Multiscale Motility of Molecular Motors

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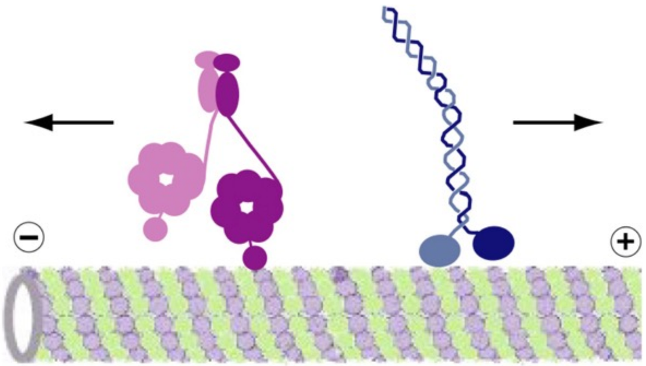
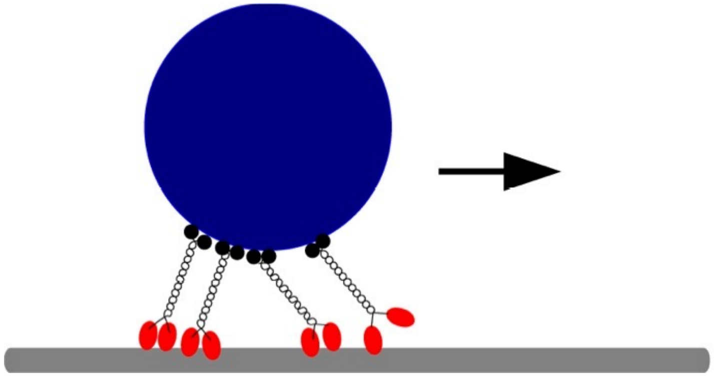
- Chemomechanics of Single Motors
- Motor Properties of Kinesin
- Cyclic Balance Conditions
- Transport by Two Molecular Motors
- Outlook

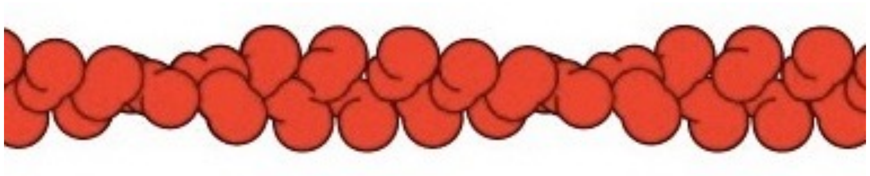
Biomolecular Machines



• Stepping motors



• Motor teams



• Actin filaments

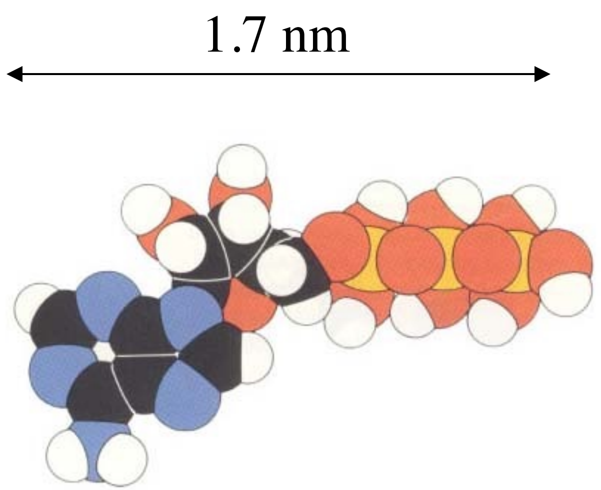
• Ribosomes

Mechano-Enzymes

- Biomolecular machines:

  Conversion of chemical energy into mechanical work

- Universal chemical energy source provided by NTP = ATP, GTP, ...



- Hydrolysis of NTP: NTP -> NDP + P
- Synthesis of NTP: NTP <- NDP + P

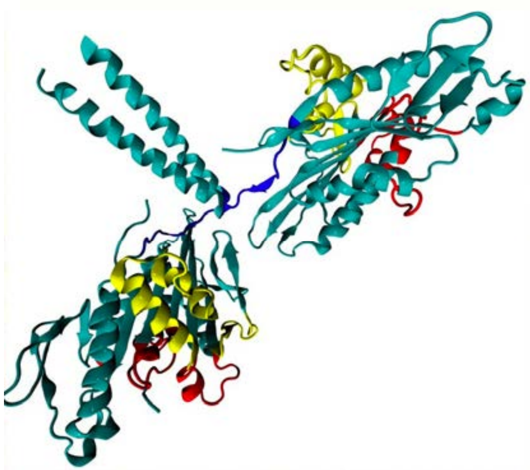
Nucleotides
NTP, NDP, P

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

Multiscale Motility of Motors

- Example: Kinesin at Microtubules

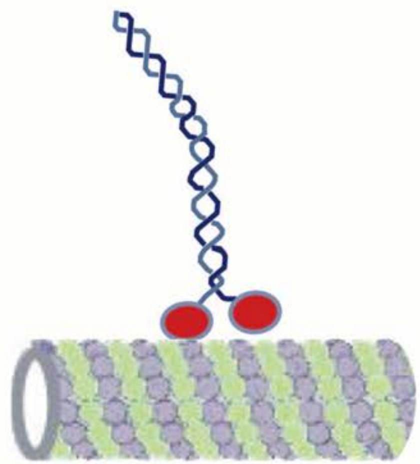
ATP Binding



Nucleotide Binding Pocket ~ 1 nm

10^{-3} s

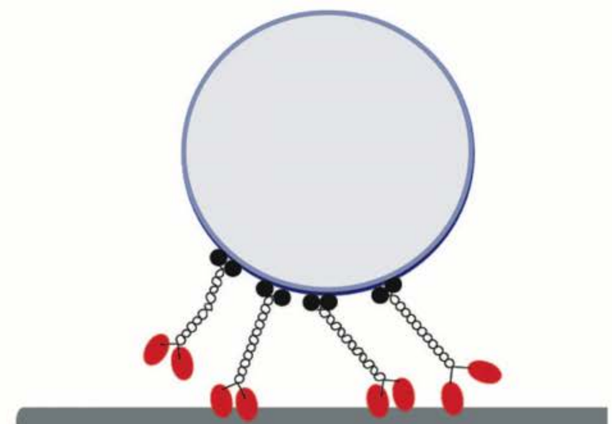
Mechanical Steps



Single head moves by 16 nm

10^{-6} s

Transport



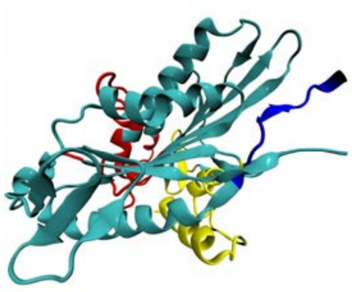
Cargo transport over cm or m !

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

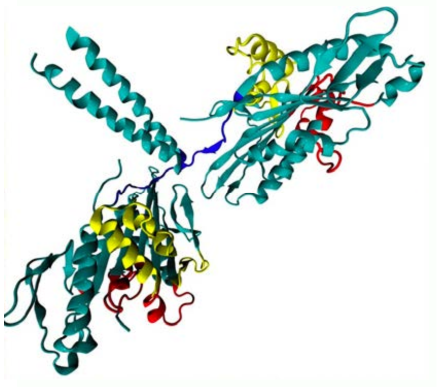
Hierarchy of Time Scales $\neq$ Hierarchy of Length Scales

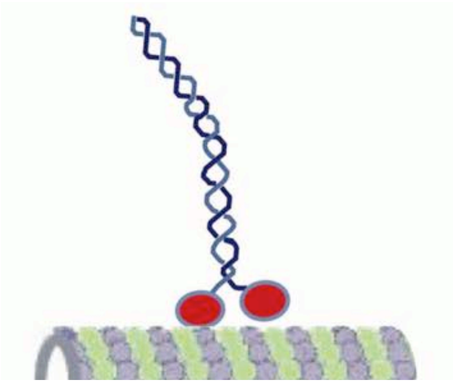
Modelling Bottom-Up

Single motor head

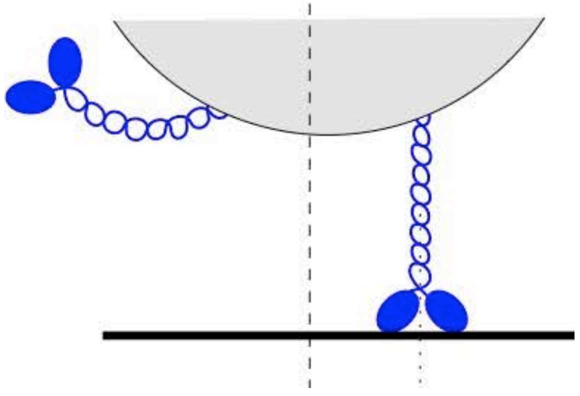


Two-headed motor





Two motors

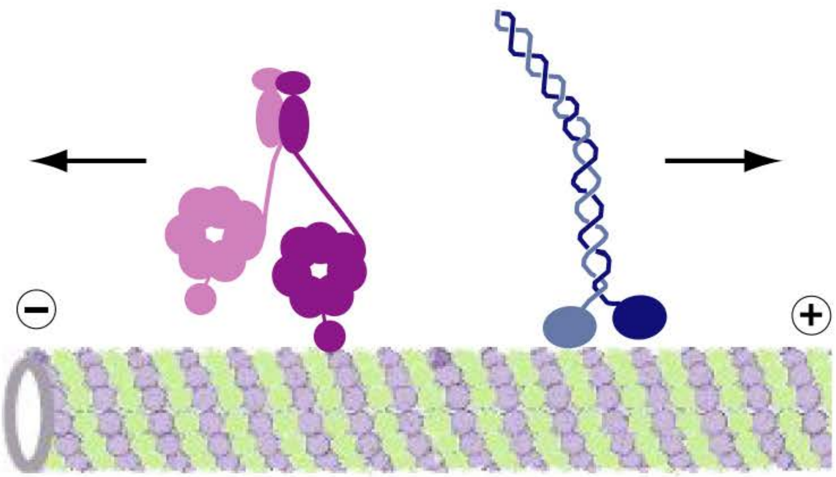


Chemomechanics of Single Motors

- Stepping motor kinesin
- Single motor domain or head
- Kinesin as (mechano-2-) enzyme
- Balance conditions for motor cycles
- Motor unbinding and run length

Stepping Motors

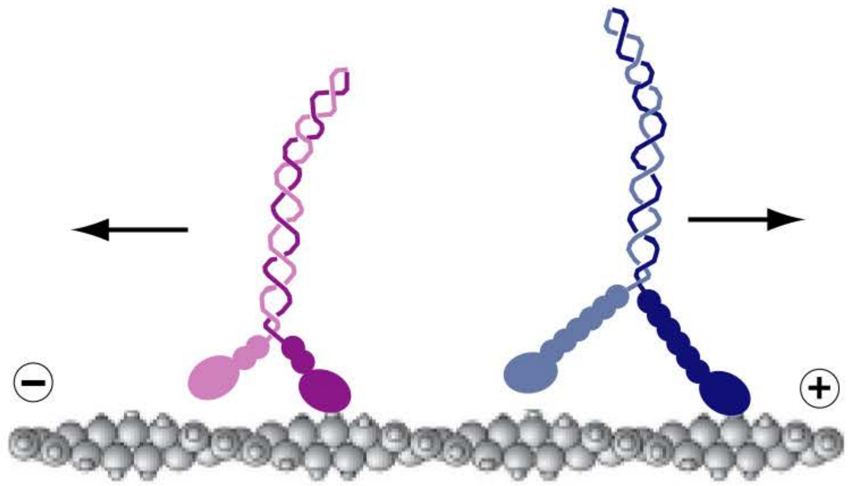
- Filament = Microtubule



Dyneins
to minus end

Kinesins
to plus end

- Filament = F-Actin



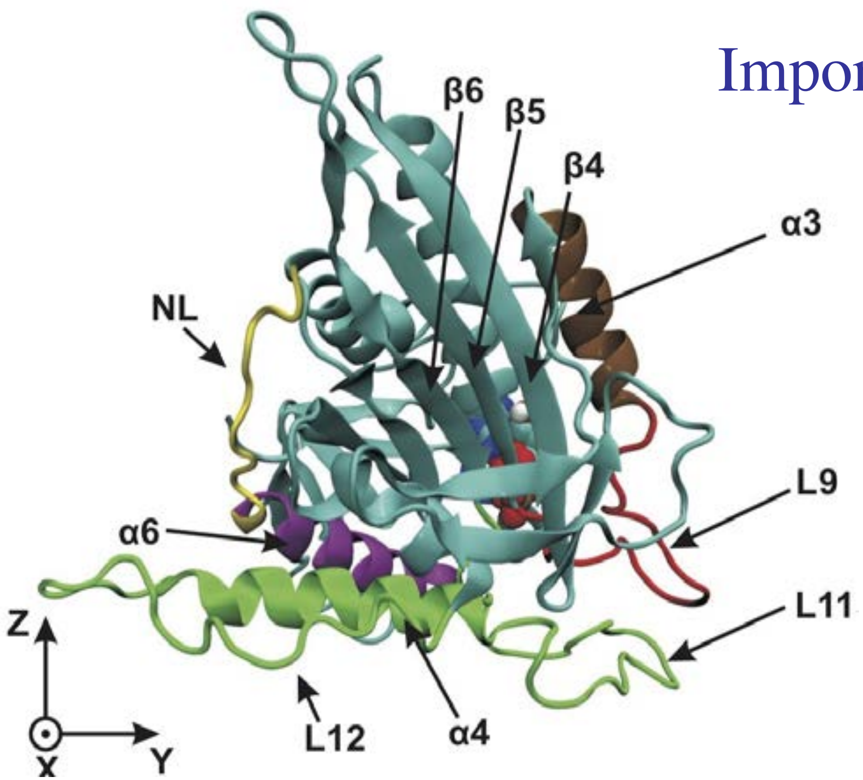
Myosin VI
to minus end

Myosin V
to plus end

- Each motor has two heads that hydrolyze ATP
- Each motor makes steps with nanometer step size

Single Motor Domain of Kinesin

Krukau, Knecht, RL, *PCCP* (2014)



Important subdomains:

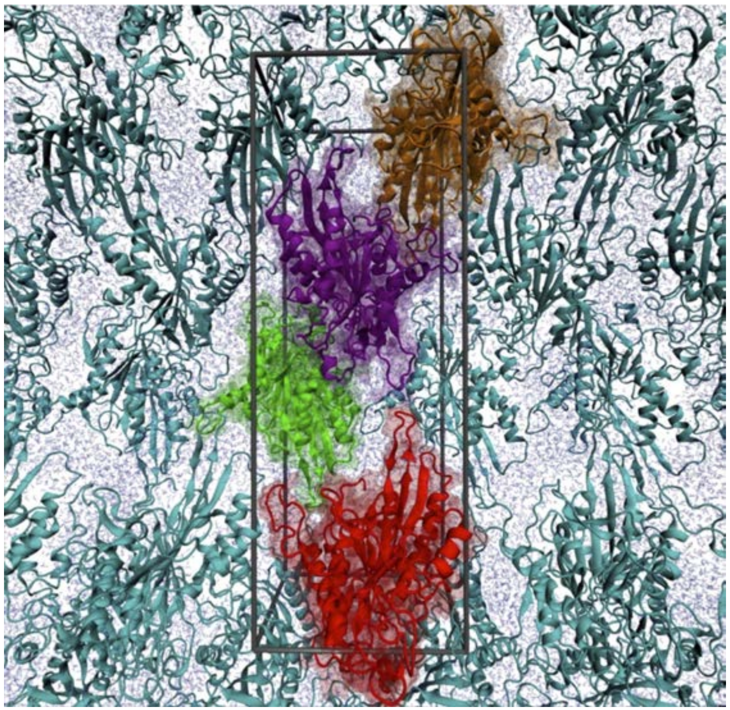
- Neck linker

  NL + α6 helix
- Nucleotide binding pocket

  L9 loop + α3 helix (+ ATP)
- Microtubule binding site

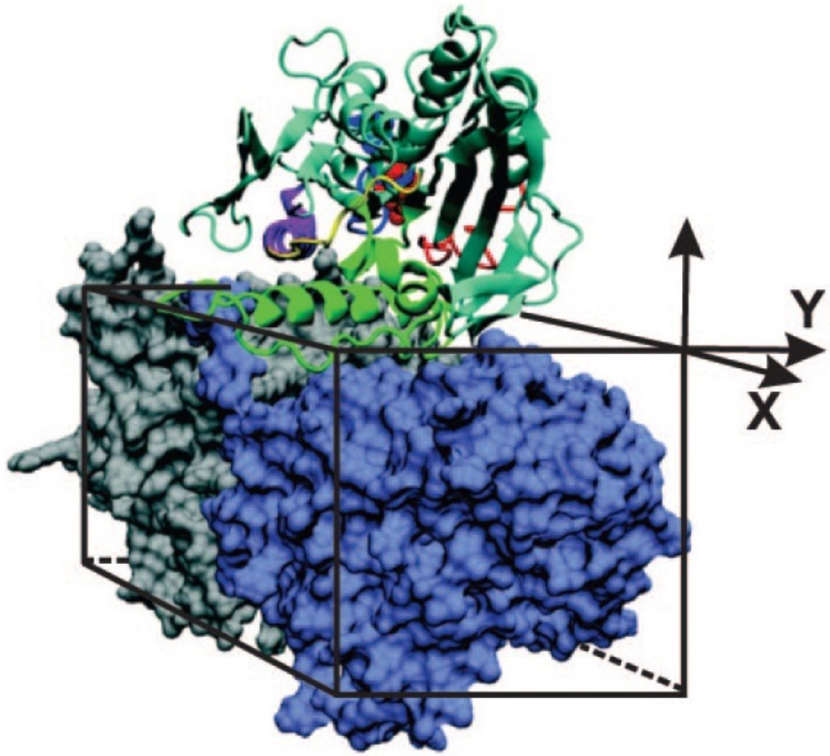
  L11 loop + α4 helix

Validation via Crystal Structure



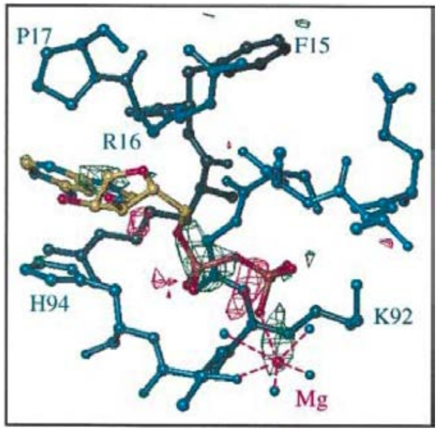
- Crystal structure of T state
- Unit cell contains 4 motor domains
- Crystal stucture as initial configuration
- Stability of structure confirmed by MD simulations over 300 ns
- Small rotations but consistent with resolution of X-ray diffractions
- Validation of simulation code

Motor attached to Tubulin Dimer



Nucleotide States of Single Head

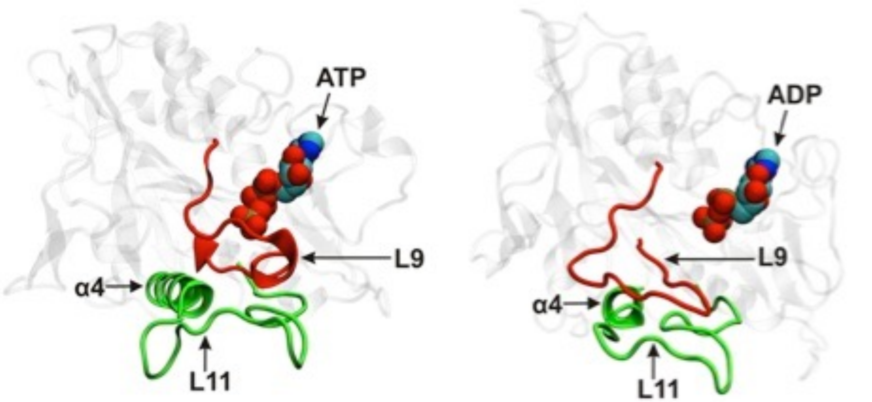
- Nucleotide Binding Pocket (NBP)



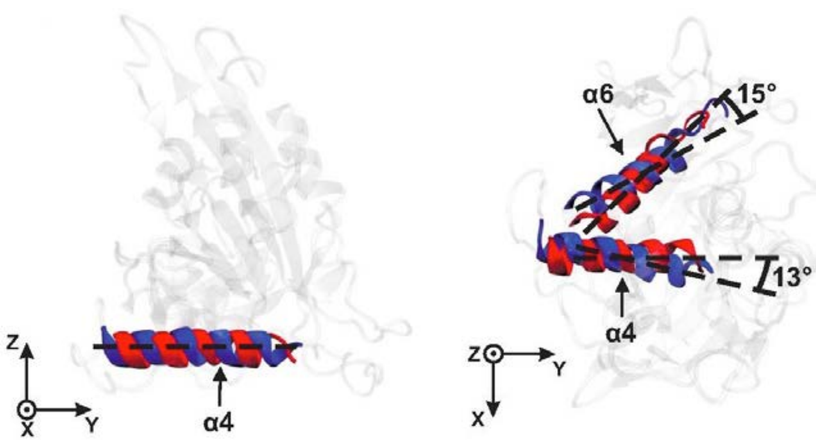
NBP can
- be occupied by ATP — T
- be occupied by ADP — D
- be empty — E

- ATP cleavage and phosphate release take about 10 ms
  => too long for brute force MD
- Dominant conformations for E, T, and D states
- Comparison of these states => conformational transitions

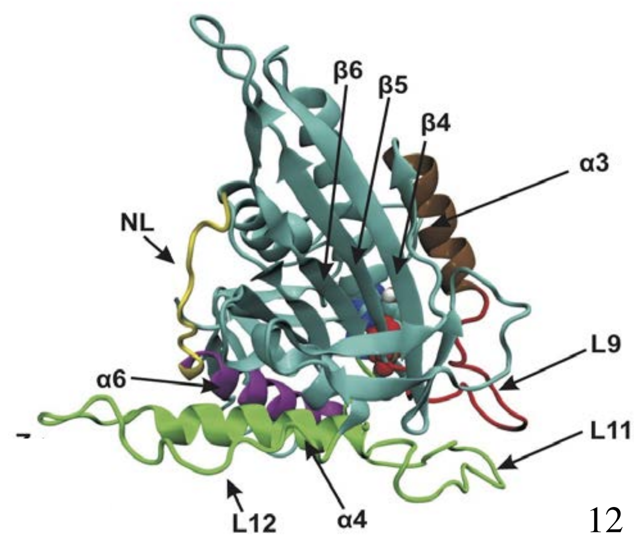
Phosphate Release: Allosteric Coupling



- Transition from T to D state
- Change in L9 loop
- Rotation of α_4 helix
- Rotation of α_6 helix
- Undocking of neck linker



red: T state,   blue: D state

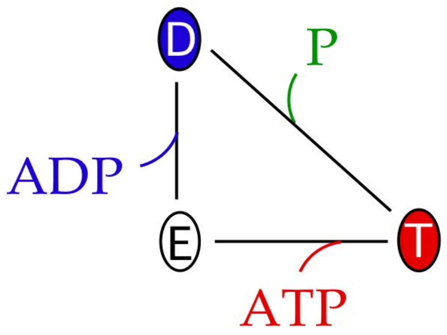


Stochastic Modelling

Liepelt, RL, *EPL 77* (2007); *Phys. Rev. Lett. 98* (2007)

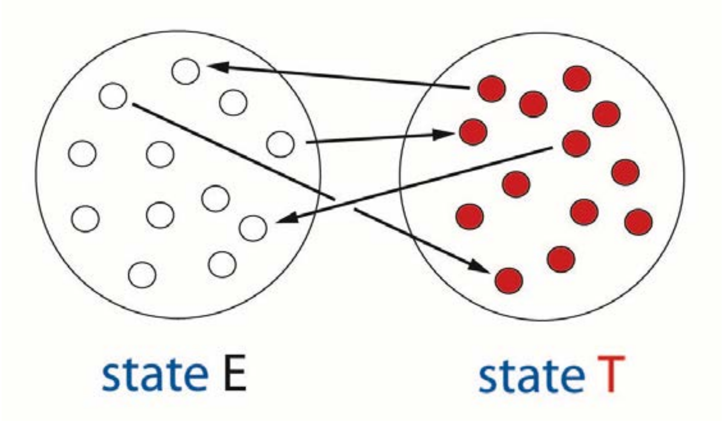
- Single head = single ATPase has 3 states:

empty: i = E
bound ATP: i = T
bound ADP: i = D



- In each state, head can attain many atomistic conformations:

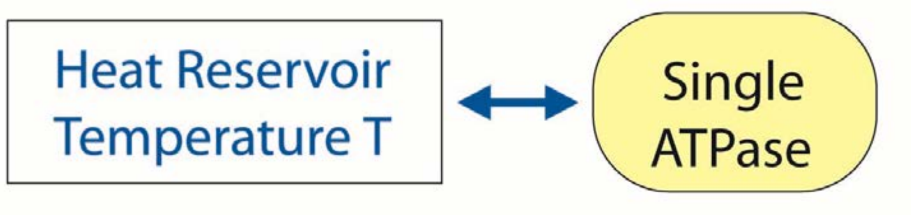
Each state i = ensemble of substates (i, k_i)



arrows = chemical transitions

Statistical Mech of Substates

- Single head coupled to heat reservoir at temperature T



- Separation of time scales:

Thermal equilibration fast compared to chemical transitions

Each state i is thermally equlibrated

- Substate (i, k_i) has energy $E(i, k_i)$
- Probability to find system in substate (i, k_i)

Boltzmann factor $\mathcal{B}(i, k_i) = \exp[- E(i, k_i) / k_B T]$

- Partition sum: $Z_i = \Sigma \; \mathcal{B}(i, k_i)$ => State properties:

Helmholtz free energy, Internal energy, Entropy

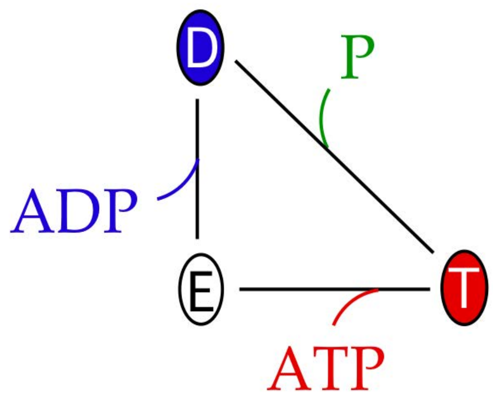
Transition Rates

- Forward and backward transitions between two states i and j
- Associated transition rates: ω_{ij} from state i to state j

  ω_{ji} from state j to state i

$\omega_{DE} = \kappa_{DE}$

$\omega_{ED} = \kappa_{ED}$ [ADP]



$\omega_{TD} = \kappa_{TD}$

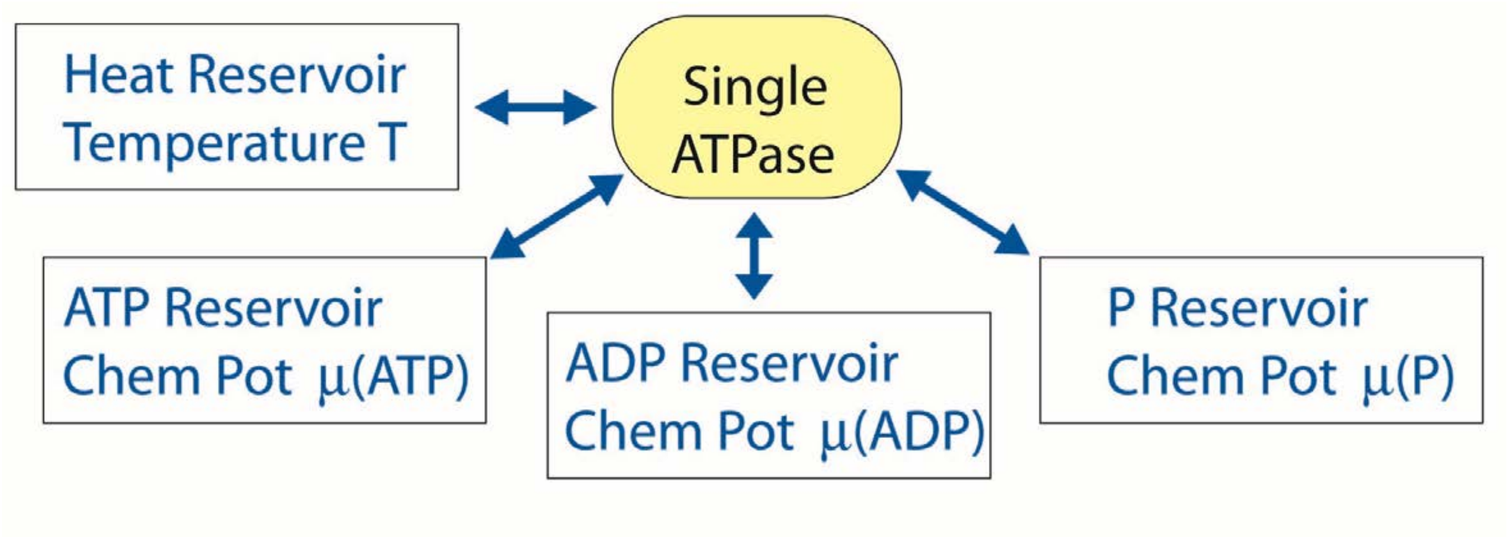
$\omega_{DT} = \kappa_{DT}$ [P]

$\omega_{ET} = \kappa_{ET}$ [ATP]

$\omega_{TE} = \kappa_{TE}$

Chemical Reservoirs

- Single head = single ATPase coupled to Chemical Reservoirs:



- Chemical reservoirs for X = ATP, ADP, and P

Activities [X] ~ molar concentrations

Chemical potentials $\mu(X) = k_B\, T \ln([X] / [X]^*)$

Activity scales $[X]^*$

Chemical (Non)Equilibrium

- ATP Hydrolysis: ATP <=> ADP + P

Chemical energy change per hydrolysed ATP :

$$\Delta\mu = \mu(ATP) - \mu(ADP) - \mu(P)$$

$$= k_B T \ln\left(K_{eq} \frac{[ATP]}{[ADP]\,[P]} \right)$$

- Equilibrium constant K_{eq} determined by three activity scales

- Chemical equilibrium $\Delta\mu = 0$

- ATP hydrolysis and synthesis for $\Delta\mu > 0$ and $\Delta\mu < 0$

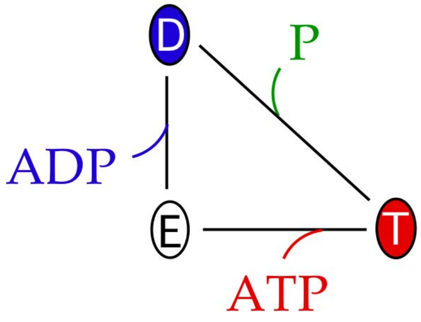
Cycles and Dicycles

- Cycle = cyclic sequence of states and edges

  Each cycle = two directed cycles = dicycles $C_\nu^{\,d}$ with d = ±

- Single motor domain or single head:

  3-state model represents a unicycle model



1 cycle
= 2 dicycles

- Hydrolysis dicycle |ETDE> : Chemical energy input: + $\Delta\mu$
- Synthesis dicycle |EDTE> : Chemical energy input: - $\Delta\mu$

Steady State Entropy Production

- Statistical (or Shannon-) entropy: $S = - k_B \sum P_i \ln(P_i)$
- Master equation for P_i : $d S / dt = \sigma_{pr} + \sigma_{fl}$
- Steady state: $d S / dt = 0 \quad \Rightarrow \quad \sigma_{pr} = - \sigma_{fl}$

$$\sigma_{pr}^{st} = \sum_{\nu} \sum_{d=\pm} \Omega^{st}(C_\nu^d) \Delta S(C_\nu^d)$$

sum over all dicycles

frequency of dicycle completion

- Entropy produced per completed dicycle:

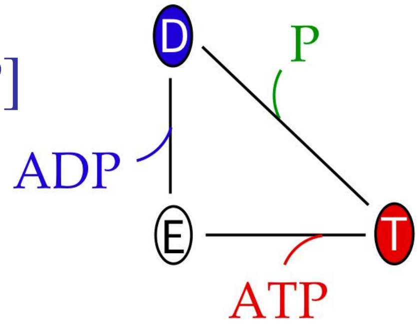
$$\Delta S\,(C_\nu^d) = k_B \ln(\Xi_\nu^d) \quad \text{with} \quad \Xi_\nu^d = \prod_{|ij>}^{\nu,d} (\omega_{ij} / \omega_{ji})$$

- Equilibrium state: $\Delta S\,(C_\nu^d) = 0$

Unicycle Model for Single Head

- Explicit dependence on concentrations [T], [P], and [D]:

$$\omega_{ED} = \kappa_{ED}\,[ADP]$$



$$\omega_{DT} = \kappa_{DT}\,[P]$$

$$\omega_{ET} = \kappa_{ET}\,[ATP]$$

$$\Xi_\nu^{\,d} = B\,\exp[\Delta\mu / k_B T] / K_{eq} \quad \text{with conc-independent B}$$

$$T\,\Delta S\,(C_\nu^{\,d}) = k_B\,\ln(\Xi_\nu^{\,d}) = k_B\,\ln(B/K_{eq}) + \Delta\mu$$

- Chemical equilibrium: $\Delta\mu = 0$, $\Delta S = 0$ => $B = K_{eq}$
- Relation between rates and equilibrium constant, simple example for balance condition

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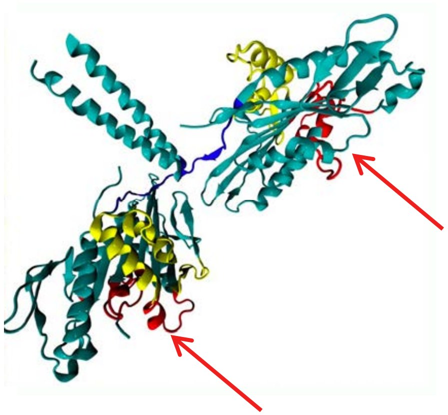
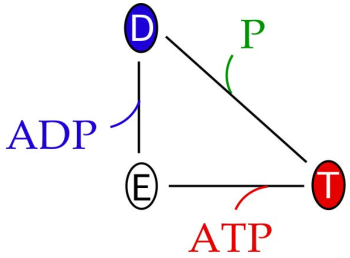
Kinesin as 2-Enzyme Complex

- Kinesin has two motor heads

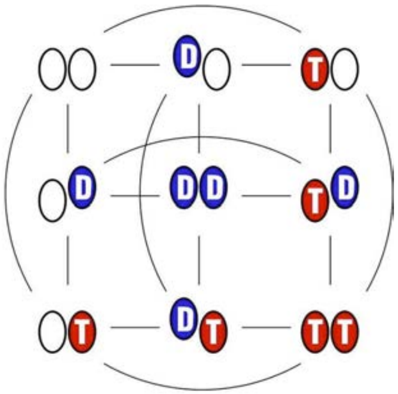


- Each head can attain 3 states E, T, D that form one chemical cycle



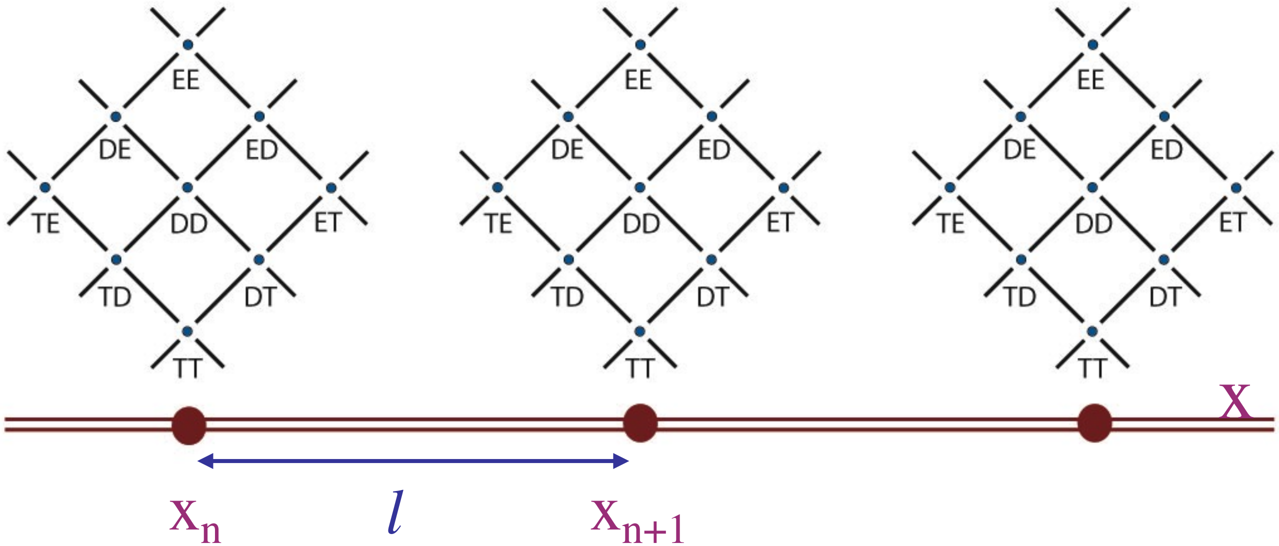
- Two heads can attain 3x3 = 9 states with 2 x 18 = 36 transitions

- States + transitions define chemical network with many cycles



Mechanical Transitions

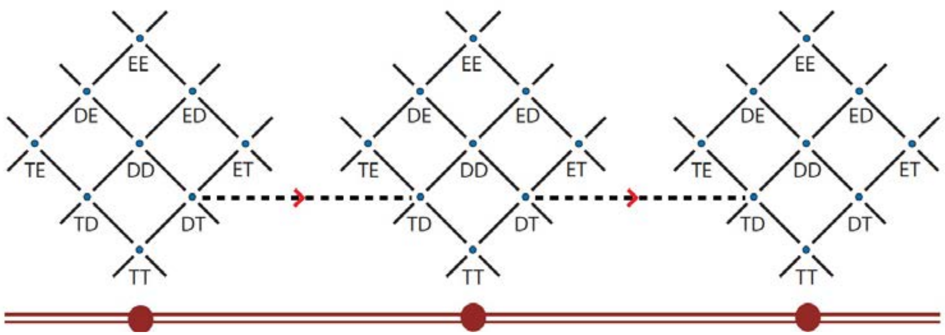
- Mechanical transitions = Spatial displacement along filament
- Discrete step size l defines lattice of motor positions:

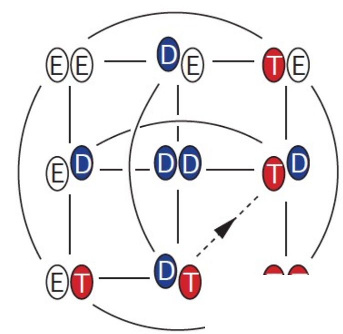


- Mechanical transitions from
  chemical state at site x_n to chemical state at site x_{n+1}

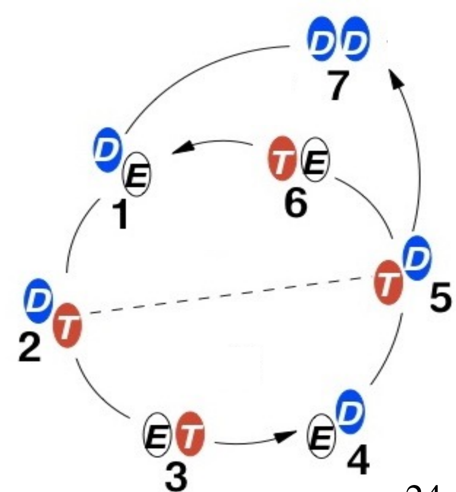
Chemomechanics of Kinesin

- Nucleotide-depending binding of kinesin:
  E, T strongly bound, D weakly bound
- Hand-over-hand motion: DT -> TD or DE -> ED



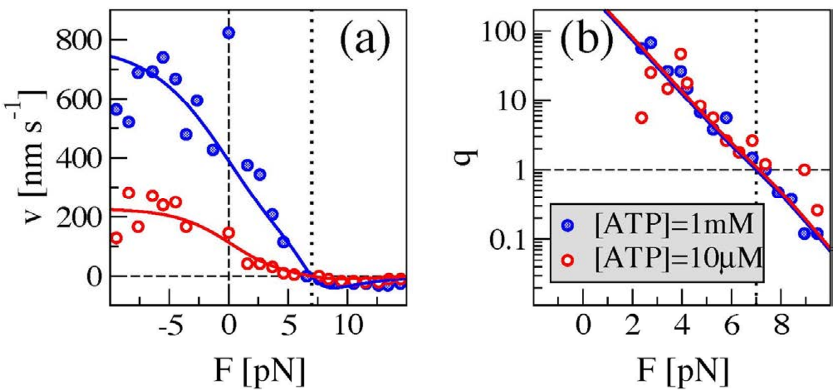


- For EE and TT, both heads are strongly
- Reduced network without EE and TT states:

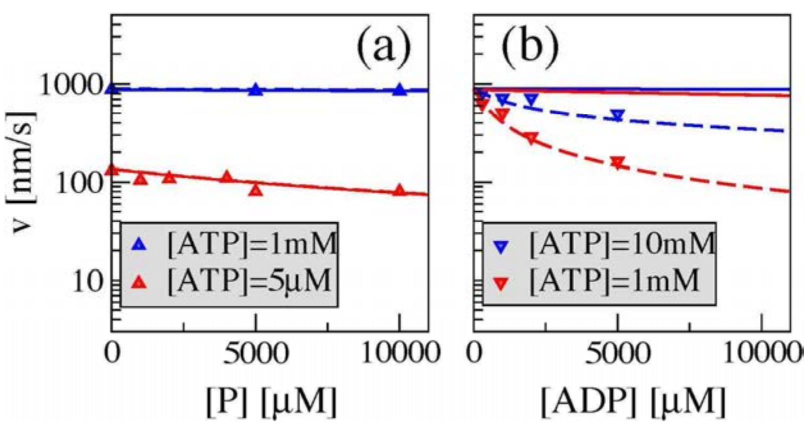


Kinesin: Theory + Experiment

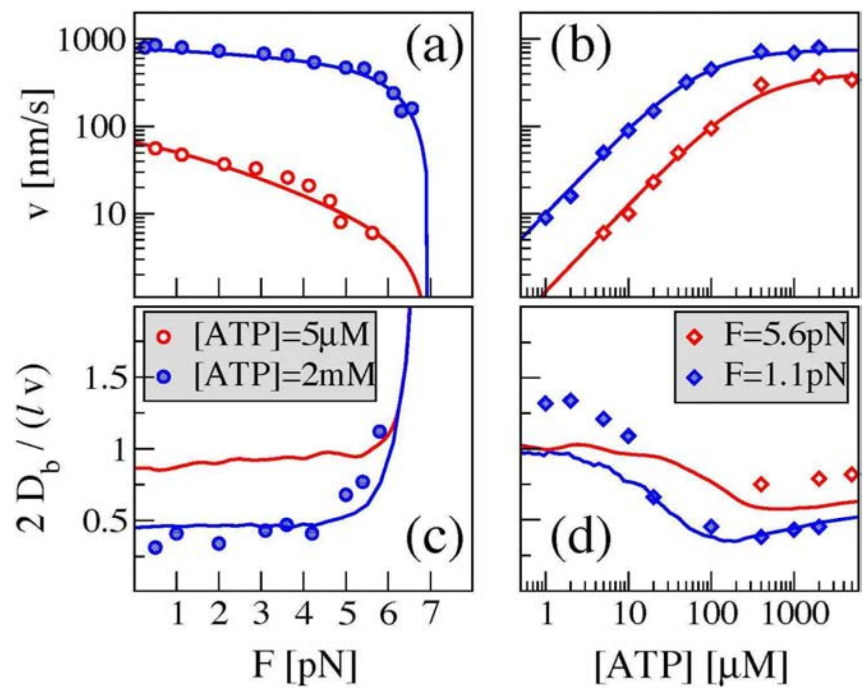
- Data of Carter, Cross (2005)



- Data of Schief et al (2004)

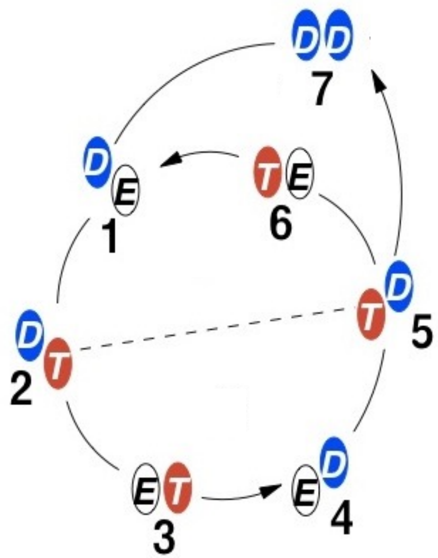


- Data of Visscher et al (1999)



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

Kinesin: Several Motor Cycles



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

Three chemomechanical motor cycles

Dominat cycle depends on Concentr of ATP, ADP, P and load force

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

- Graph theory: three fundamental cycles => three independent conditions on ω–products Ξ_ν^{d}

Balance Conditions for Cycles

- Entropy produced per completed dicycle $\mathcal{C}_\nu^{\,d}$ in steady state:

$$\Delta S\,(\mathcal{C}_\nu^{\,d}) \;=\; k_B\;\ln(\,\Xi_\nu^{\,d}\,) \qquad \text{with}\;\; \Xi_\nu^{\,d} = \prod_{|ij>}^{\nu,d} (\omega_{ij}/\omega_{ji})$$

- Balance condition for each dicycle $\mathcal{C}_\nu^{\,d}$:

$$k_B T\;\ln(\,\Xi_\nu^{\,d}\,) \;=\; \Delta\mu(\mathcal{C}_\nu^{\,d}) - W_{me}(\mathcal{C}_\nu^{\,d}) = -\,k_B T\;\ln(\,\Xi_\nu^{\,-d}\,)$$

Relation between kinetics and thermodynamics
Thermodynamics imposes constraints on kinetics

- Special case: Enzymes without mechanical work — Haldane (1965)

- No dependence on state functions U_i , S_i , G_i

Classification of Cycles

- Balance condition for each directed cycle C_ν^d :

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

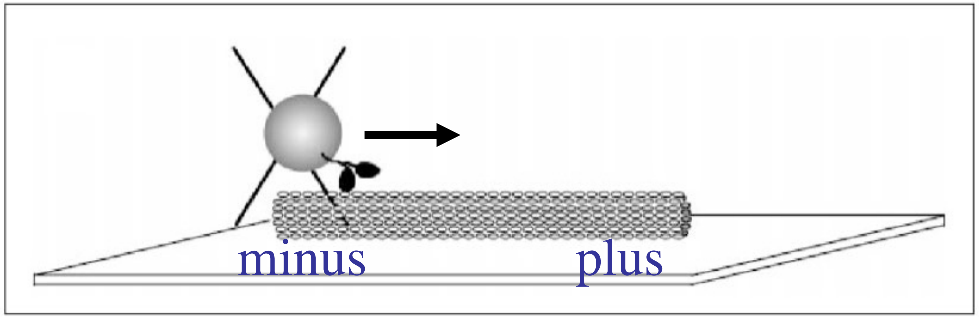
Classification of cycles:

- Detailed balance: $\Delta\mu(C_\nu^d) = 0$ and $W_{me}(C_\nu^d) = 0$
- Mech nonequilibrium: $\Delta\mu(C_\nu^d) = 0$ and $W_{me}(C_\nu^d) \neq 0$
- Chem nonequilibium: $\Delta\mu(C_\nu^d) \neq 0$ and $W_{me}(C_\nu^d) = 0$
- Chemomech coupling: $\Delta\mu(C_\nu^d) \neq 0$ and $W_{me}(C_\nu^d) \neq 0$

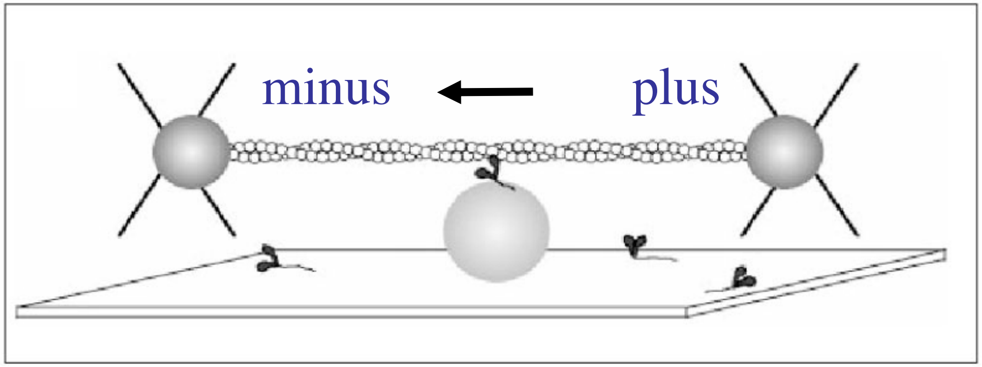
Force as Control Parameter

- Motors attached to beads, force applied to beads via laser trap

- Bead assay:



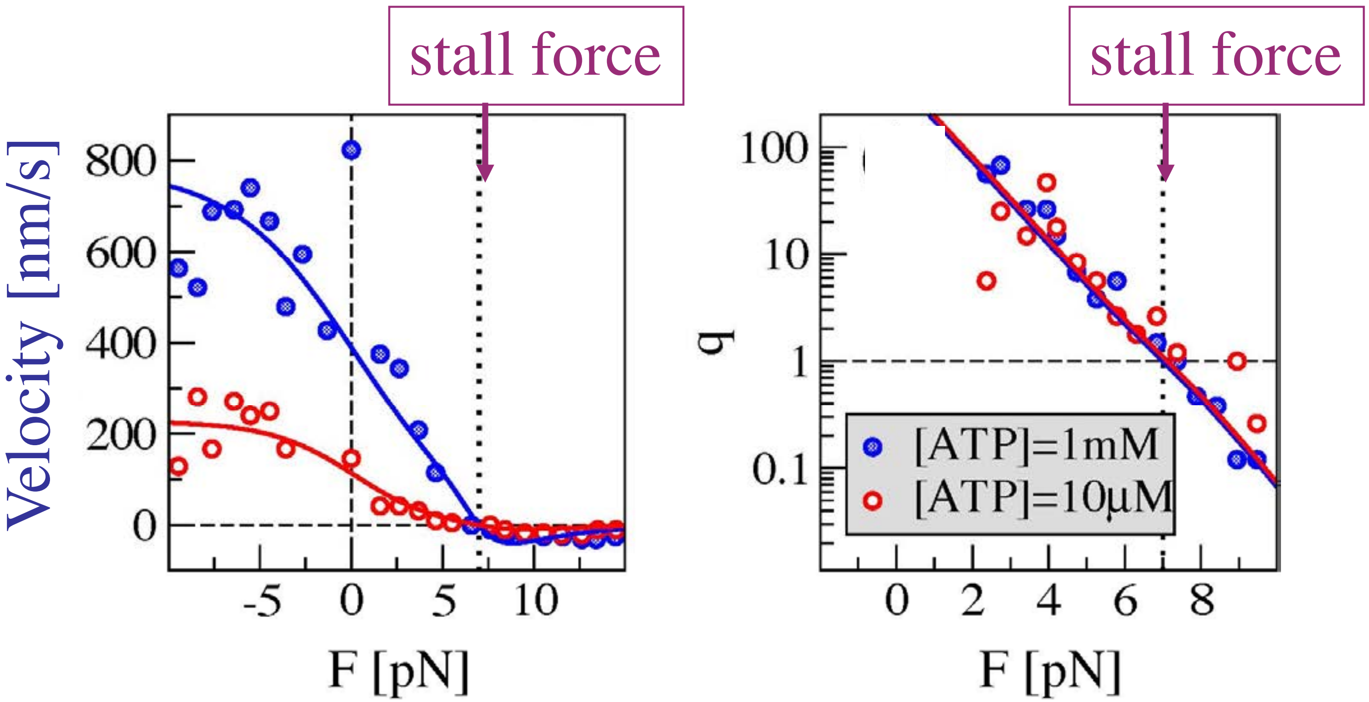
- Gliding assay:



Force-Velocity Relationship

• Motor velocity v decreases with increasing force F

• Velocity vanishes at stall force F_s



q = ratio of forward to backward steps

Exp. data: Carter and Cross, *Nature* (2005)

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

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 ω–products:

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

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

- Cycle contains a single mechanical transition |ab> :

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

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

Unbinding of Kinesin

- Thermal noise leads to unbinding of single motor from filament

- Unbinding rate ε_{si} is F-dependent:

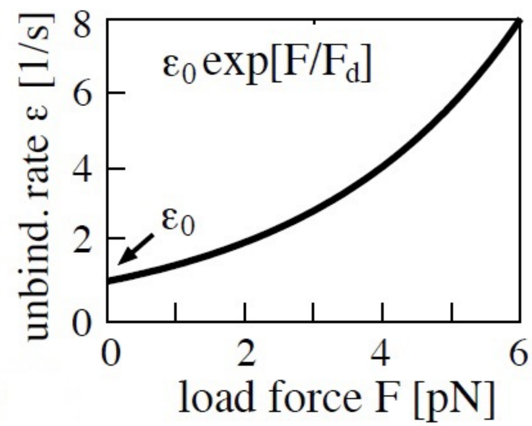
  $\varepsilon_{si} \sim \exp(F/F_d)$

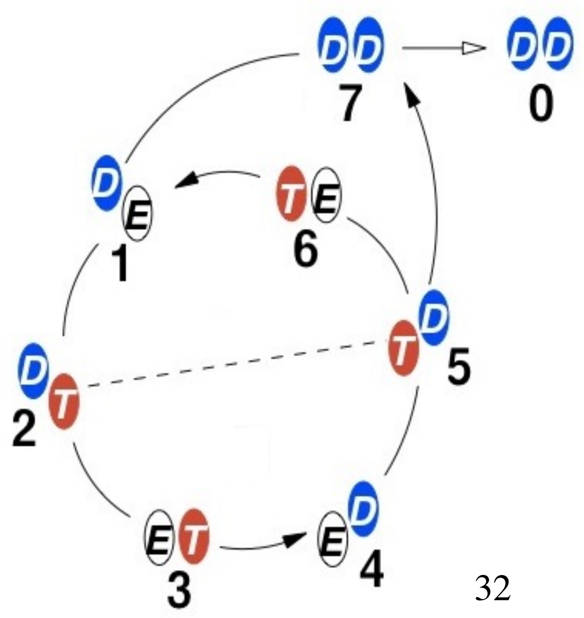
  Detachment force F_d

- Chemomechanical network with

  bound states i = 1, ..., 7

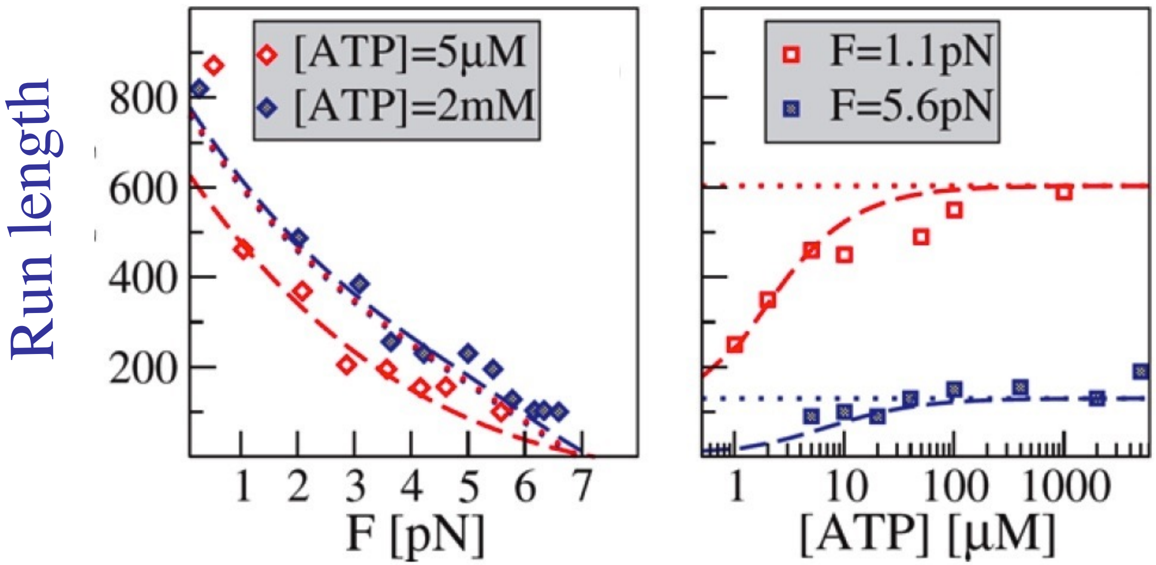
  plus unbound state i = 0





Run Length of Kinesin

- (Average) run time = inverse unbinding rate
- (Average) run length = velocity · run time = v / ε_{si}
- Agreement with experimental data on kinesin-1:



Run length decreases with increasing force F and increases with increasing [ATP]

Exp. data: Schnitzer et al, *Nat, Cell. Biol.* (2000)

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

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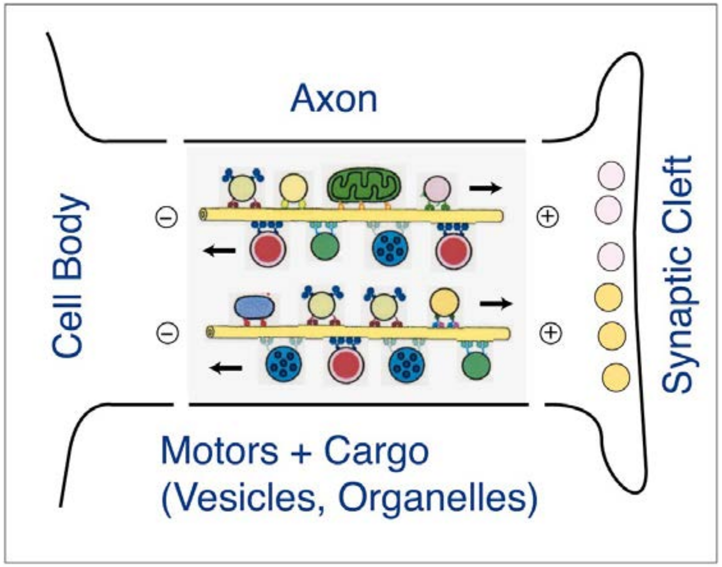
Intracellular Cargo Traffic

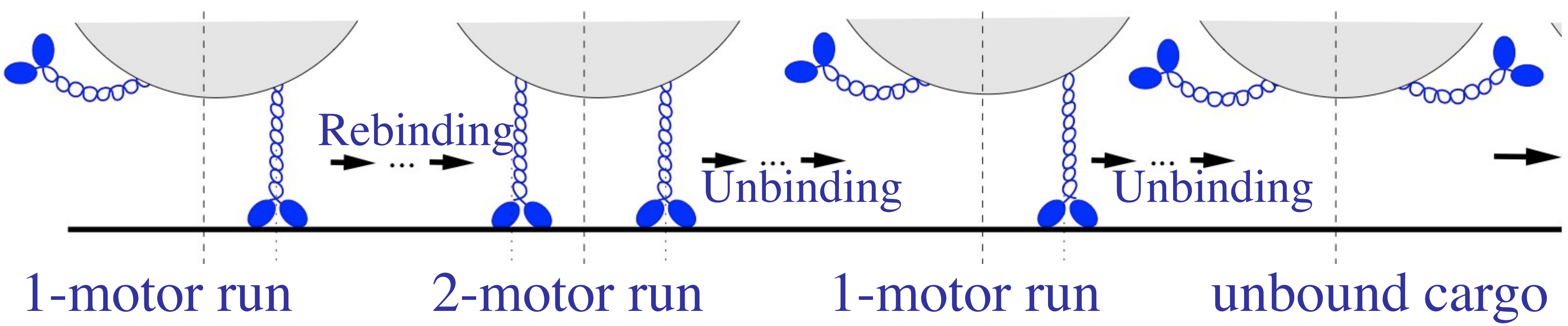
- Example: Neuron, axon, and synapse



- Each cargo pulled by several motors:
  - Uni-directional transport by one motor team
  - Bi-directional transport by two motor teams

Transport by Two Molecular Motors

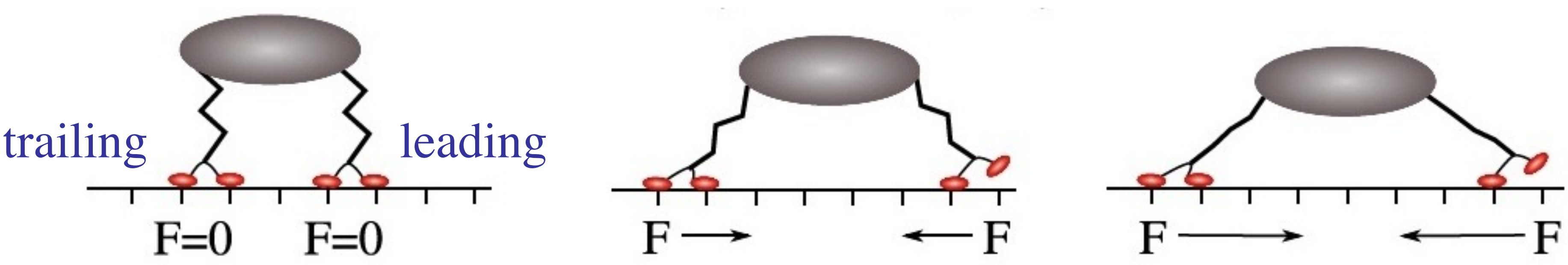
- Cargo pulled by two (identical) motors
- Each motor has a finite run length
- Start with cargo pulled by one motor = 1-motor run
- Rebinding of unbound motor: 1-motor run becomes 2-motor run



- Unbinding of bound motor: 2-motor run becomes 1-motor run
- Unbinding of remaining motor: unbound cargo

Elastic Coupling between Motors

- Motor stalks attached to common cargo
- Both motors step stochastically (forward steps to the right)



relaxed springs,
mutual force F = 0

step by leading motor,
built-up of force F

several steps by
leading motor

- Effective spring with spring constant K

  Extension ΔL leads to mutual force $F = K\,\Delta L$

- New force scale: Strain force $F_K = K \cdot$ step size

Theoretical Description

Keller et al, *J. Stat. Phys.* (2013)

- Motors as mechano-enzymes:

  Each motor has two motor heads that hydrolye ATP

  Use chemomechanical network for single motor

  Case study: kinesin

Berger et al, *Phys. Rev. Lett.* (2012)

- Motors as stochastic steppers:

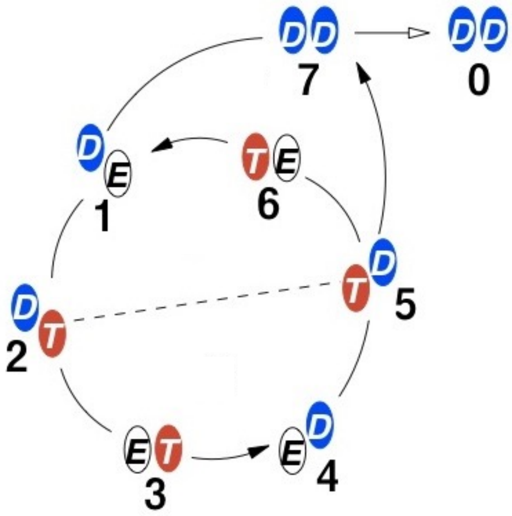
  Each motor steps forward and backward, unbinds and rebinds

  Use experimental force-velocity relationship

  Available for different kinesins, dyneins ...

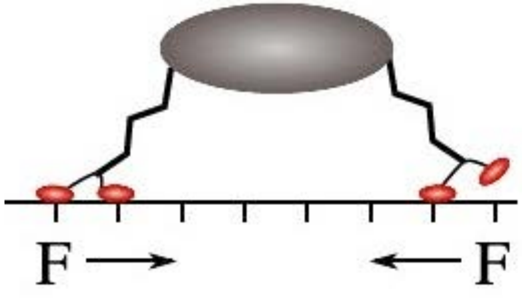
Motors as Mechano-Enzymes

- Each motor can attain
  seven bound states i = 1, ..., 7
  plus unbound state i = 0

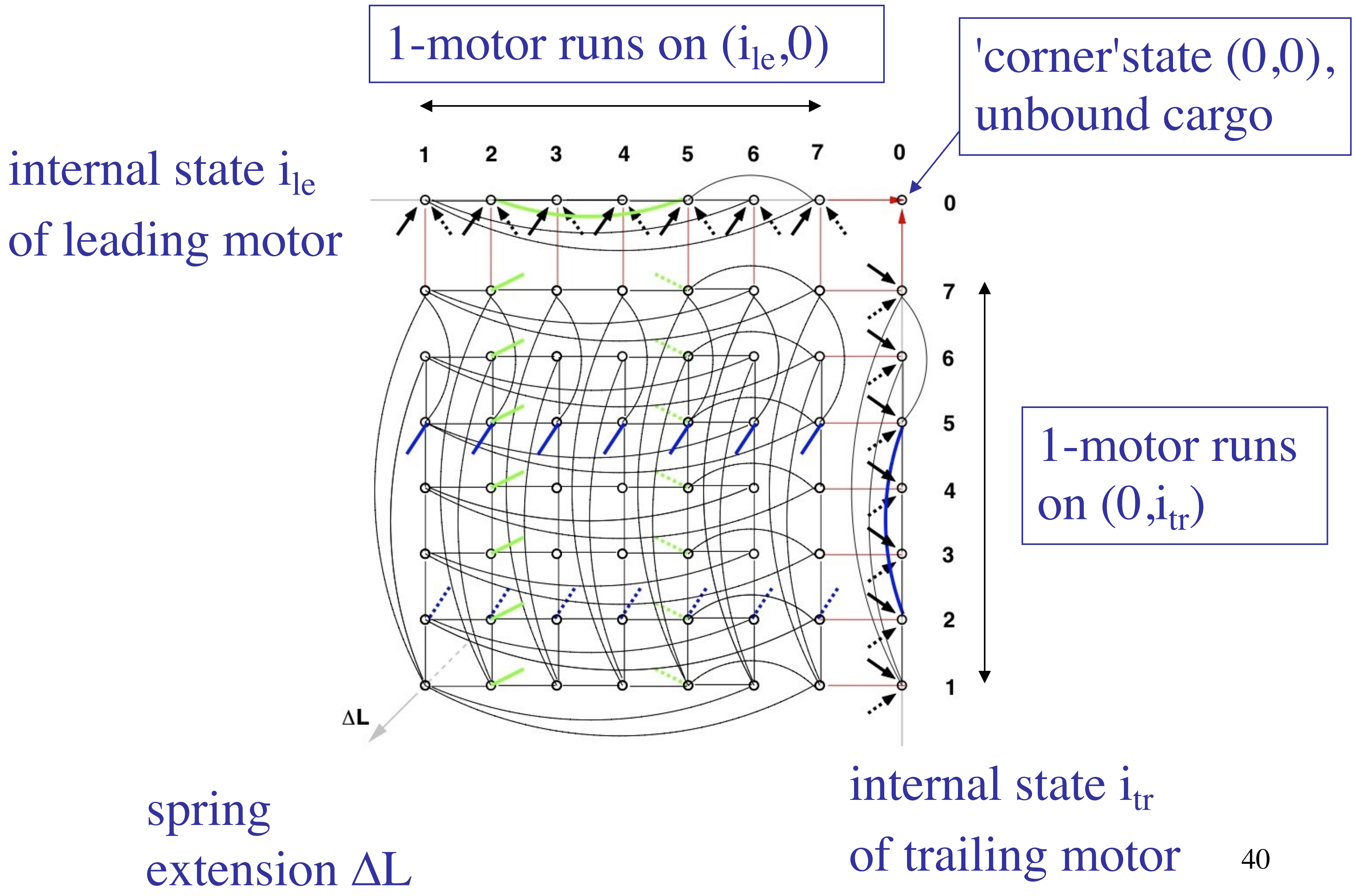


- Two internal coordinates
  leading motor $i_{le} = 1, ..., 7, 0$
  trailing motor $i_{tr} = 1, ..., 7, 0$

- Third coordinate = spring extension
  $\Delta L = F/K$

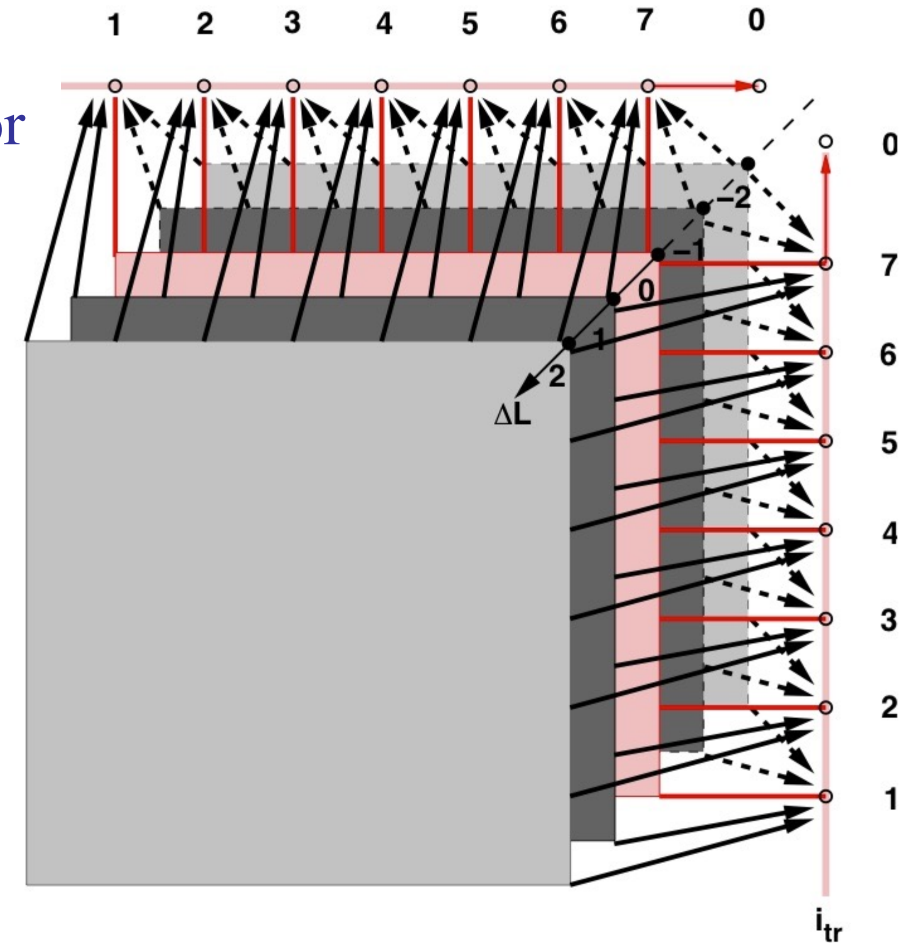


Subspace of Motor Couple with ΔL = 0



State Space of Motor Couple

internal state i_{le}
of leading motor



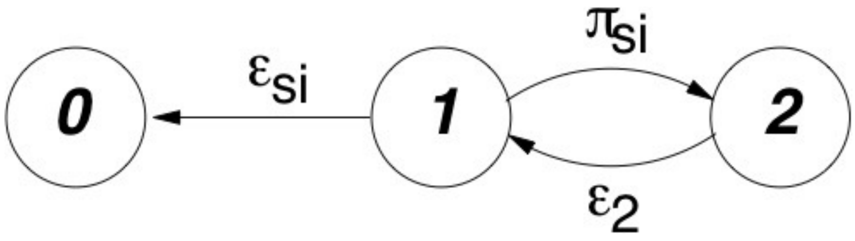
ΔL-planes with
constant ΔL

internal state i_{tr}
of trailing motor

neighboring ΔL-planes
connected by mechanical steps

Only Two Additional Parameters

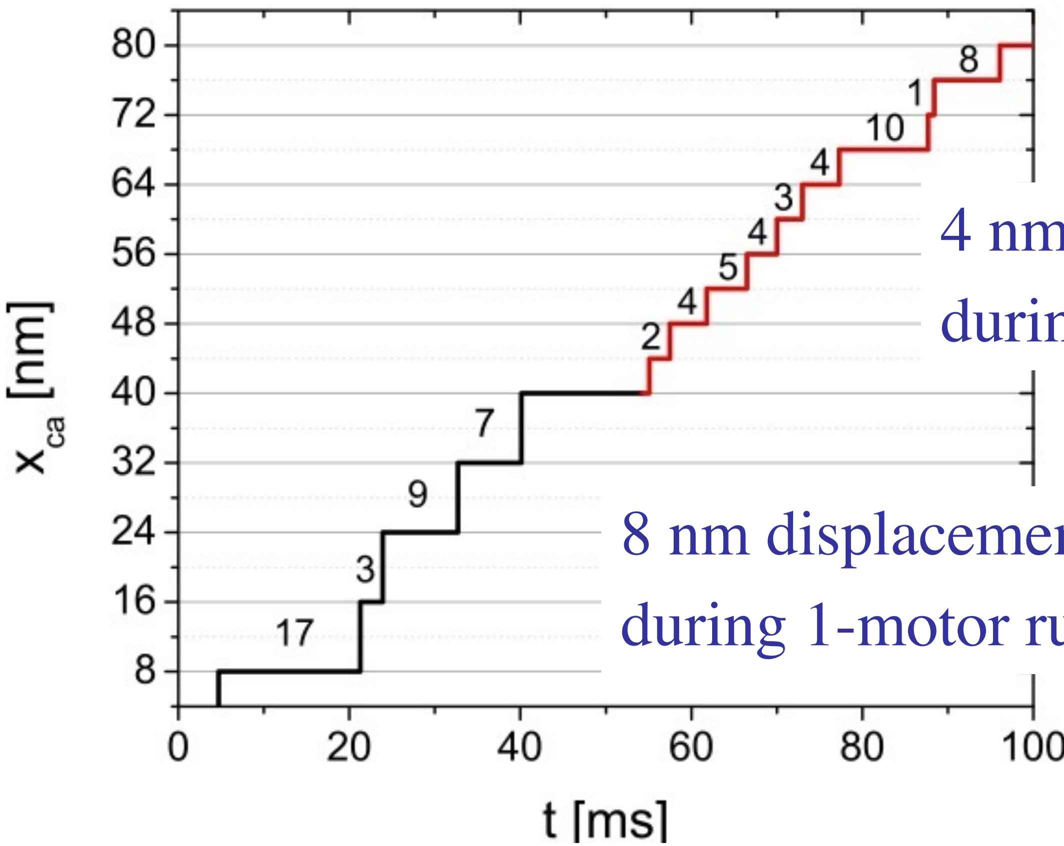
- Complex network but only two additional parameters, apart from single motor properties:
  - Spring constant K of effective spring
  - Rebinding rate π_{si} of single, unbound motor
- Rebinding rate π_{si} can be determined from statistical properties of 1-motor runs



- Spring constant K can be determined from statistical properties of 2-motor runs

Cargo Trajectories

- Determine spatial displacemens of cargo
- Cargo position for 2-motor runs: $\bar{x}_{ca} = \frac{1}{2}(x_{le} + x_{tr})$

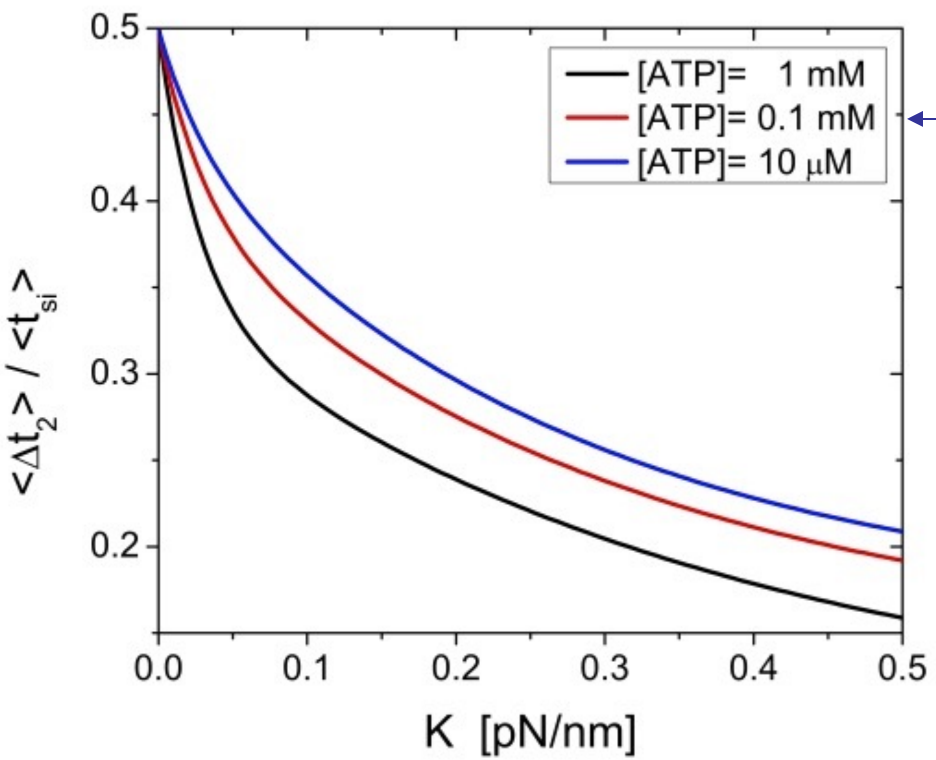


4 nm displacements during 2-motor runs

8 nm displacements during 1-motor runs

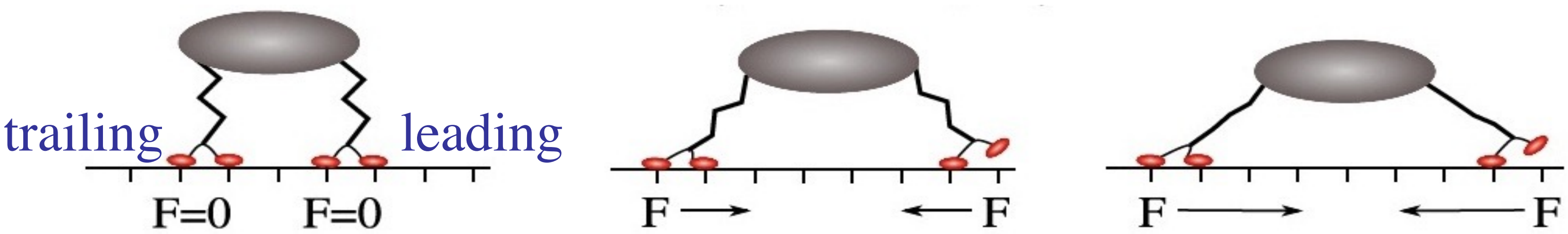
Cargo Trajectories II

- Average run time of 1-motor runs => rebinding rate π_{si}
- Average run time of 2-motor runs => spring constant K



dependence on ATP-concentration

Motors as Stochastic Steppers



- Reduced state space with coordinate ΔL only
- Single step leads to strain force F_K
- Slow build-up of elastic strain:
  Spontaneous unbinding of one motor
- Fast build-up of elastic strain:
  Force-induced unbinding or
  Force-induced stalling of one motor

Different Transport Regimes

- Compare strain force F_K with detachment force F_d and stall force F_s

- Weak coupling regime:

  F_K small compared to both F_s and F_d

  Spontaneous unbinding of one motor

- Force-induced unbinding regime for $F_d \ll F_s$

  F_K is comparable to F_d but small compared to F_s

- Force-induced stalling regime for $F_s \ll F_d$

  F_K is comparable to F_s but small compared to F_d

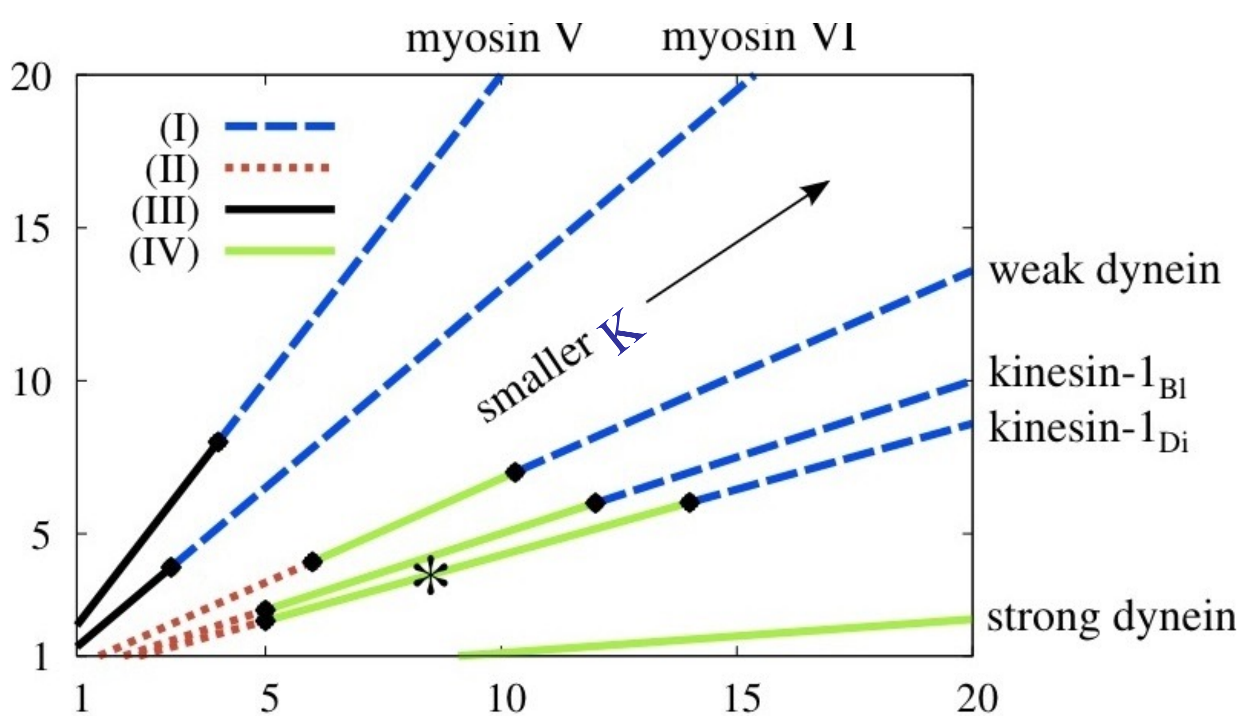
- Strong coupling regime: $F_K \approx F_d \approx F_s$

Variation of Elastic Coupling

- Effective spring constant K as control parameter
- Variation in value of K:

  Different motor species explore different transport regimes

reduced detachment force F_d / F_K



reduced stall force F_s / F_K

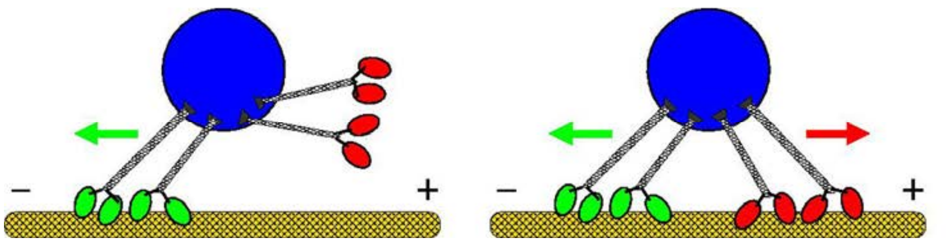
Cargo Transport in General

- Transport by N>2 identical motors

Klumpp and RL, *PNAS* (2005)

- Transport by two antagonistic motor teams, Stochastic tug-of-war

Müller et al, *PNAS* (2008)

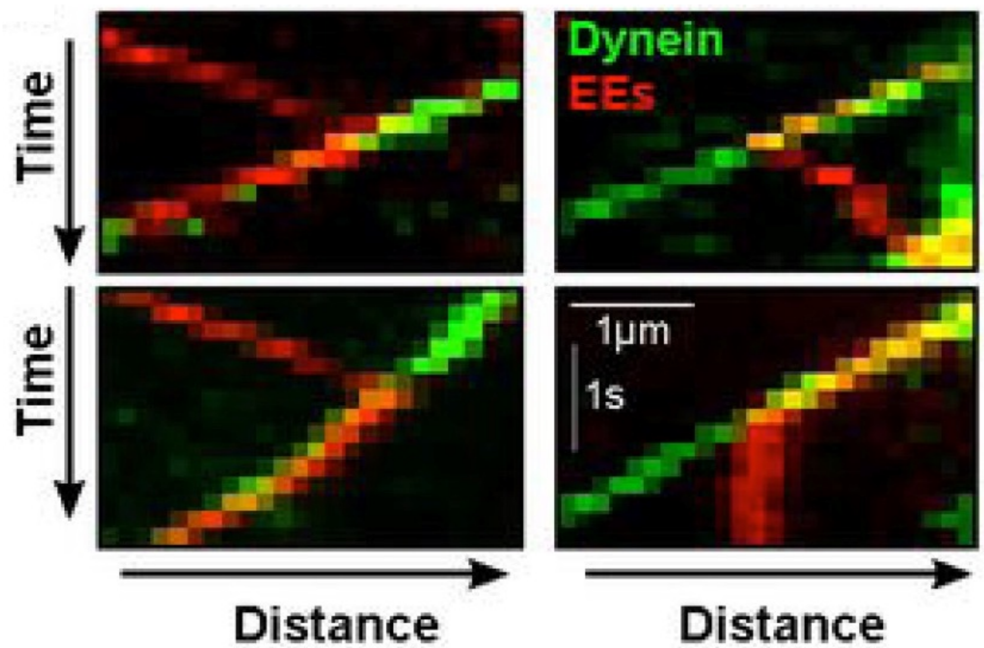


Tug-of-War for Endosomes

- Dictyostelium:
  Several dyneins against one kinesin
  Elongation of cargo during slow movements

Soppina ... Mallik
PNAS (2009)

- Fungus (Ustilago maydis):
  Binding and release of dynein

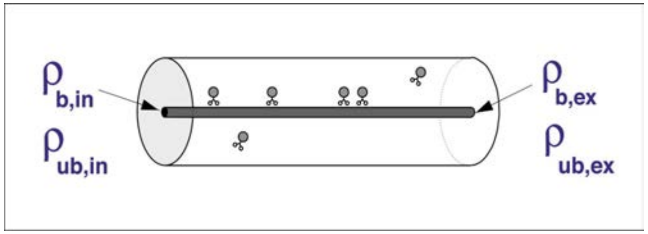


Schuster .... Steinberg,
PNAS (2011)

Motor Traffic: Patterns and Phase Transitions

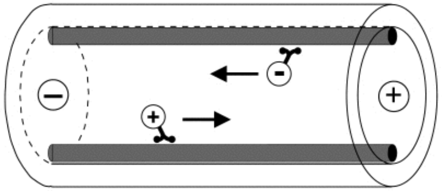
J. Stat. Phys. ***113*** *(2003)*

- Tube with two open boundaries:
  MT transitions related to ASEP phases



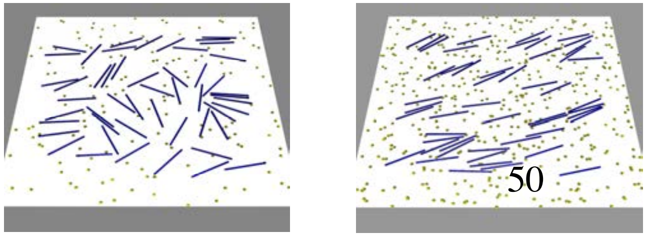
Europhys. Lett. ***66*** *(2004)*

- Traffic of two motor species in tubes:
  Symmetry breaking MT transition



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

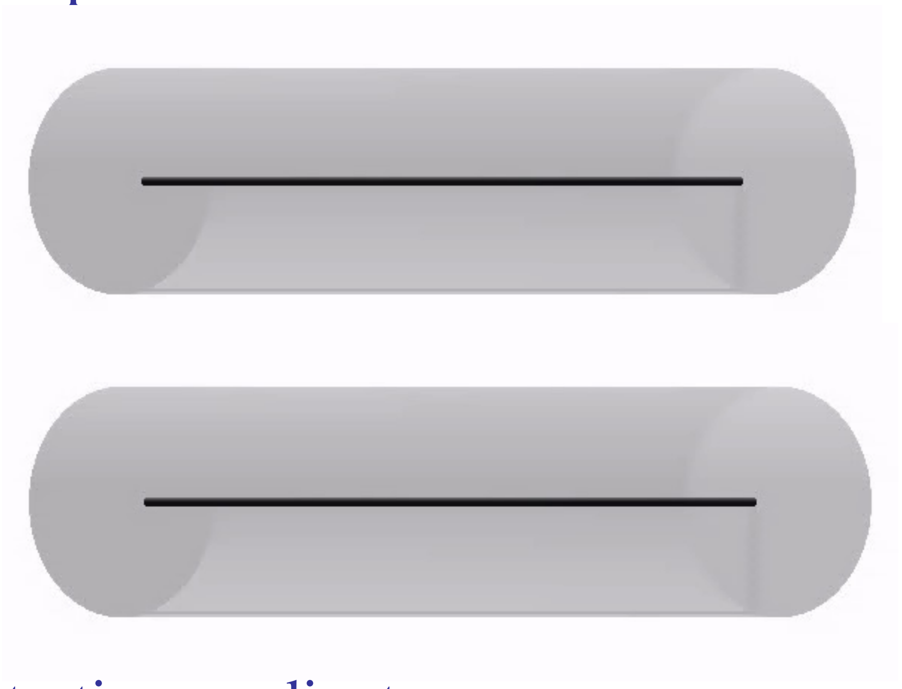
- Traffic of filaments along substrates:
  Isotropic-nematic MT transition



Traffic in a half open tube

M. Müller et al, J. Phys. CM 17 (2005)

- Half open tube:
  left boundary open, reservoir of motors = 'cell body'
  right boundary closed = 'Synapse'



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

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

Concentration gradients
created by motors

Max Planck Institute of Colloids and Interfaces
Active Night Life !