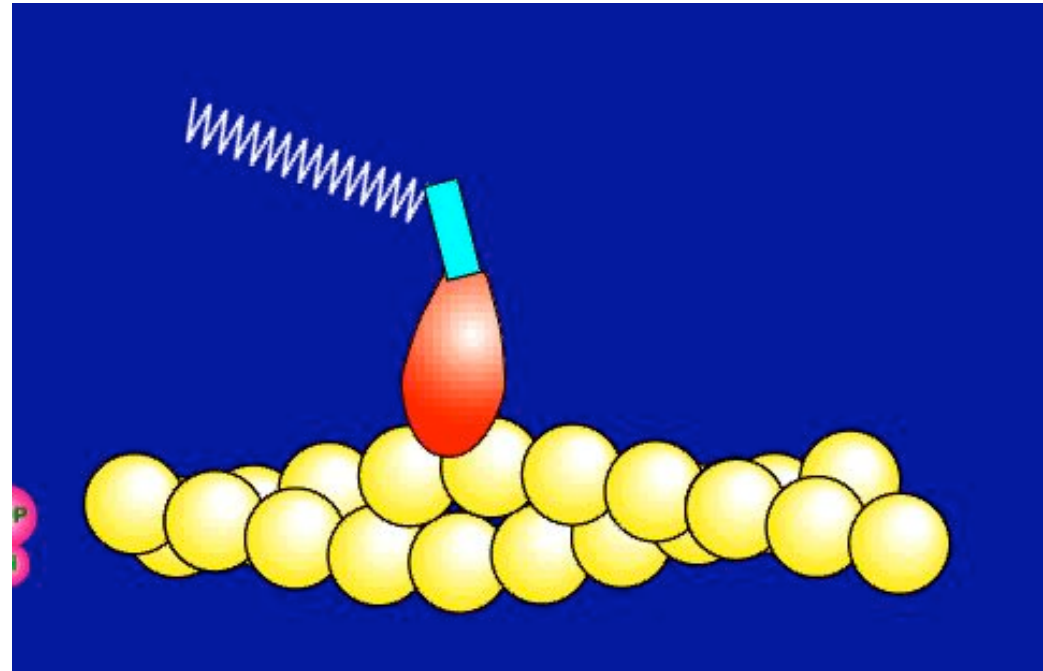
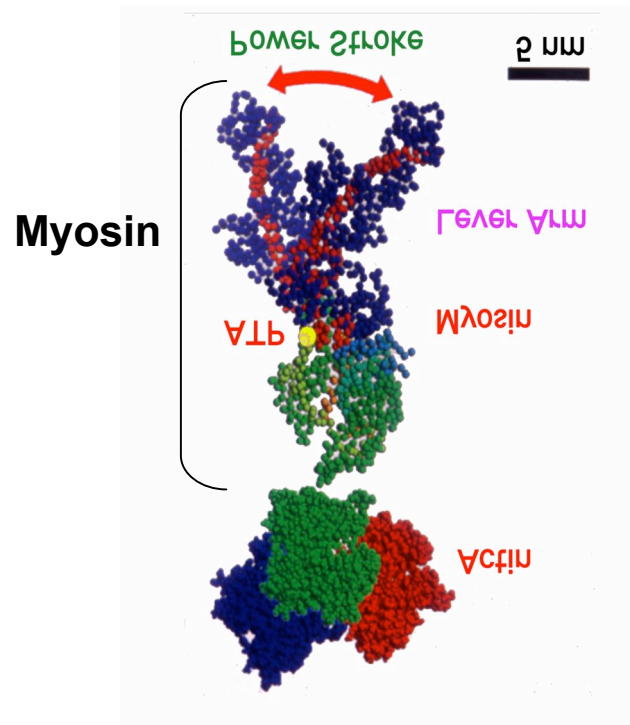
Potential slope \rightarrow  Bias potential energy = 10 kT (50% of ΔG_{ATP})

How is the potential slope generated?

Steric effect based on the compatibility between the orientations of myosin head and actin binding sites.

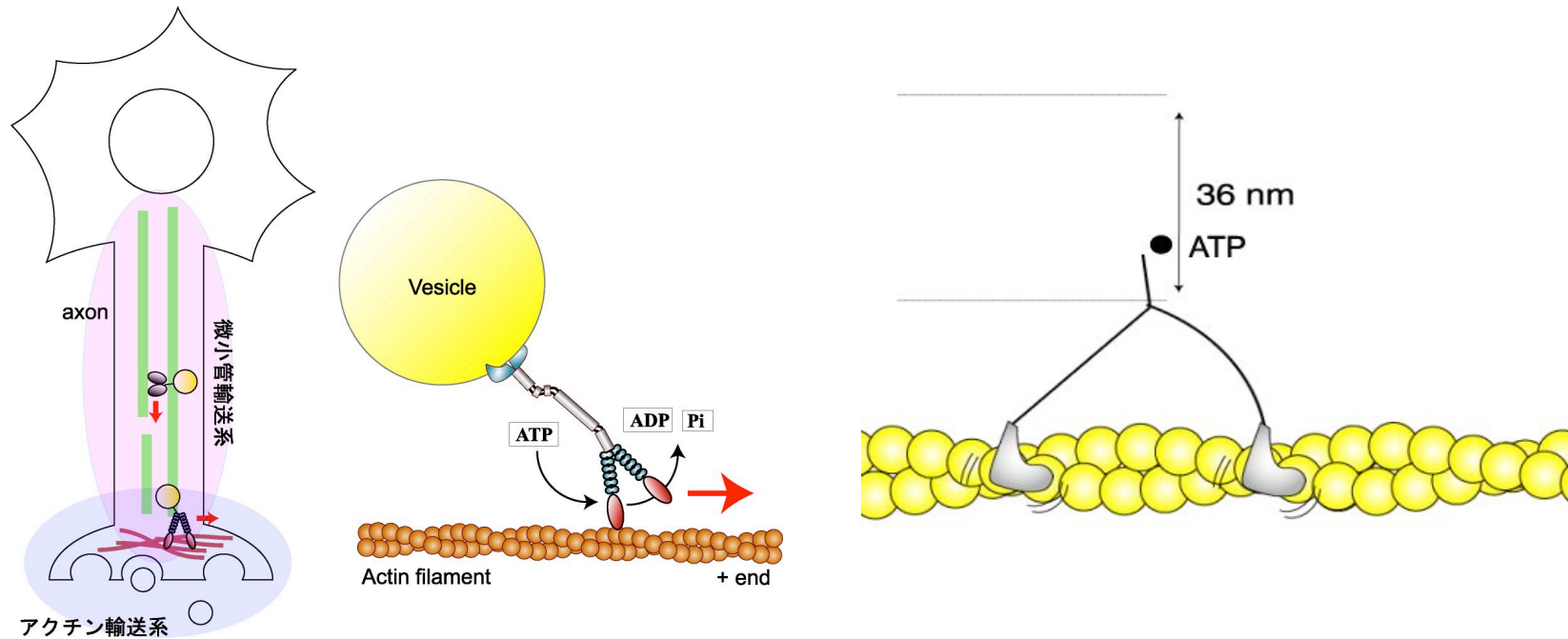


What is the role of the lever arm?



The neck domain acts as a strain-dependent gate for controlling the timing of the start and stop of movement coupled to the ATPase reaction. The tilting of the neck domain may contribute to isometric force.

Unconventional myosin-V is also moved by biased Brownian motion

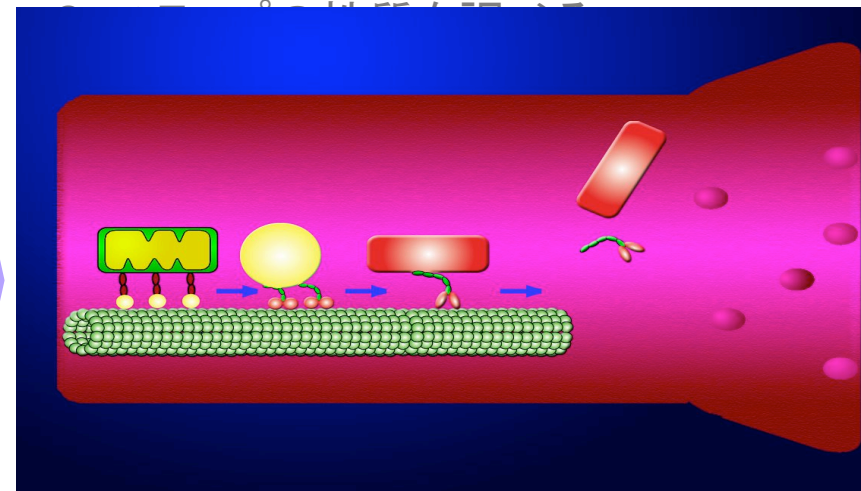
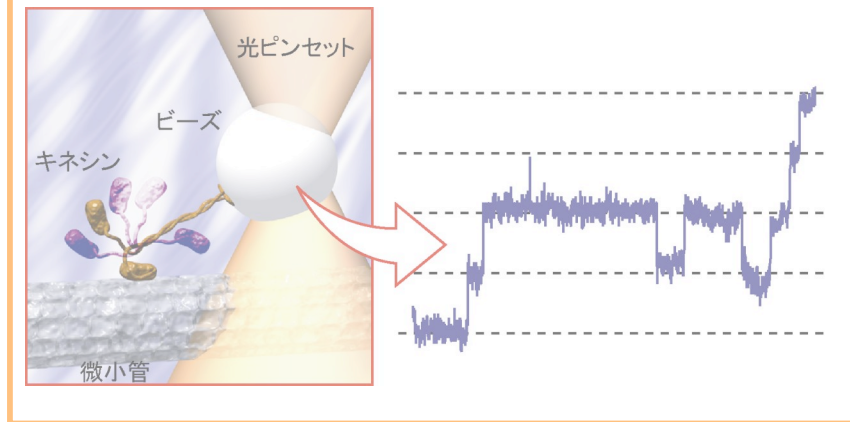


Okada et al. BBRC 2007
Komori, Nishikawa et al.
Nishikawa et al. in preparation

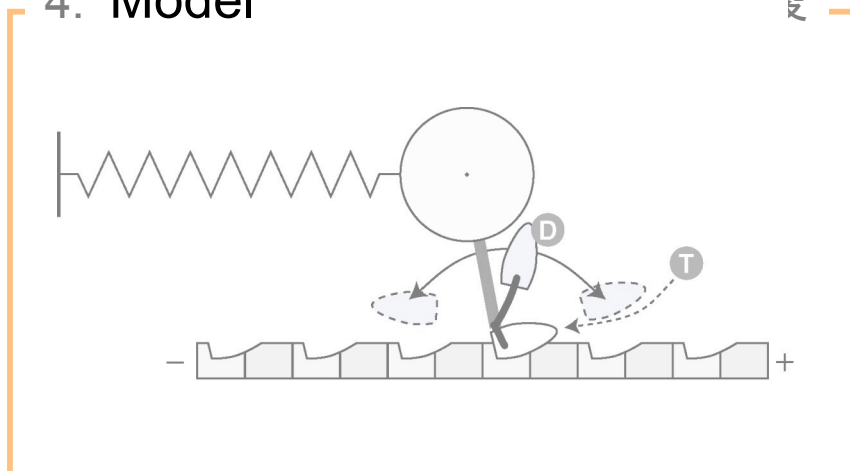
Kinesin moves by rectifying Brownian steps.

Nishiyama, Higuchi et al. Nat. Cell Biol. 2002;
Taniguchi, et al. Nature Chem.Biol. 2005

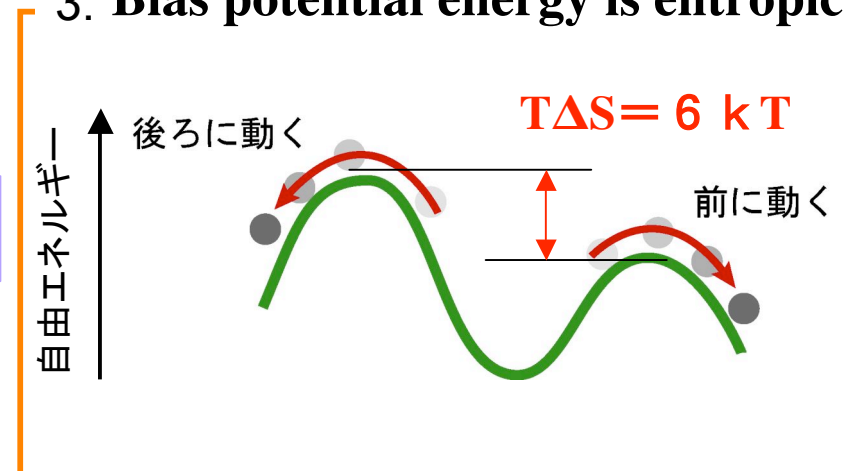
1. Single molecule nanometry



4. Model



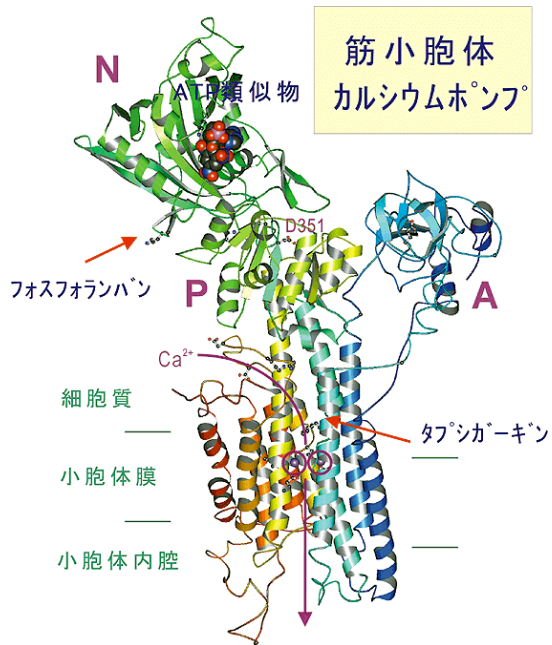
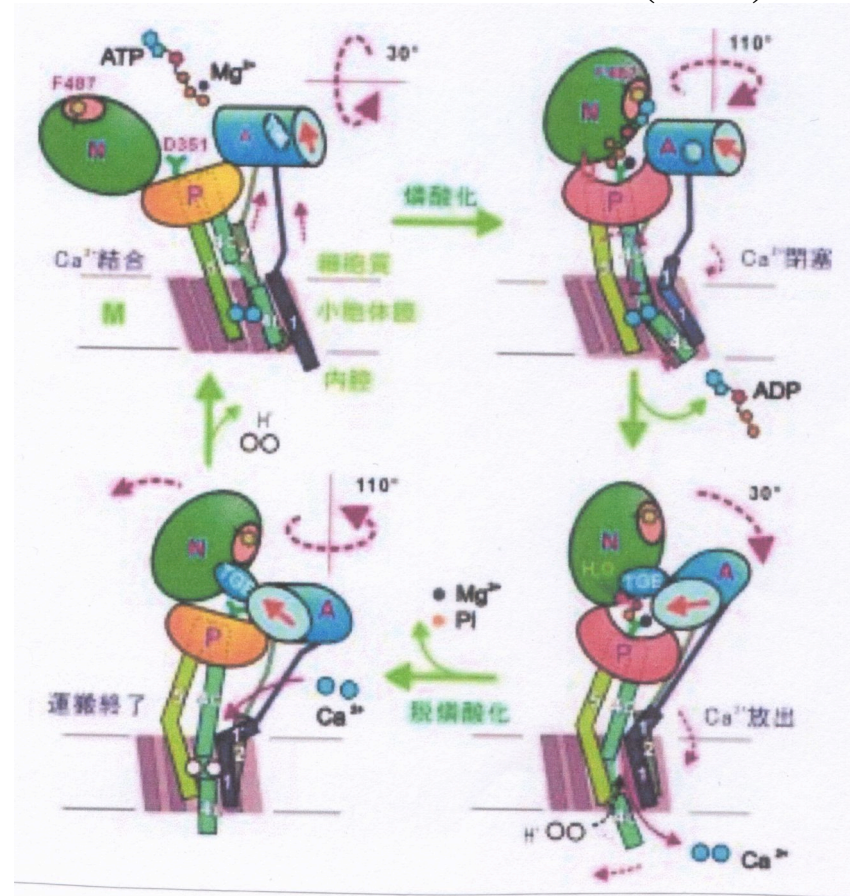
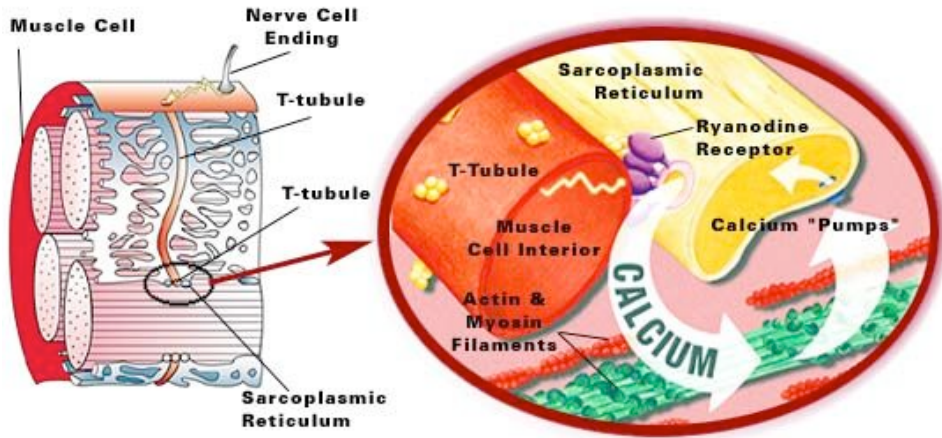
3. Bias potential energy is entropic



Calcium pump transports Ca^{2+} by Brown ratchet mechanism.



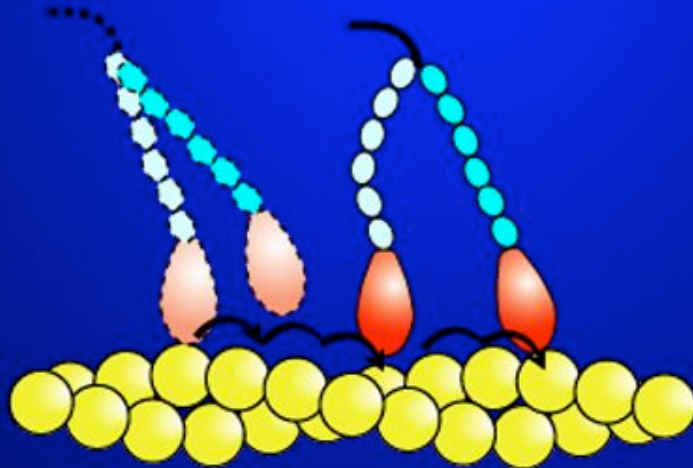
C. Toyoshima et al.
 Nature (Article),
 405(2000)
 418(2002)
 430(2004)
 432(2004)



Ca^{2+} is transported by rectified Brownian rotation of a large domain.

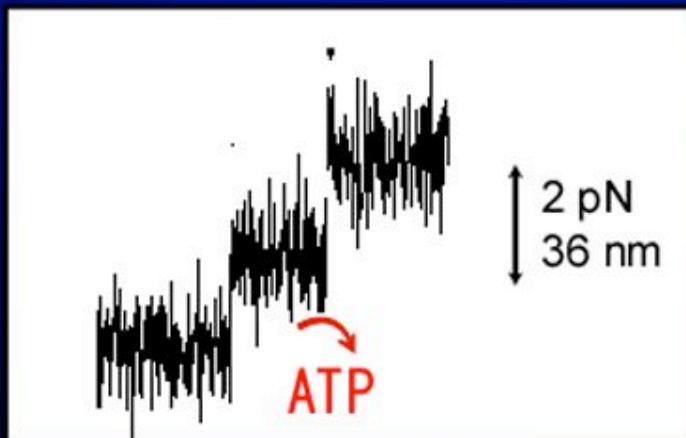
Efficiency of energy conversion

(Myosin V)



$$E = 2 \text{ pN} \times 36 \text{ nm} \\ = 72 \times 10^{-21} \text{ J/ATP}$$

$$\Delta G_{\text{ATP}} \doteq -80 \times 10^{-21} \text{ J/mol}$$



**Maximum
Efficiency >50%**

Energy as low as thermal noise is converted at >50% efficiency

Conclusion

Unique operation of the molecular motor:

The molecular motor does not overcome but rather use Brownian motion

- 1) Origin of movement is Brownian motion**
- 2) Chemical energy from ATP is used to bias (rectify) Brownian motions in a one direction.**
- 3) Maximum efficiency of energy conversion is $>50\%$ at energy level as small as thermal noise.**

**The biological molecular machines use
Brownian motions (thermal noise).**



**1) The molecular machine can thus operate at energy
as low as thermal noise with high efficiency.**

**2) But, the operations are
stochastic and ambiguous.
(Negative factor for manmade device)**



**Adaptivity and
flexibility in
biosystems**



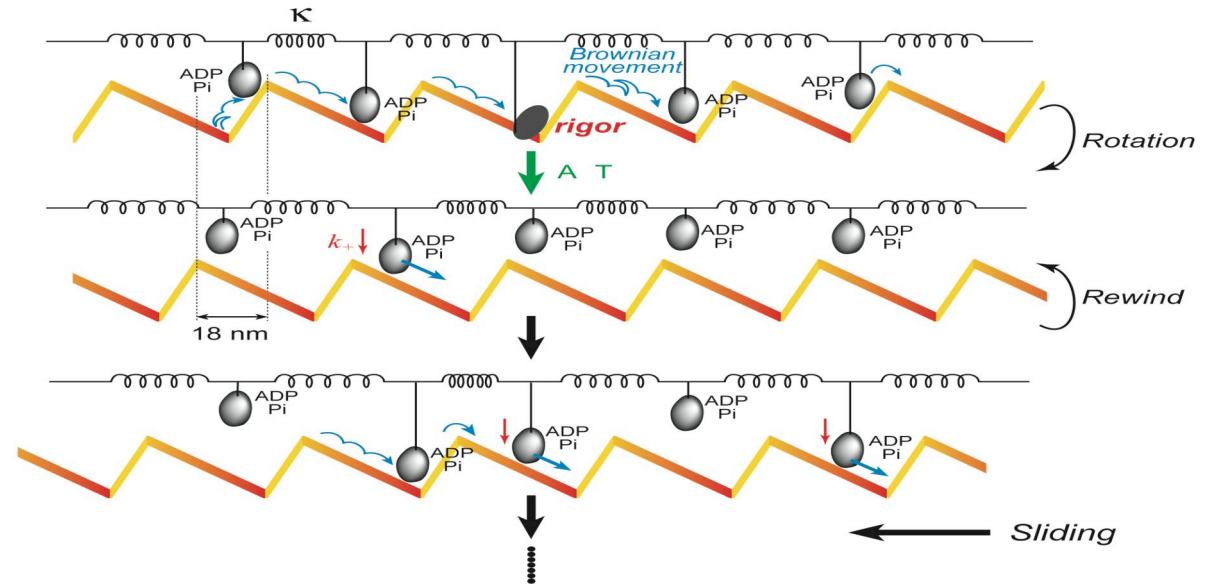
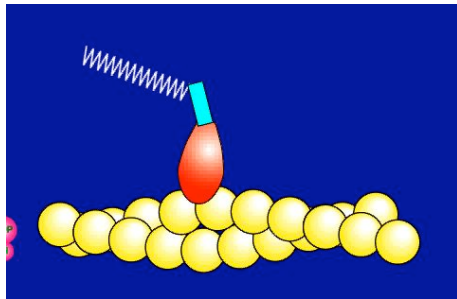
**Cooperative operations of stochastic molecular machines
allow dynamic and adaptive biological systems.**

Computer simulation of cooperative action between myosin motors undergoing stochastic Brownian steps.

Single molecule



System



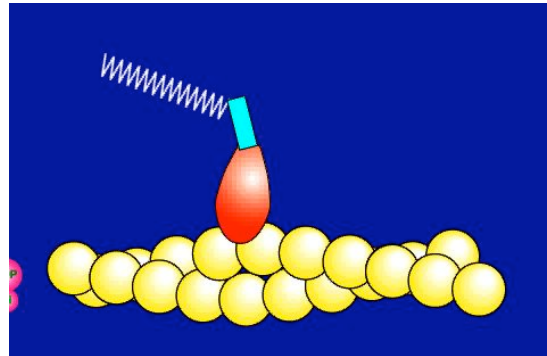
Langevin equation

$$0 = -\rho \frac{dx_i}{dt} - \frac{dU(x_i, t)}{dx} + F(t) - A_i$$

$$A_i = \kappa(x_i - x_{i-1}) - \kappa(x_{i+1} - x_i)$$

Kitamura, Tokunaga, Iwane, Esaki,
et al. Biophysics, 2005

Flexible and adaptive motions of muscle are achieved by stochastic nature of molecular motors.

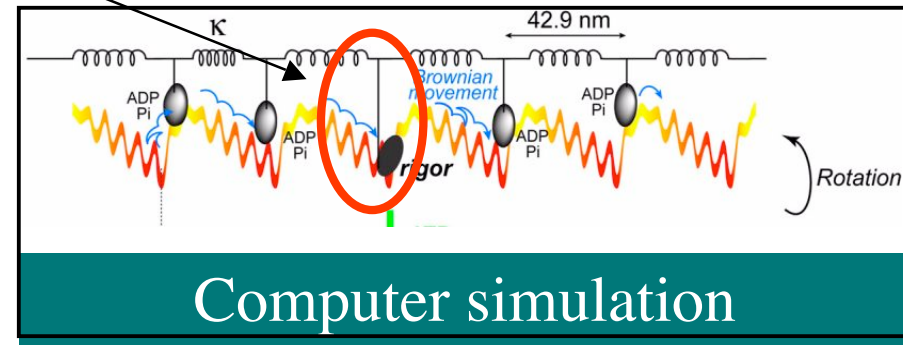
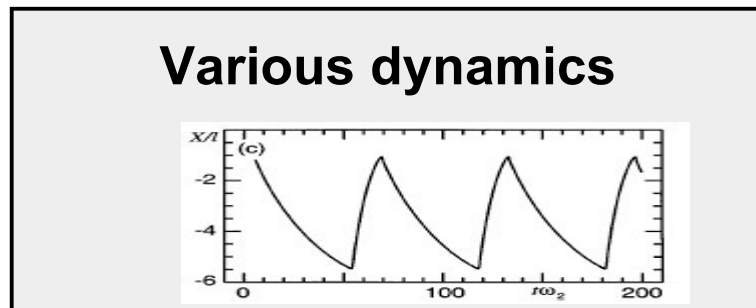


Element

Stochastic and ambiguous

Assembly

System



System composed of stochastic and ambiguous elements can modulate its motion flexibly depending on the conditions.

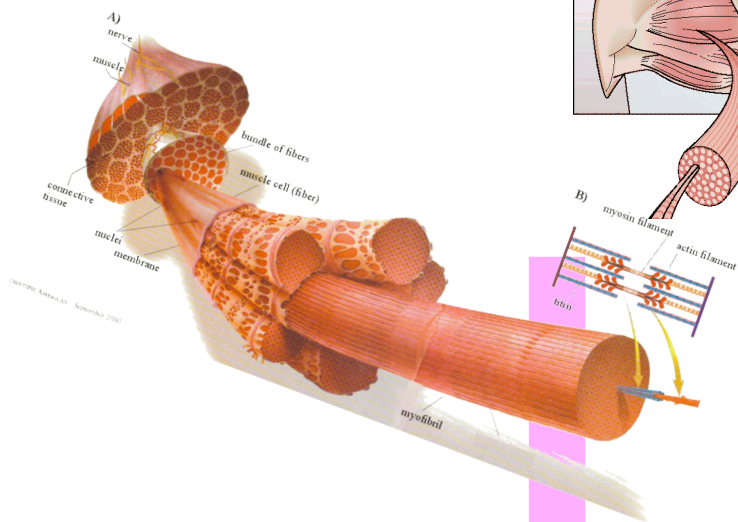
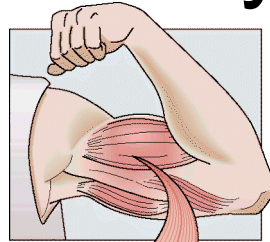


Artificial muscle

Simokawa et al.

Development of muscle-type actuators

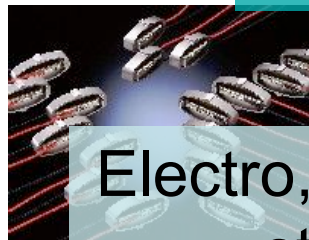
Muscle



System

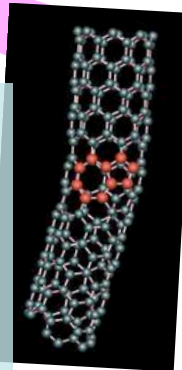


Material



Electro, ferroelectric
strictive

Piezoelectric



Robotics

Biomedical devices

Present

Large scale control



Deterministic devices



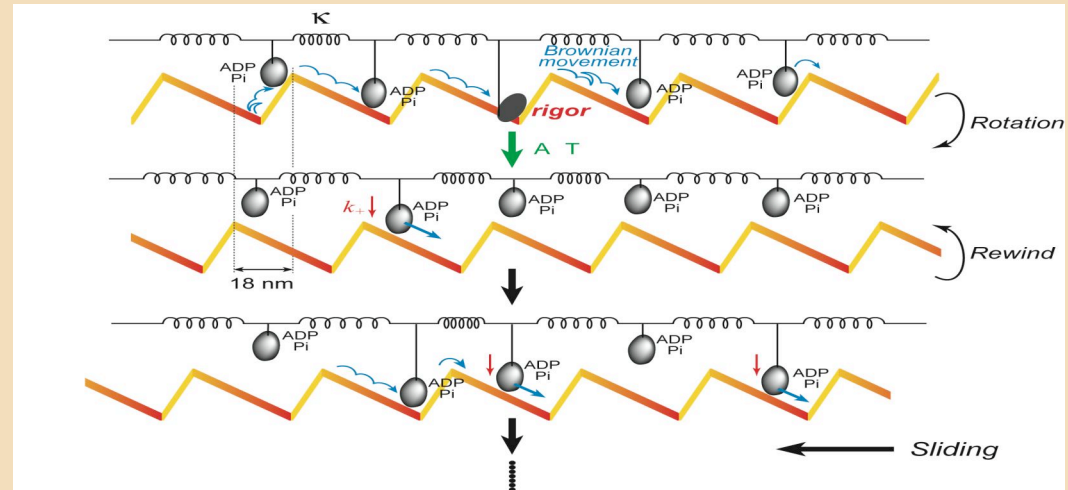
Various actions

Our proposal

Simple control



Stochastic devices

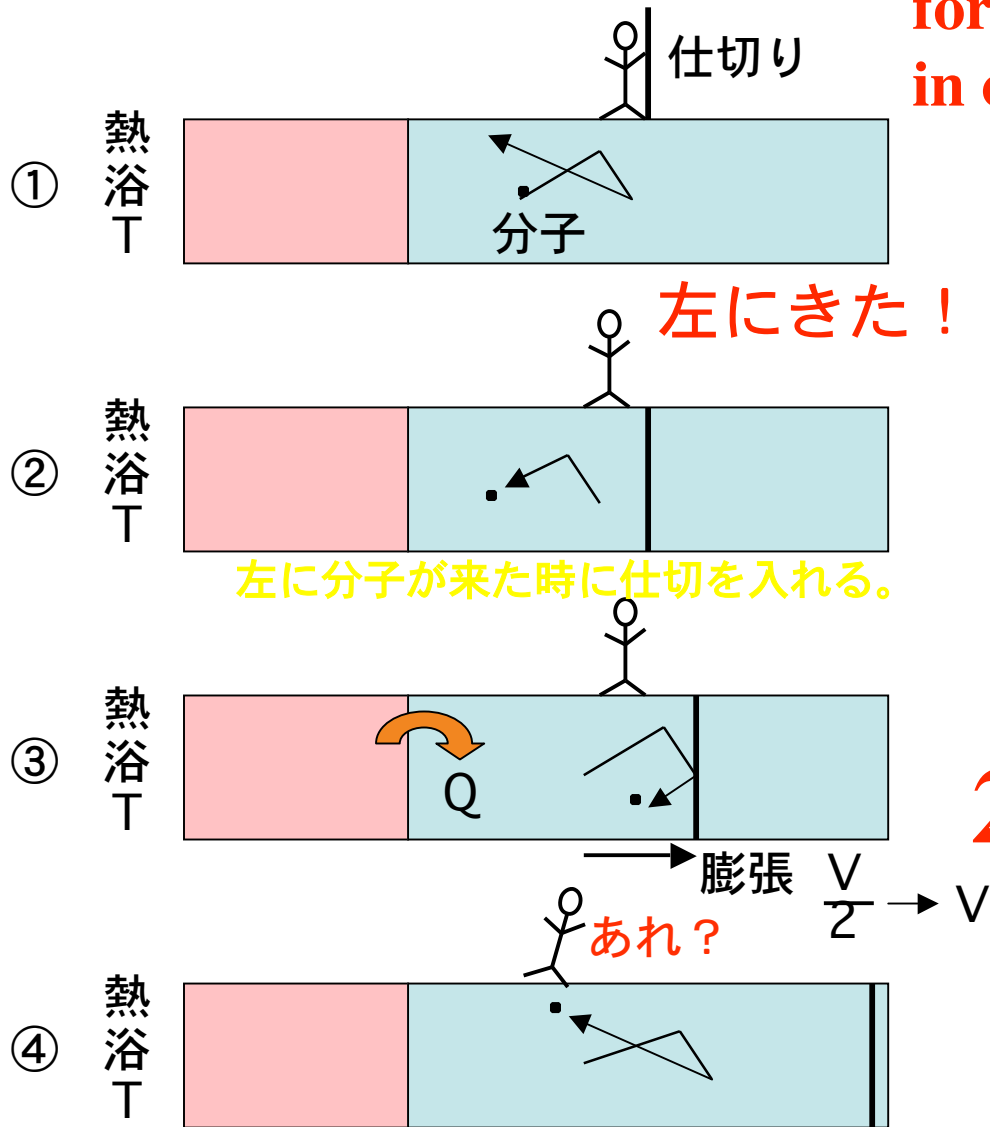


Flexible actions

Information and entropy

Szilard's piston Demon

How much is the energy required for processing one bit data in case of computer?



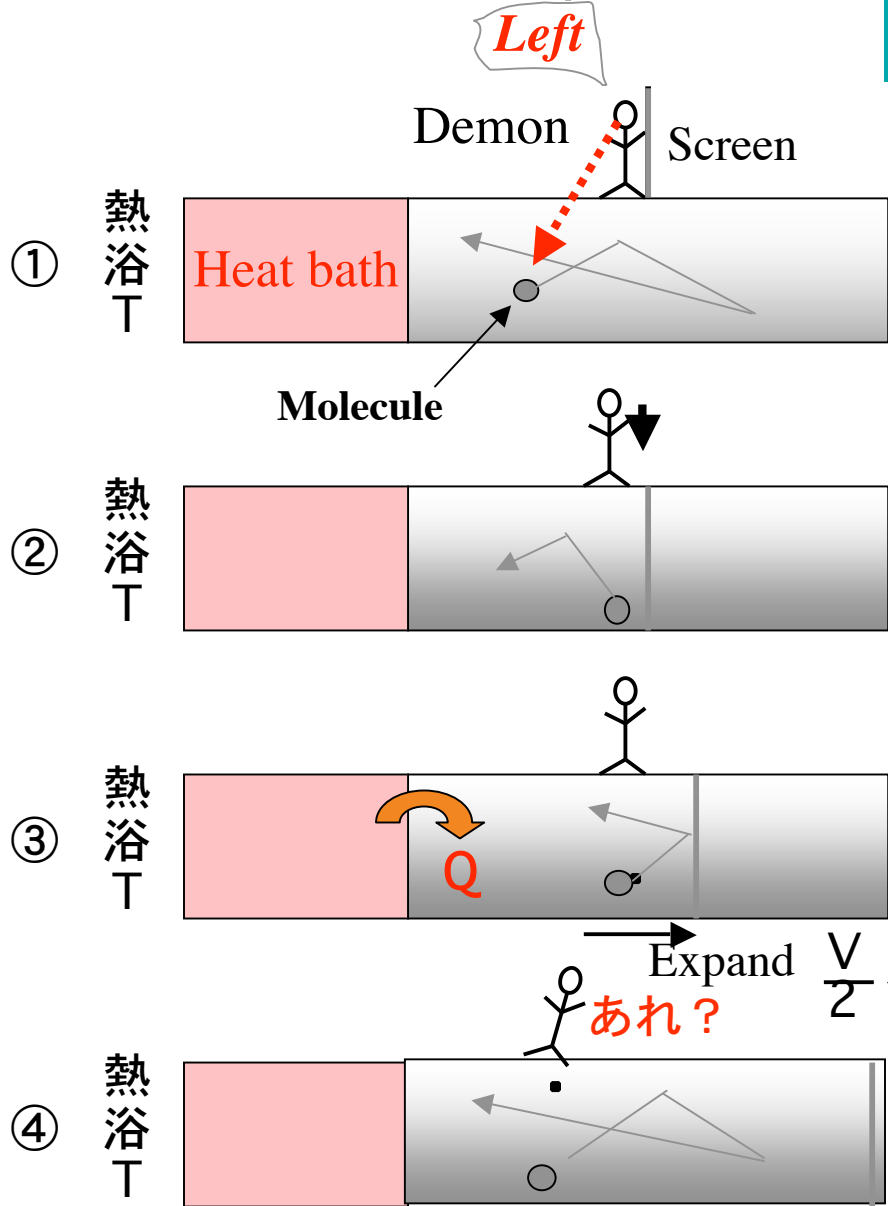
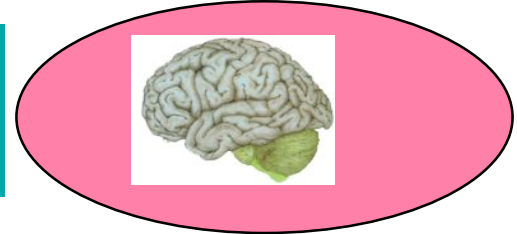
$20 \times 10^6 k_B T / \text{bit}$



Information and entropy

Szilard's piston Demon

If Brownian motion is used,



- ② → ④ Volume is expanded
- ③ Temperature is decreased,
- ④ So heat Q is transferred to the box.
- ⑤ The temperature is kept constant.

$$Q \text{ は } Q = k_B T \int_{V/2}^V p dv$$

$$= k_B T \ln 2 \text{ である。}$$

$$Q = k_B T \ln 2 = 0.7 k_B T / \text{bit}$$

(Cf, Computer: $20 \times 10^6 k_B T / \text{bit}$)

シャノンの情報量のDef. $I = -\ln P = -k_B T \ln P = -S$ (エントロピー)

*Single Molecule Analysis of Chemotaxis
in Dictyostelium Cells*

*Thermal noise is positive
for signal processing in cells?*

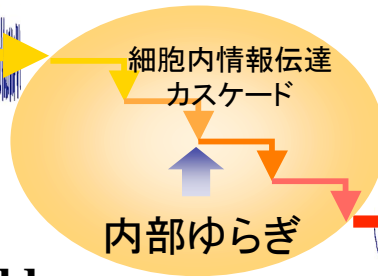
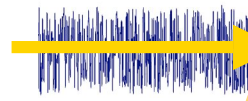
Fluctuations and Signal Transduction

Ueda, Sako, ...ty, (2001). Science. 294

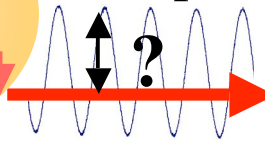
Gradient of electric field

+ noise

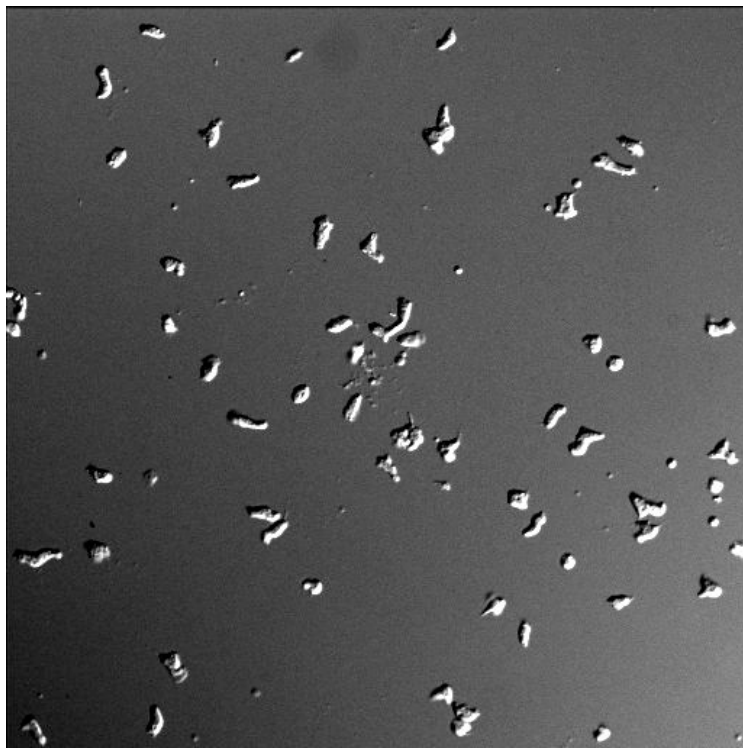
Cell



Response



Gradient of electric field



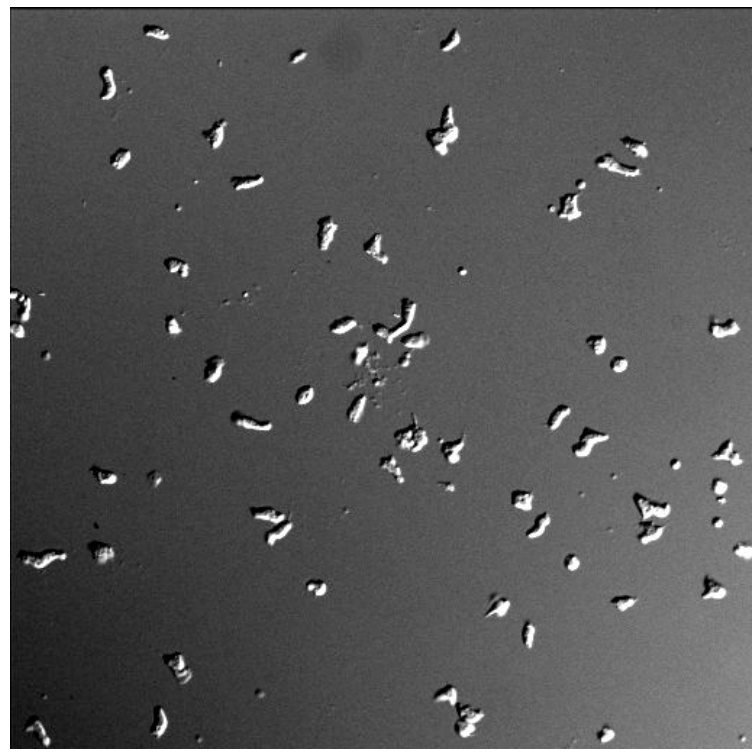
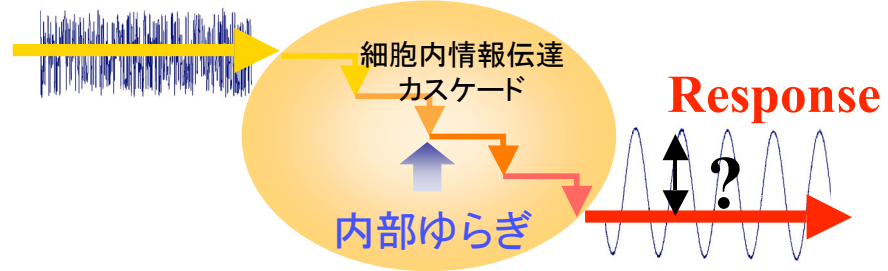
← Ameba

The gradient of electric field is several-fold as small as the membrane potential noise, but ameba can move toward the - voltage.

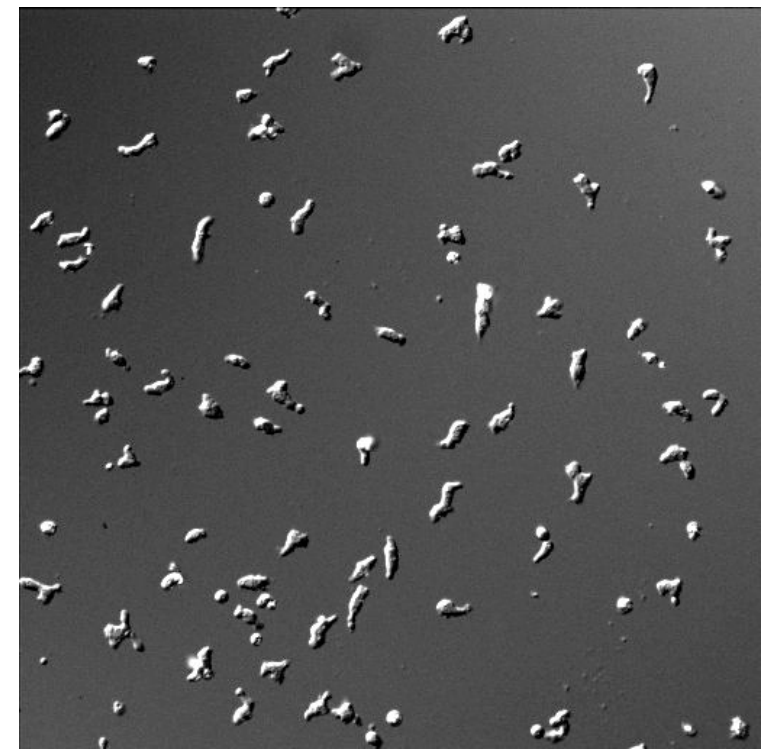
$2\text{mV}/10\mu\text{m}$

Gradient of electric field
+ noise

Cell

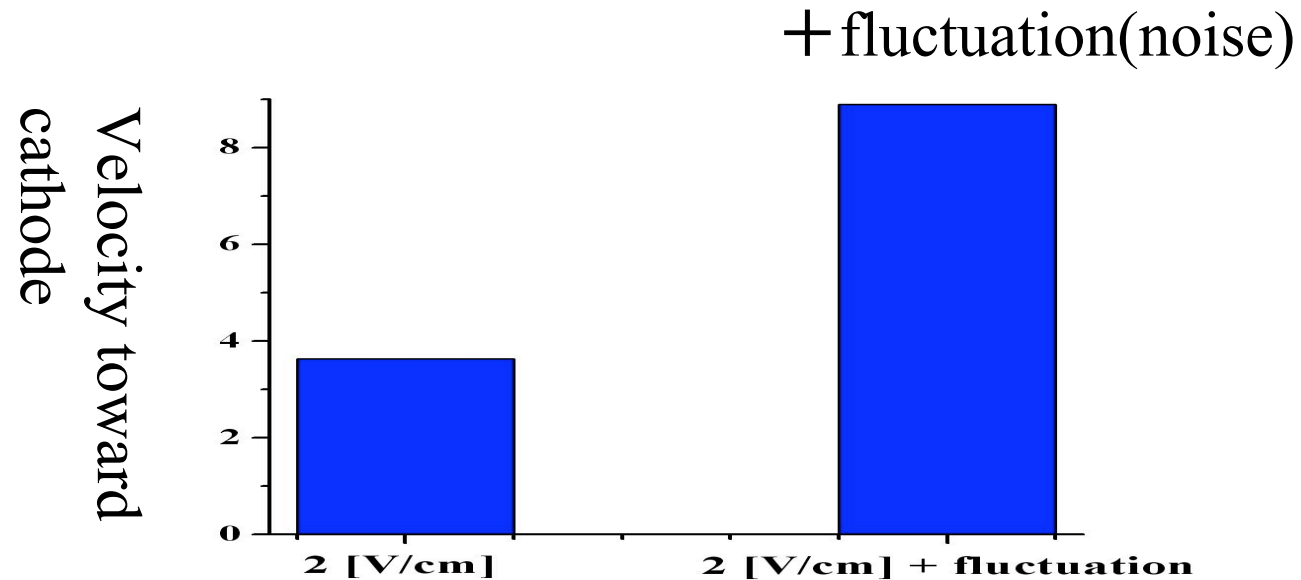


2mV/10 μ m



+ 1mV/10 μ m Noise

Galvanic taxis efficiency in the presence of noise



Membrane potential = 47mV

Applied voltage = 2mV/membrane

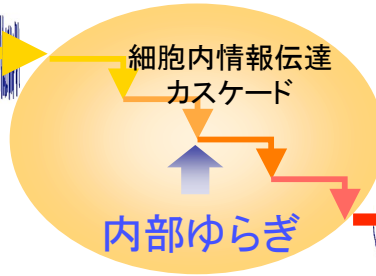
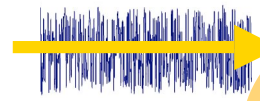
Fluctuation = 1mVp-p/membrane

Sato, Ueda, et al. in preparation

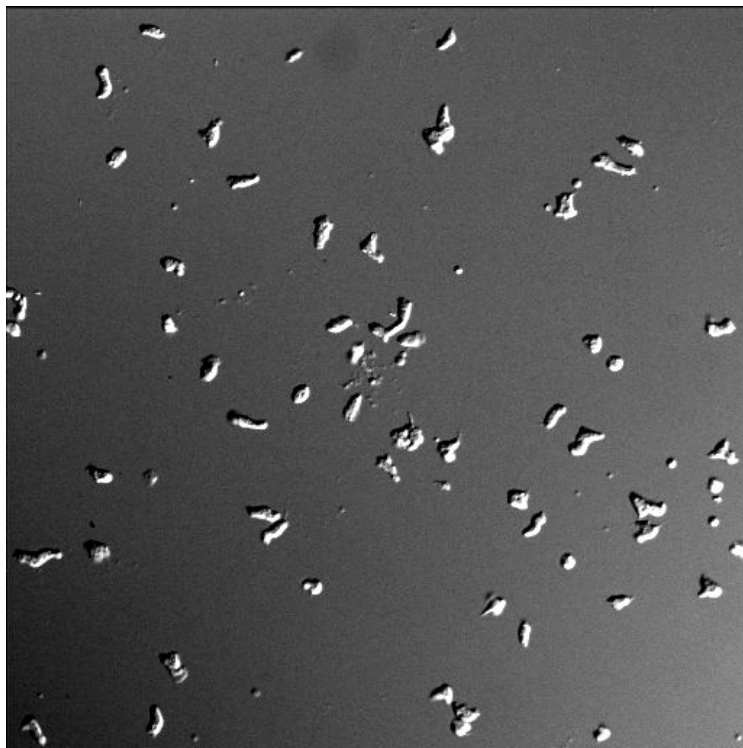
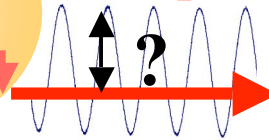
Noise is useful for taxis!!

Gradient of electric field
+ noise

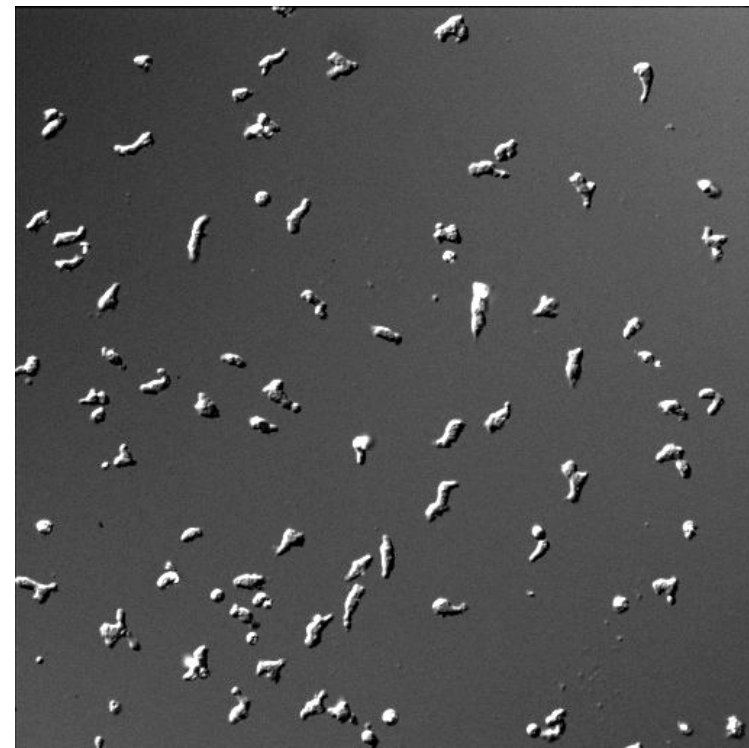
Cell



Response

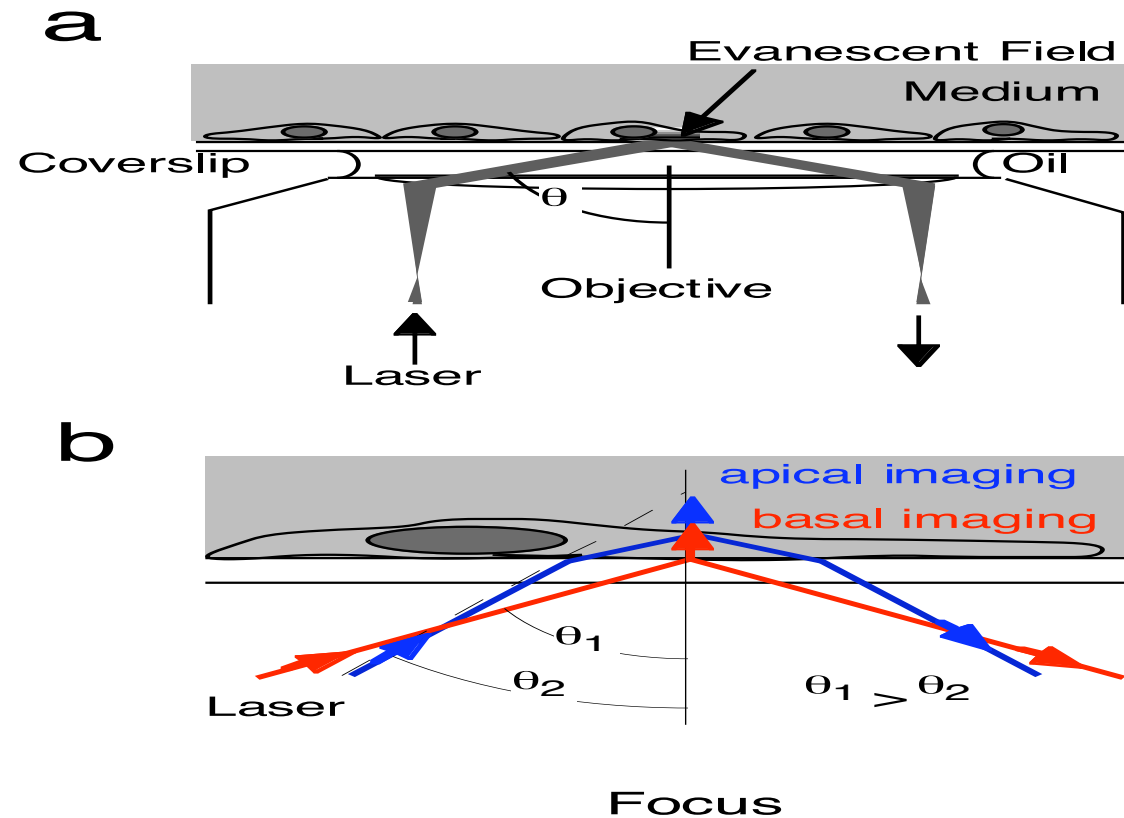


2mV/10μm



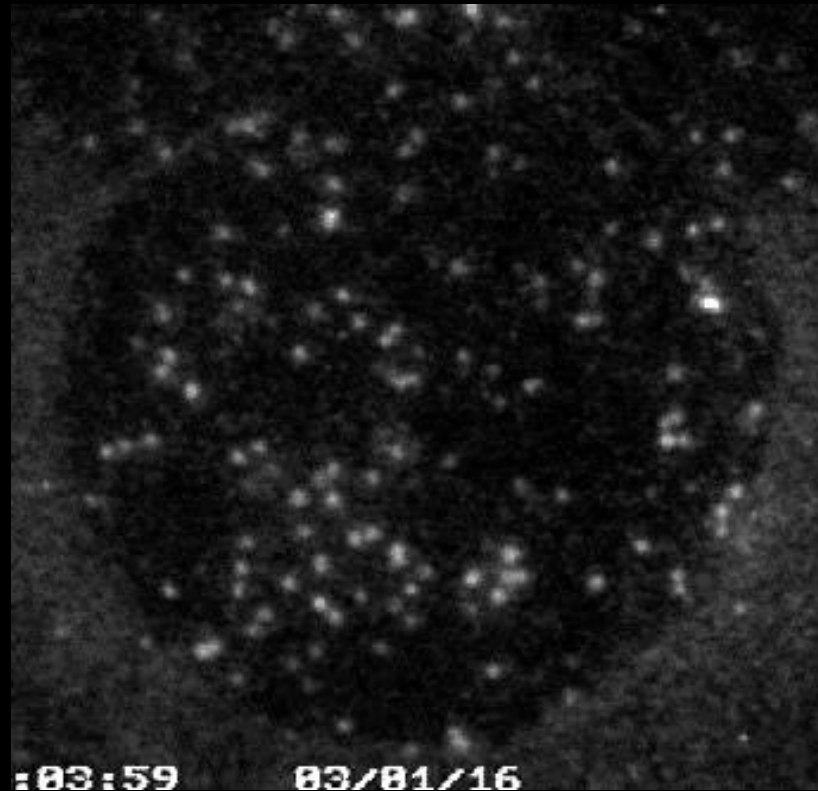
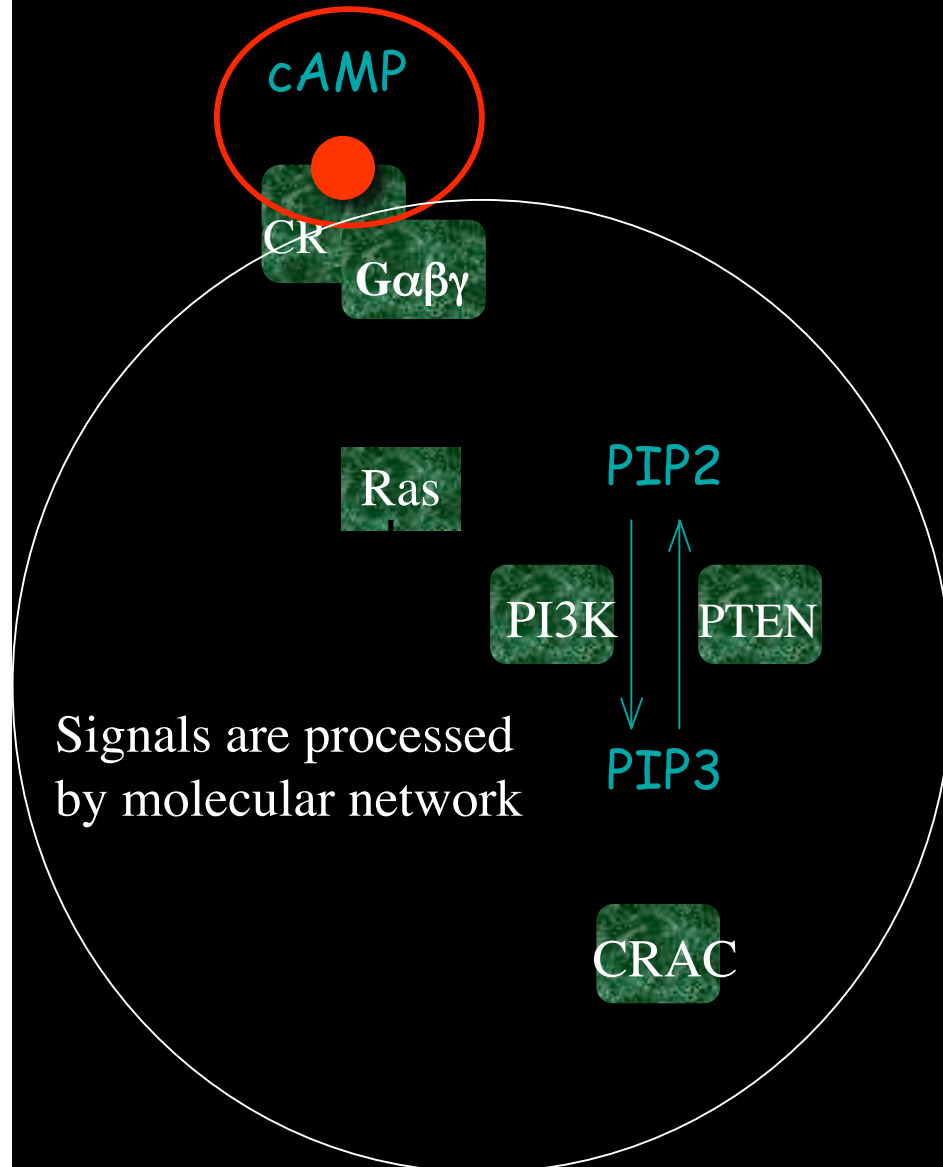
+ 1mV/10μm Noise

TIRF microscopy for visualization of single molecule signaling events in living cells



Sako Y, ...TY, Nature Cell Biol. 2, 2000
Sako, Y, TY, Nature Rev. 2003

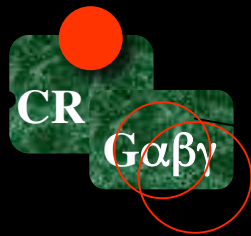
Single molecule imaging of signal processing in living cells



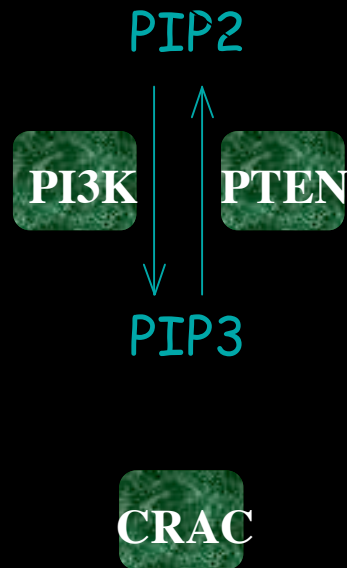
Ueda et al. (2001) Science 294, 864-867

G-protein on the membrane

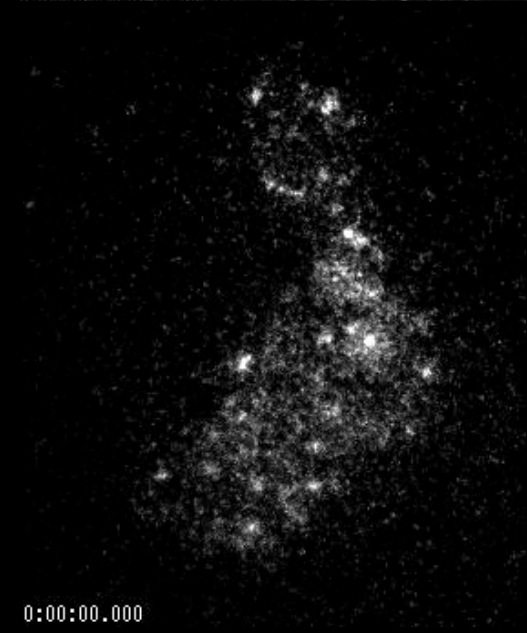
cAMP



Ras



Gα

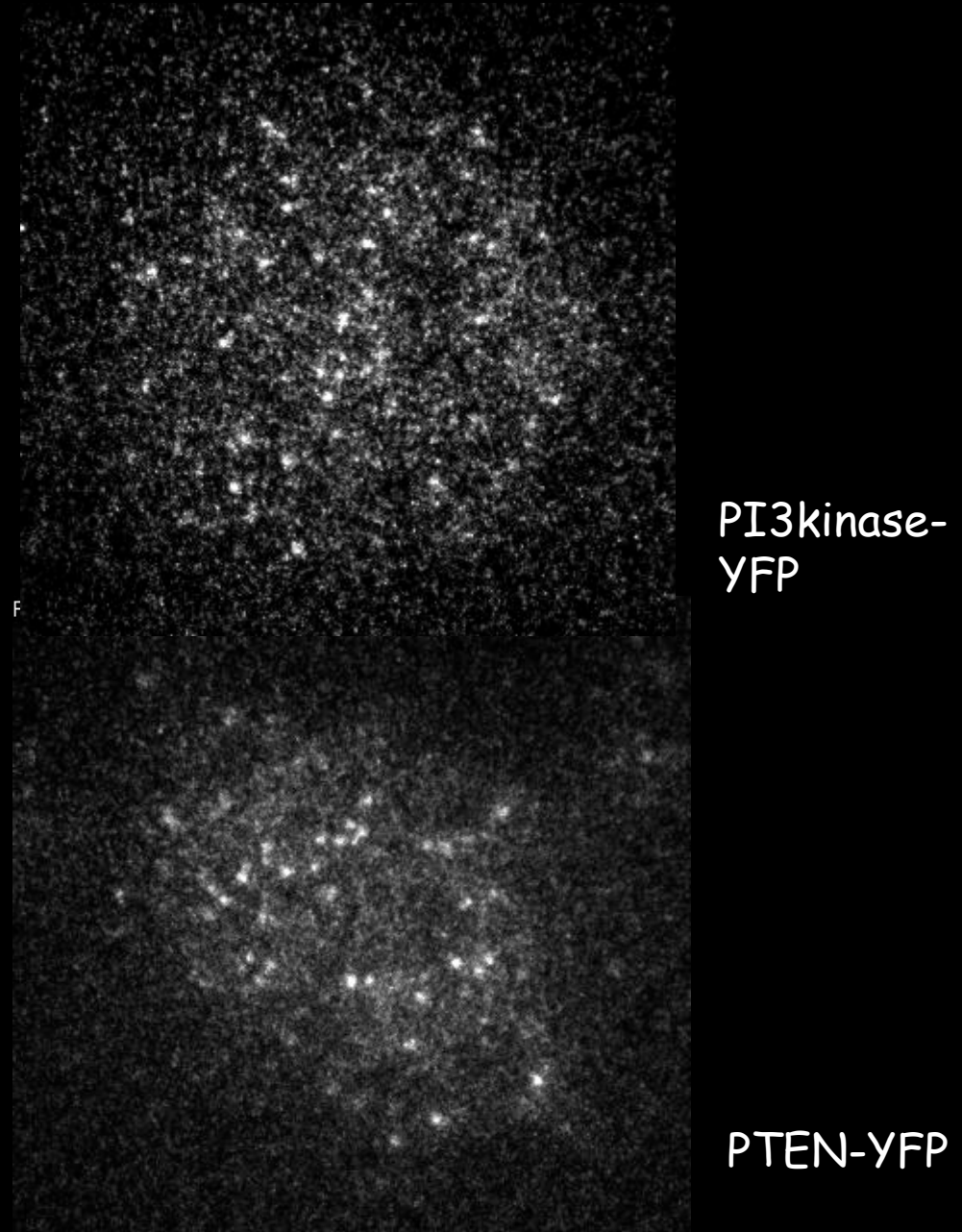
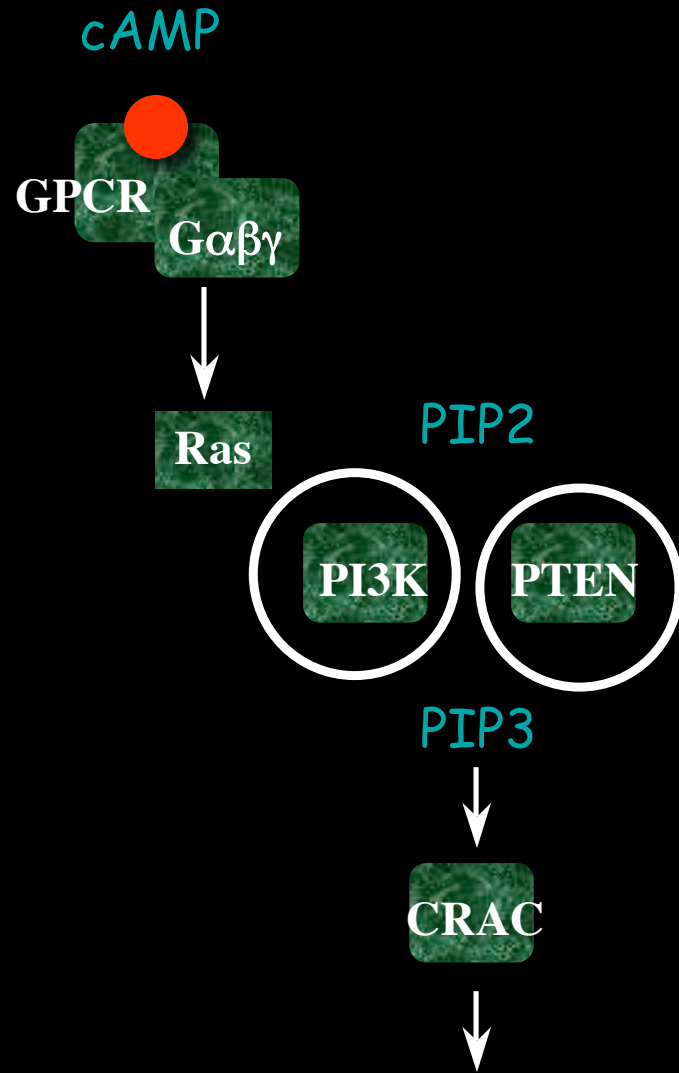


Gβ

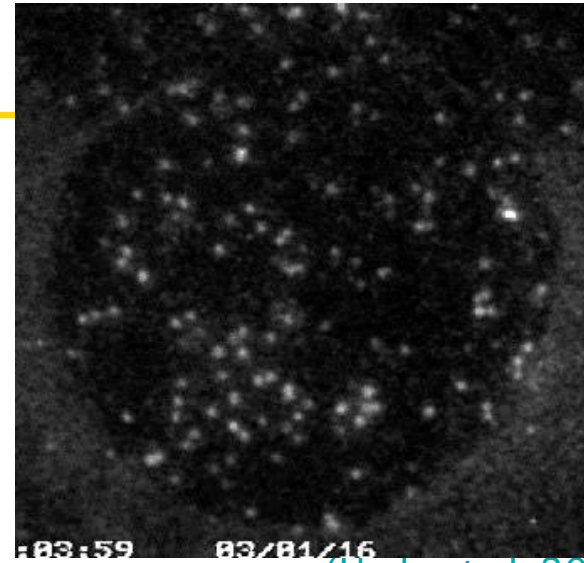
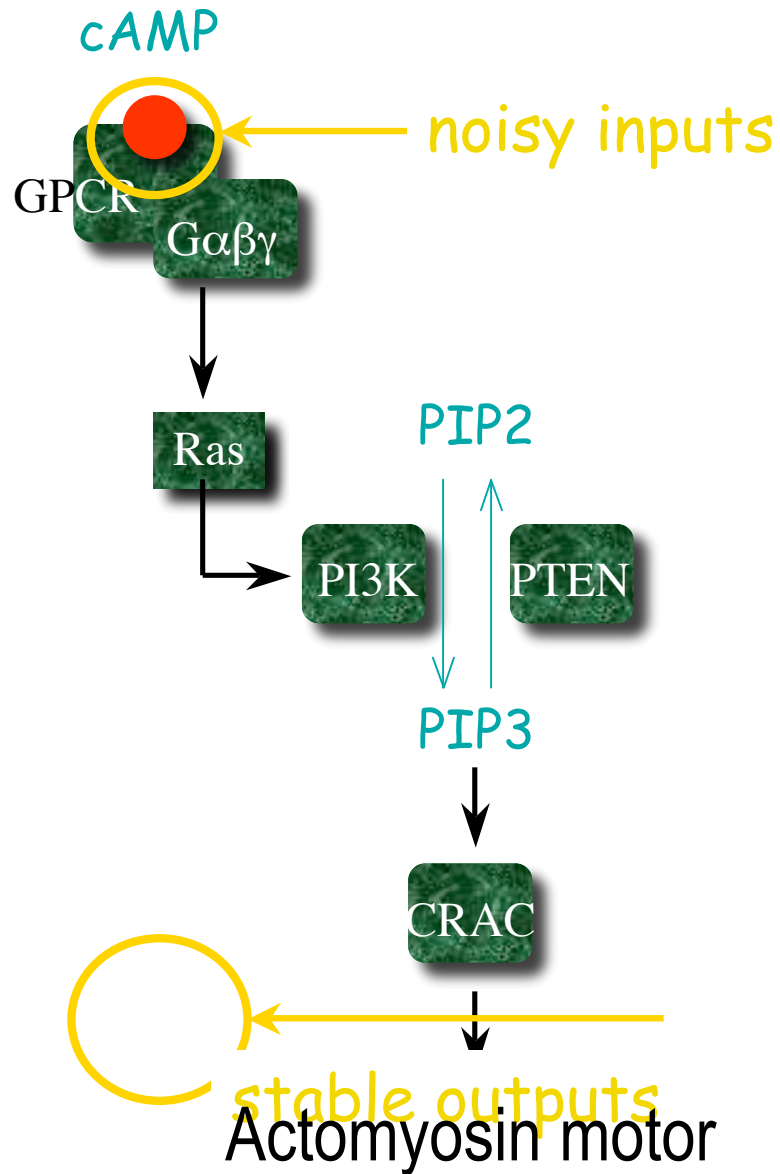
Miyanaga et al. submitted

0:00:00.000

Imaging of PI3 kinase and PTEN



How noisy signals are processed to generate stable response in chemotactic signaling system?



(Ueda et al. 2001)

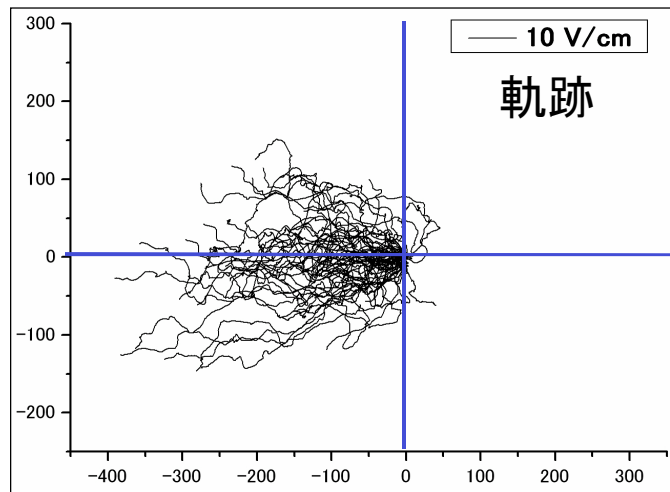
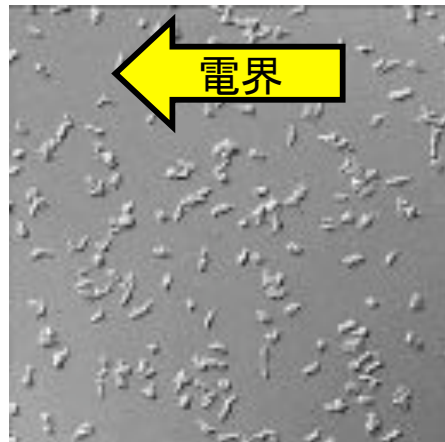


(Parent & Devreotes, 1999)

Processor that does not shut out but accepts thermal noise

生物は、激しい擾乱の中で
情報を得て生活している

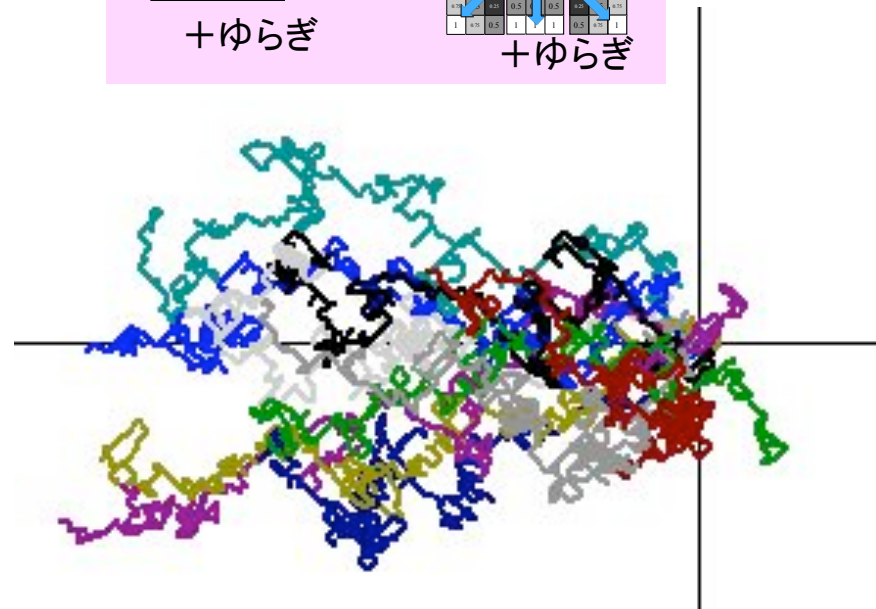
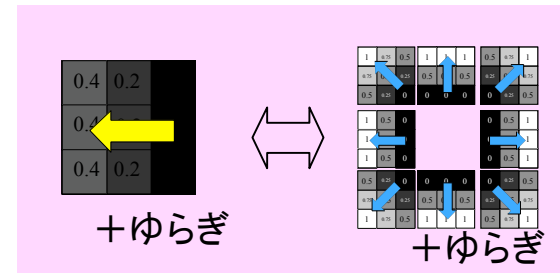
アメーバの走電性



(a) 粘菌の走電性実験



「ゆらぐ」微弱な入力パターンと
「ゆらぐ」記憶パターンのマッチングによる
確率的情報演算



(b) ゆらぎ演算による出力結果

共同研究企業提供

Dynamic visual perception

**Fluctuations play an essential
role in flexible visual
perception by human brain**

Murata et al.

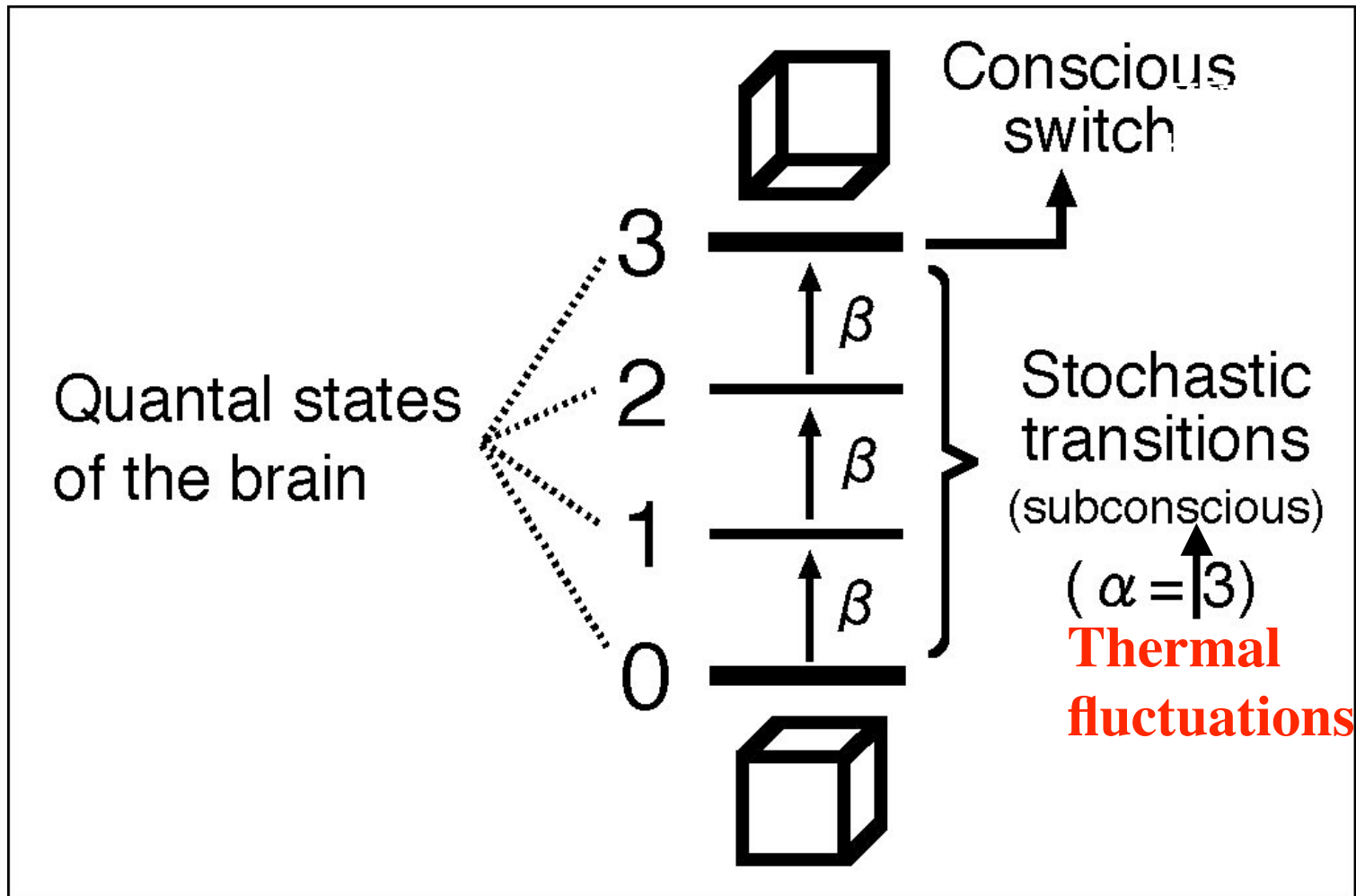
Perception of
ambiguous
figure
多義図形



Man
and
lady

DALI “The image disappears” 1938

Ambiguous figure perception is driven by spontaneous fluctuations

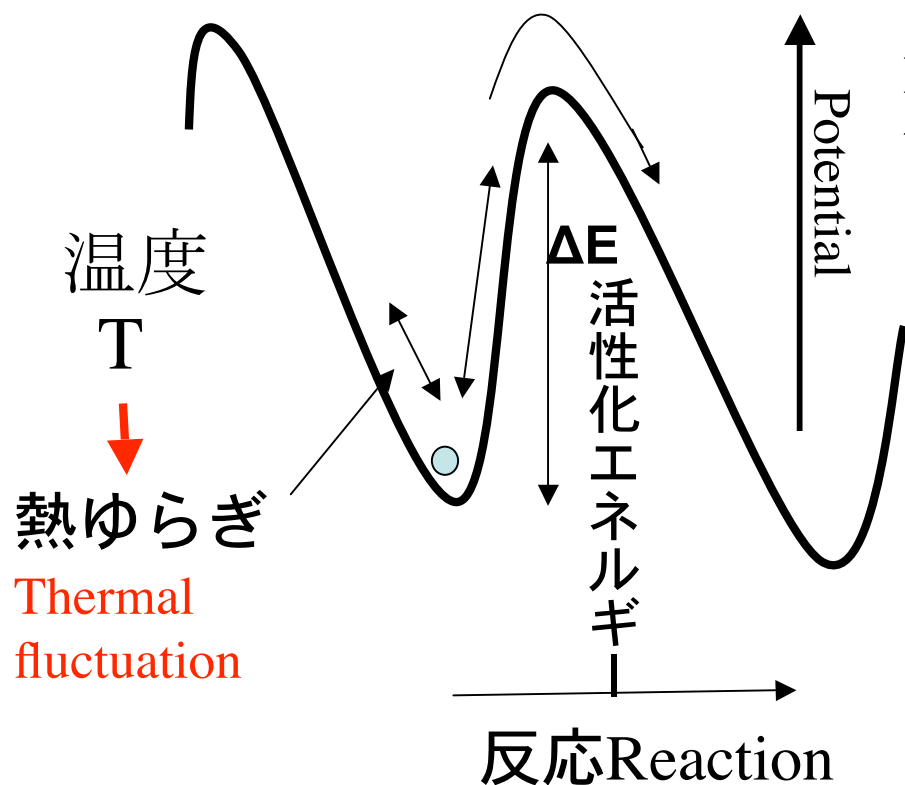


Hidden Figure Perception



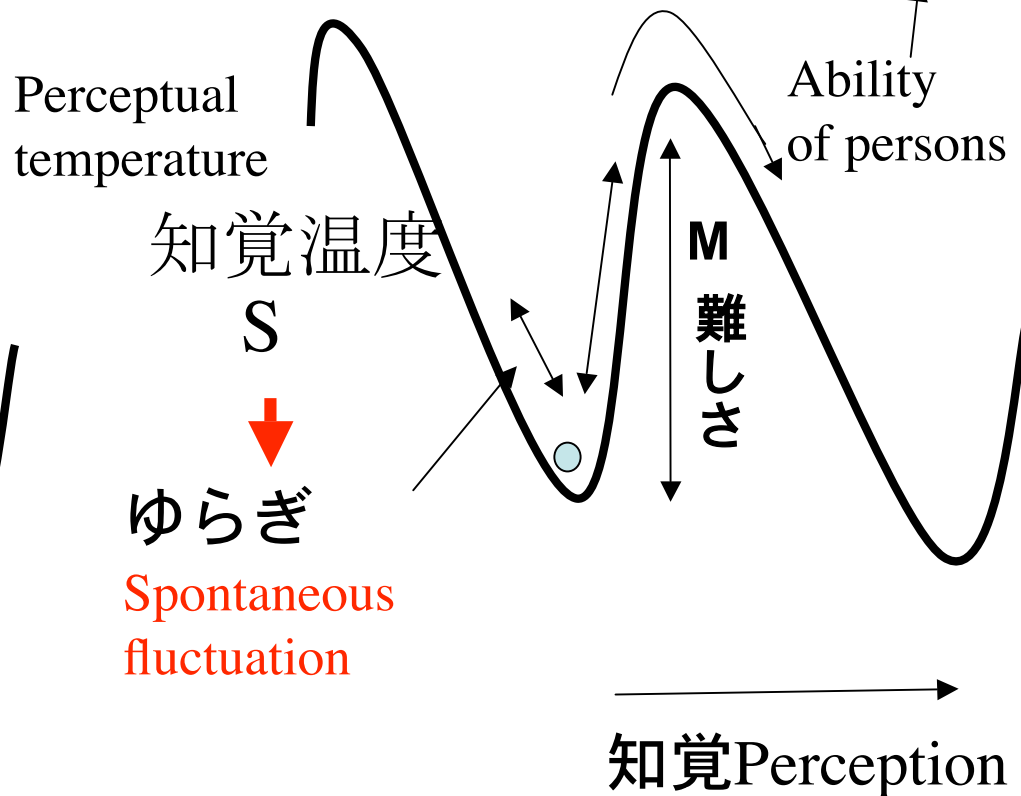
Enzymatic reaction 酵素反応

$$v = v_0 \exp(-\Delta E/k_B T)$$



Perceptual reaction 知覚反応

$$v = 1/t = C \exp(-M/S)$$



Perception is driven by fluctuations !

Dynamic visual perception

**Fluctuations play an essential
role in flexible visual
perception by human brain**

Murata et al.

What is difference between biological and manmade machines?

Biological system utilizes fluctuations for economizing energy and resulting stochastic operations are used for flexible function.

