RICE UNIVERSITY

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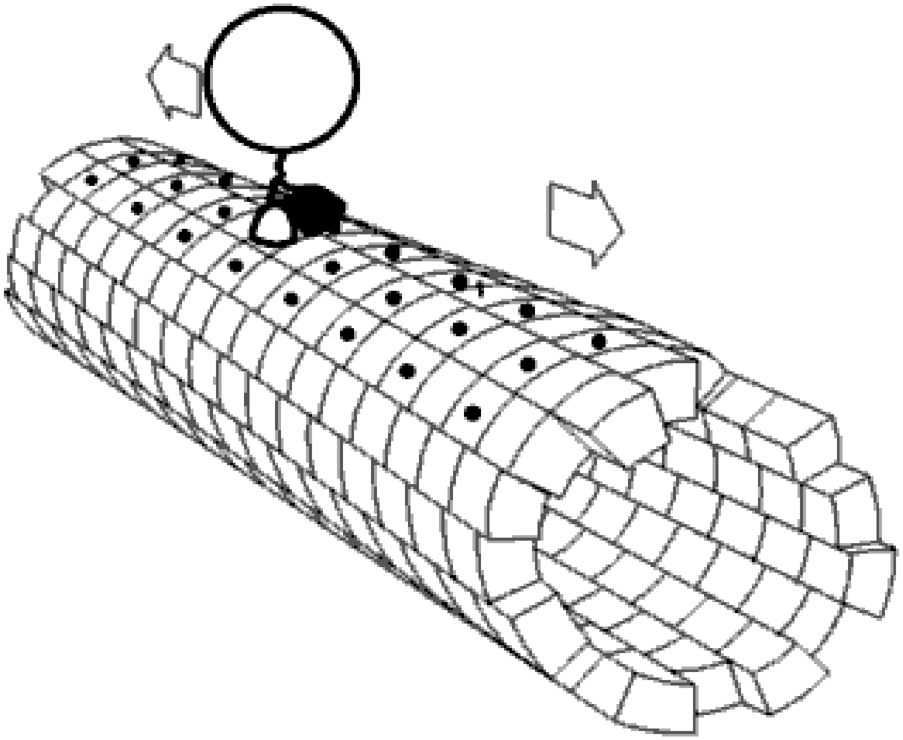
COUPLING OF TWO MOTOR PROTEINS: A NEW MOTOR CAN MOVE FASTER

Motor Proteins

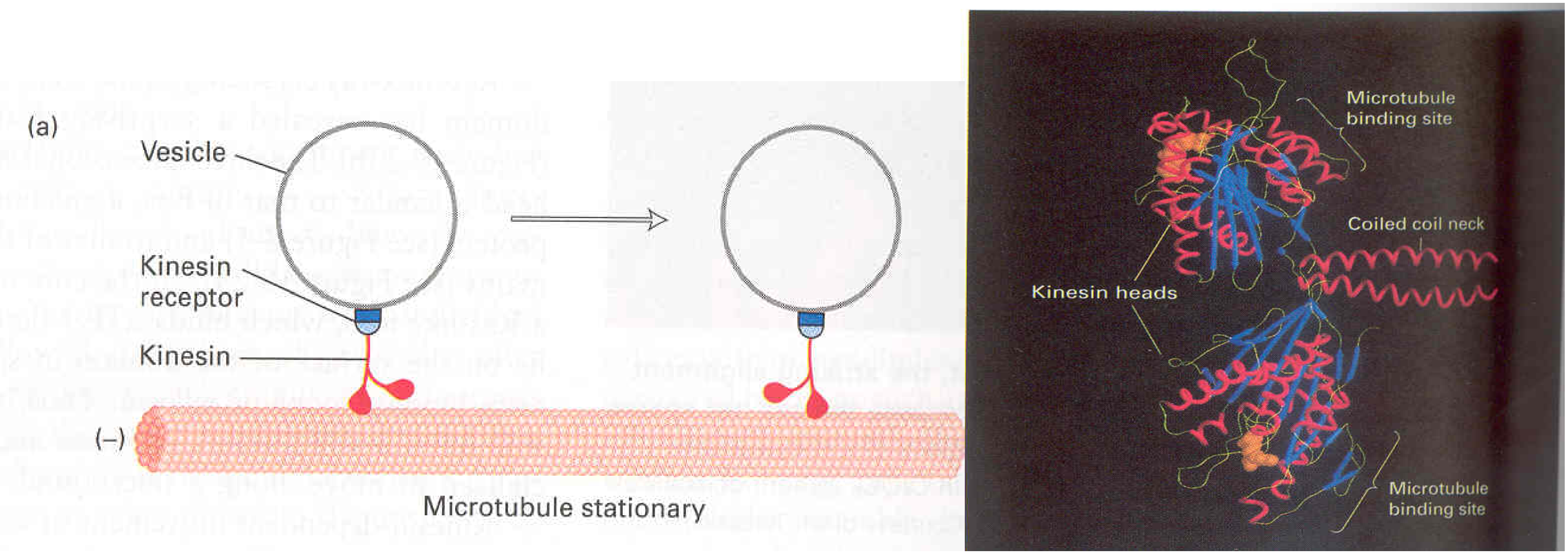
Enzymes that convert the chemical energy into mechanical work

Functions: cell motility, cellular transport, cell division and growth, muscles, …



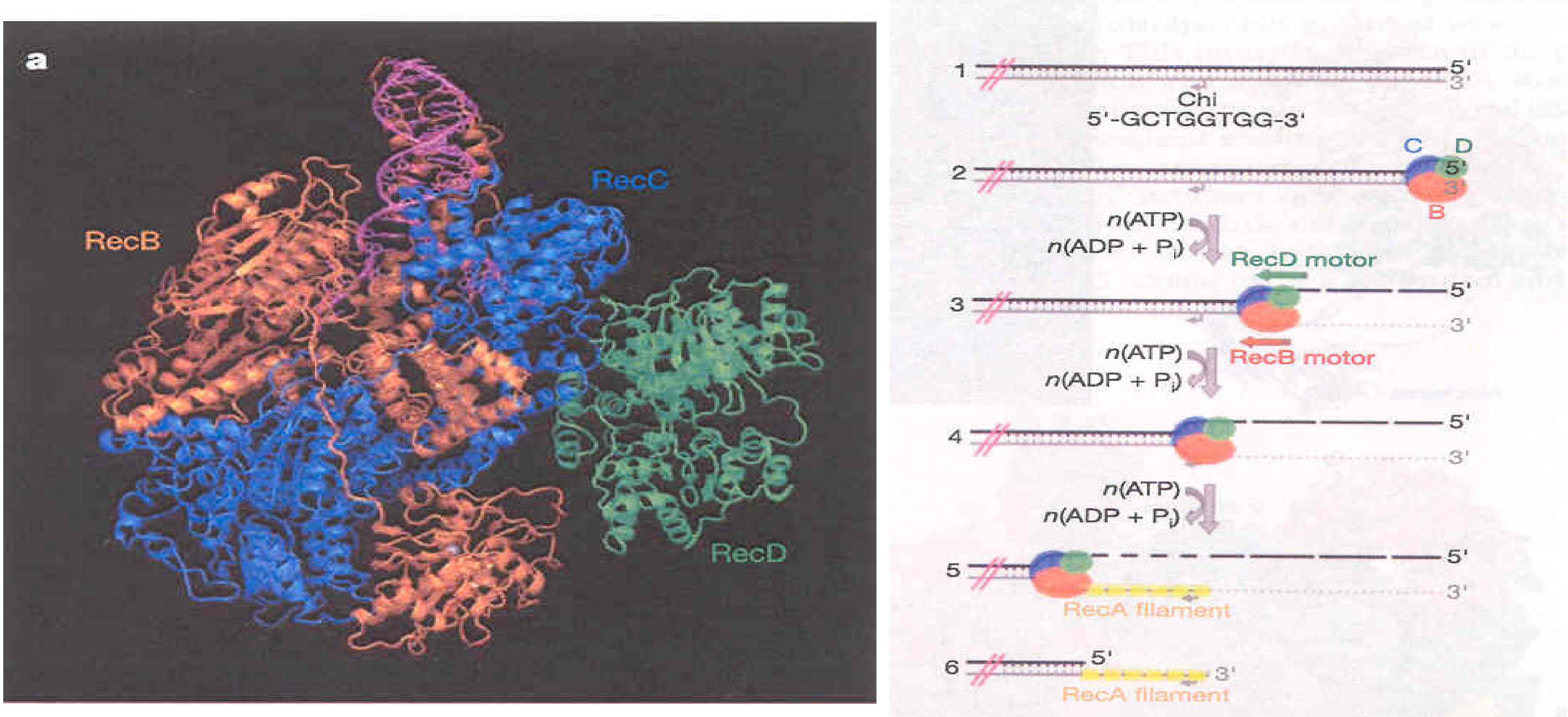
Courtesy of Marie Curie Research Institute, Molecular Motor Group

Motor Proteins: Examples



KINESINS – linear processive motor proteins, move along microtubules, important for transport of vesicles and organelles, cell motility

Motor Proteins: Examples



M.R. Singleton, et al., *Nature*, **432**, 187 (2004).

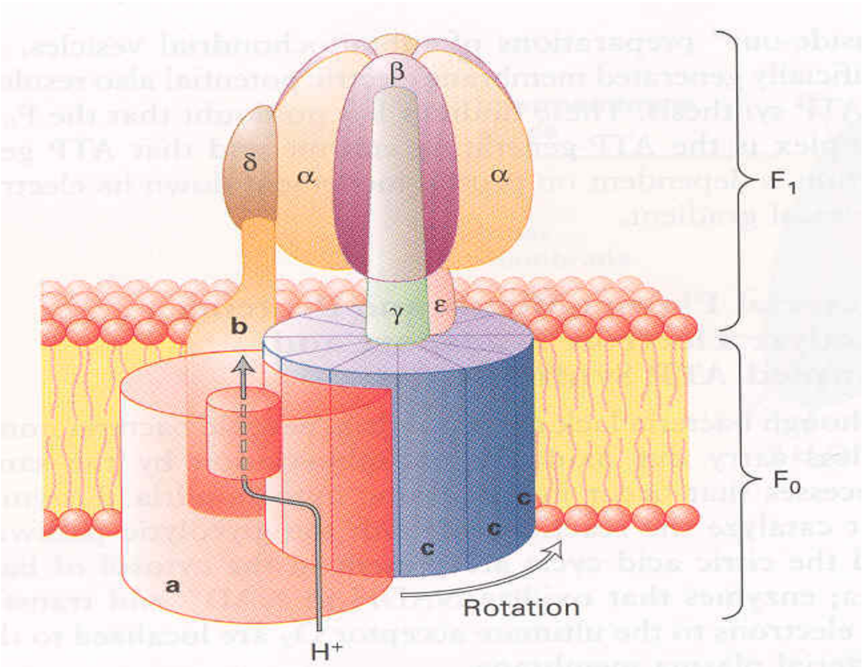
HELICASES – linear processive motor proteins that repair DNA breaks and defects, also important for RNA and DNA replication

Motor Proteins: Examples

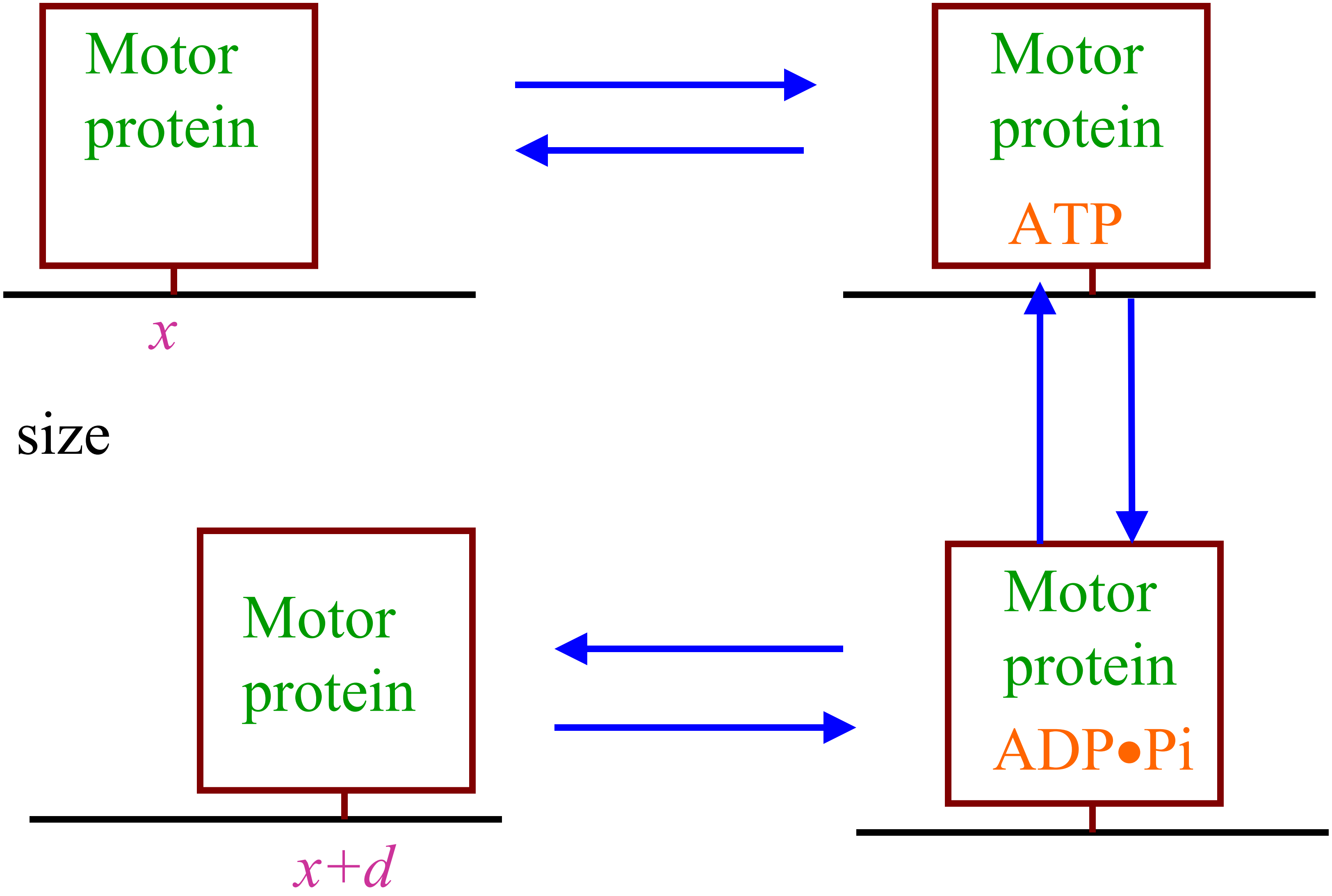
F0-F1 ATP synthase –

Rotary motor proteins

Synthesizes ATP by utilizing the gradient in proton concentration, or uses ATP for proton flux



Motor Proteins: Chemistry



d- step size

Motor Proteins: Properties

Non-equilibrium systems

Velocities: 0.01-100 μm/s (for linear processive)

Step Sizes: 0.3-40 nm

Forces: 1-60 pN

Fuel: hydrolysis of ATP, or related compounds, or polymerization

Efficiency: 50-100% (!!!)

Power – like jet engine

Directionality- move mainly in one direction

Diversity

Motor Proteins

Photo Copyright Faerberg Leonid

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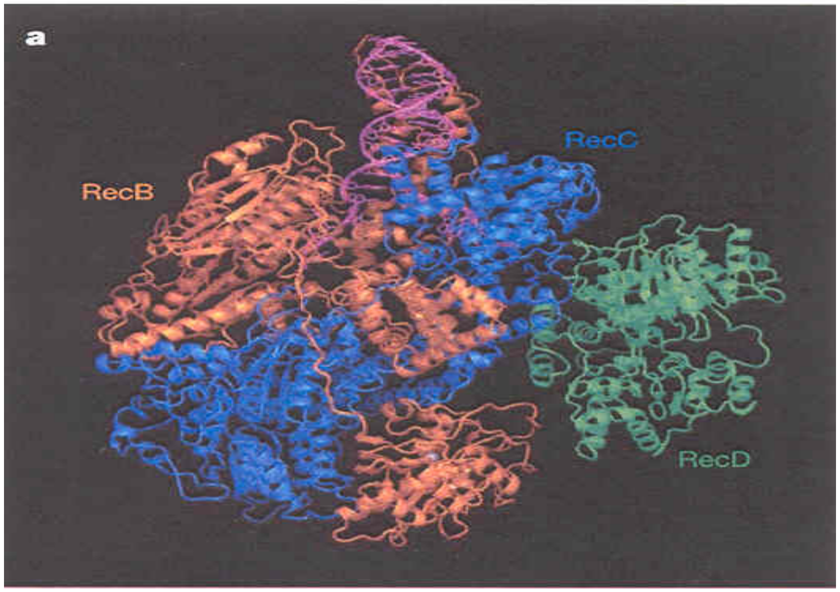
Motor Proteins

Fundamental Problems:

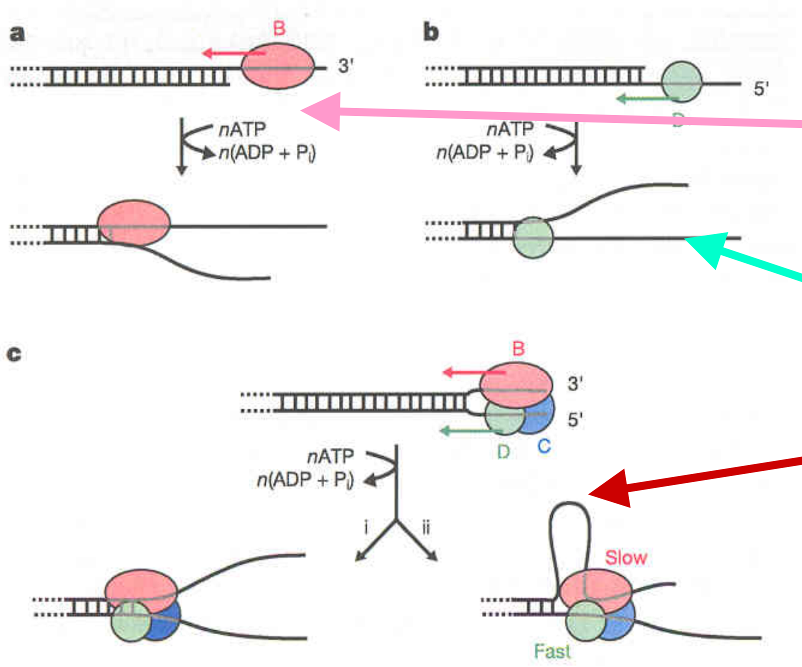
1) How the chemical energy is transformed into the mechanical motion?
2) How many different mechanisms of motor proteins motion? "Physicists *versus* biologists"
3) Why many motor proteins are complexes of already functional subunits?
4) …

RecBCD helicase



RecBCD consists of 3 subunits: RecB and RecD are active helicases; RecC- connects them

Experiments: translocation velocities at high [ATP]



V(RecB)=73 bp/s

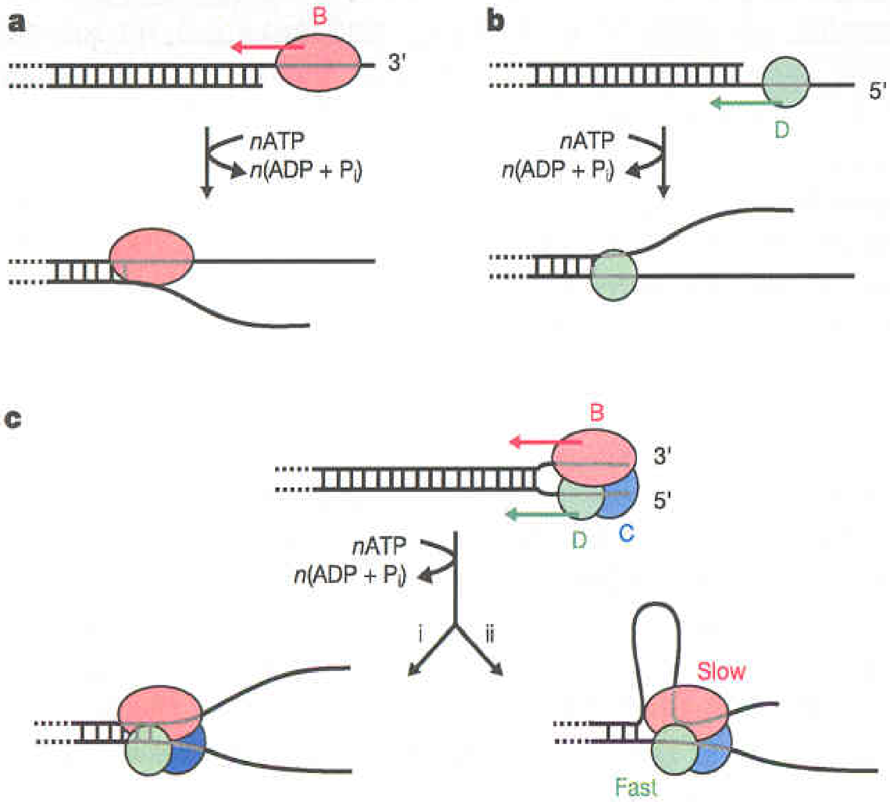
V(RecD)=300 bp/s

V(RecBCD)=370 bp/s !!!

Counter-intuitive!

THEORETICAL PROBLEM:

Complex motor protein particles (made of two or more active domains) can move faster than the individual motor subunits!



Our idea: interaction energy between the subunits affect the overall dynamic and biochemical properties

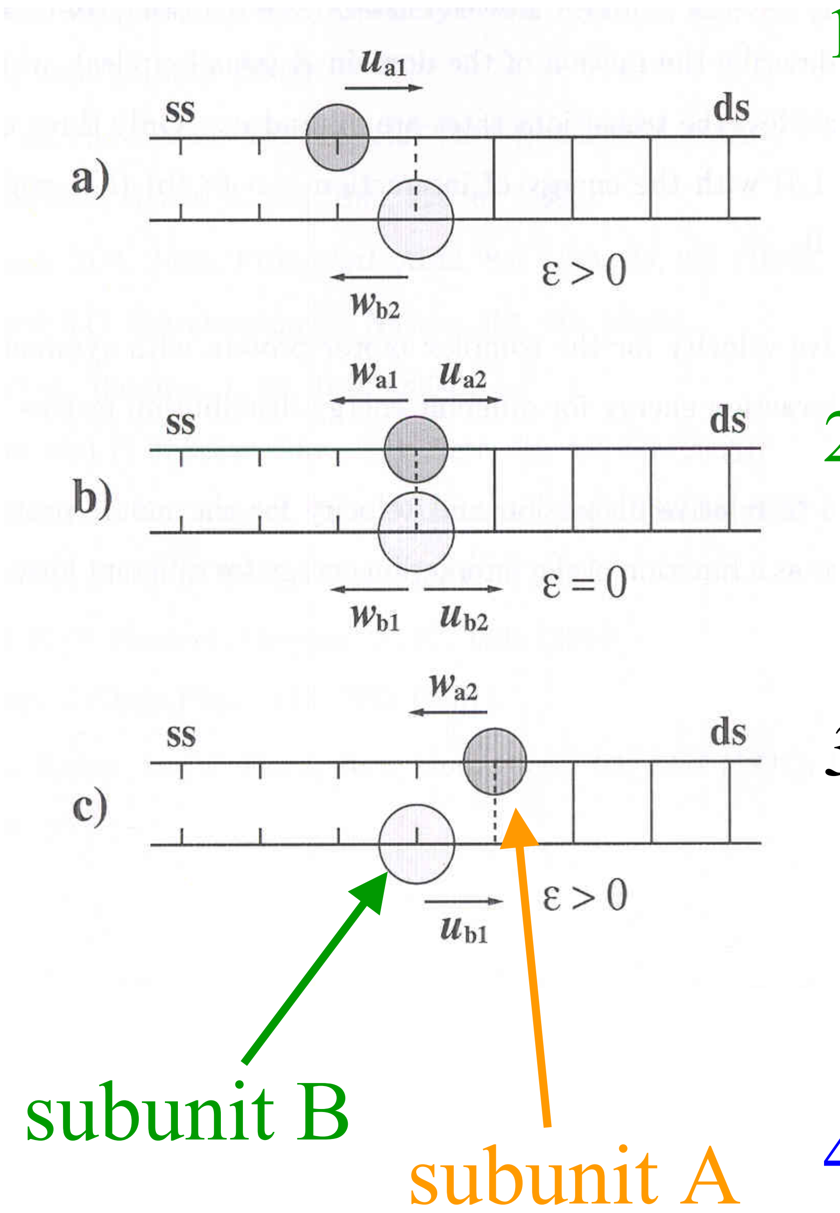
Our goal: to develop a quantitative model of inter-domain interaction

Theoretical Modeling of Motor Proteins Dynamics:

main approaches:

1) Thermal Ratchets
2) Multi-state Chemical Kinetic (Stochastic) Models
3) Molecular and Brownian Dynamics computer simulations

Model for Coupled Proteins:



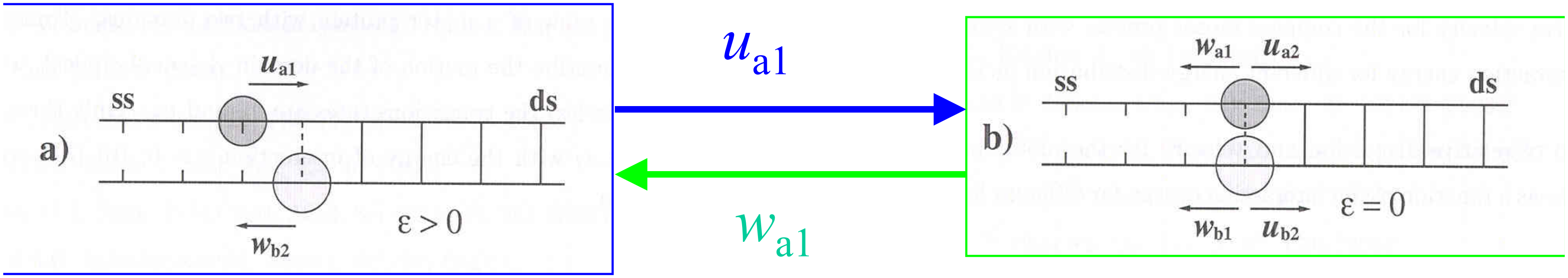
1) Motor protein: consists of two subunits moving along different molecular tracks (double- or single-stranded DNA)

2) 3 possible configurations: vertical configuration has $\varepsilon=0$, other configurations $\varepsilon>0$

3) u,w – transition rates; ε-energy of interaction, $\varepsilon > 2\,k_BT$ – energy to break the bond between nucleotides

4) No sequence dependence

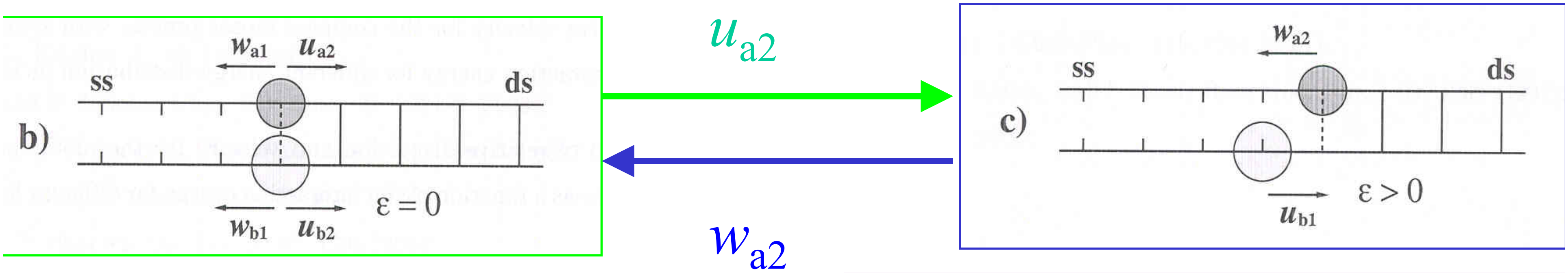
Model:



detailed balance relation:

$$\frac{u_{a1}}{w_{a1}} = \frac{u_a}{w_a}\exp\left(+\frac{\varepsilon}{k_B T}\right)$$

u_a, w_a-rates when $\varepsilon=0$

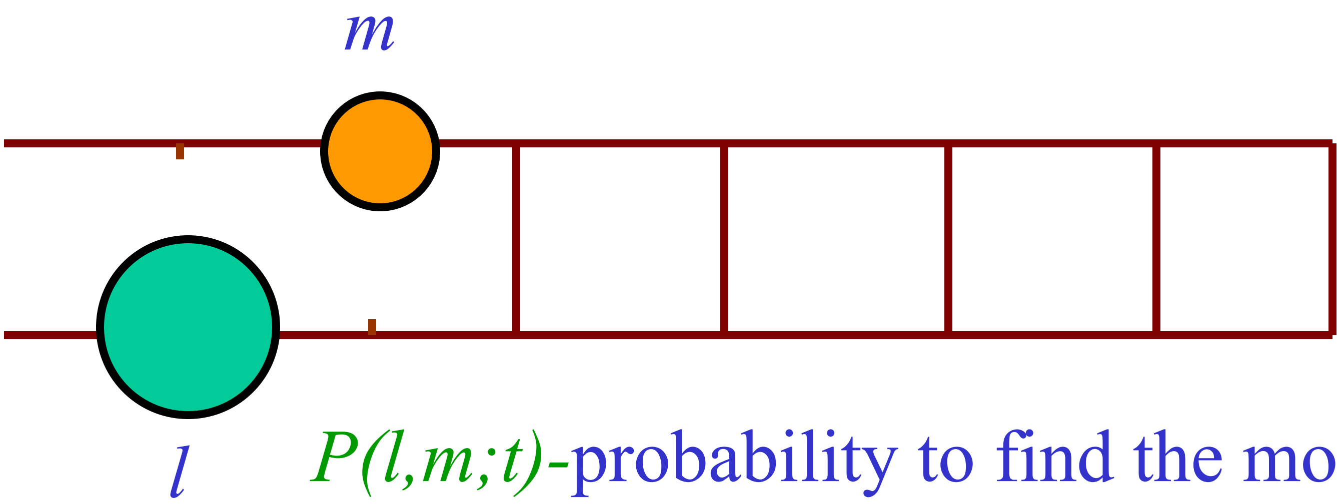


detailed balance relation:

$$\frac{u_{a2}}{w_{a2}} = \frac{u_a}{w_a}\exp\left(-\frac{\varepsilon}{k_B T}\right)$$

motion of domain A

Model:



$P(l,m;t)$-probability to find the molecule in the configuration with the domain A at the site m, the domain B at the site l, at time t

Dynamics is described by Master equations:

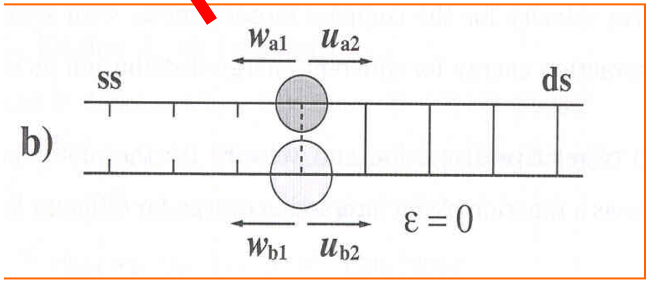
$$\frac{dP(l+1,l;t)}{dt} = u_{a2}P(l,l;t) + w_{b1}P(l+1,l+1;t) - (u_{b1}+w_{a2})P(l+1,l;t)$$

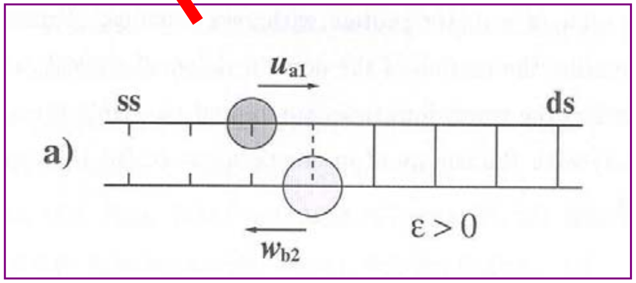
$$\frac{dP(l-1,l;t)}{dt} = u_{b2}P(l-1,l-1;t) + w_{a1}P(l,l;t) - (u_{a1}+w_{b2})P(l-1,l;t)$$

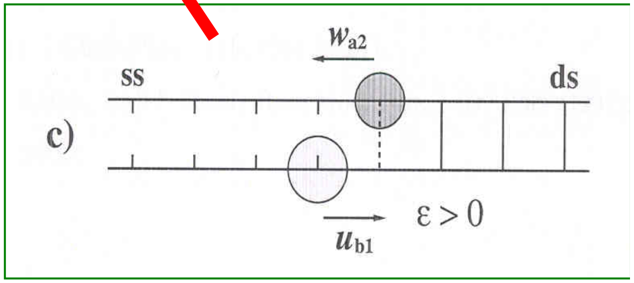
Model:

Define:

$$P_0(t) = \sum_{l=-\infty}^{l=+\infty} P(l,l;t), \quad P_1(t) = \sum_{l=-\infty}^{l=+\infty} P(l-1,l;t), \quad P_1^{'}(t) = \sum_{l=-\infty}^{l=+\infty} P(l+1,l;t)$$







Vertical configurations

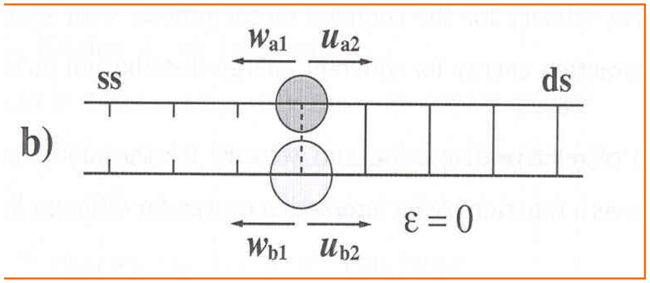
Non-vertical configurations

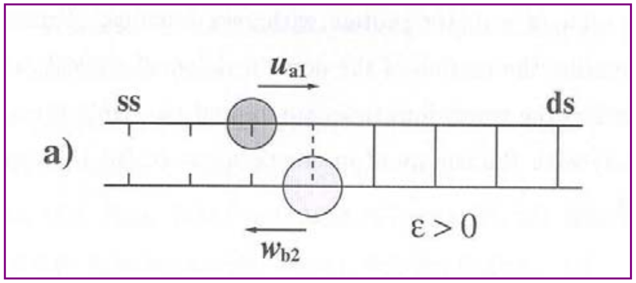
normalization condition

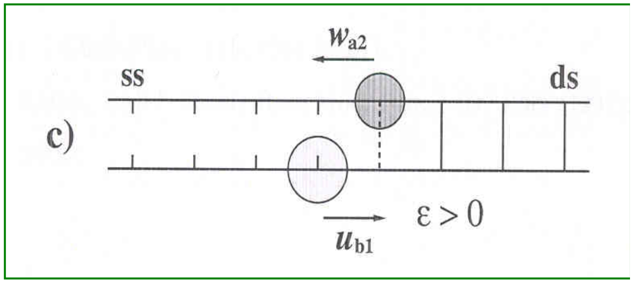
$$\boxed{P_0(t) + P_1(t) + P_1^{'}(t) = 1}$$

Solutions:

Stationary-state solutions:







$$P_0 = \frac{1}{1 + \alpha + \beta}$$

$$P_1 = \frac{\beta}{1 + \alpha + \beta}$$

$$P_1' = \frac{\alpha}{1 + \alpha + \beta}$$

Where: $\alpha = \dfrac{u_{a2} + w_{b1}}{u_{b1} + w_{a2}}, \quad \beta = \dfrac{u_{b2} + w_{a1}}{u_{a1} + w_{b2}}$

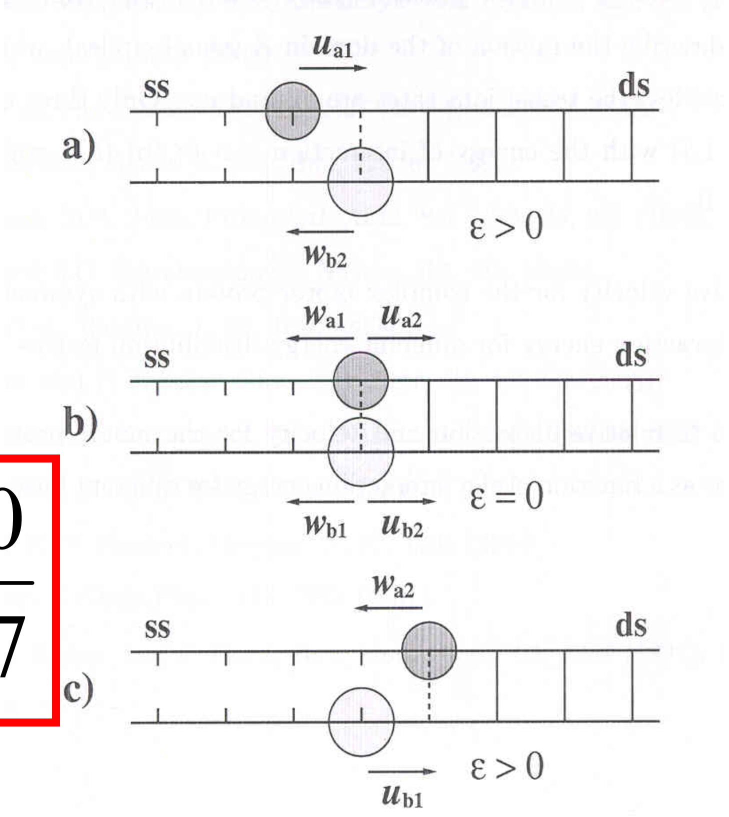
Dynamic properties: velocity, dispersion

$$V = \frac{1}{1 + \alpha + \beta}(u_{a2} + u_{b2} - \alpha w_{a2} - \beta w_{b2})$$

Analysis. Simple Cases:

If no interaction (ε=0):

$$u_1 = u_2 = u \qquad w_1 = w_2 = w$$



$$\boxed{\frac{V(\varepsilon=0)}{V_0} = \frac{2}{3}, \quad \frac{1}{3} \le \frac{D(\varepsilon=0)}{D_0} \le \frac{10}{27}}$$

where:

$$V_0 = u - w, \qquad D_0 = \frac{u+w}{2}$$

for free particles – without restrictions on possible configurations

Analysis:

Asymmetric domains and ε>0

Recall-detailed balance relations:

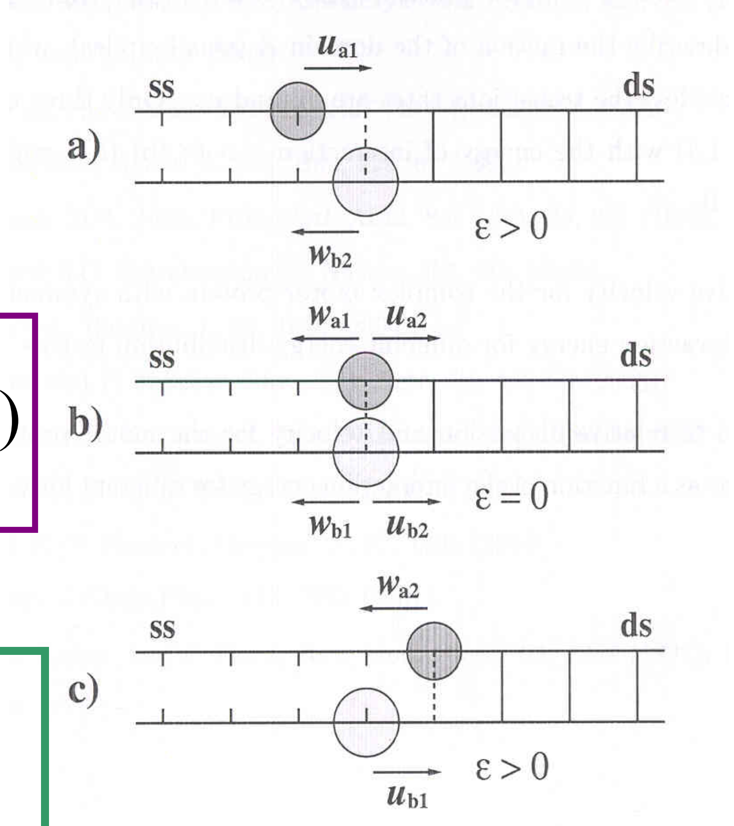
$$\frac{u_{j1}}{w_{j1}} = \frac{u_j}{w_j}\exp(+\frac{\varepsilon}{k_B T})$$

$$\frac{u_{j2}}{w_{j2}} = \frac{u_j}{w_j}\exp(-\frac{\varepsilon}{k_B T})$$

for $j=a,b$

$$u_{j1} = u_j \gamma^{1-\theta_{j1}} \quad w_{j1} = w_j \gamma^{-\theta_{j1}}$$

$$u_{j2} = u_j \gamma^{-\theta_{j2}} \quad w_{j2} = w_j \gamma^{1-\theta_{j2}}$$



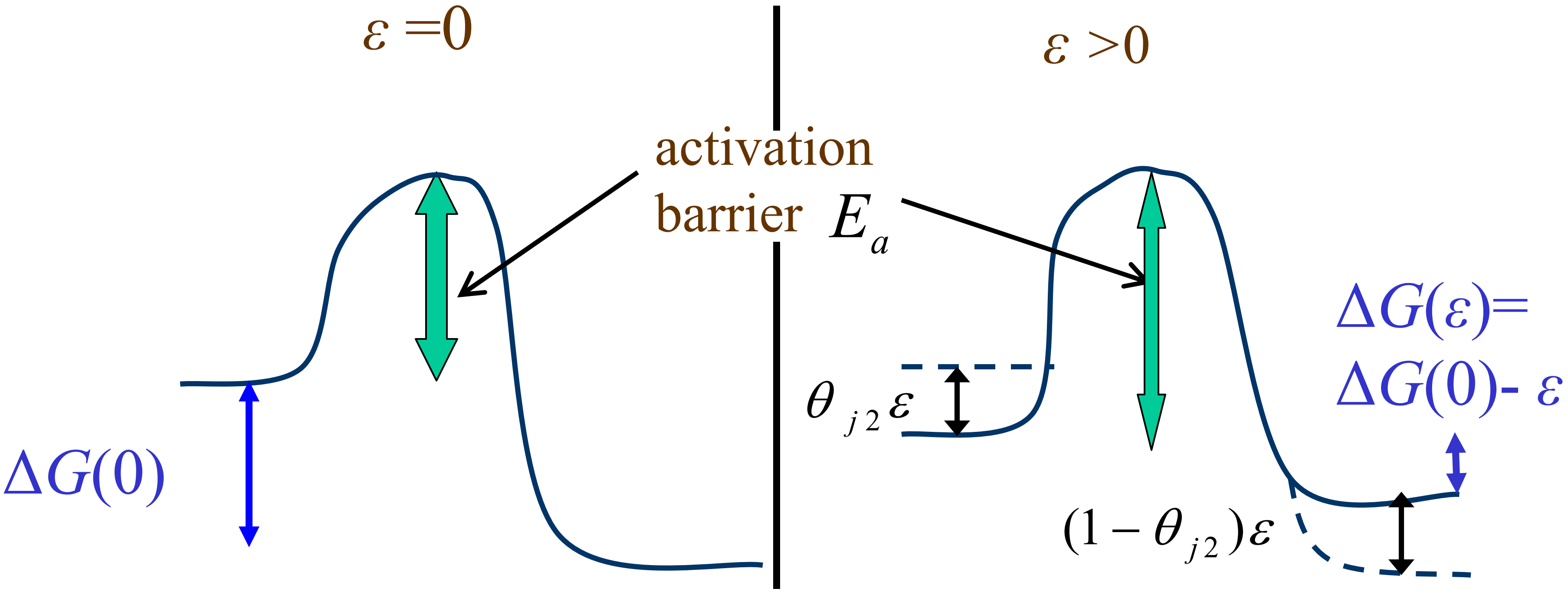
$$\gamma = \exp(\frac{\varepsilon}{k_B T})$$

$0<\theta_{j1}$, $\theta_{j2}<1$- energy distribution factors

Analysis:

Energy distribution factors $0<\theta_{j1},\ \theta_{j2}<1$

$$u_{j2}(\varepsilon) = u_j(0)e^{\frac{-\theta_{j2}\varepsilon}{k_B T}} \qquad w_{j2}(\varepsilon) = w_j(0)e^{\frac{(1-\theta_{j2})\varepsilon}{k_B T}}$$



$\varepsilon = 0$

$\varepsilon > 0$

activation barrier E_a

$\Delta G(0)$

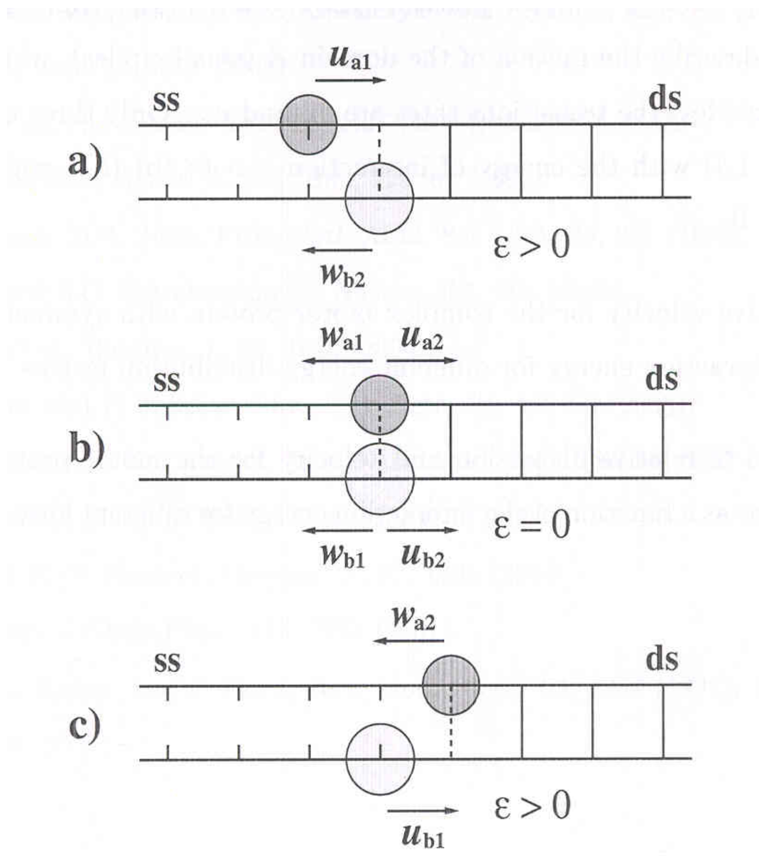
$\theta_{j2}\varepsilon$

$(1-\theta_{j2})\varepsilon$

$\Delta G(\varepsilon) = \Delta G(0) - \varepsilon$

$$u_{j2} \cong e^{-E_a / k_\mathrm{B} T}$$

Analysis:



Consider symmetric domains:

A=B, Assume $\theta_{jk} = \theta$

Relative velocity:

$$\boxed{r_V = \frac{V}{V_0} = \frac{2\gamma^{1-\theta}}{2+\gamma}}$$

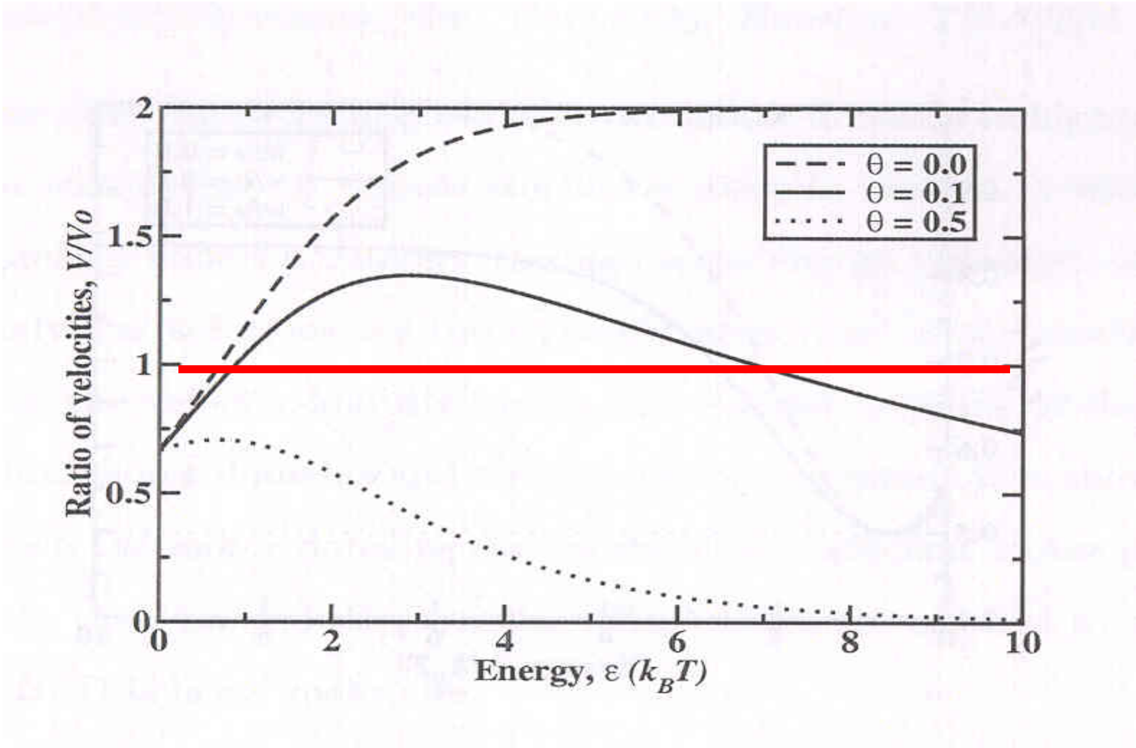
Velocity of free non-interacting particle

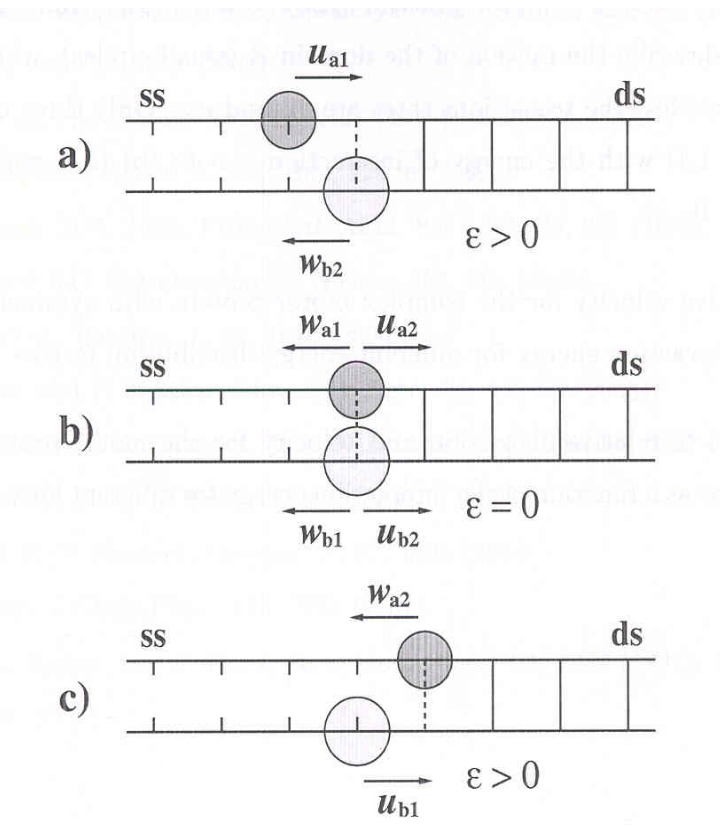
$$\boxed{\gamma = \exp\left(\frac{\varepsilon}{k_B T}\right)}$$

interaction parameter

Analysis:

Relative velocity:

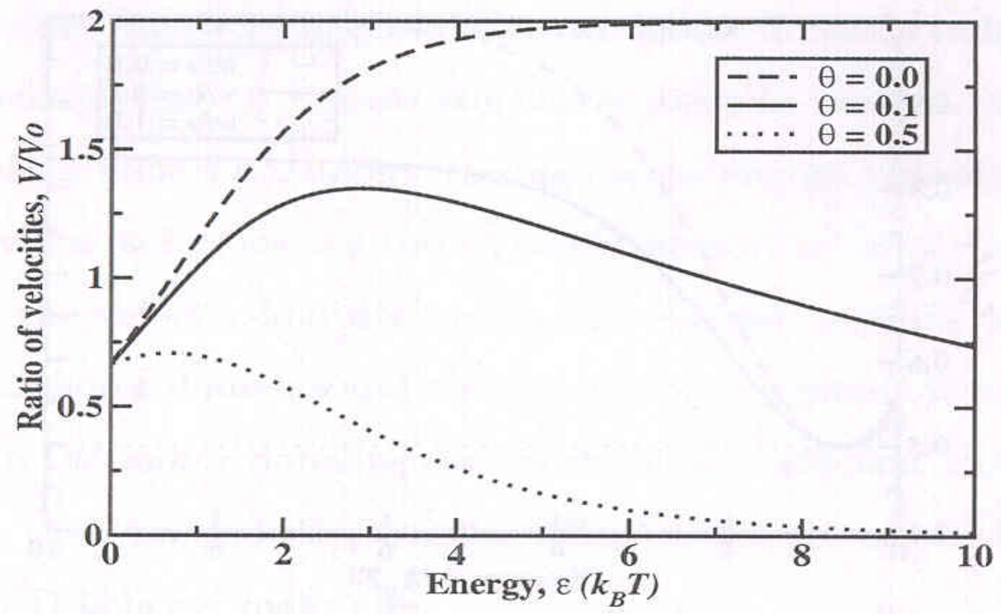


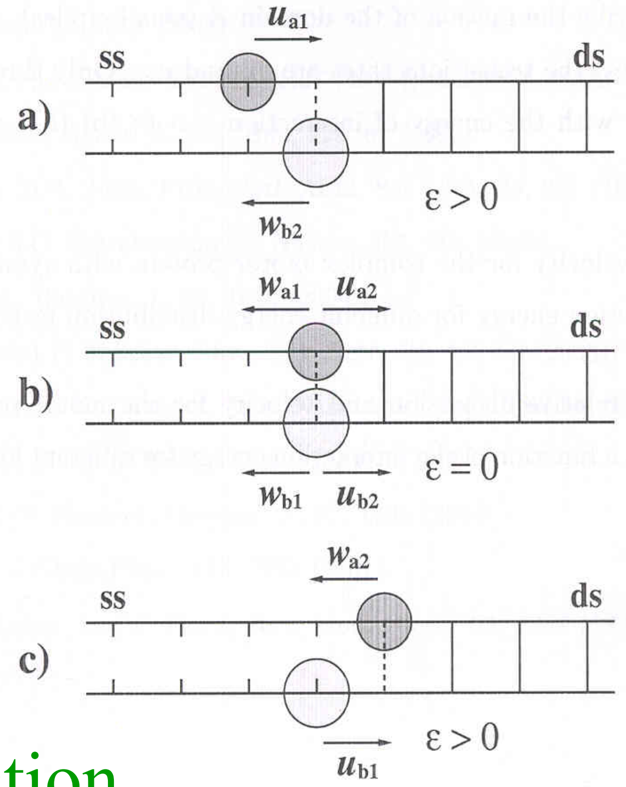


For $0<\theta<0.23$ there is a range of interaction energies ε when the velocity of the motor protein's complex is **FASTER** than the velocities of free particles**!!!**

Note: maximal $r_V=2$!

Analysis:





Effect of inter-domain interaction

$$V = \frac{1}{2}\left\{(u_{a1} - w_{b2})P_1 + (u_{a2} + u_{b2} - w_{a1} - w_{b1})P_0 + (u_{b1} - w_{a2})P_1'\right\}$$

increases with ε

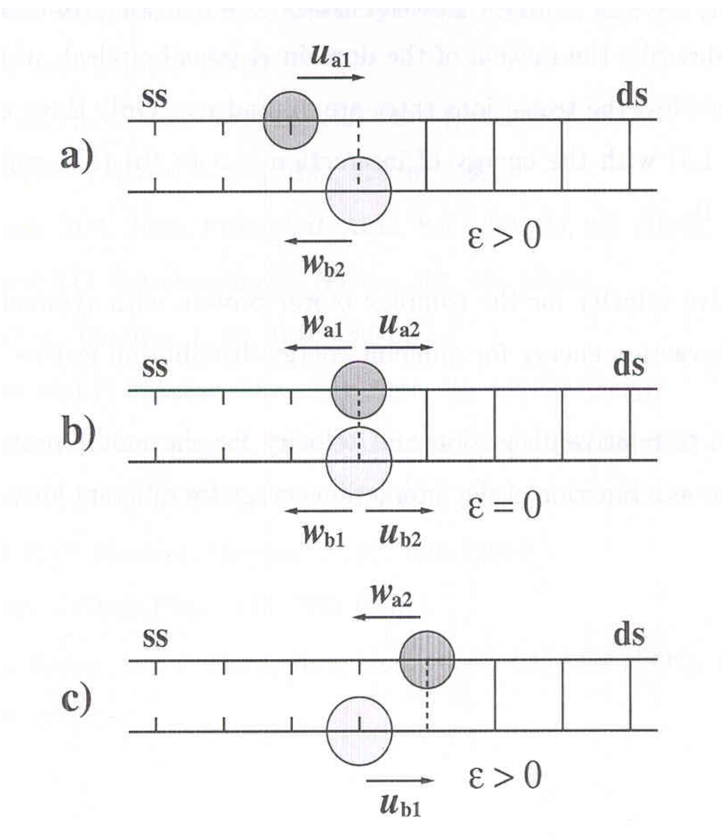
decreases with ε

Analysis:

Relative dispersion for the motor protein molecule with symmetric domains (A=B)

$$r_D = \frac{D}{D_0} = \frac{2\gamma^{1-\theta}}{2+\gamma} g(u, w; \gamma)$$

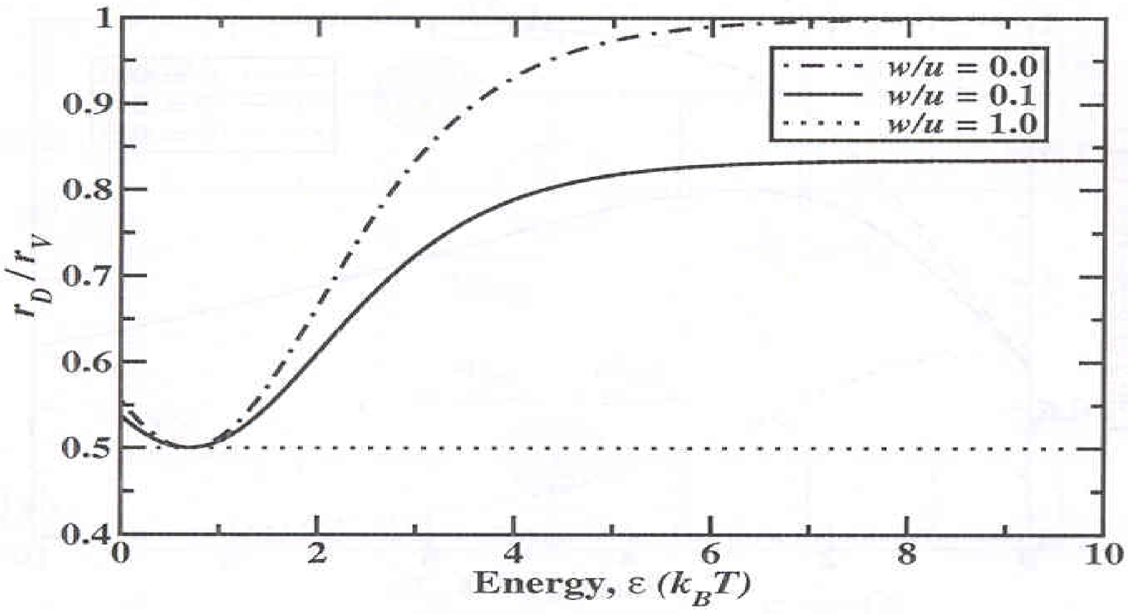
$$0.5 \le g(u, w; \gamma) < 1$$

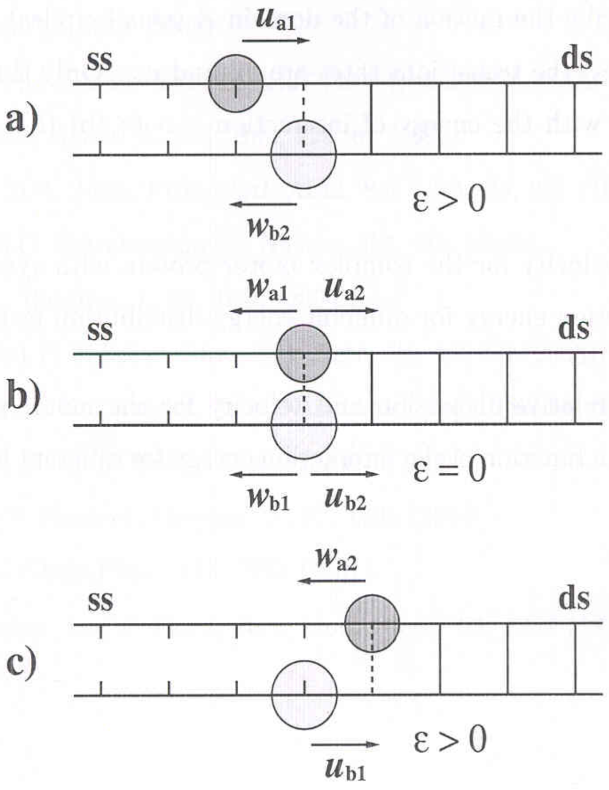


Interaction between domains might decrease or increase fluctuations

Analysis:

Comparison of velocity and dispersion





Interaction energy effects velocity more than dispersion

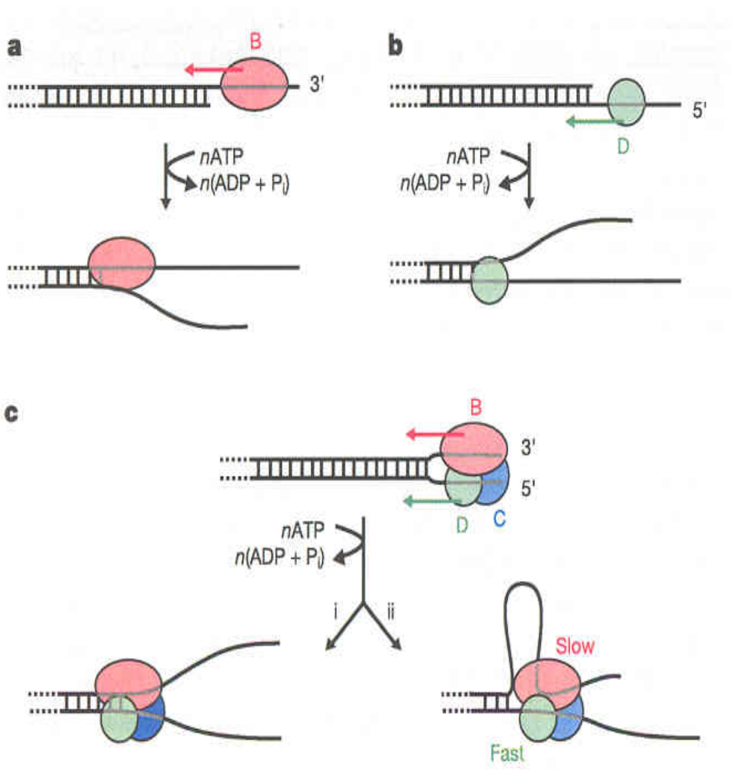
Case r_V>1 and r_D<1 – very efficient motor protein!

Application for Helicases:

From experiments: $w_{ji} \approx 0$

$$\boxed{V \approx \frac{(u_a + u_b)\,\gamma^{1-\theta}}{\gamma + \left(\frac{u_a}{u_b} + \frac{u_b}{u_a}\right)}}$$

RecB RecD



RecBCD

u_a=73 bp/s - velocity of free RecB

u_b=300 bp/s- velocity of free RecD

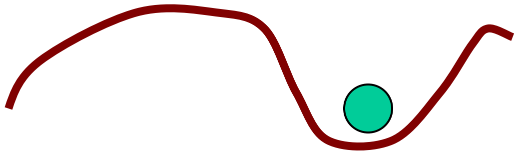
Assume $\theta \approx 0$

V(RecBCD)=370 bp/s for $\varepsilon \approx 6\, k_B T$

RecBCD is working at almost maximal efficiency

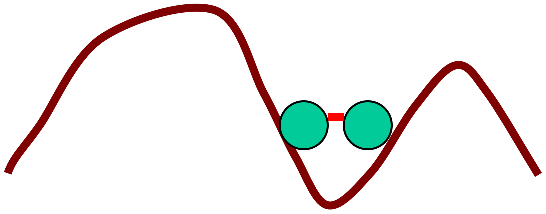
Explanations:

Single subunit, V_1

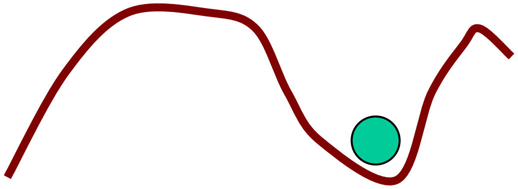


Coupled motor protein

$V > V_1, V_2$



Single subunit, V_2



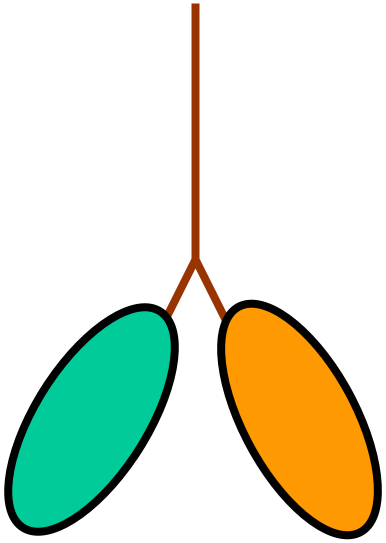
Analogy with molecular orbitals

Energy landscapes of the motor proteins are changed when they are coupled

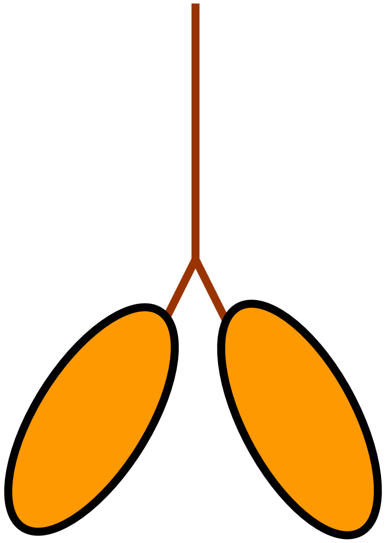
Other Applications:

Investigation of dynamics of heterodymeric kinesins KIF3A/B

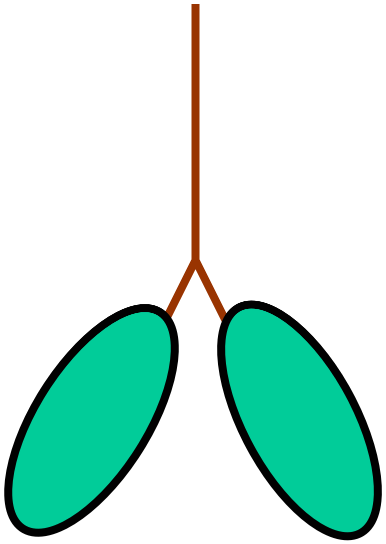
W. Hancock *et al.*, *Biophys. J.* **87**, 1795 (2004)



KIF3A/B -wild type

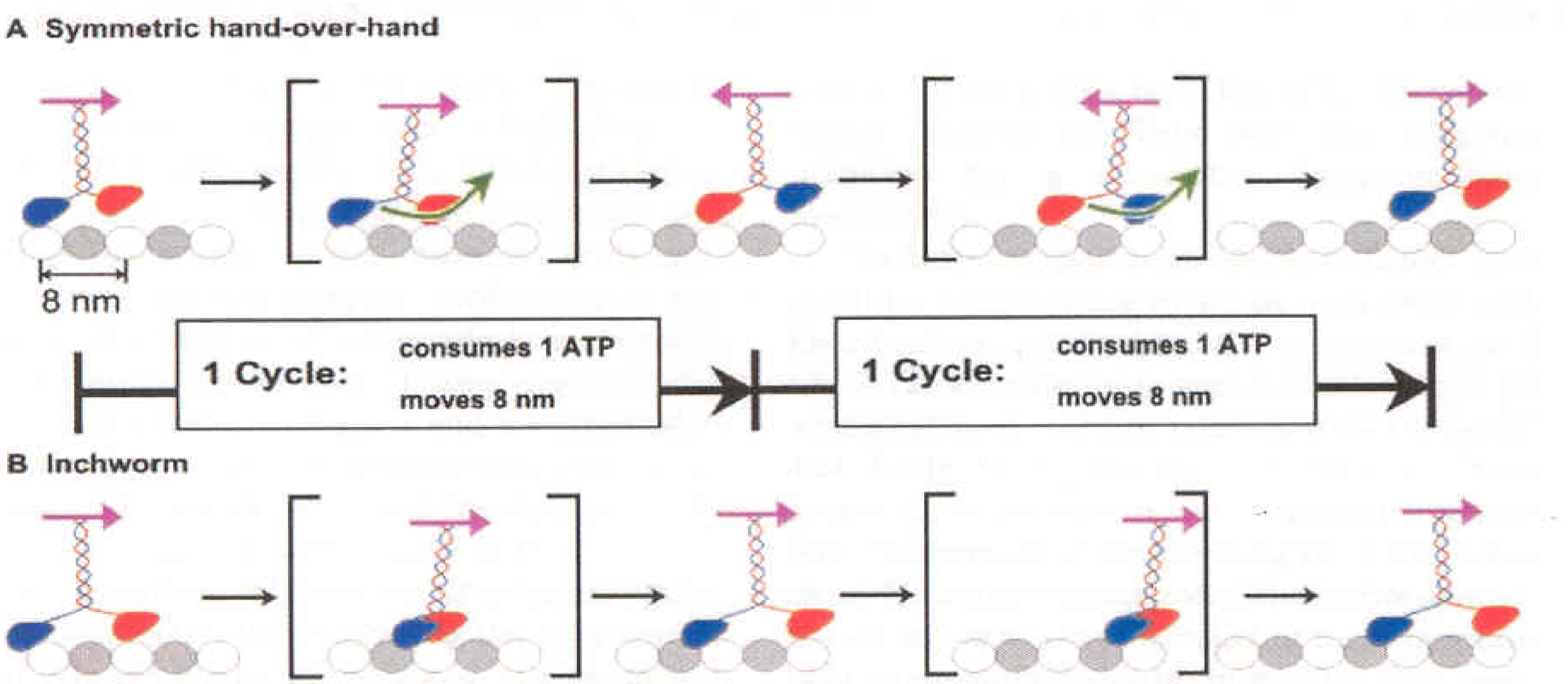


KIF3A/A-chimera



KIF3B/B-chimera

Mechanism of Motility

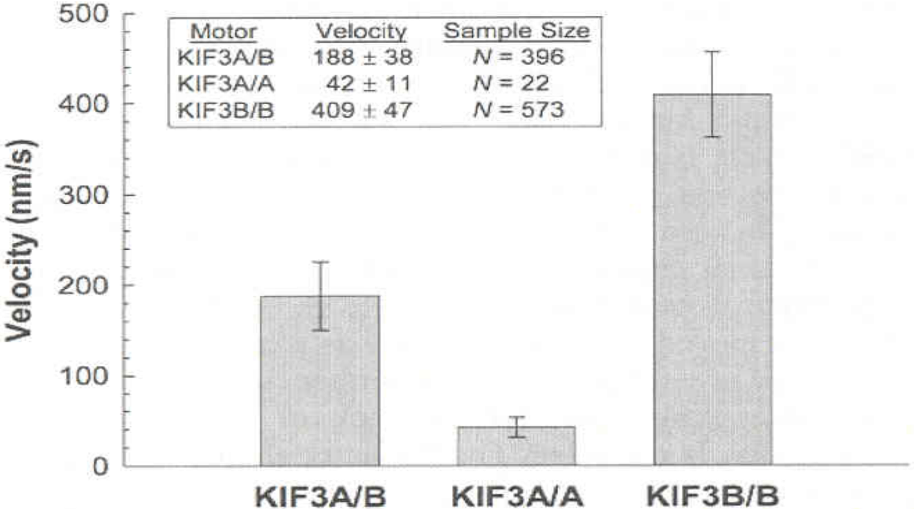


It is shown experimentally that for kinesins and myosins – hand-over-hand mechanism

Other Applications:

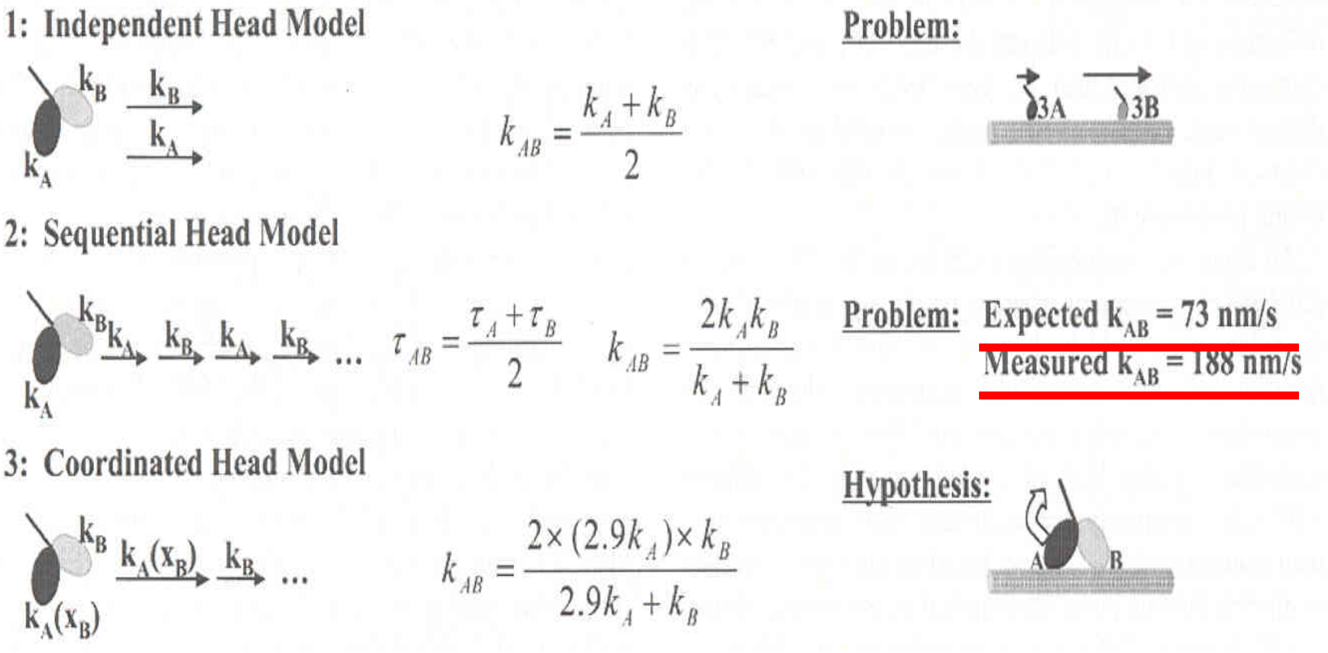
Velocities of wild-type KIF3A/B and chimeric KIF3 motor proteins

$$\boxed{V_{\text{dimer}} = \frac{2V_AV_B}{V_A+V_B}}$$



Motor	Velocity	Sample Size
KIF3A/B	188 ± 38	$N = 396$
KIF3A/A	42 ± 11	$N = 22$
KIF3B/B	409 ± 47	$N = 573$

Models:



1: Independent Head Model

$$k_{AB} = \frac{k_A+k_B}{2}$$

Problem:

2: Sequential Head Model

$$\xrightarrow{k_A} \xrightarrow{k_B} \xrightarrow{k_A} \xrightarrow{k_B} \dots$$

$$\tau_{AB} = \frac{\tau_A+\tau_B}{2} \qquad k_{AB} = \frac{2k_Ak_B}{k_A+k_B}$$

Problem: Expected k_{AB} = 73 nm/s

Measured k_{AB} = 188 nm/s

3: Coordinated Head Model

$$\xrightarrow{k_A(x_B)} \xrightarrow{k_B} \dots$$

$$k_{AB} = \frac{2\times(2.9k_A)\times k_B}{2.9k_A+k_B}$$

Hypothesis:

Coordination= interaction?

Other Applications:

Properties of homodimeric and heterodimeric kinesins

Proc. Natl. Acad. Sci. USA, **99**, 16058-16063 (2002)

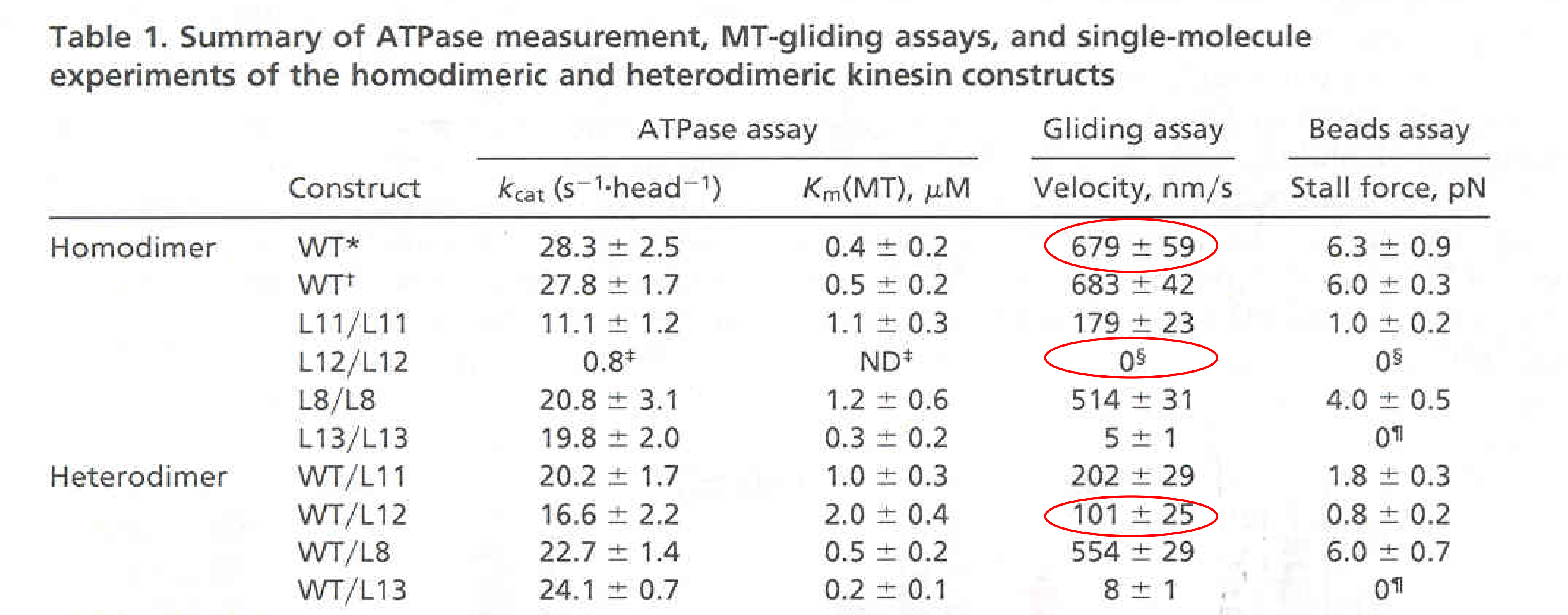


Table 1. Summary of ATPase measurement, MT-gliding assays, and single-molecule experiments of the homodimeric and heterodimeric kinesin constructs

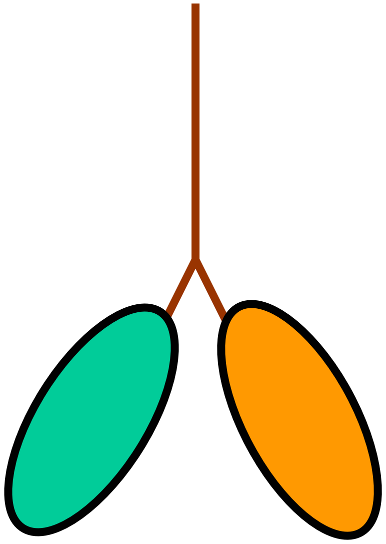
		ATPase assay		Gliding assay	Beads assay
	Construct	k_{cat} (s^{-1}·head^{-1})	K_m(MT), μM	Velocity, nm/s	Stall force, pN
Homodimer	WT*	28.3 ± 2.5	0.4 ± 0.2	679 ± 59	6.3 ± 0.9
	WT†	27.8 ± 1.7	0.5 ± 0.2	683 ± 42	6.0 ± 0.3
	L11/L11	11.1 ± 1.2	1.1 ± 0.3	179 ± 23	1.0 ± 0.2
	L12/L12	0.8‡	ND‡	0§	0§
	L8/L8	20.8 ± 3.1	1.2 ± 0.6	514 ± 31	4.0 ± 0.5
	L13/L13	19.8 ± 2.0	0.3 ± 0.2	5 ± 1	0¶
Heterodimer	WT/L11	20.2 ± 1.7	1.0 ± 0.3	202 ± 29	1.8 ± 0.3
	WT/L12	16.6 ± 2.2	2.0 ± 0.4	101 ± 25	0.8 ± 0.2
	WT/L8	22.7 ± 1.4	0.5 ± 0.2	554 ± 29	6.0 ± 0.7
	WT/L13	24.1 ± 0.7	0.2 ± 0.1	8 ± 1	0¶

It cannot be explained by independent hand-over-hand model!

Coupling is important!

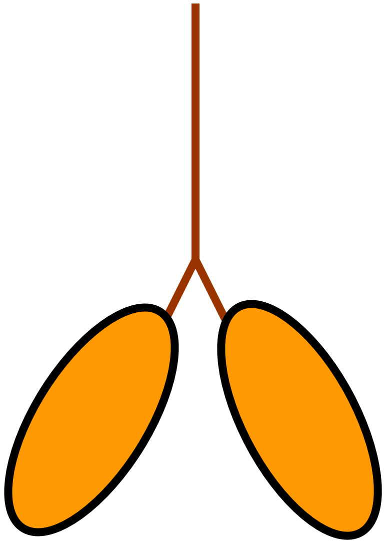
Other Applications:

Can be described by a 2-state model with different interactions between different domains



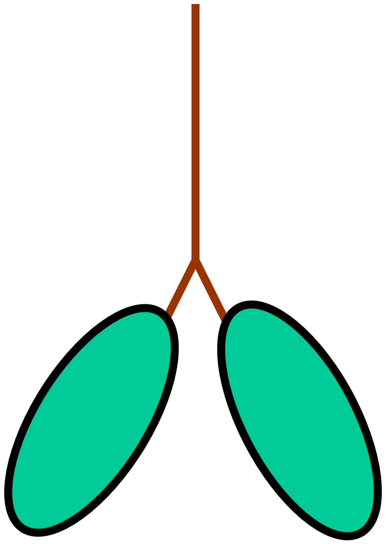
KIF3A/B -wild type

ε(A-B)



KIF3A/A-chimera

ε(A-A)



KIF3B/B-chimera

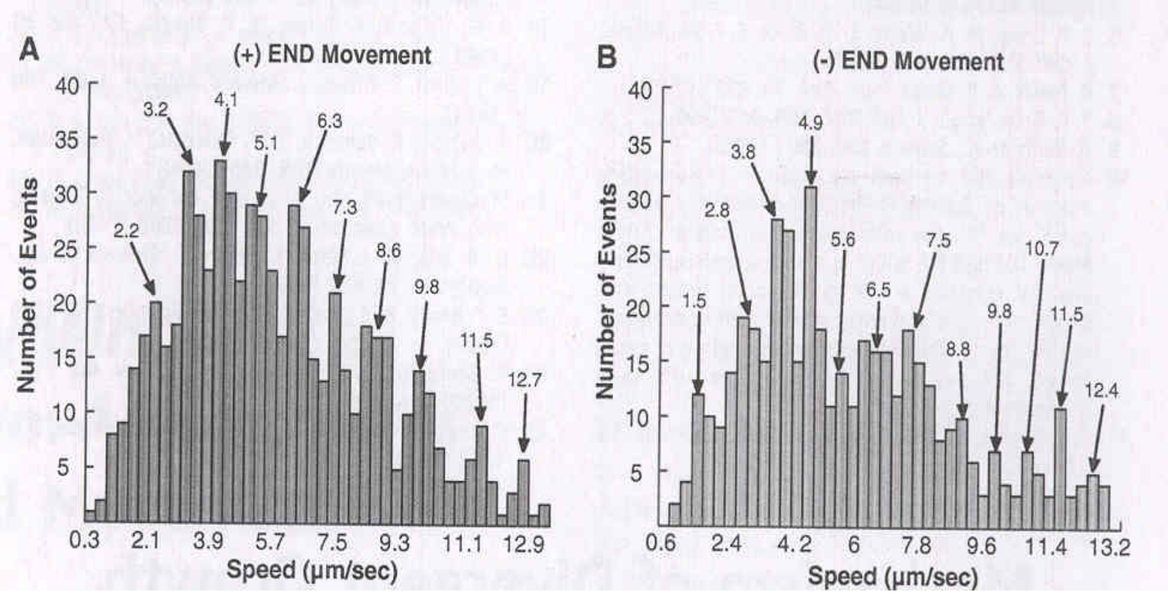
ε(B-B)

Experiments:

In vivo transport of organelles by dyneins and kinesins

Fluorescence Imaging investigation

C. Kural et al., *Science*, **308**, 1469-1472 (2005)

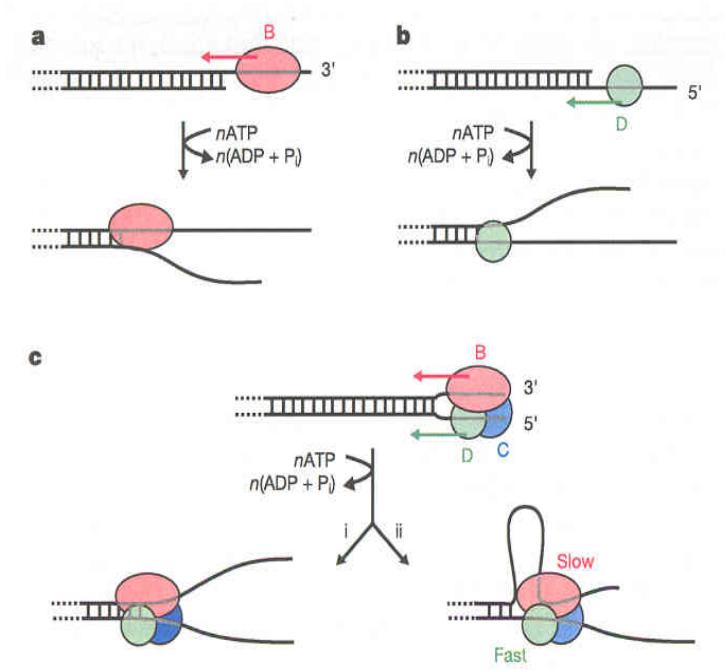


Observation of multiple peaks in velocities of organelles – multiple kinesins or dyneins work together!

In vivo speed of 1 kinesin – 1.5 µm/s

In vivo speed of 1 dynein – 1.7 µm/s

Future Directions and Improvements:



1) Include sequence dependence
2) More realistic potentials (spring, etc.) of interactions
3) Intermediate conformations and states
4) DNA elasticity and inter-domain protein flexibility
5) Interactions and dynamics of *N* motor proteins

CONCLUSIONS

- A possible quantitative mechanism of inter-domain interaction and cooperation in motor proteins is proposed
- Main idea– coupling between motor subunits changes the energy landscapes
- Predictions: proteins made of several functional subunits might move faster and fluctuate less than free particles
- The mechanism is successfully applied for helicases
- 2 dynamic regimes for different couplings

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Phys. Rev. E **73**, 031922 (2006)