Chemomechanical Coupling and Multiscale Motility of Molecular Motors

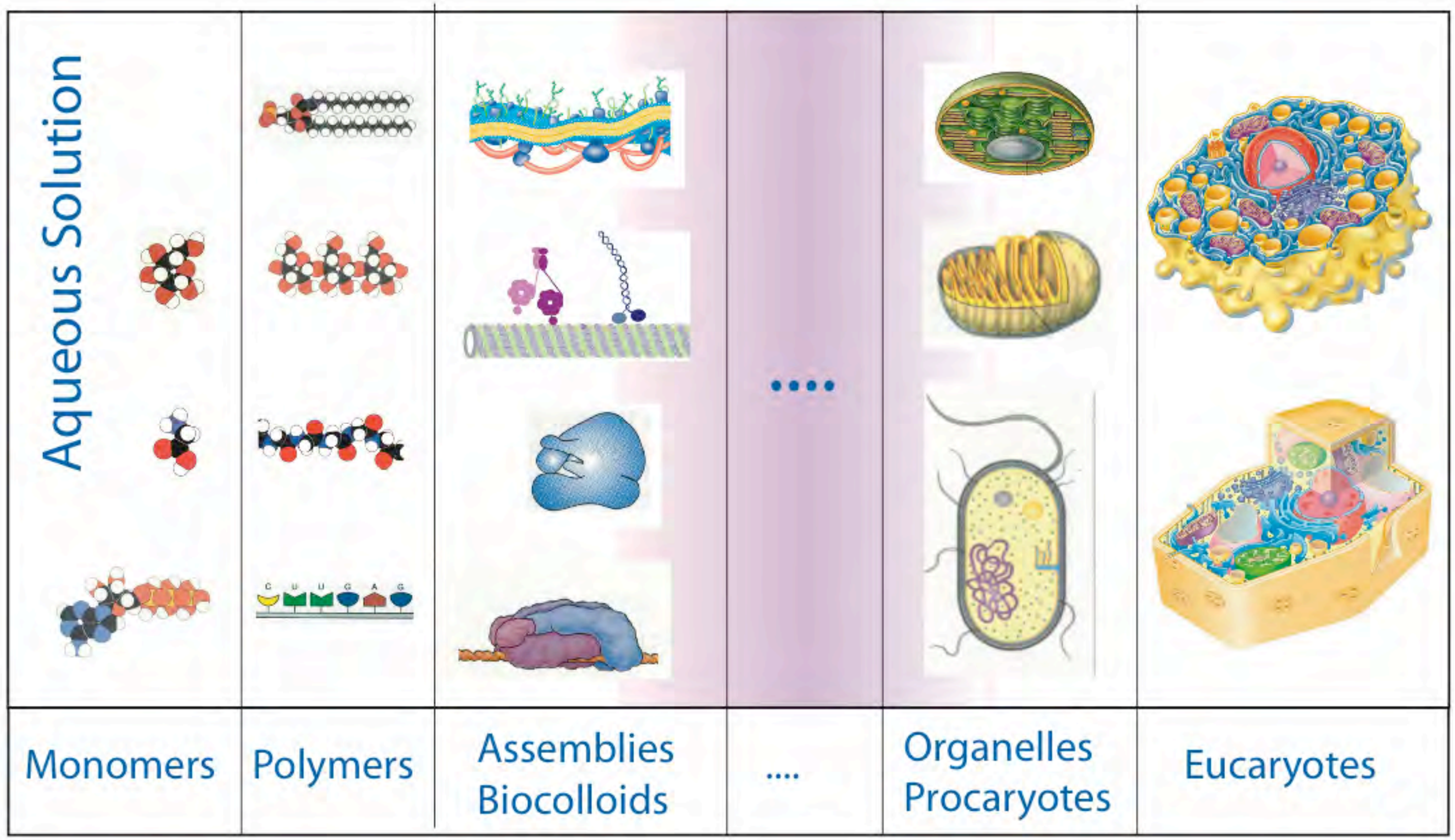
Reinhard Lipowsky

MPI of Colloids and Interfaces, Theory & Bio-Systems

- Introduction
- Chemomechanical (CM) Coupling
- Example: CM Coupling for kinesin
- Multiscale Motility:
  Cargo transport by motor teams
- Outlook on related processes

Bio-Systems in the Nanoregime

Hierarchy of Nanostructures, Bottom-Up:



Transition regime

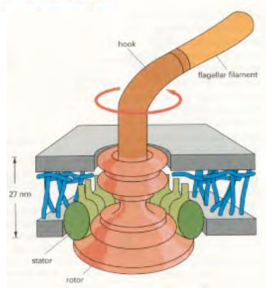
... Matter Life ...

Molecular Machines

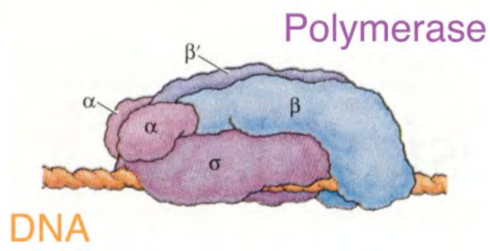
"Every Motion has its Motor"

Protein Oligomers + Substrate:

- Nano-Motors:
  - Stepping motors: Kinesin, Dynein, ...
  - Rotary motors: Bacterial flagellae

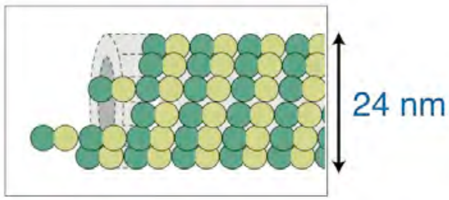


- Nano-Pumps: Na-K-Pump, ...
- Nano-Assemblers: Polymerases, ...



Assembly of Many Proteins:

- Growing filaments

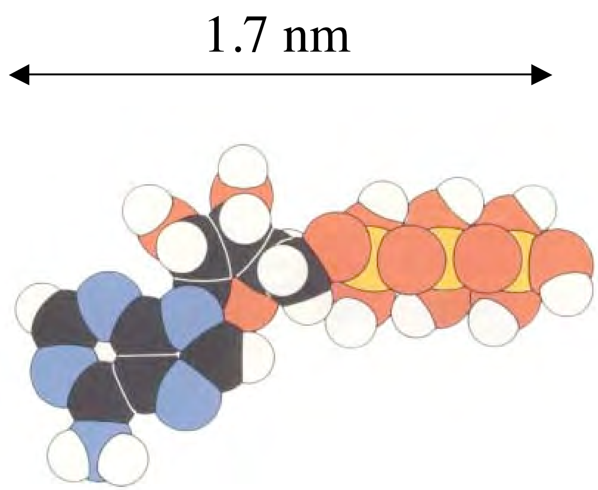


Chemomechanical Coupling

- Molecular machines:

  Conversion of chemical energy into mechanical work

- universal chemical energy source provided by ATP:



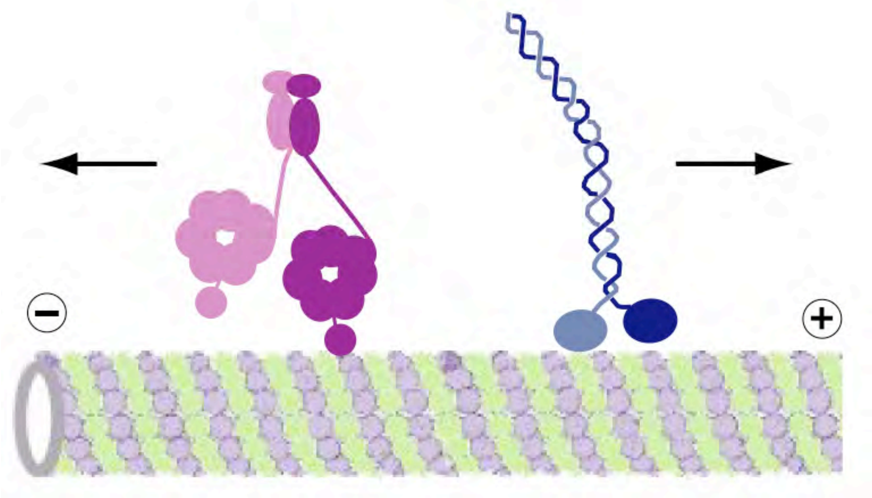
- Hydrolysis of ATP: ATP -> ADP + P
- Synthesis of ATP: ADP + P -> ATP

Nucleotides
ATP, ADP, P

"Human body hydrolyses 60 kg of ATP per day!"

Stepping Motors

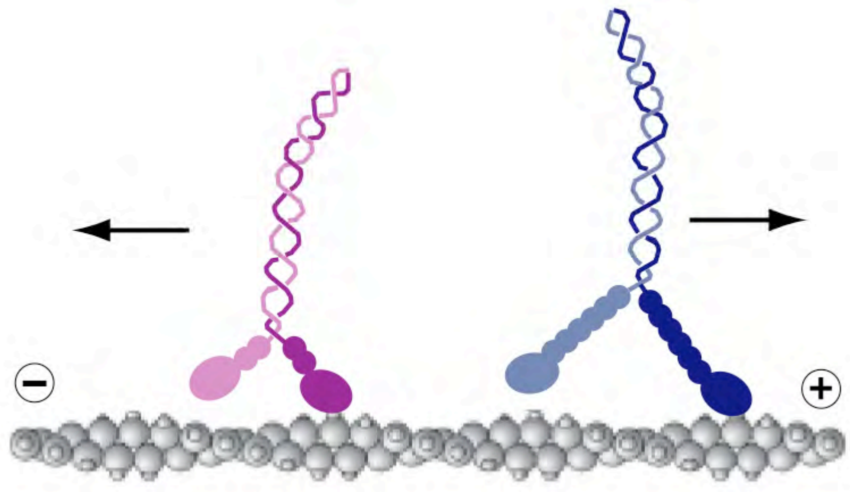
• Filament = Microtubule



Dyneins
to minus end

Kinesins
to plus end

• Filament = F-Actin



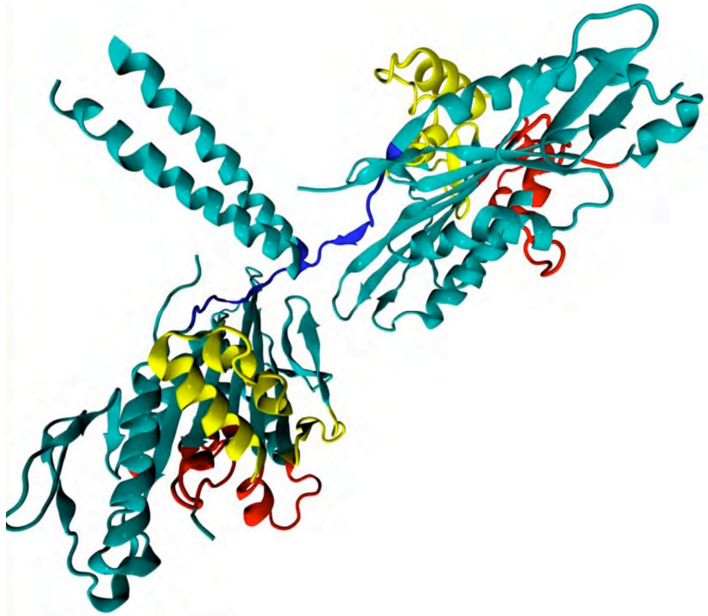
Myosin VI
to minus end

Myosin V
to plus end

• Filaments are polar: Plus- und Minus-Ends (no charges)
• No load: Each motor steps into a prefered direction
• Each motor has two heads that hydrolyze ATP
• Each motor makes discrete steps with fixed step size

Kinesin: Molecular Dimensions

• Two Heads:

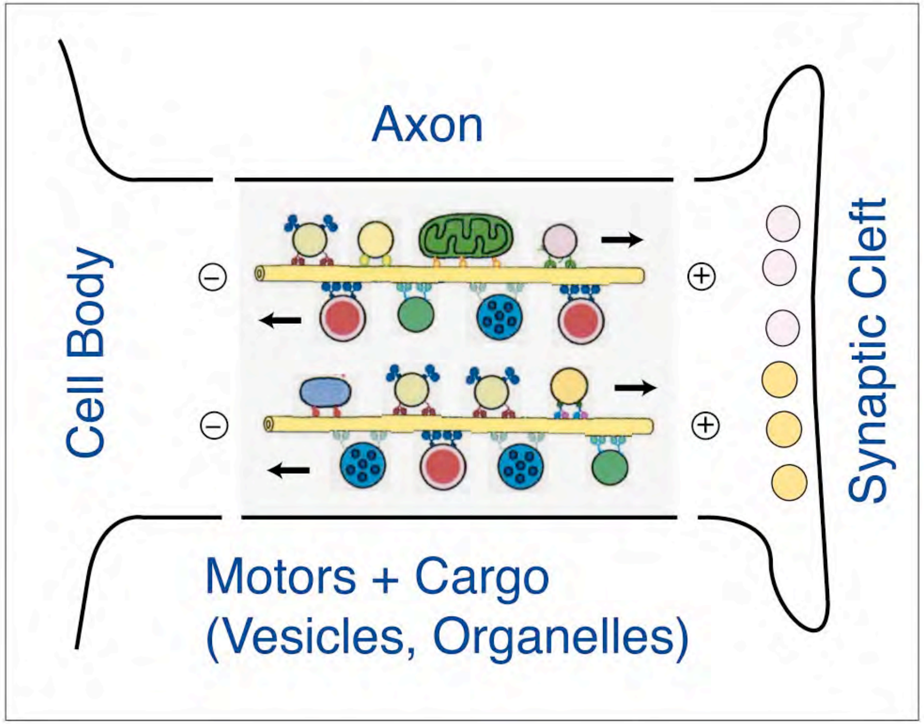


Red: Nucleotide binding
Yellow: Microtubule binding

• Discrete steps: 8 nm for center-of-mass, 16 nm for single head
• Hand-over-hand: trailing head moves in front of leading head

Kinesin: Macroscopic Transport

- Example: Neuron, Axon, and Synapse



- Axon between spine and finger tip is ~ half a meter !
- Cooperative cargo transport by several motors

Multiscale Motility

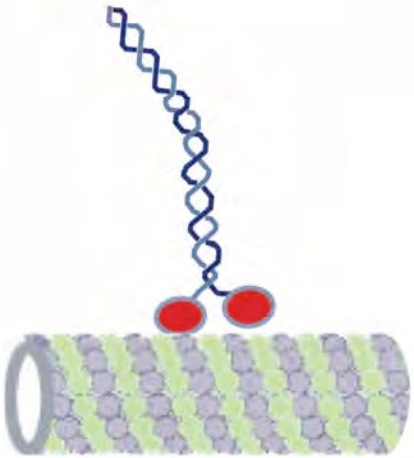
- Example: Kinesin Dimers at Microtubules

ATP Binding

Nucleotide Binding Pocket ~ 1 nm

10^{-3} s

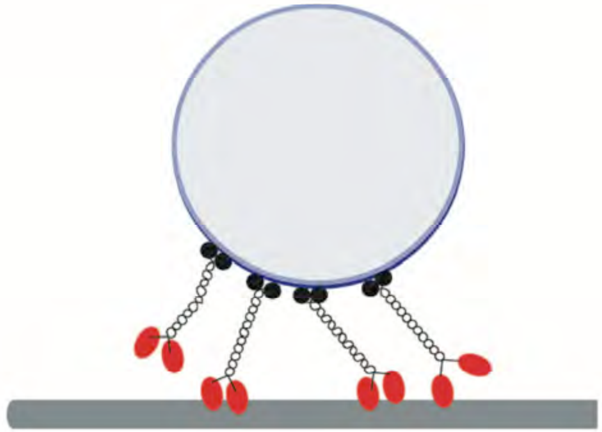
Mechanical Steps



Single head moves by 16 nm

10^{-6} s

Transport



Cargo transport over cm or m !

10^{4} - 10^{6} s

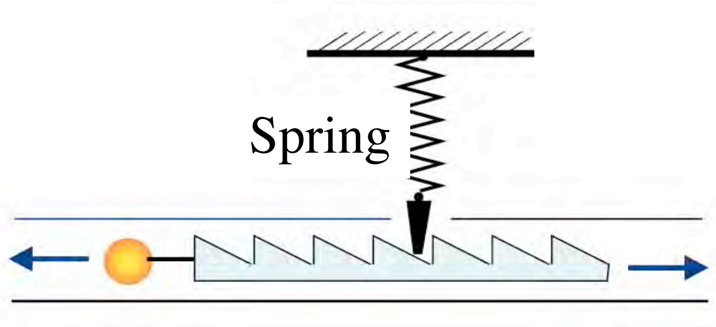
Hierarchy of Time Scales $\neq$ Hierarchy of Length Scales

- Introduction
- Chemomechanical Coupling
- Example: CM Coupling for kinesin
- Multiscale Motility:

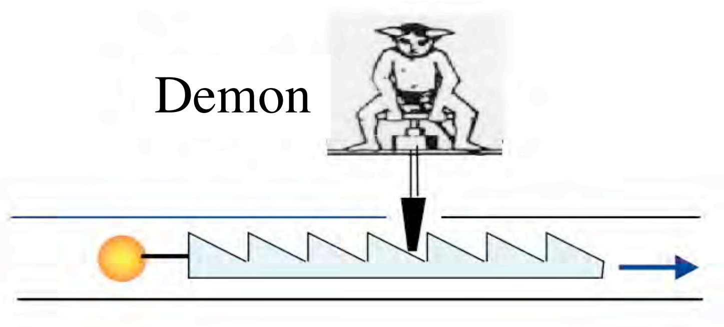
  Cargo transport by motor teams
- Outlook on related processes

CM Coupling: Different Views

- Directed motion in spite of thermal noise
- Rectification of thermal fluctuations?
- Smoluchowski Ratchet



- Maxwell Demon



- Bio-Systems: Demon = Molecular mechanics coupled to chemical nonequilibrium

- Ratchet view: Mechanics first

  Motor as Brownian particle with internal states

- Network view: Chemistry first

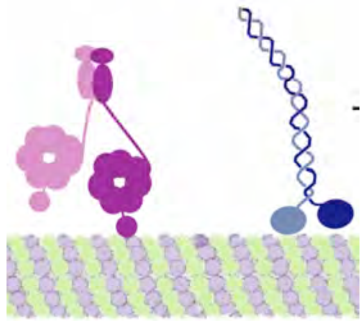
  Motor as enzyme with mechanical transitions

Motors as Enzymes

- ATPase = Enzyme that hydrolyzes ATP –> ADP + P
- Motor = ATPase with several catalytic domains

  M = # catalytic domains ≤ # ATP binding sites

- Examples:



Kinesin: $M = 2$

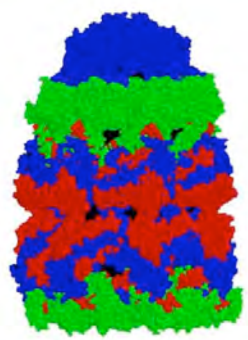
Myosin V: $M = 2$

Dynein: $M = 2 - 4 \leq 8$

....

F1 ATPase: $M = 3 < 6$

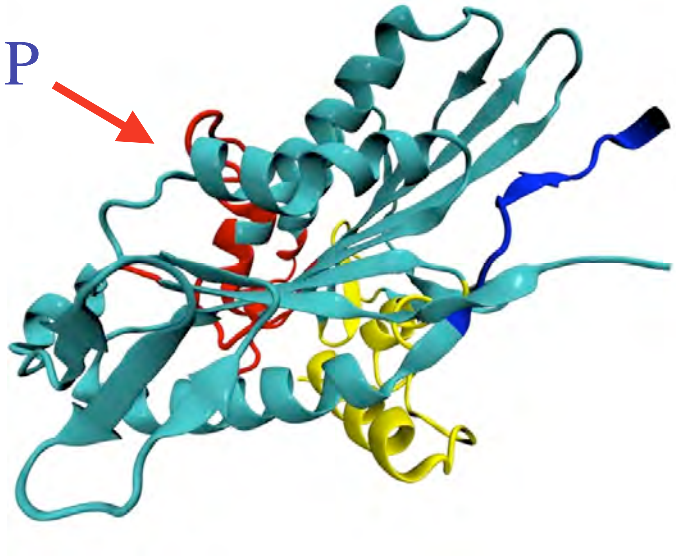
GroEl : $M = 7 < 14$



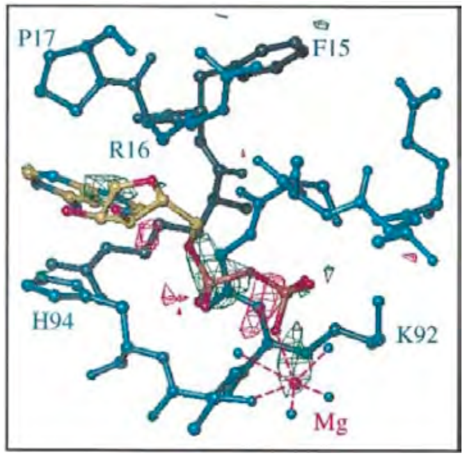
Single Motor Head or Single Enzymatic Domain

- Example: Single Head of Kinesin (M = 1)

NBP



- Nucleotide Binding Pocket (NBP)



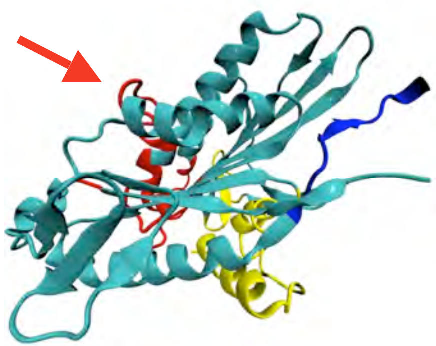
Müller ... Mandelkow, *Biol. Chem.* ***380*** *(1999)*

Different nucleotide states:

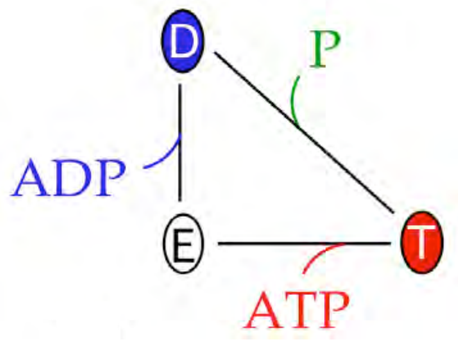
NBP can be occupied by ATP or ADP or may be empty

Chemical Network: Single Head

- Single head of kinesin:



empty (E)
bound ATP (T)
bound ADP (D)



- Each edge = 2 directed edges = forward + backward transition

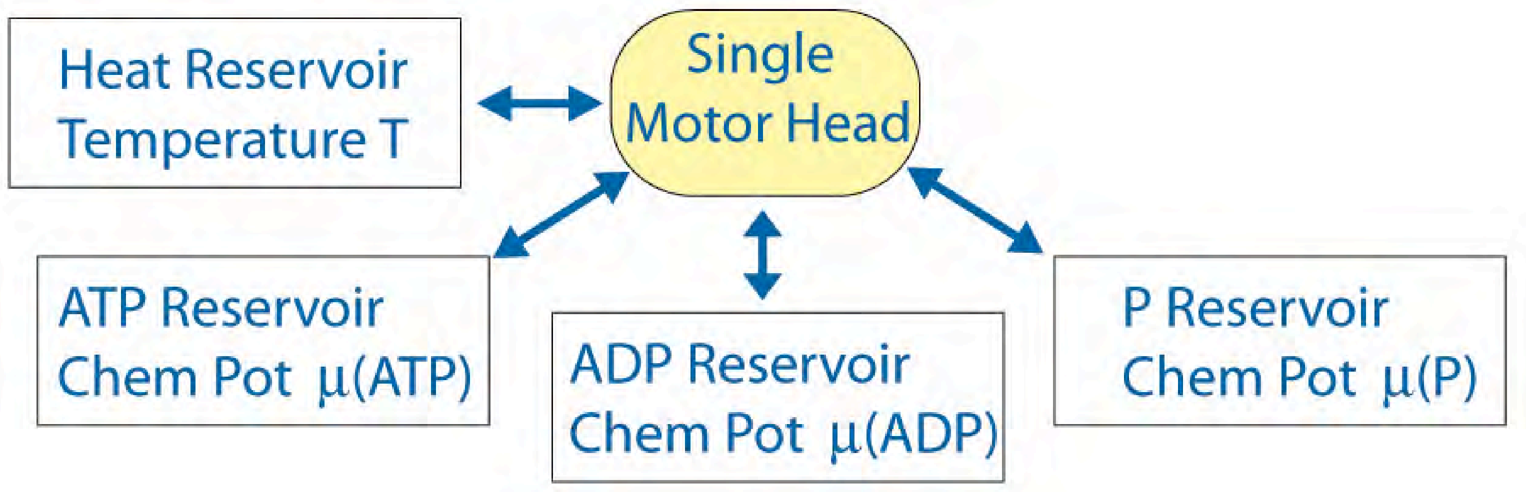
|DE> = ADP release — |TD> = ATP hydrolysis + P release
|ED> = ADP binding — |DT> = ATP synthesis + P binding

- Binding of X = ATP, ADP, P: Energy change by $+\mu(X)$
- Release of X = ATP, ADP, P: Energy change by $-\mu(X)$

$\mu(X)$ = Chemical potential

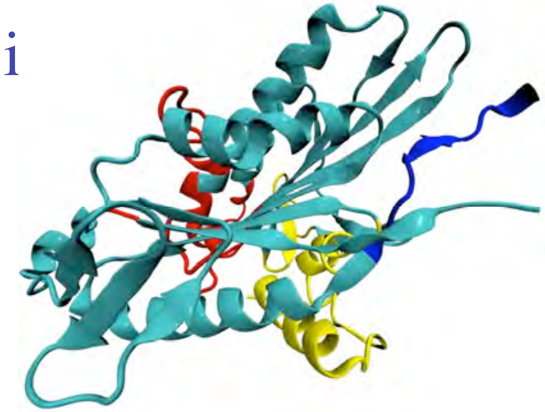
Thermodynamics of Single Head

- Single Motor Head plus Reservoirs:



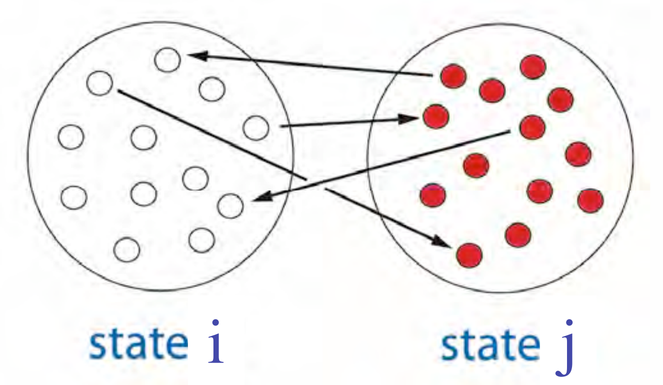
- Isothermal enzymatic process at fixed temperature T
- Binding and release of X = ATP, ADP, and P
- Chemical energy change $\Delta\mu = \mu(ATP) - \mu(ADP) - \mu(P)$
- Nonzero $\Delta\mu$ describes chemical nonequilibrium !

Ensemble of Substates



- Motor head at temperature T: Each state i contains many atomic configurations
- Each motor state i = ensemble of substates (i, k_i)
- State properties:

  Internal energy U_i
  Entropy S_i
  Free energy S_i



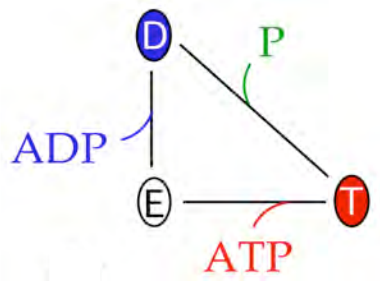
- Transition |ij> from i to j :
  Transition between substates
- Transition rates: Forward rate ω_{ij} from state i to state j
  Backward rate ω_{ji} from state j to state i

Cycles and Dicycles

- Cycle = cyclic sequence of states and edges

  Each cycle = two directed cycles = dicycles C_ν^d with $d = \pm$

Single head
= Single ATPaase:



1 cycle
= 2 dicycles

- Hydrolysis dicycle |ETDE> :

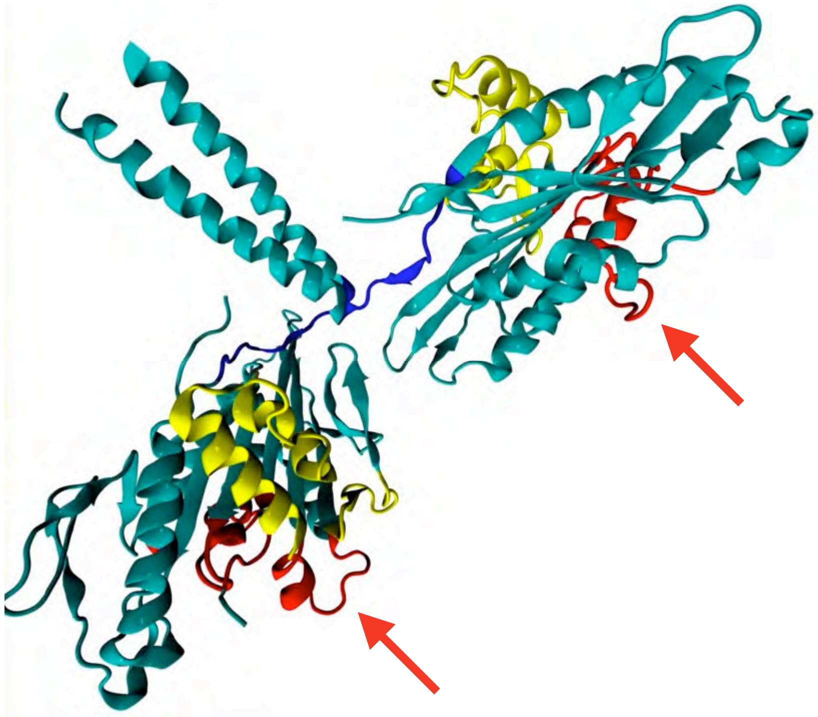
  Chemical energy change: $\mu(ATP) - \mu(P) - \mu(ADP) = + \Delta\mu$

- Synthesis dicycle |EDTE> :

  Chemical energy change: $\mu(ADP) + \mu(P) - \mu(ATP) = - \Delta\mu$

Two Motor Heads

- Stepping motors have two nucleotidebinding pockets (NBP) that act as two catalytic domains



Different nucleotide states:

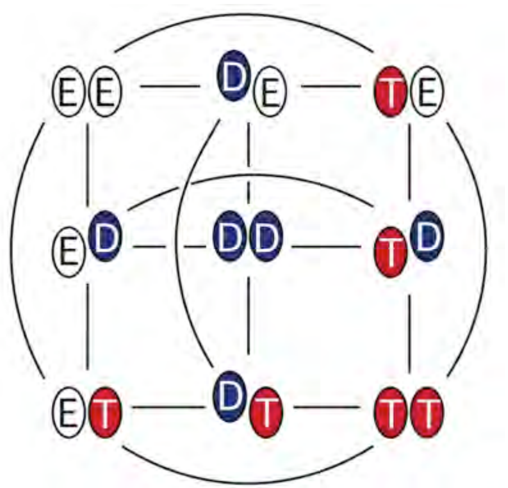
Each of the two NBPs may be occupied by ATP or ADP or may be empty

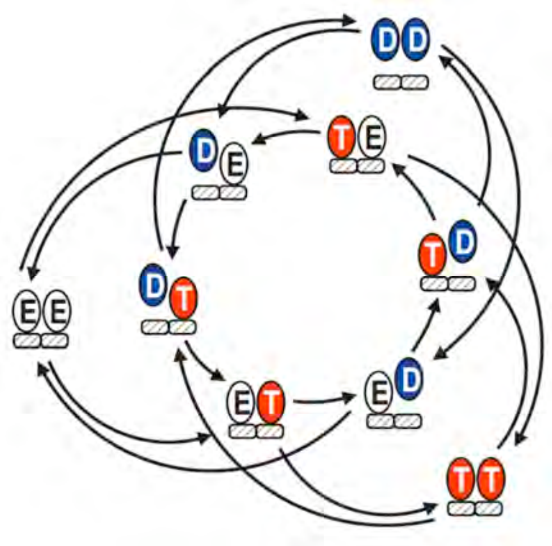
Chemical Network: Two Heads

Liepelt and RL, *EPL* **77** *(2007); J. Stat. Phys.* ***130*** *(2008)*

- Two heads = catalytic domains: $3^2 = 9$ states EE, DE, ...

  18 edges, 36 chemical transitions, 36 transition rates

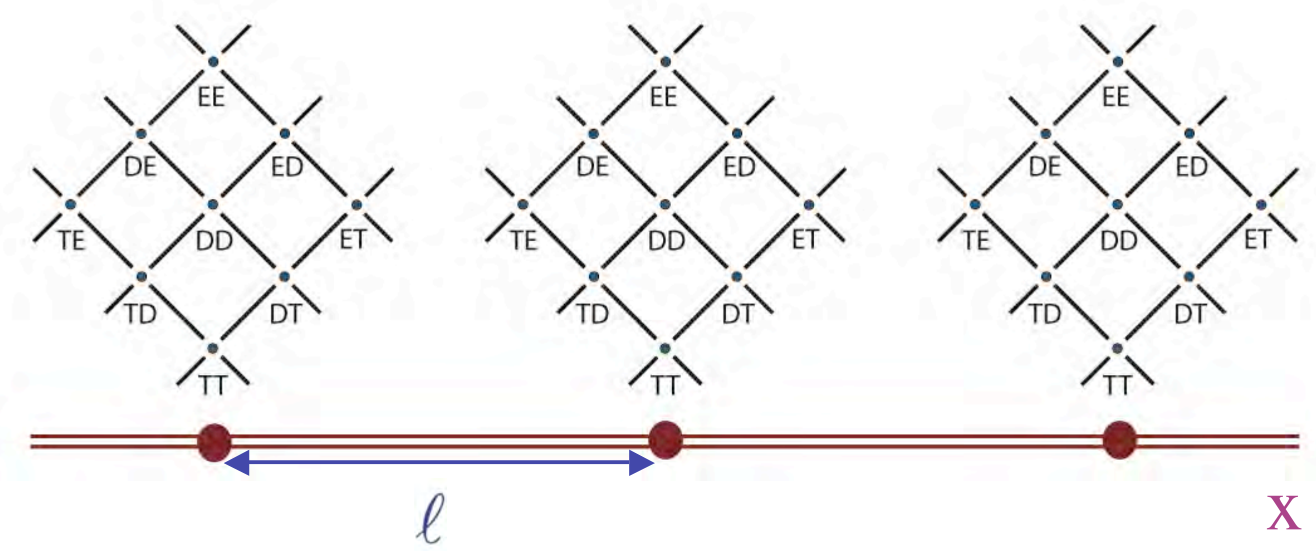




More than 200 cycles !

Chemomechanical Networks

- Mechanical transitions = Spatial displacement along filament
- Spatial coordinate x parallel to the filament
- Motor makes successive discrete steps of step size ℓ
- Periodically placed copies of chemical network:



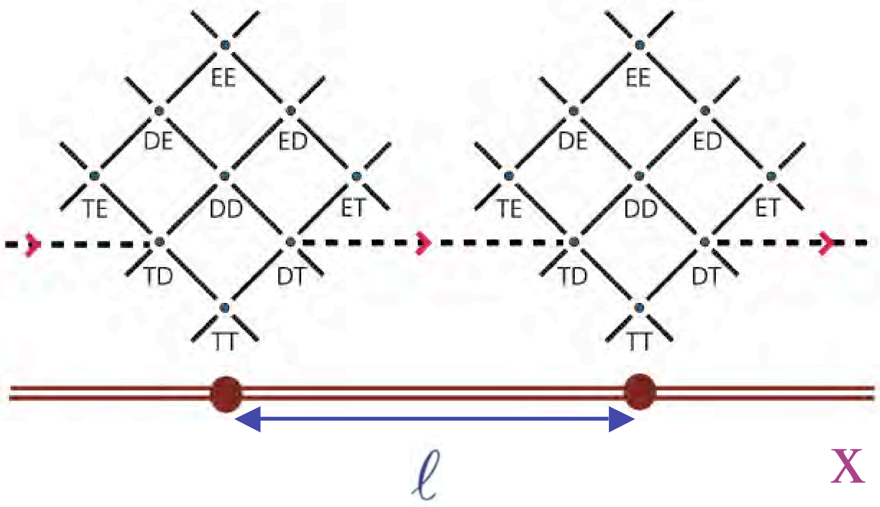
- In principle: Different x-coordinates for different chemical states

Simplifications for Stepping Motors

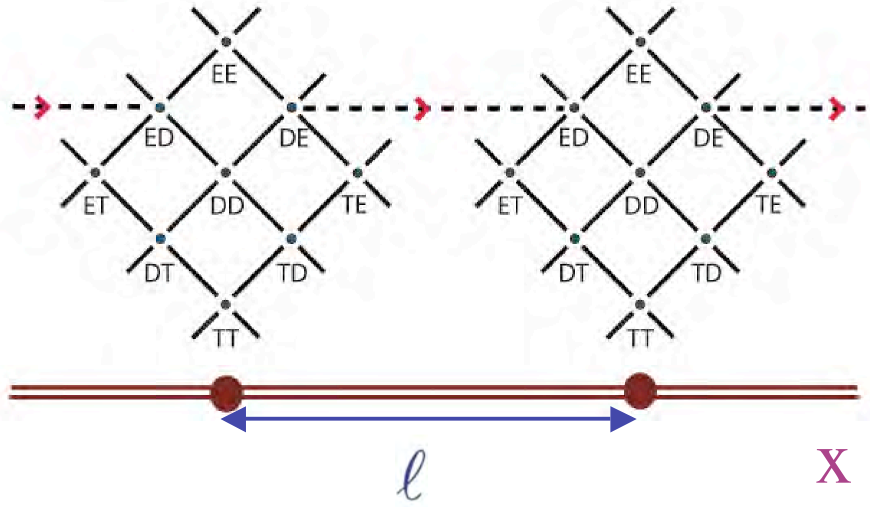
- Mechanical transitions fast compared to chemical transitions:
  Mechanical transitions without chemical transitions
- Different affinities of different nucleotide states to filament:
  Mechanical transitions emanate from a weakly bound state
- In general: One step or several substeps

- Kinesin: No substeps, D weakly bound, T and E strongly bound

DT -> TD



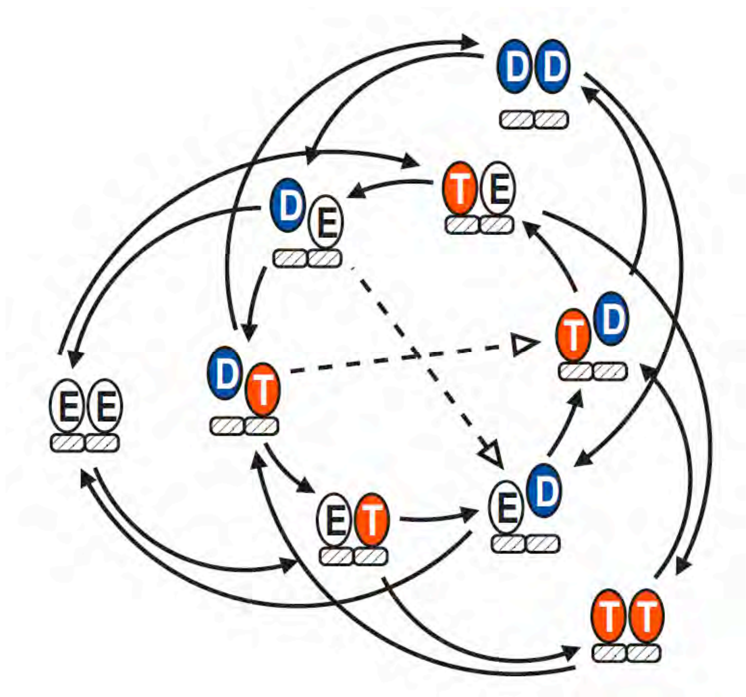
DE -> ED



Compact CM Networks

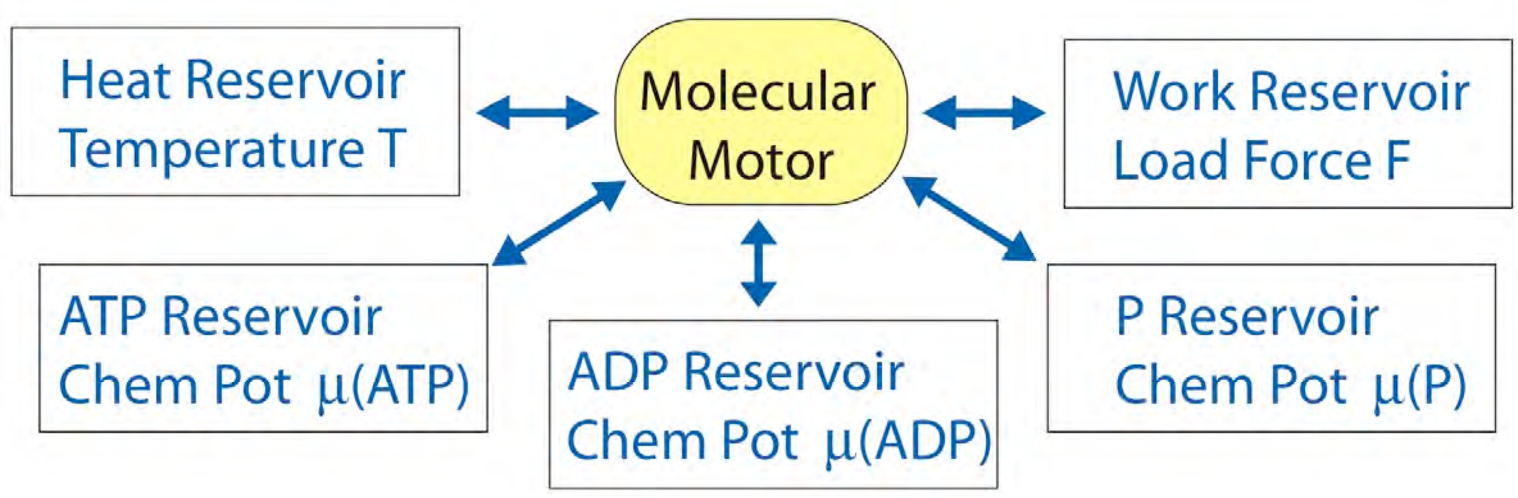
- More convenient representation:

  One copy of CM network plus periodic boundary conditions



Thermodynamics of Motor

- Motor molecule coupled to several reservoirs:



- Isothermal motor activity at fixed temperature T
- Chemical energy change $\Delta\mu = \mu(ATP) - \mu(ADP) - \mu(P)$
- Mechanical work $W_{me} = \ell F$ during spatial displacement ℓ

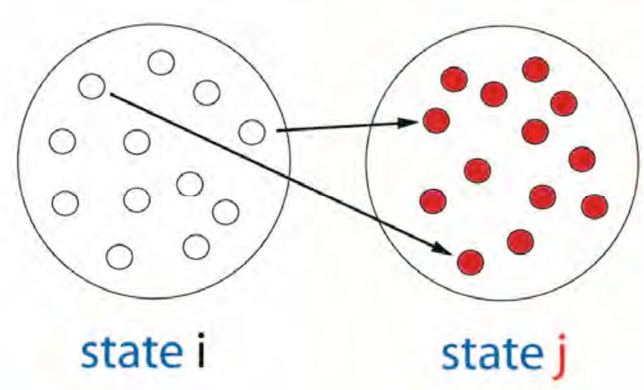
Energy and Entropy Changes

RL et al , *J. Stat. Phys.* ***135*** *(2009)*

- Motor can change its energy U_i by
  - chemical energy μ (nucleotide binding + release)
  - heat Q released by the motor
  - mechanical work W performed by the motor

- Energy change during transition |ij>

$$U_j - U_i = \mu_{ij} - Q_{ij} - W_{ij}$$



- Entropy change during |ij> :

$$\Delta S_{ij} = \underbrace{S_j - S_i}_{\text{System}} + \underbrace{Q_{ij} / T}_{\text{Reservoir}}$$

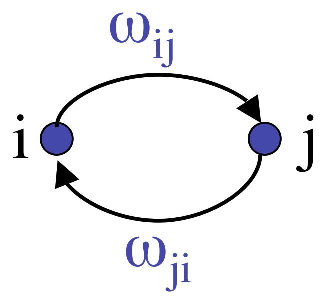
- Free energy change: $H_i = U_i - T\,S_i$

$$H_j - H_i = \mu_{ij} - W_{ij} - T\,\Delta S_{ij}$$

Constrained Equilibrium

- Free energy change: $H_j - H_i = \mu_{ij} - W_{ij} - T\,\Delta S_{ij}$

- Subsystem consisting of states i and j
  and associated transitions |ij> and |ji>



Transition rates ω_{ij} and ω_{ji}

- Constrained equilibrium and detailed balance:

$$H_j - H_i = \mu_{ij} - W_{ij} - k_B T \ln (\omega_{ij} / \omega_{ji})$$

- Entropy change during transition |ij>

$$\Delta S_{ij} = k_B \ln (\omega_{ij} / \omega_{ji})$$

- Alternative but more restricted derivation:
  Markov processes and entropy production

Cyclic Balance Conditions

- Summation of energy and entropy changes along completed dicycle C_ν^d

- Released heat: $Q(C_\nu^d) = \sum Q_{ij} = \mu(C_\nu^d) - W(C_\nu^d)$

- Produced entropy I: $T\,\Delta S(C_\nu^d) = \sum T\,\Delta S_{ij} = Q(C_\nu^d)$

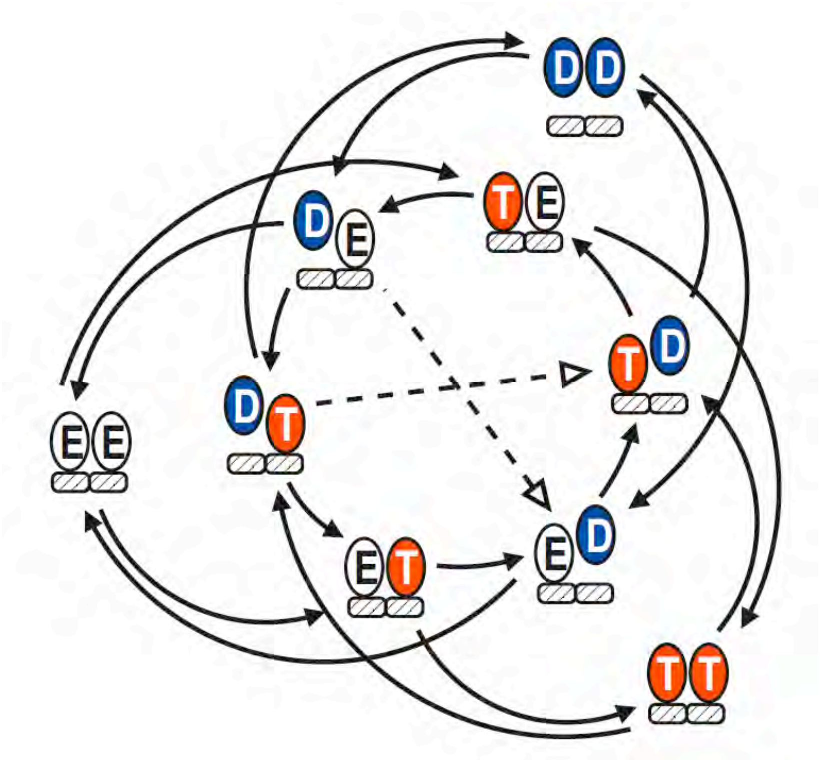
- Produced entropy II: $T\,\Delta S(C_\nu^d) = k_B T \ln(\Xi_\nu^d)$

  with $\Xi_\nu^d = \prod_{|ij\rangle}^{\nu,d} (\omega_{ij}/\omega_{ji})$

$$k_B T \ln(\Xi_\nu^d) = \mu(C_\nu^d) - W(C_\nu^d) = Q(C_\nu^d)$$

Relation between kinetics and thermodynamics
Thermodynamics imposes constraints on kinetics

Example: Stepping Motors



More than 200 cycles !

Classification of Cycles

- Balance condition for each directed cycle C_ν^d :

$$k_B T \ \ln(\Xi_\nu^d) \ = \ \mu(C_\nu^d) - W(C_\nu^d)$$

Classification of cycles:

- Detailed balance: $\mu(C_\nu^d) = 0$ and $W(C_\nu^d) = 0$
- Mech nonequilibrium: $\mu(C_\nu^d) = 0$ and $W(C_\nu^d) \neq 0$
- Chem nonequilibium: $\mu(C_\nu^d) \neq 0$ and $W(C_\nu^d) = 0$
- Chemomech coupling: $\mu(C_\nu^d) \neq 0$ and $W(C_\nu^d) \neq 0$

Force Dependence

- Force (F) dependence of transition rates ω_{ij} :

$$\omega_{ij} = \omega_{ij,0} \; \Phi_{ij}(F) \qquad \text{with} \quad \Phi_{ij}(0) = 1$$

- Factorization of Ξ factors:

$$\Xi = \prod_{|ij>}^{\nu,d} (\omega_{ij} / \omega_{ji}) = \Xi_0 \; \Xi_F$$

$$\Xi_F = \prod_{|ij>}^{\nu,d} (\Phi_{ij} / \Phi_{ji}) = \exp(- W_{me} / k_B T)$$

- Cycle contains a single mechanical transition |ab> :

$$\Phi_{ab}(F) / \Phi_{ba}(F) = \exp(- W_{me} / k_B T) = \exp(- \ell F / k_B T)$$

$$\Phi_{ij}(F) / \Phi_{ji}(F) = 1 \qquad \text{for } |ij> \neq |ab>$$

Summary: CM Coupling

- Different views: Ratchets versus networks
- Chemical networks of nucleotide states
- Examples:

  1 and 2 motor heads = 1 and 2 enzymatic domains
- Chemomechanical networks:

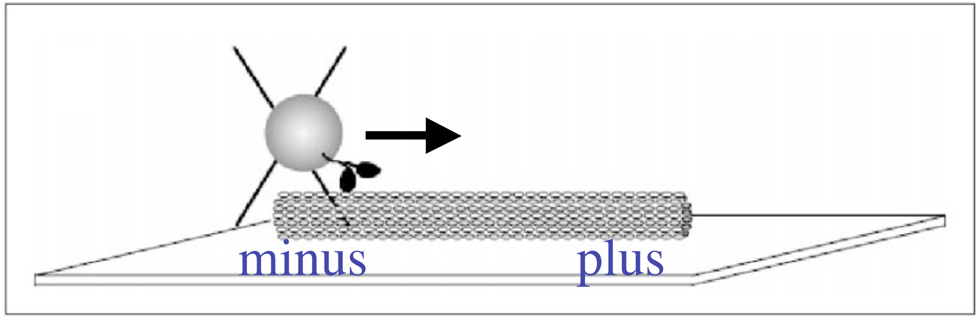
  Spatially periodic copies of chemical networks
  connected by mechanical transitions
- Free energy landscape of motor states
- Balance conditions and classification of motor cycles

- Introduction
- Chemomechanical Coupling
- ☞ Example: CM Coupling for kinesin
- Multiscale Motility:

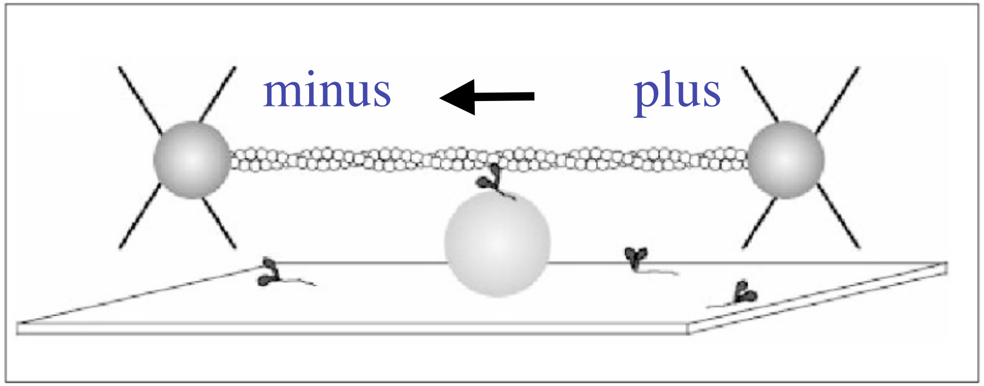
  Cargo transport by motor teams
- Outlook on related processes

Single Motor Experiments

- Bead assay: Mobile motor



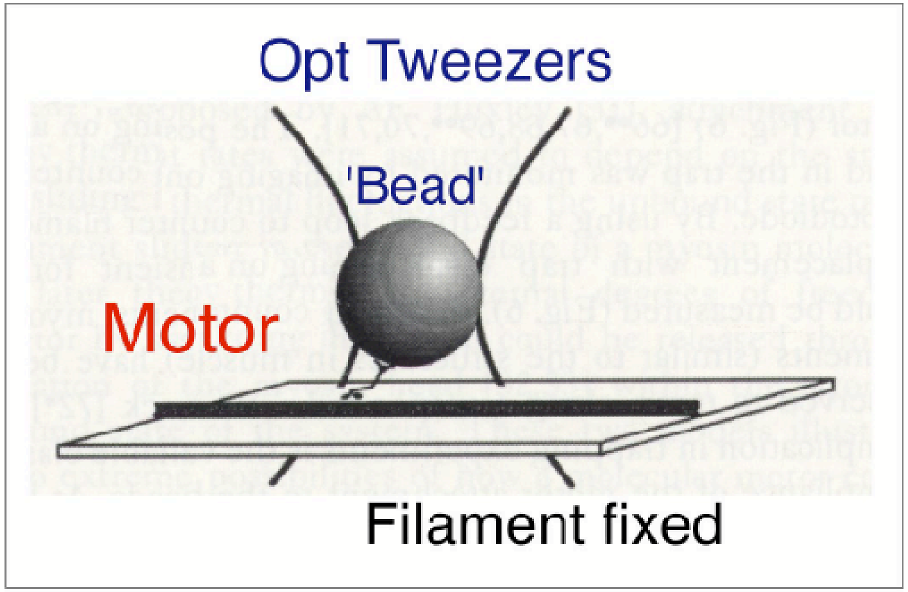
- Gliding assay: Mobile filament



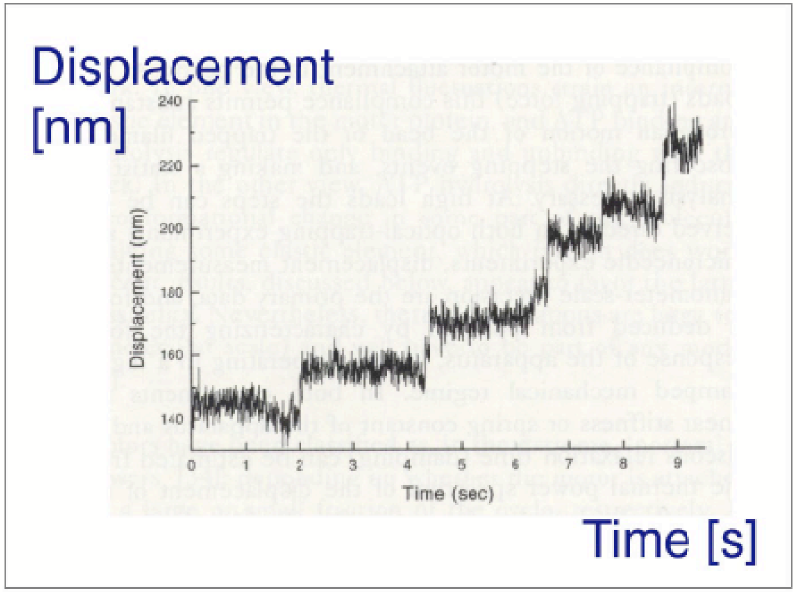
- Polar filament
- Plus and minus end
- Force generation of motor
  => relative displacement
  (actio = reactio)
- Bead assay:
  Motor moves to plus
- Gliding assay:
  Filament shifted to minus

Kinesin: Mechanical Stepping

- Bead Assay:



- Discrete Steps:



*Svoboda et al, Nature **365** (1993)*

- Kinesin's center-of-mass moves by 8 nm
- Each head moves by 16 nm (hand-over-hand motion)
- Hydrolysis of one ATP per step (tight coupling)

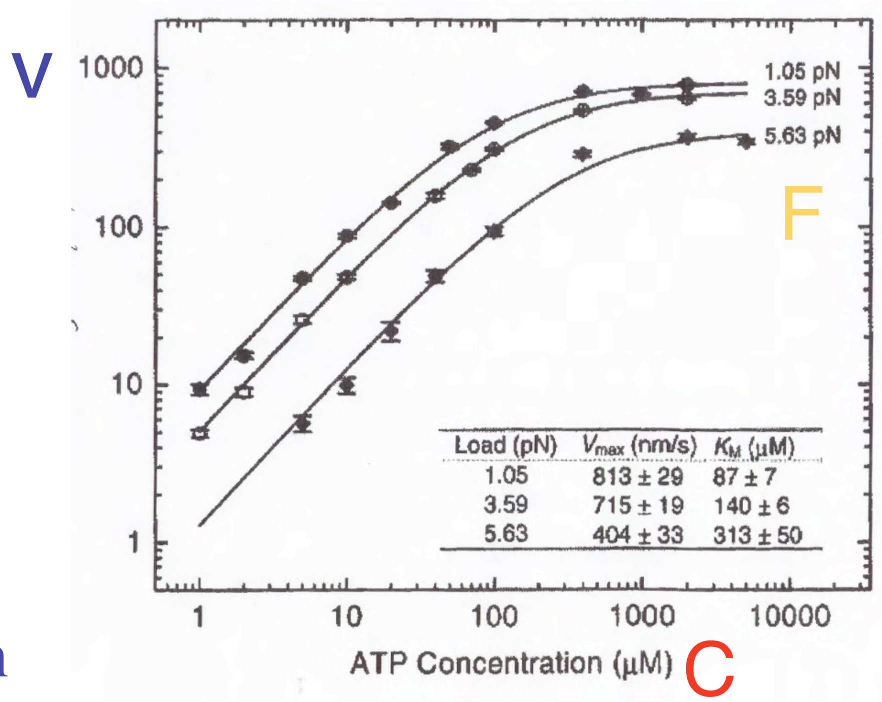
[ATP] Dependence of Velocity

- Velocity v as a function of concentration [ATP] = C and external force F

$$v(C,F) \simeq v_{sat}(F)\,\frac{C}{C_*(F)+C}$$

'Michaelis-Menten Relation'

Visscher et al, Nature ***400*** *(1999)*



- Simple functional dependence on two variables C and F

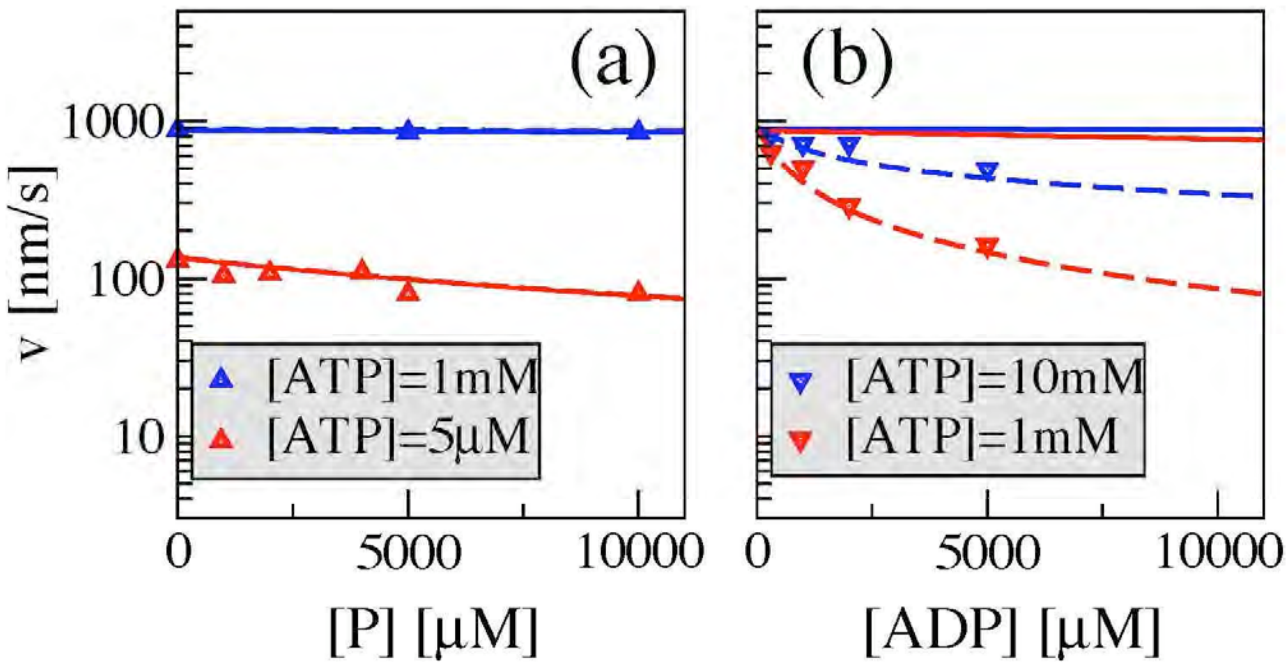
- Predicted by a large class of motor models

RL, Phys. Rev. Lett. 85 (2000)

[ADP] and [P] Dependence

*Schief et al, PNAS **101** (2004)*

- Motor velocity decreases slowly with increasing [P]
- Motor velocity decreases strongly with increasing [ADP]

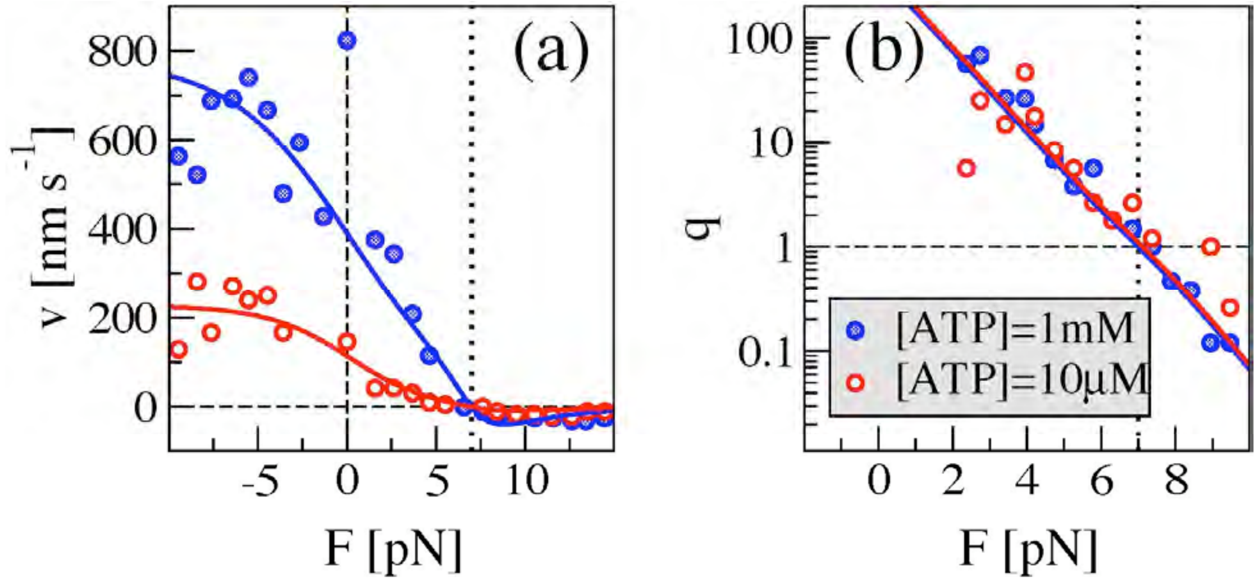


Load Force Dependence

Nishiyama ... Yanagida, *Nat. Cell Biol.* ***4*** *(2002)*

Carter and Cross, *Nature* ***435*** *(2005)*

Resisting Load Force F > 0

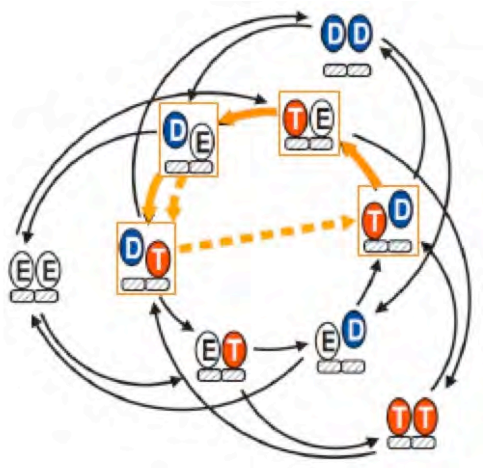


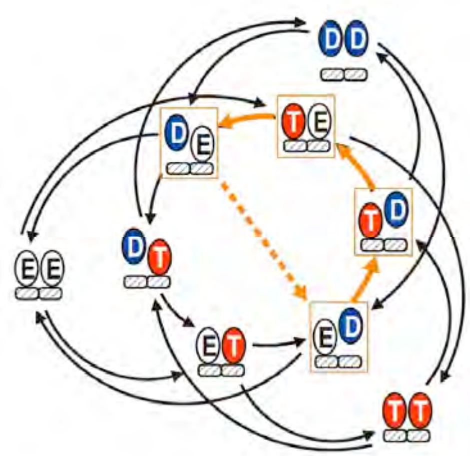
- Kinesin generates about 7 pN = stall force F_s
- Kinesin makes processive backwards steps
- Mechanical steps are very fast (faster than 15 μs)

Kinesin: Proposed Motor Cycles

- Many different unicycle models
- Two examples from experimental groups:

Mori et al,
Nature (2007)



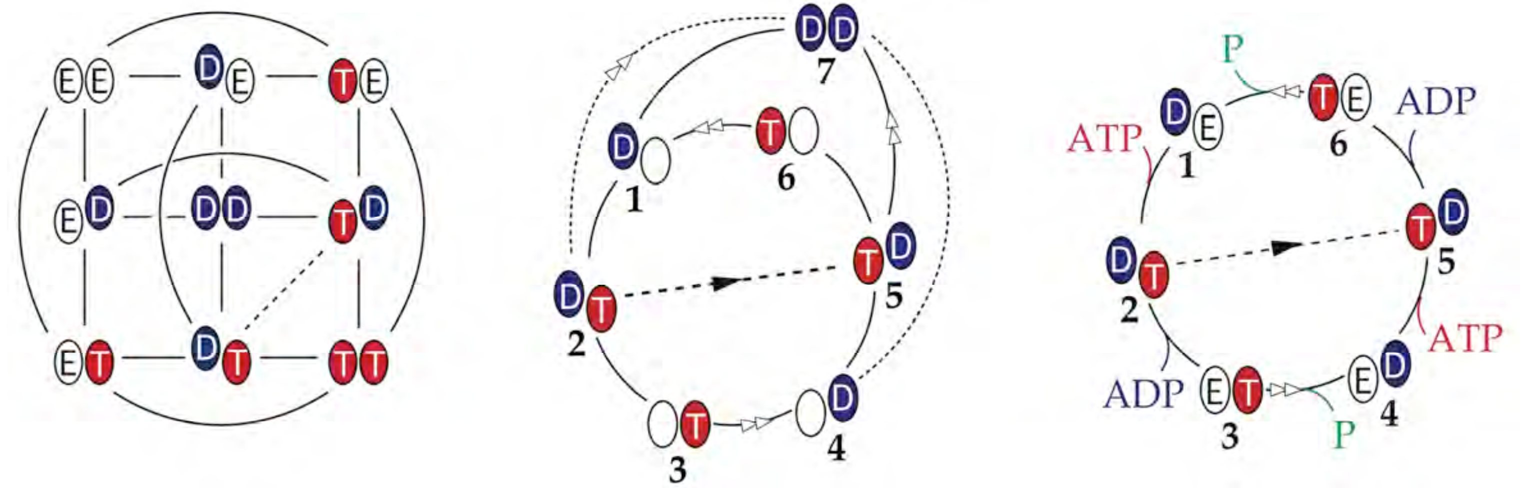


Alonso et al,
Science (2007)

- Theory: Unicycle models by Fisher and Kolomeisky
- Basic Problem: Backstepping coupled to ATP synthesis
  but no synthesis for small ADP concentrations!

Network of CM Motor Cycles

Liepelt and RL , *Phys. Rev. Lett.* ***98*** *(2007)*

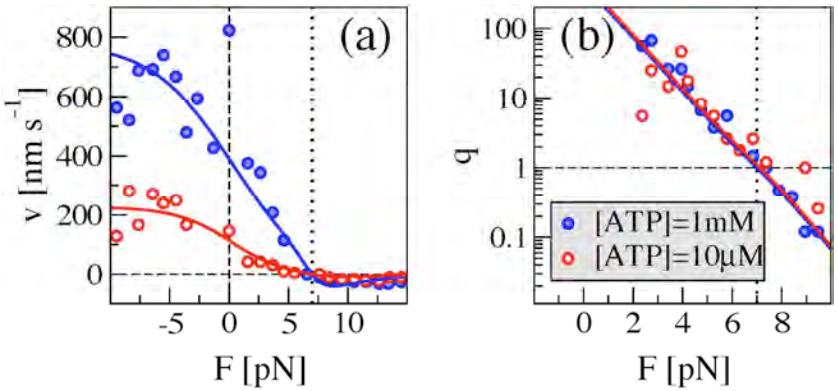


Three chemomechanical motor cycles:

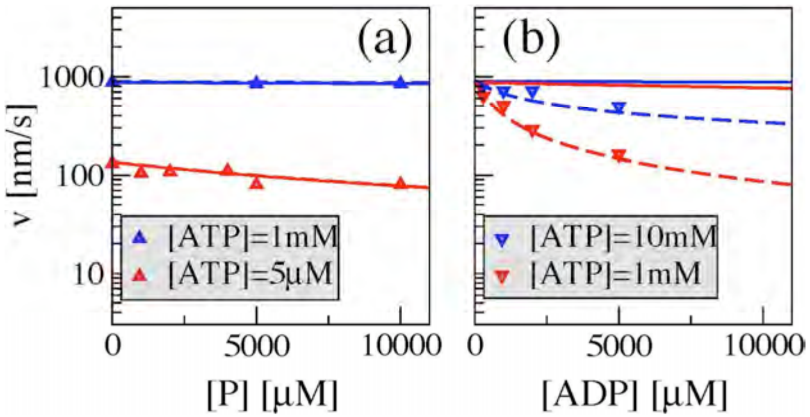
- Small ADP and P, small load force F: dicycle |25612>
- Small ADP and P, large load force F: dicycle |52345>
- Large ADP, small load force F: dicycle |25712>

Kinesin: Theory + Experiment

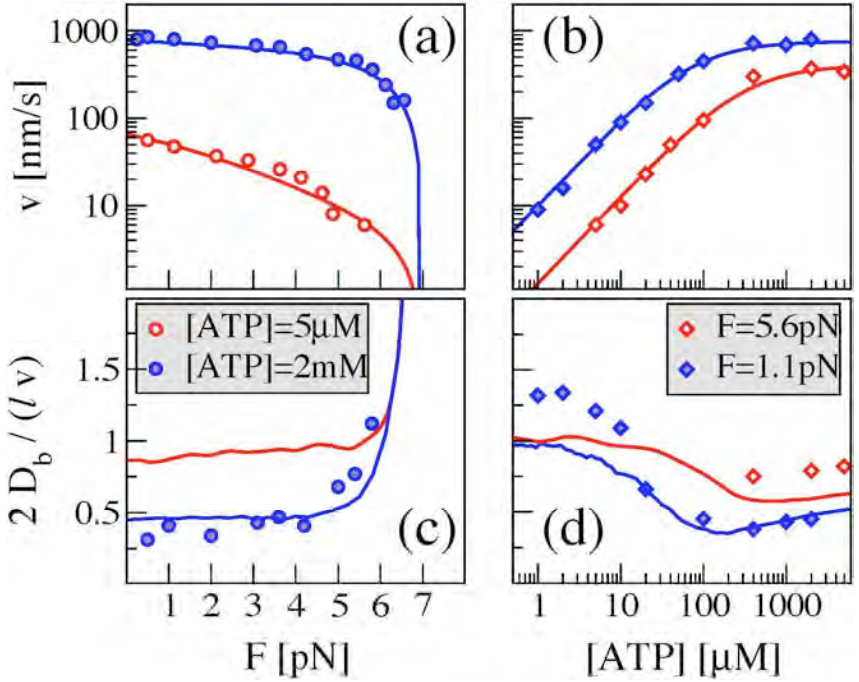
- Data of Carter, Cross (2005)



- Data of Schief et al (2004)



- Data of Visscher et al (1999)



- Data of Schnitzer et al (2000) on run length as a function of force and [ATP]

Summary: Processive Motion of Single Motors

- Network representation provides
  unification of experimental results

- Kinesin characterized by
  network of chemomechanical cycles

- Same representation applicable to any motor
  with chemomechanical coupling!

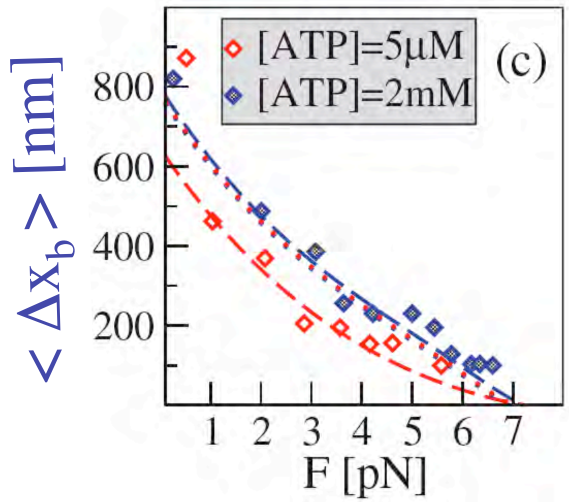
- Introduction
- Chemomechanical Coupling
- Example: CM Coupling for kinesin
- Multiscale Motility:
  Cargo transport by motor teams
- Outlook on related processes

Run Length and Unbinding Rate

RL, Klumpp, and Niewenhuizen, *Phys. Rev. Lett.* ***87*** *(2001)*

- Thermal noise => motor eventually kicked off from filament
- Average run length $< \Delta x_b >$

  Unbinding rate $\varepsilon \sim 1/ < \Delta x_b >$



- External load force F

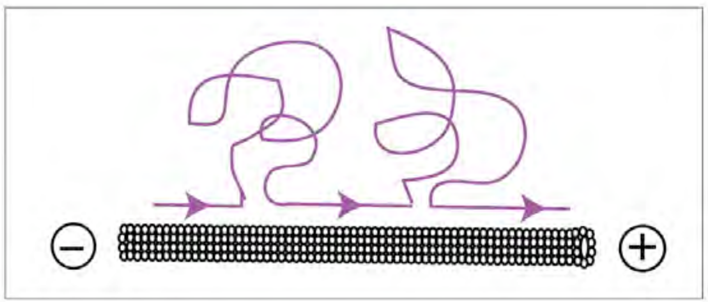
  Unbinding rate $\varepsilon(F) \sim \exp(F/F_d)$

  Detachment force F_d

- Kinesin: $< \Delta x_b > \approx 1$ µm, $F_d \approx 3$ pN

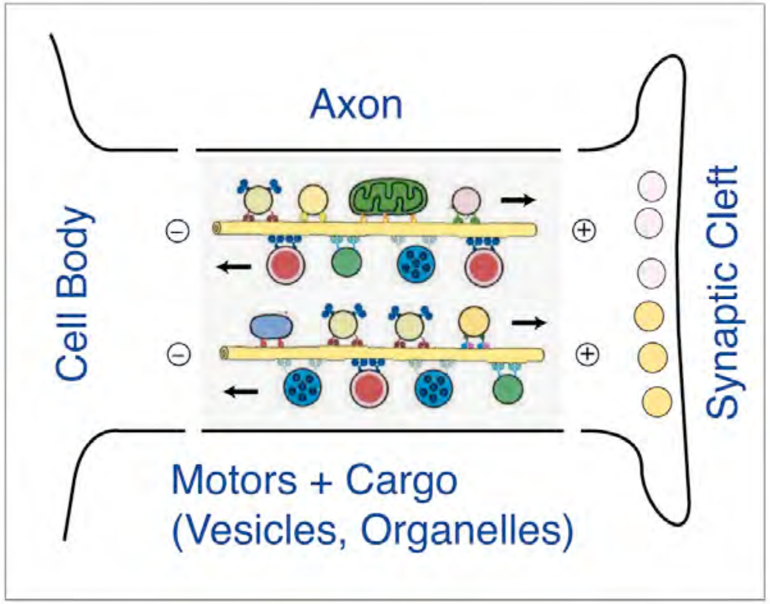
- Length scales >> run length :

  Alternating sequence of directed stepping and unbound diffusion



Intracellular Cargo Transport

- Example: Neuron, Axon, and Synapse



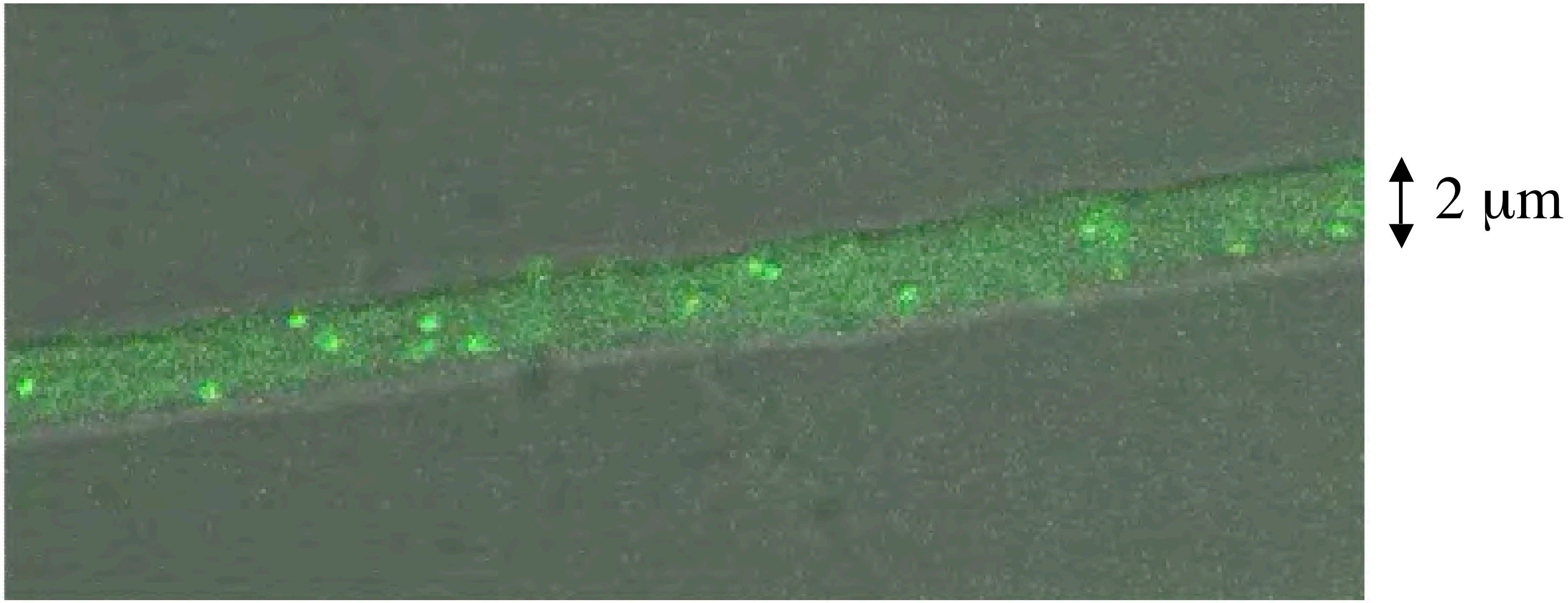
Cargo transport by several motors:

- Uni-directional transport by single motor species
- Bi-directional transport by two motor species

Axonal Cargo Transport

- Example: Transport of viruses in chick neurons

  Virus capsid labeled by GFP



Smith et al, *PNAS*. ***98*** *(2001)*

Single Motor Transport

- Unbinding rate ε

$$\varepsilon(F) = \varepsilon_0 \ \exp(F / F_d)$$

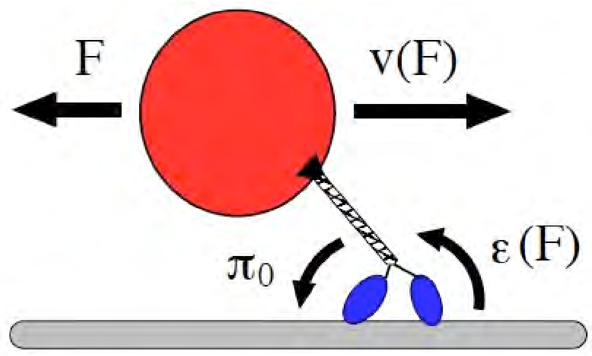
Detachment force F_d

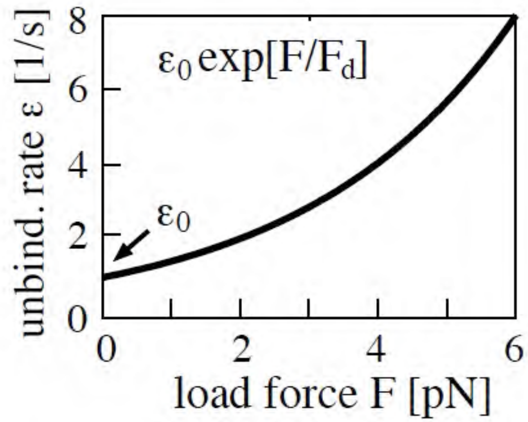
- Binding rate π_0
- Parametrization of velocity v:

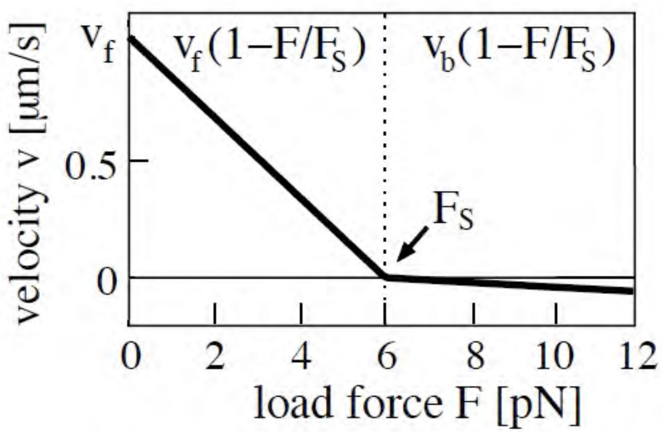
forward velocity v_f at zero load

stall force F_s at which v vanishes

backward velocity scale v_b







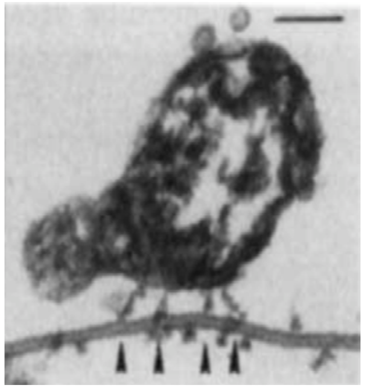
Uni-directional Transport

*S. Klumpp, RL: PNAS **102** (2005)*

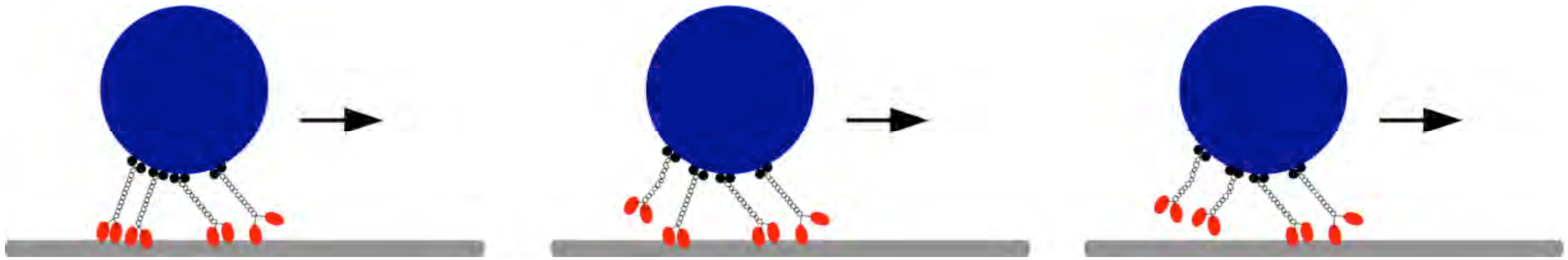
- N identical motors firmly attached to cargo particle (vesicle, organelle)
- Thermal noise:

  Each motor unbinds and rebinds from filament

  => Number k≤ N of active motors is not fixed but fluctuates



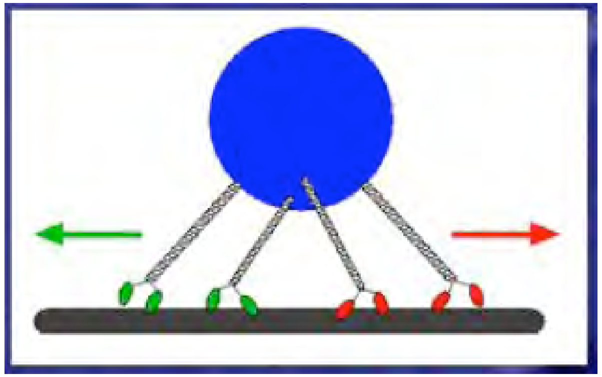
Ashkin et al. Nature 348 (1990)



Bi-Directional Transport

*M. Müller, S. Klumpp, RL, PNAS **105** (March 2008)*

• Cargo with two antagonistic types of motors:



Green minus motors pull to the left
Red plus motors pull to the right

• Experimental observations reveal complex behavior:

Different types of trajectories with and without pauses

Mutations of one motor type affect both directions!

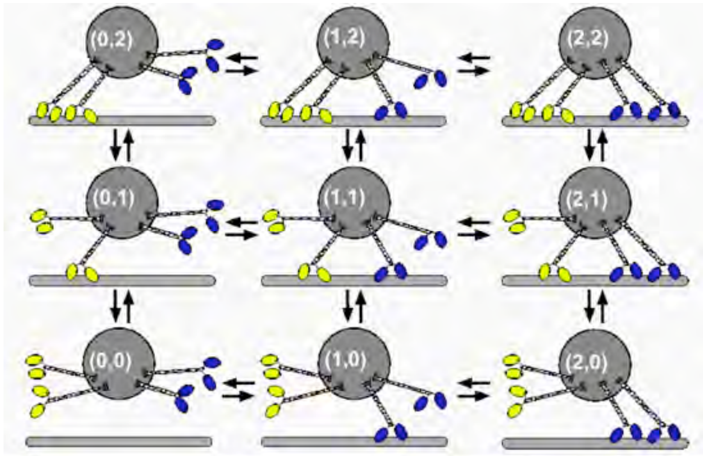
=> Speculations about coordination complex ?

Stochastic Tug-of-War

- Thermal noise: # of minus and plus motors fluctuates in time
- Cargo states with (n_-, n_+) active motors, $n_- \leq N_-$ and $n_+ \leq N_+$

Example: $(N_-, N_+) = (2,2)$

2-dimensional lattice of cargo states



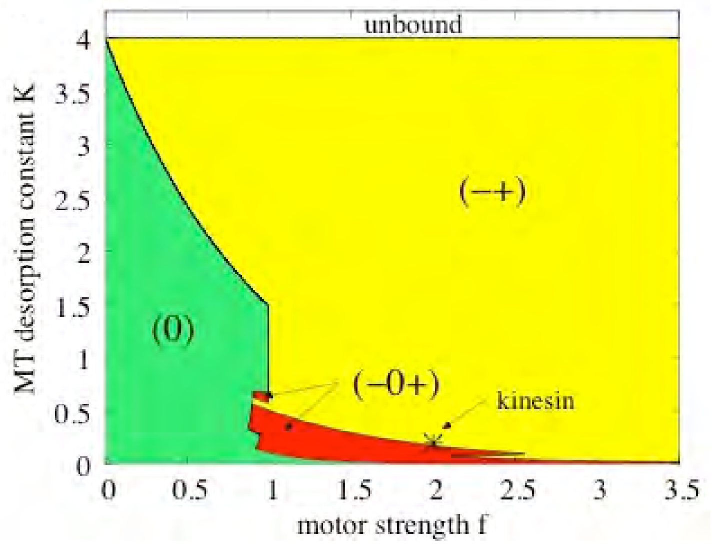
- Uni-directional transport for $N_- = 0$ or $N_+ = 0$
- All cargo states with $n_- > 0$ and $n_+ > 0$:

  Plus motors pull on minus motors and vice versa

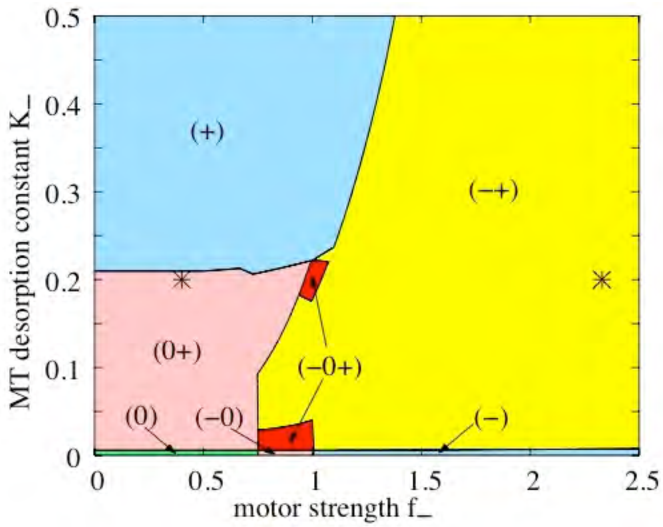
  => nontrivial force balance

Tug-of-War: Steady States

• Symmetric case:



• Asymmetric case:



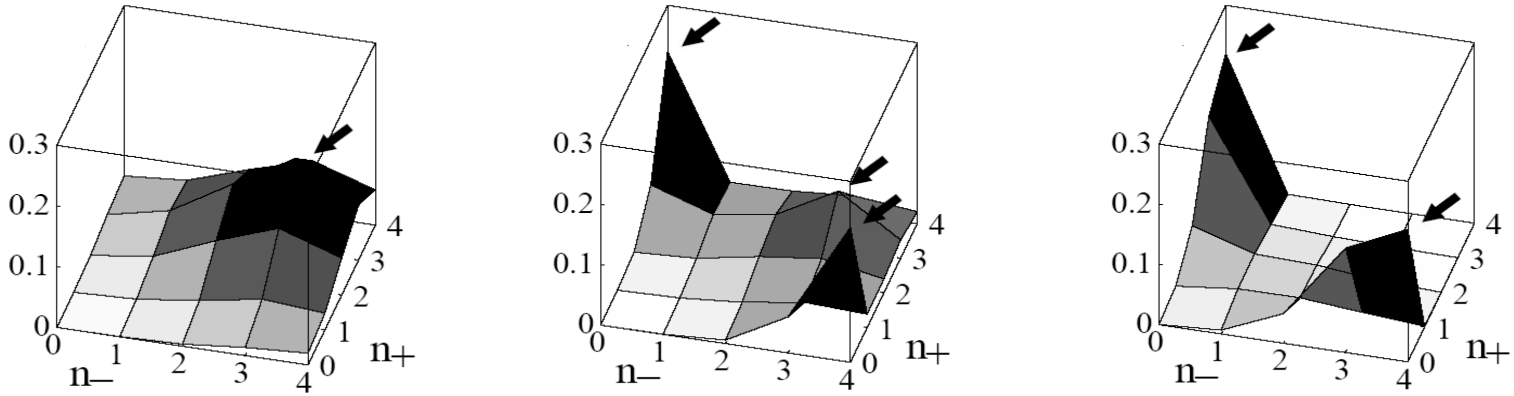
• Steady state distributions with 1, 2, or 3 maxima

• Asymmetric case: 7 different steady states

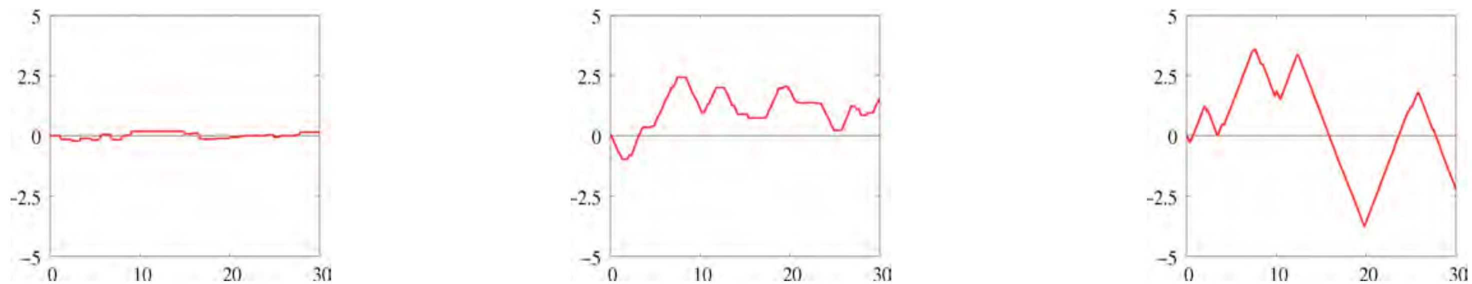
All experimental observations can be explained by small changes in single motor parameters !

Example: 4 against 4 Motors

- Steady state distributions:



- Typical trajectories of cargo:



No motion

Bi-directional with pauses

Bi-directional without pauses

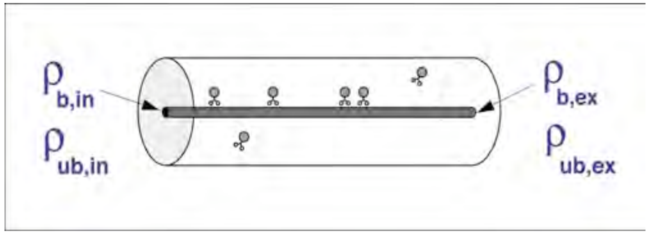
Related Topics I

Motor Traffic and Phase Transitions:

J. Stat. Phys. ***113*** *(2003)*

- Tube with two open boundaries:

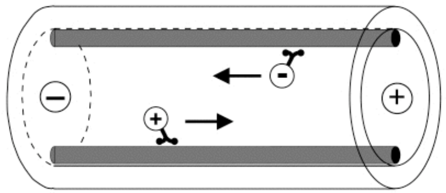
  TP transitions related to ASEP phases



Europhys. Lett. ***66*** *(2004)*

- Traffic of two motor species in tubes:

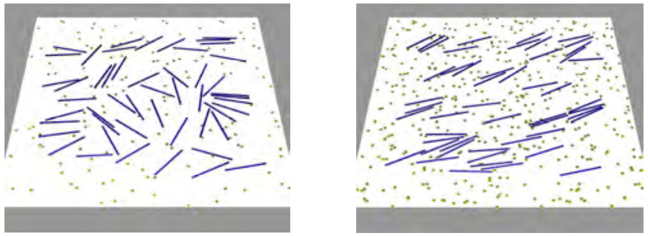
  Symmetry breaking TP transition



Phys. Rev. Lett. ***96*** *(2006)*

- Traffic of filaments along substrates:

  Isotopic nematic TP transition



Traffic in a half open tube

M. Müller, J. Phys. CM ***17*** *(2005)*

- Axon-like boundary condition = half open tube

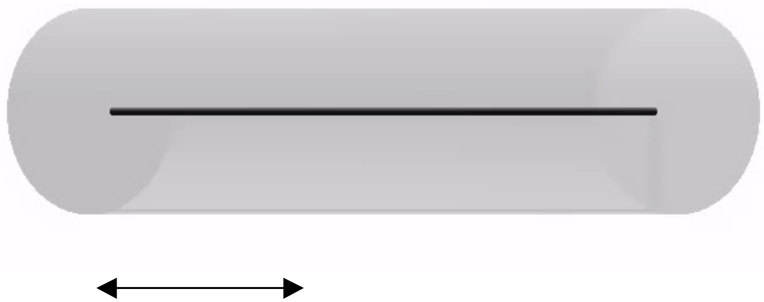
  left boundary open, reservoir of motors = 'cell body'

  right boundary closed = 'Synapse'

- (+) Motors (kinesins) moving to the right:

traffic jams

- (–) Motors (dyneins) moving to the left



limited entry

Jam length L_*

Related Topics II

- Molecular dynamics of Kinesin

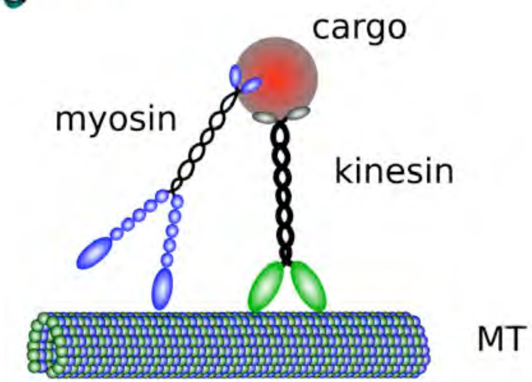
- Chemomech coupling for Myosin V

  Connection to ratchet regime

- Transport by Kinesin plus Myosin V

  One motor acts as diffusing anchor

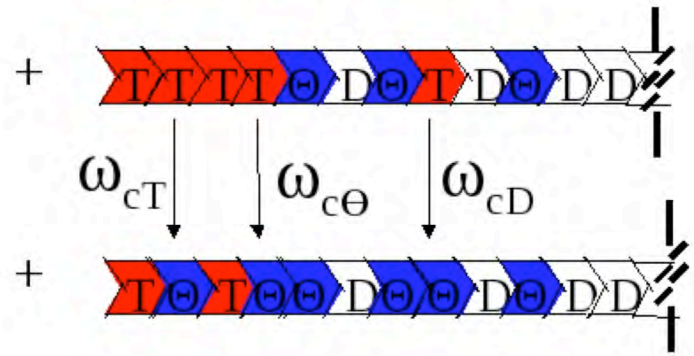
  Berger et al, *EPL* ***87*** *(2009)*



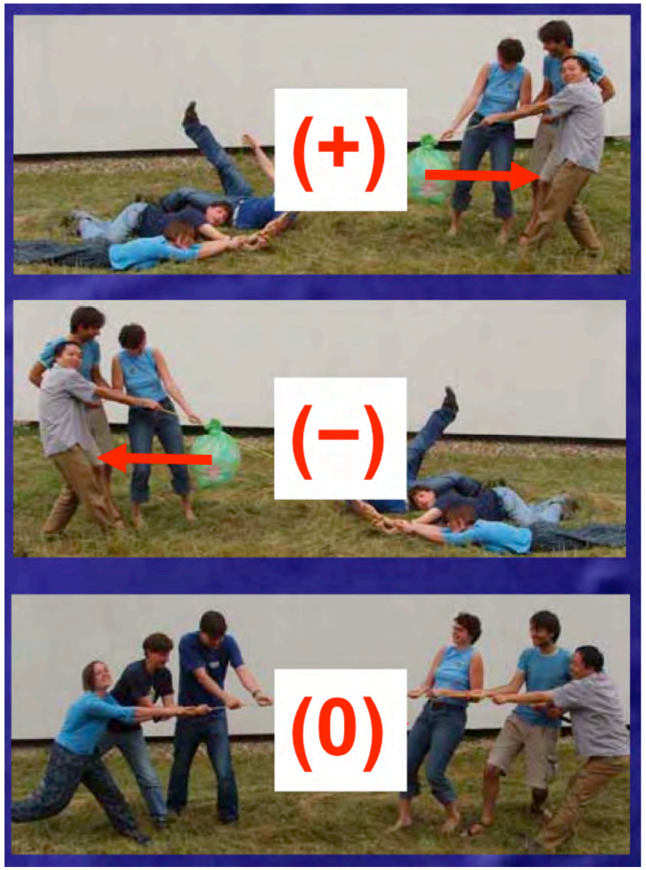
- Chemomech coupling for

  actin (de)polymerization

Xin Li et al, *Phys. Rev. Lett.* ***103*** *(2009)*



Coworkers



Stepping Motors, Theory:

Neha Awasthi
Florian Berger
Veronika Bierbaum
Yan Chai
Corina Keller
Stefan Klumpp
Aliaksei Krukau
Steffen Liepelt
Melanie Müller
Angelo Valleriani

Stepping Motors, Experiment:

Janina Beeg
Rumiana Dimova
Karim Hamdi

Actin Filaments:

Jan Kierfeld
Pavel Kraikivski
Xin Li
Thomas Niedermayer