Chemomechanical Coupling of Molecular Motors

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MPI of Colloids and Interfaces, Potsdam

- Introduction: Motility on many scales
- Chemomechanical (CM) Coupling
  - Network representations of enzymes and motors
  - Thermodynamics of motors
  - Energy and entropy changes
- Balance Conditions for Motor Cycles
- Example: CM Coupling for Kinesin
- Outlook on Multiscale Motility

Related Talks and Posters

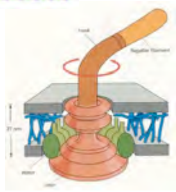
- Steffen Liepelt:
  "Chemomechanical networks of molecular motors"
- Veronika Bierbaum:
  "Energy transduction by myosin V"
- Florian Berger:
  "Cooperative transport by active and passive motors"
- Stefan Klumpp:
  "Transcription of ribosomal RNA"

Molecular Machines

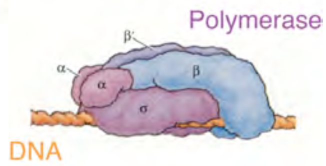
"Every Motion has its Motor"

Protein Oligomers + Substrate:

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

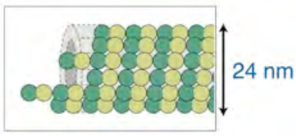


- Nano-Pumps: Na-K-Pump, ...
- Nano-Assemblers: Polymerases, ...



Assembly of Many Proteins:

- Growing filaments

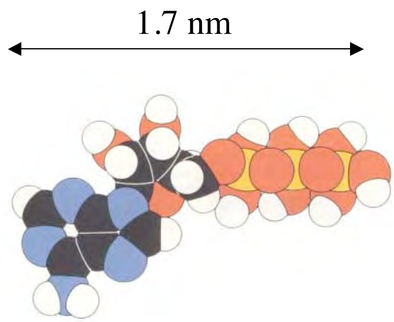


Chemomechanical Coupling

- Molecular machines:

  Conversion of chemical energy into mechanical work

- Universal chemical energy source provided by ATP:



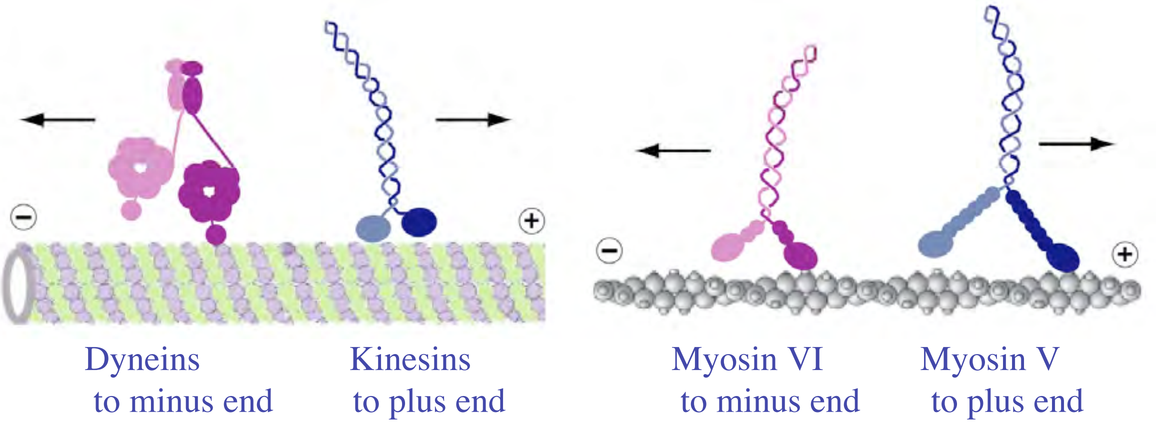
- Hydrolysis of ATP: ATP -> ADP + P
- Synthesis of ATP: ADP + P -> ATP

Nucleotides
ATP, ADP, P

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

Stepping Motors

- Filament = Microtubule
- Filament = F-Actin



- 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

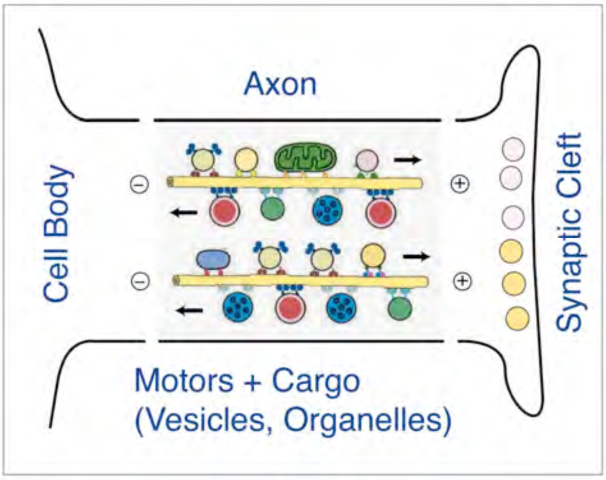
- Two Heads:

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

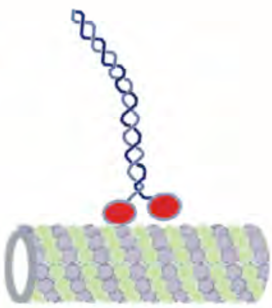


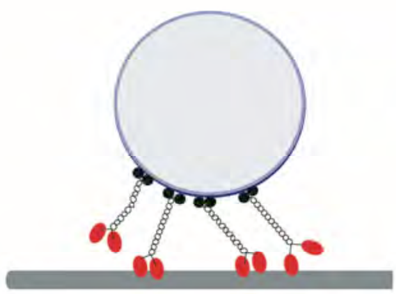
- Axon between spine and finger tip is ~ half a meter !
- Cooperative cargo transport by several motors

Multiscale Motility

- Example: Kinesin Dimers at Microtubules

ATP Binding | Mechanical Steps | Transport





Nucleotide Binding Pocket ~ 1 nm

10^{-3} s

Single head moves by 16 nm

10^{-6} s

Cargo transport over cm or m !

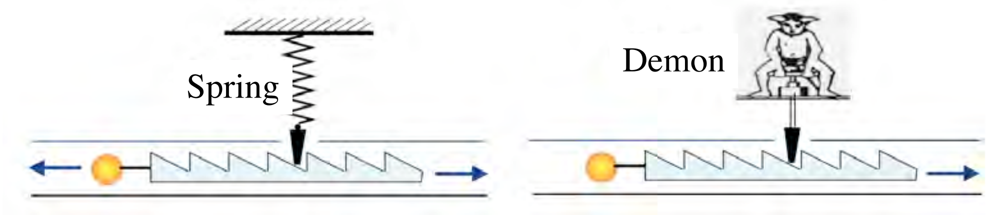
10^{4} - 10^{6} s

Hierarchy of Time Scales ≠ Hierarchy of Length Scales

- Introduction
- Chemomechanical Coupling
- Example: CM Coupling for Kinesin
- Outlook on Multiscale Motility

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 — Jülicher et al, *RMP* ***69*** (1997)

  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

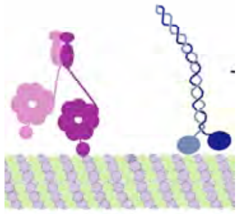
  Myosin V: M = 2

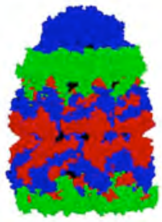
  Dynein: M = 2 - 4 ≤ 8

  ....

  F1 ATPase: M = 3 < 6

  GroEl : M = 7 < 14

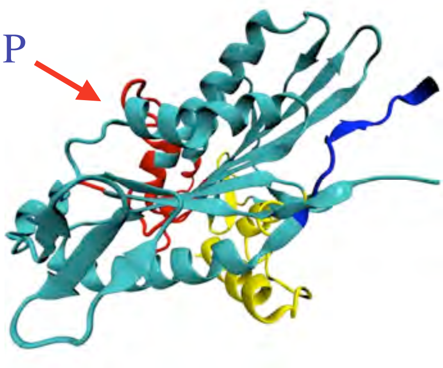




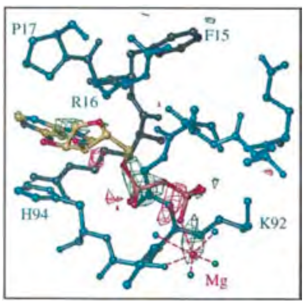
Single Motor Head or Single Enzymatic Domain

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

NBP



- Nucl Binding Pocket (NBP)



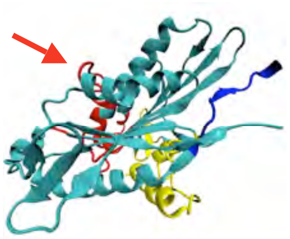
Müller ... Mandelkow, *Biol. Chem.* ***380*** (1999)

Different nucleotide states:

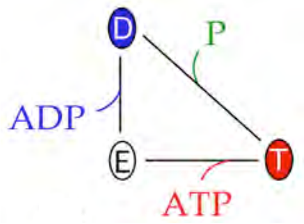
NBP can be occupied by ATP or ADP or may be empty

Chemical Network: Single Head

- Single head of kinesin:



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



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

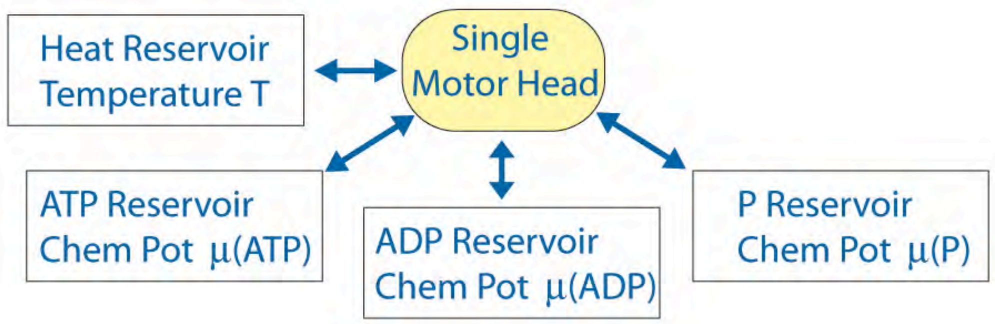
|DE> = ADP release |TD> = ATP hydrolysis + P release
|ED> = ADP binding |DT> = ATP synthesis + P binding

- 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

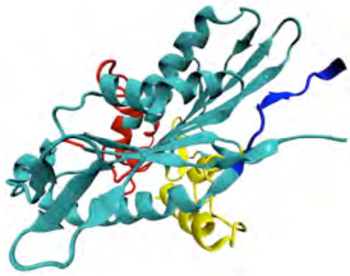
- Single Motor Head plus Reservoirs:



- 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 !

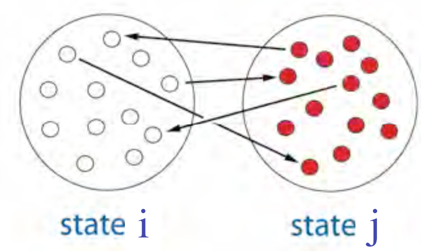
Ensemble of Substates

- Motor head at temperature T: Each state i contains many atomic configurations
- Each motor state i = ensemble of thermally equilibrated substates (i, k_i)
- cf. Transition state theory, Kramers theory



RL, Liepelt: *J. Stat. Phys.* **130** (2008)

- State properties: Internal energy U_i
  Entropy S_i
  Free energy H_i
- Transition |ij> from i to j :
  Transition between substates

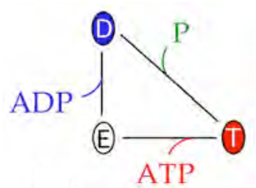


- Transition rates: Forward rate ω_{ij} from state i to state j
  Backward rate ω_{ji} from state j to state i

Cycles and Dicycles

- Cycle = cyclic sequence of states and edges
  Each cycle = two directed cycles = dicycles C_ν^d with d = ±

Single head
= Single ATPaase:

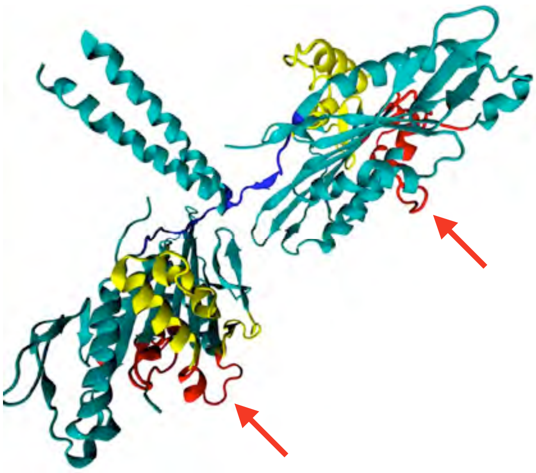


1 cycle
= 2 dicycles

- 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:

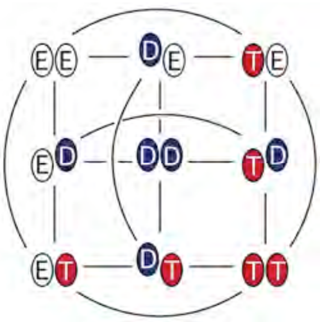
Each of the two NBPs may be occupied by ATP or ADP or may be empty

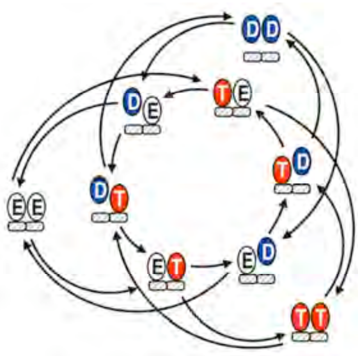
Chemical Network: Two Heads

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

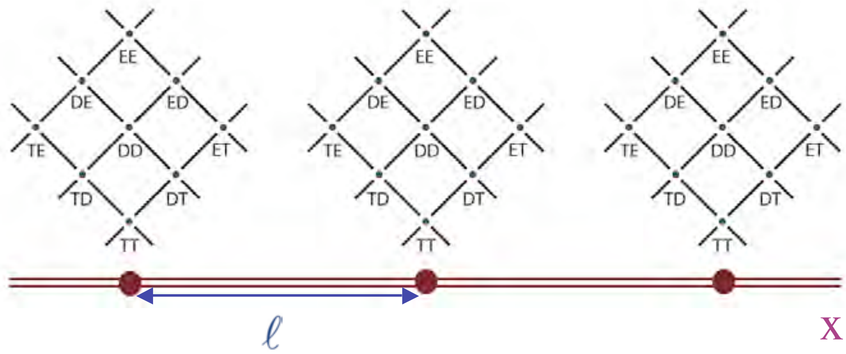




More than 200 cycles !

Chemomechanical (CM) 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 (unit cells) of chemical network:



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

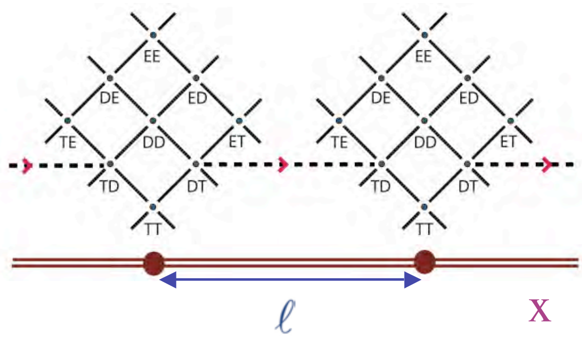
RL et al , *J. Stat. Phys.* ***135*** (2009)

Simplifications for Stepping Motors

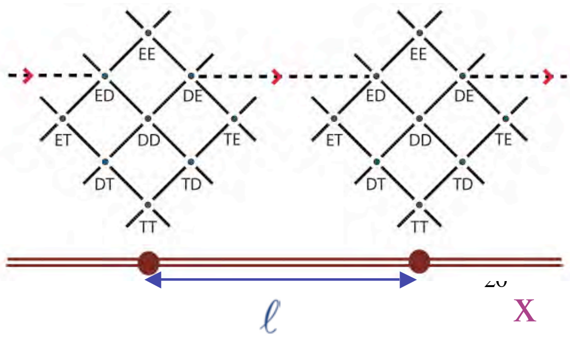
- Mechanical transitions fast compared to chemical transitions:
  Mechanical transitions without chemical transitions
- Different affinities of different nucleotide states to filament:
  Mechanical transitions emanate from a weakly bound state
- In general: One step or several substeps

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

DT -> TD



DE -> ED



CM Network for Myosin V

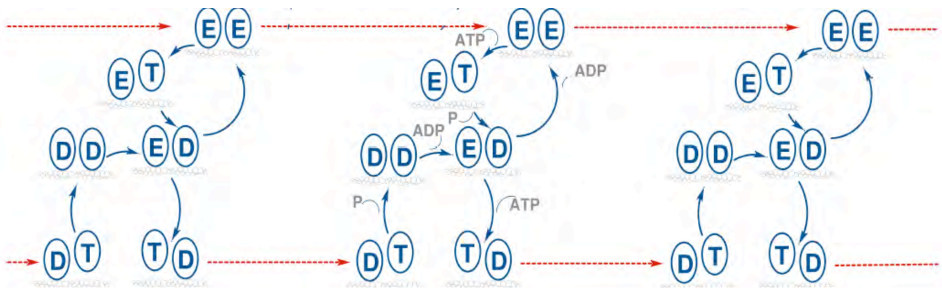
- Two stepping modes:

  (1) CM stepping coupled to ATP

  (2) Force-induced stepping without ATP

Gebhardt ... Rief
PNAS ***103*** (2006)

- Unification of different stepping modes:



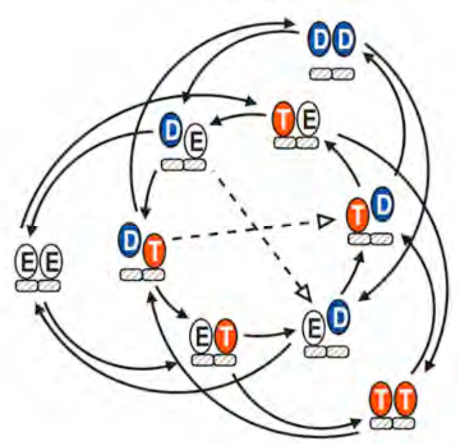
Force-induced stepping

CM Stepping

=> see poster by V. Bierbaum <=

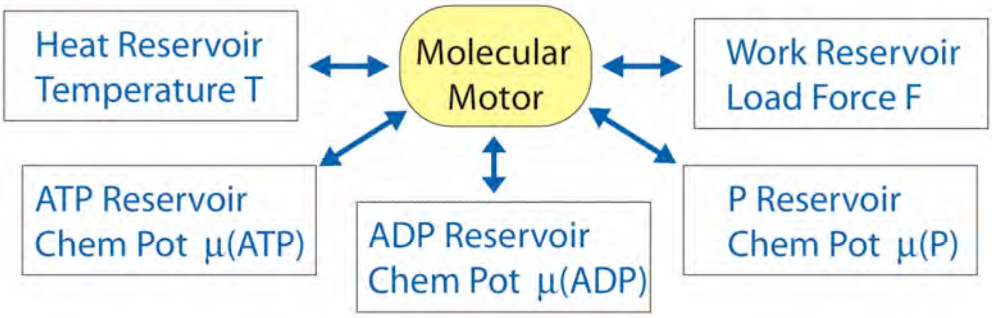
Compact CM Networks

- More convenient representation:
  One copy or unit cell 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

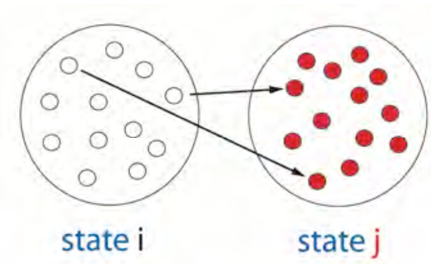
RL, Liepelt: *J. Stat. Phys*. ***130*** (2008)

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: $H_i = U_i - T\,S_i$

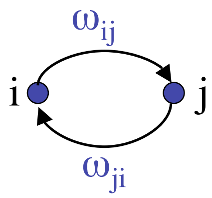
$$H_j - H_i = \mu_{ij} - W_{ij} - T\,\Delta S_{ij}$$

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

Constrained Equilibrium

• 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:

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

Without mechanical work, $W_{ij} = 0$: Hill and Simmons, *PNAS* ***73*** (1976)

• Entropy change during transition |ij> :

$$\Delta S_{ij} = k_B \ln (\omega_{ij} / \omega_{ji}) = S_j - S_i + Q_{ij} / T$$

Only one substate, $S_j = S_i$: Seifert, *PRL* ***95*** (2005)

Landscapes of State Functions

• Internal energy: $U_j - U_i = \mu_{ij} - Q_{ij} - W_{ij}$

• Internal entropy: $S_j - S_i = k_B T \ln (\omega_{ij} / \omega_{ji}) - Q_{ij} / T$

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

• No assumptions about motor dynamics apart from existence of transition rates ω_{ij}

• State functions U_i , S_i , and H_i are somewhat elusive: both difficult to calculate and hard to measure

Cyclic Balance Conditions

Liepelt and RL, *EPL* **77** (2007)

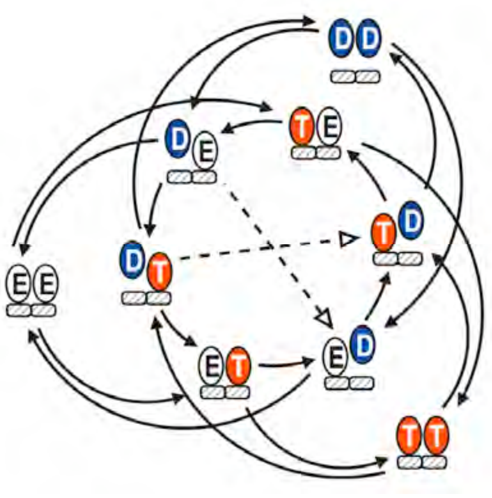
- Summation completed dicycle C_ν^d
- Released heat: $Q(C_\nu^d) = \sum Q_{ij} = \mu(C_\nu^d) - W(C_\nu^d)$
- Produced entropy I: $T \Delta S(C_\nu^d) = \sum T \Delta S_{ij} = Q(C_\nu^d)$
- Produced entropy II: $T \Delta S(C_\nu^d) = k_B T \ln(\Xi_\nu^d)$

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

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

Relation between kinetics and thermodynamics
Thermodynamics imposes constraints on kinetics

Example: Stepping Motors



More than 200 cycles !

Liepelt and RL , *Phys. Rev. E.* **79** (2009)

Classification of Cycles

- Balance condition for each directed cycle $C_\nu{}^d$:

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

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 $W(C_\nu{}^d) \neq 0$

RL, Liepelt: *J. Stat. Phys.* ***130*** (2008)

Force Dependence

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

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

- 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> :

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

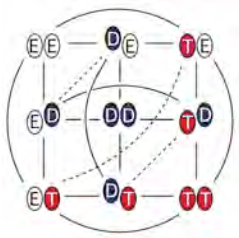
$$\Phi_{ij}(F) / \Phi_{ji}(F) = 1 \quad \text{for } |ij> \ \neq \ |ab>$$

Note: In general, additional dependencies on F !

Liepelt and RL, *EPL* ***77*** (2007)

Motor Dynamics

- Continuous time Markov process on state space
- Master equation for probability P_i :



$$d P_i / dt = - \sum_j \, [P_i \, \omega_{ij} - P_j \, \omega_{ji}]$$

- Steady state properties can be calculated by linear algebra or diagrammatic method (Kirchhoff, Hill)
- Local excess fluxes $\Delta J_{ij} = P_i \, \omega_{ij} - P_j \, \omega_{ji}$ for steady state determine motor properties as measured in single mol exp
- Example 1: Motor velocity $v = \sum^{f}_{|ij>} \ell_{ij} \, \Delta J_{ij}$
- Example 2: Hydrolysis rate $h = \sum^{h}_{|ij>} \Delta J_{ij}$

=> Operation modes, efficiency; see talk by S. Liepelt <=

Liepelt and RL , *Phys. Rev. E.* ***79*** (2009)

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- Chemomechanical Coupling
- ☞ Example: CM Coupling for Kinesin
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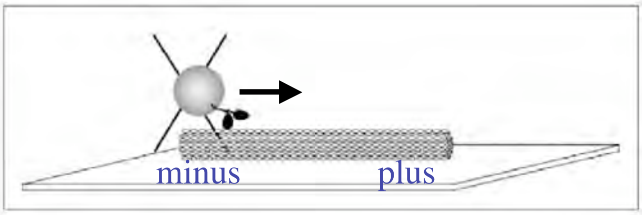
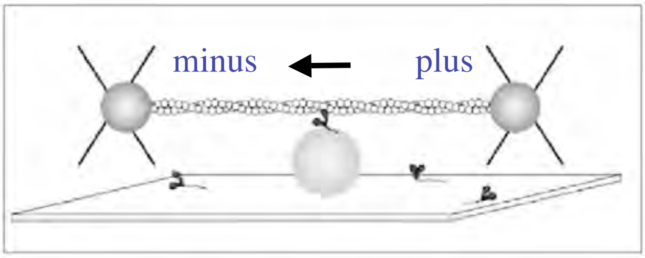
Single Motor Experiments

- Bead assay: Mobile motor



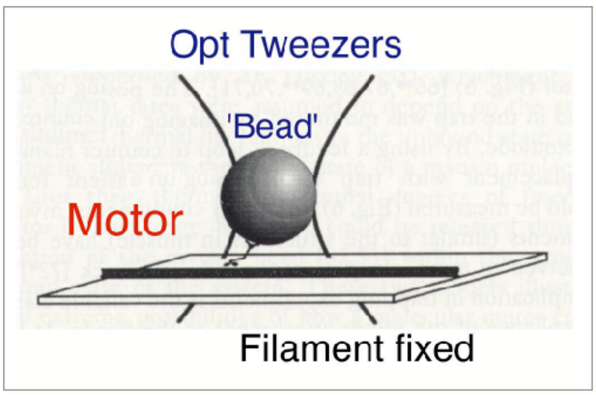
- Gliding assay: Mobile filament



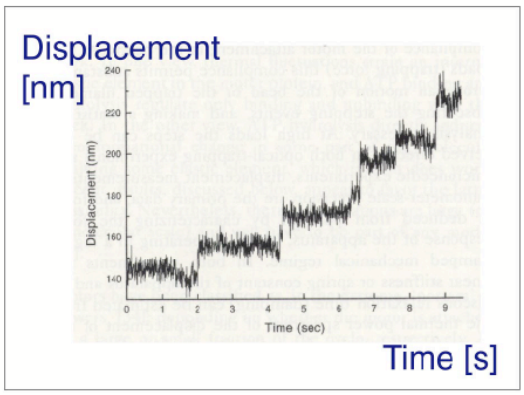
- Polar filament
- Plus and minus end
- Force generation of motor
  => relative displacement
  (actio = reactio)
- Bead assay:
  Motor moves to plus
- Gliding assay:
  Filament shifted to minus

Kinesin: Mechanical Stepping

- Bead Assay:



- Discrete Steps:



Svoboda ... Block, *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

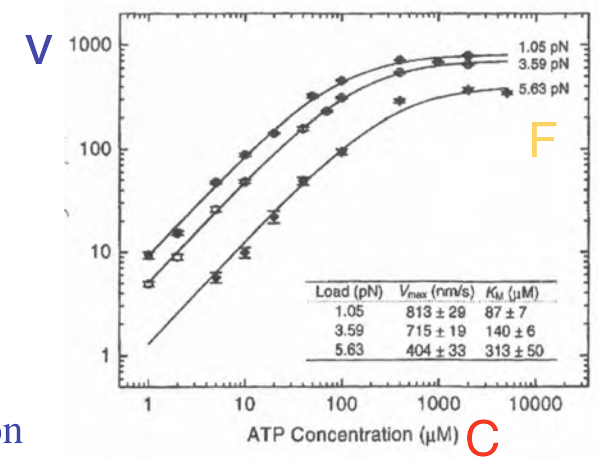
- Velocity v as a function of concentration [ATP] = C and external force F

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

'Michaelis-Menten Relation'

- Simple functional dependence on two variables C and F
- Predicted by a large class of motor models RL, *PRL.* ***85*** (2000)

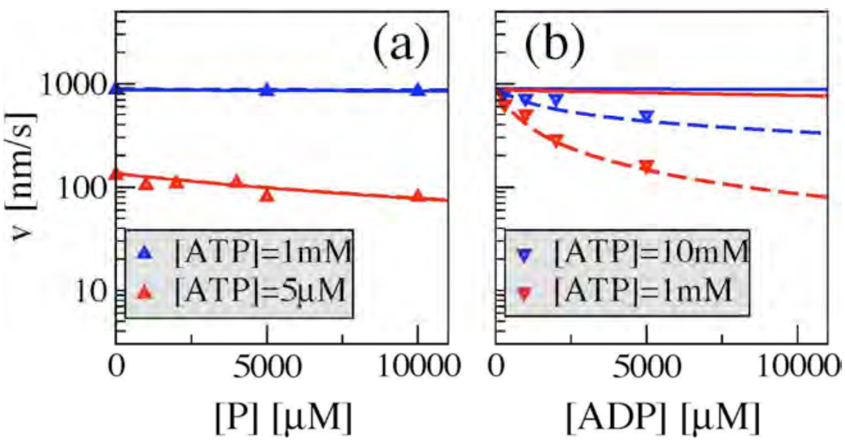
Visscher et al, *Nature* ***400*** (1999)



[ADP] and [P] Dependence

Schief … Howard, *PNAS* ***101*** (2004)

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

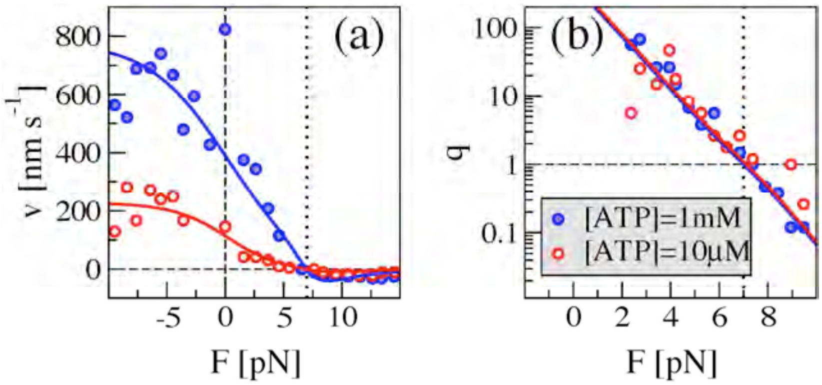


Load Force Dependence

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

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

Resisting Load Force F > 0

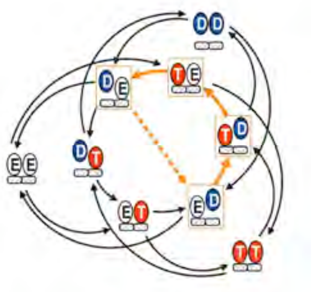


- Kinesin generates about 7 pN = stall force F_s
- Kinesin makes processive backwards steps
- Mechanical steps are very fast (faster than 15 μs)

Kinesin: Proposed Motor Cycles

- Two examples from experimental groups:

Mori ... Vale, *Nature* ***450*** (2007)



Alonso ... Cross, *Science* ***316*** (2007)

- Theory: Variety of unicycle models

Fisher and Kim, *PNAS* ***102*** (2005)

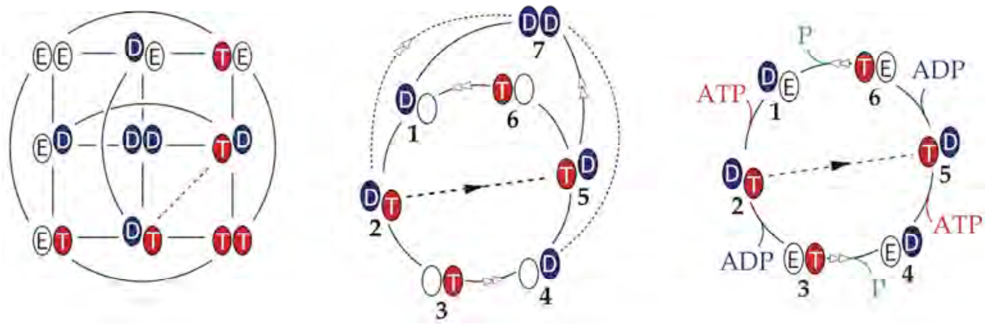
- Basic artefact of all unicycle models:

  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)

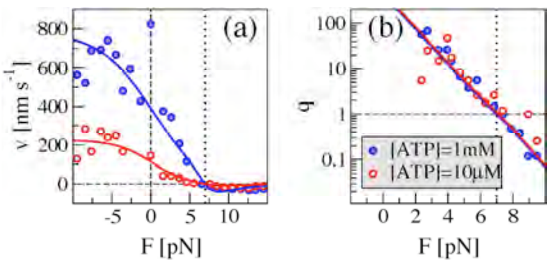


Three chemomechanical motor cycles:

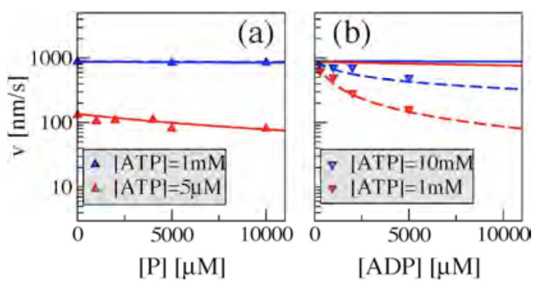
- Small ADP and P, small load force F: dicycle |25612>
- Small ADP and P, large load force F: dicycle |52345>
- Large ADP, small load force F: dicycle |25712>

Kinesin: Theory + Experiment

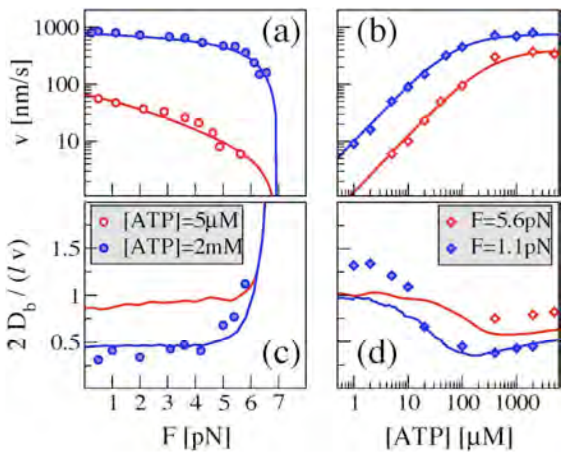
- Data of Carter, Cross (2005)



- Data of Schief et al (2004)



- Data of Visscher et al (1999)

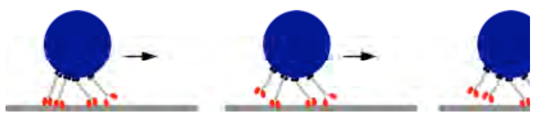


- Data of Schnitzer et al (2000) on run length as a function of force and [ATP]

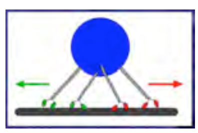
- Introduction
- Chemomechanical Coupling
- Example: CM Coupling for Kinesin
- ☞ Outlook on Multiscale Motility

Motility on Larger Scales

- Single motors: Run length and motor walks
- Macroscopic cargo transport by several motors
- Uni-directional transport by one motor team



- Bi-directional transport by two motor teams



Stochastic Tug-of-war

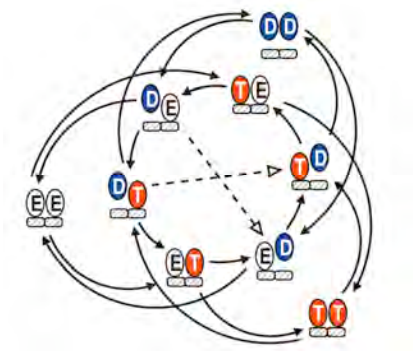
- Traffic of motors and cargoes

=> Talk on Friday

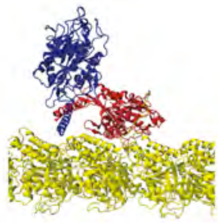
Motility on Smaller Scales

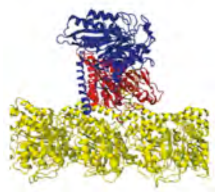
- All-atom MD simulations:
  kinesin + tubulin

- Free energy landscape
  of nucleotide states:

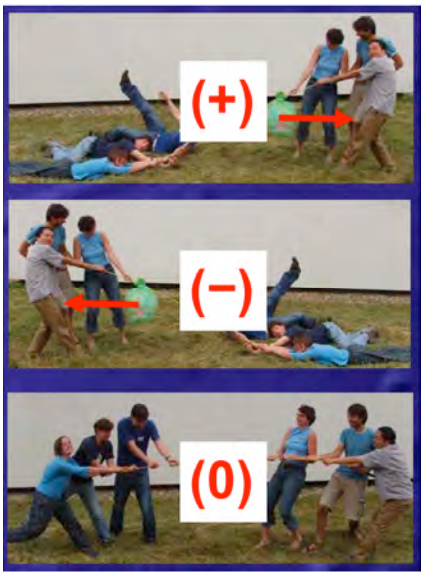


- Mechanical stepping:
  DT -> TD ?





Coworkers



Stepping Motors, Theory:

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