Cooperative Transport of Cargo by Molecular Motors

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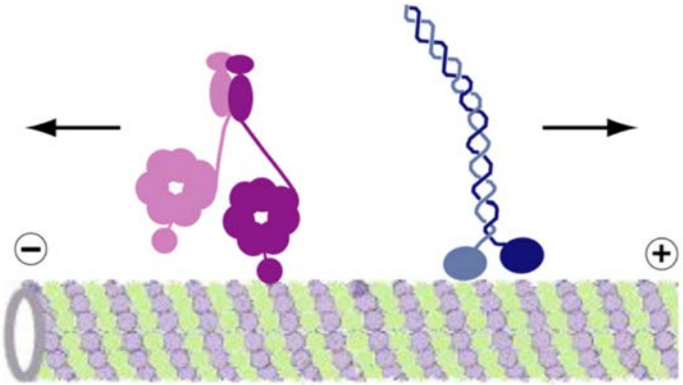
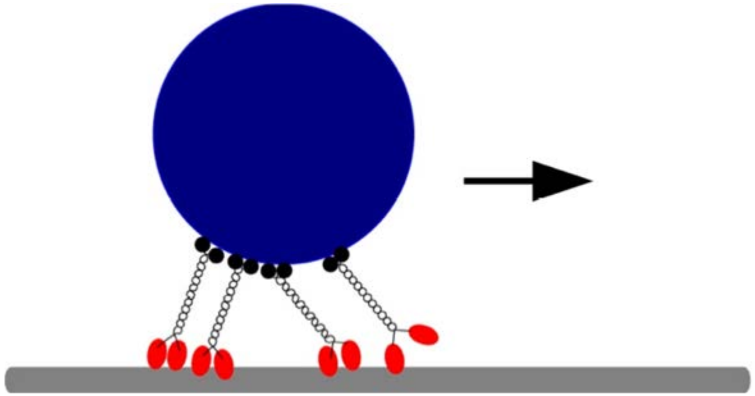
- Multiscale Motility
- Single motor properties
- Cooperative Cargo Transport
- Tug-of-war with elastic coupling
- Outlook: Motor Traffic

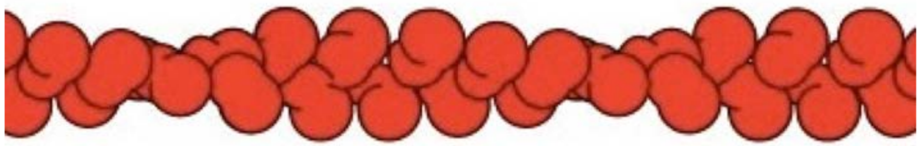
Biomolecular Machines



- Stepping motors



- Motor teams



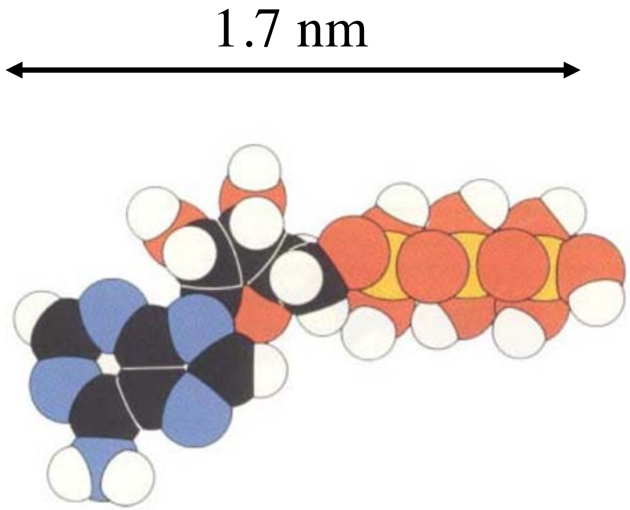
- Actin filaments

- 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!"

Chemical (Non)Equilibrium

- Molecular motors work at constant temperature
- Non-equilibrium processes driven by chemical unbalance
- ATP Hydrolysis: ATP <=> ADP + P

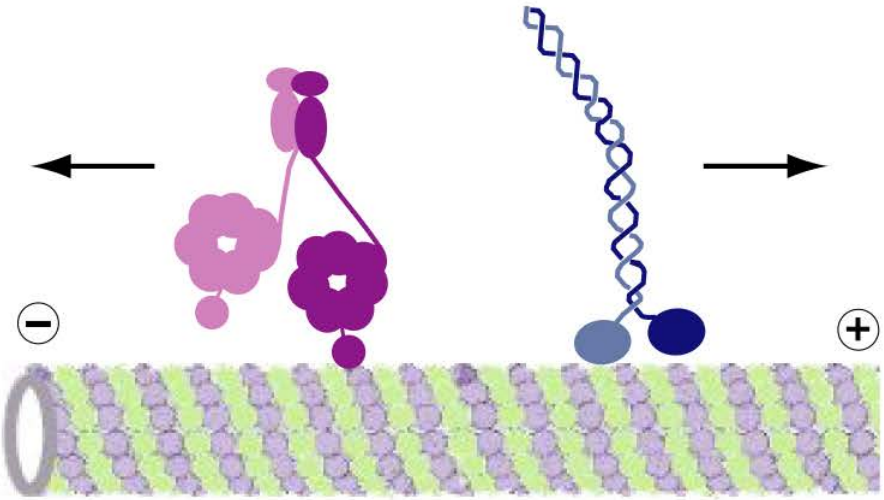
Reaction free enthalpy
per hydrolysed ATP :

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

- Equilibrium constant K_{eq}
- Chemical equilibrium $\Delta\mu = 0$
- ATP hydrolysis for $\Delta\mu > 0$
- ATP synthesis for $\Delta\mu < 0$

Stepping Motors

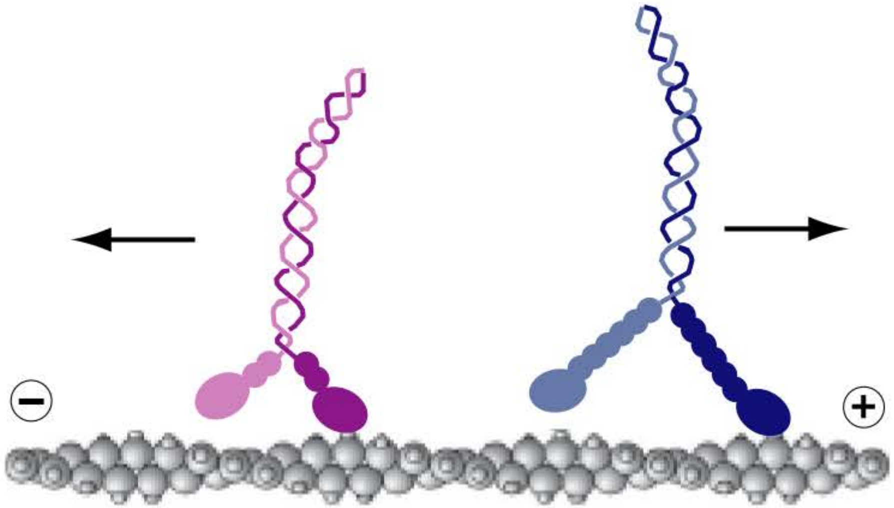
- Filament = Microtubule



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 motor domains = motor heads
- Each head acts as an ATPase, i.e., as an enzyme that catalyzes the hydrolysis of ATP
- Each motor makes steps with nanometer step size

Multiscale Motility of Motors

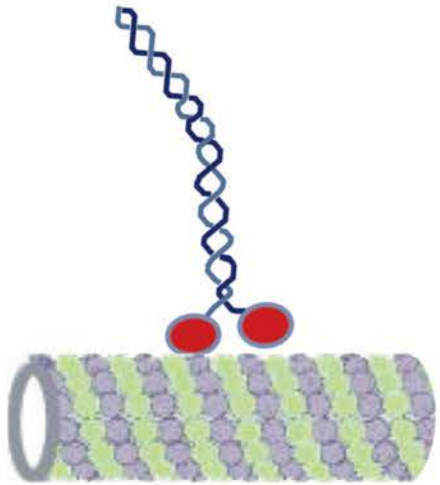
- Example: Kinesin at Microtubules

ATP Binding + hydrolysis

Nucleotide Binding Pocket ~ 1 nm

10^{-3} s

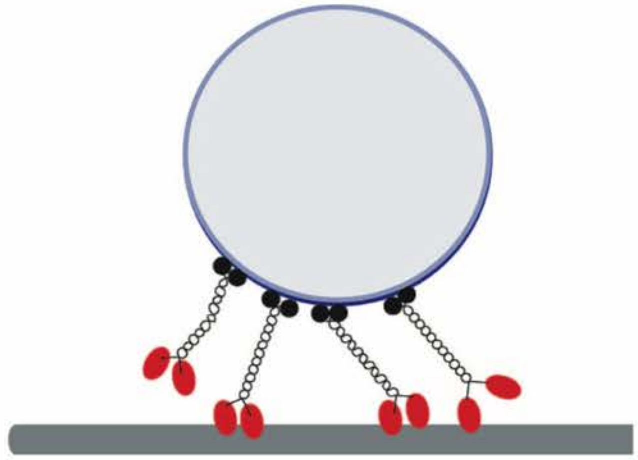
Mechanical step



Single head moves by 16 nm

10^{-6} s

Transport

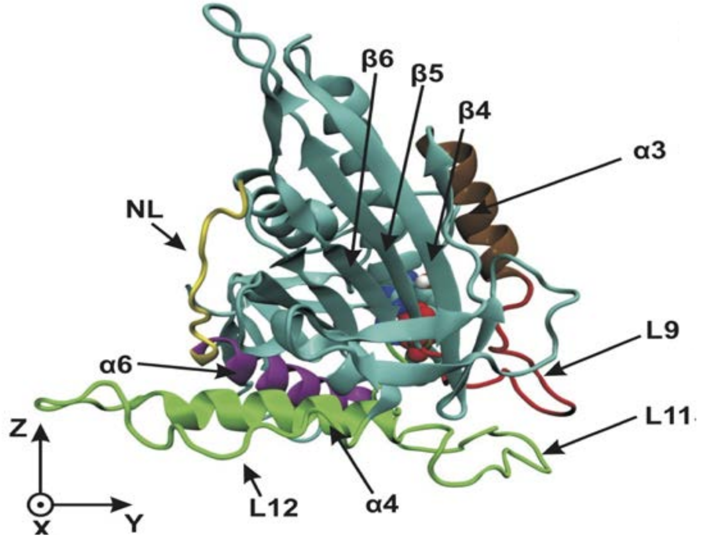


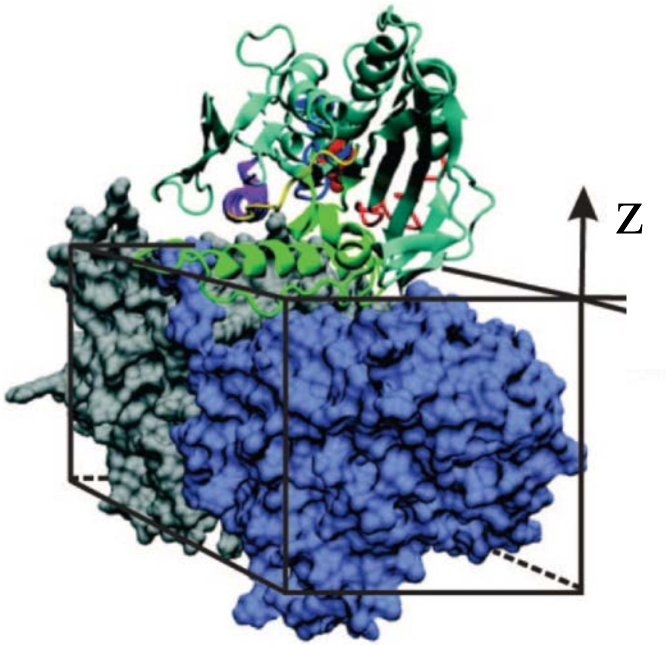
Cargo transport over cm or m !

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

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

Single Motor Head of Kinesin





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

Important subdomains:

- Nucleotide binding: L9 + α3
- Microtubule binding: L11 + α4
- Neck linker: NL + α6 helix

Allosteric coupling between subdomains:

- Conform changes of loops L9 and L11
- Rotation of helices α3 and α4

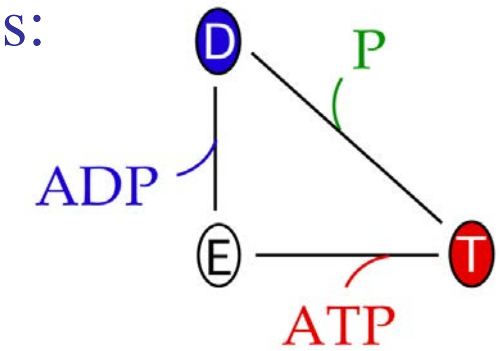
Different allosteric coupling in the absence and presence of tubulin

Stochastic Modelling: Single Head as 1-Site ATPase

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

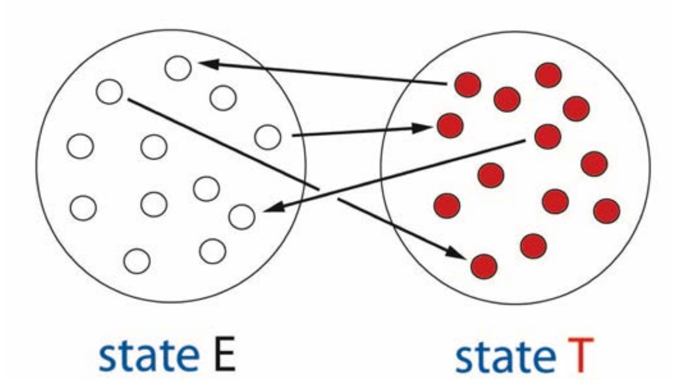
- Single head = single ATPase has 3 states:

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



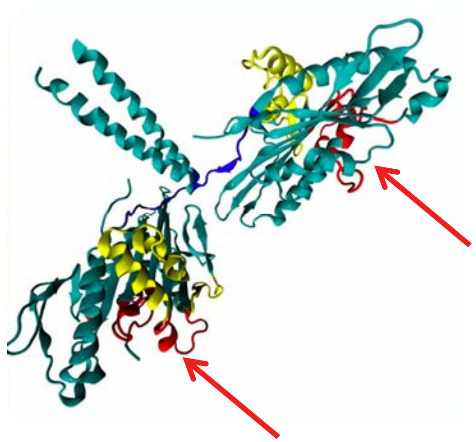
- In each state, head can attain many atomistic conformations:

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

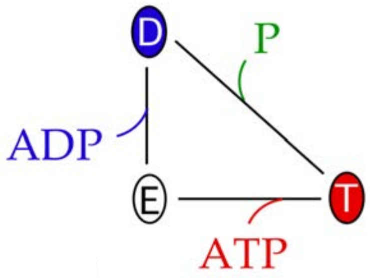


arrows = chemical transitions

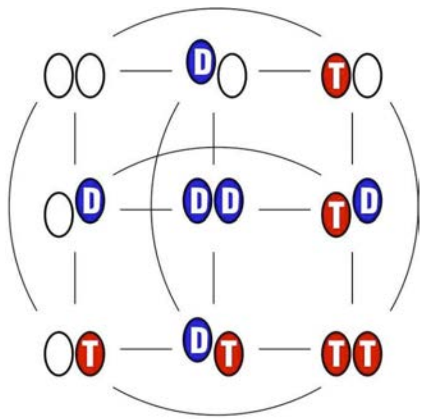
Kinesin as 2-Site ATPase



- Kinesin has two motor heads
- Each head can attain 3 states E, T, D that form one chemical cycle



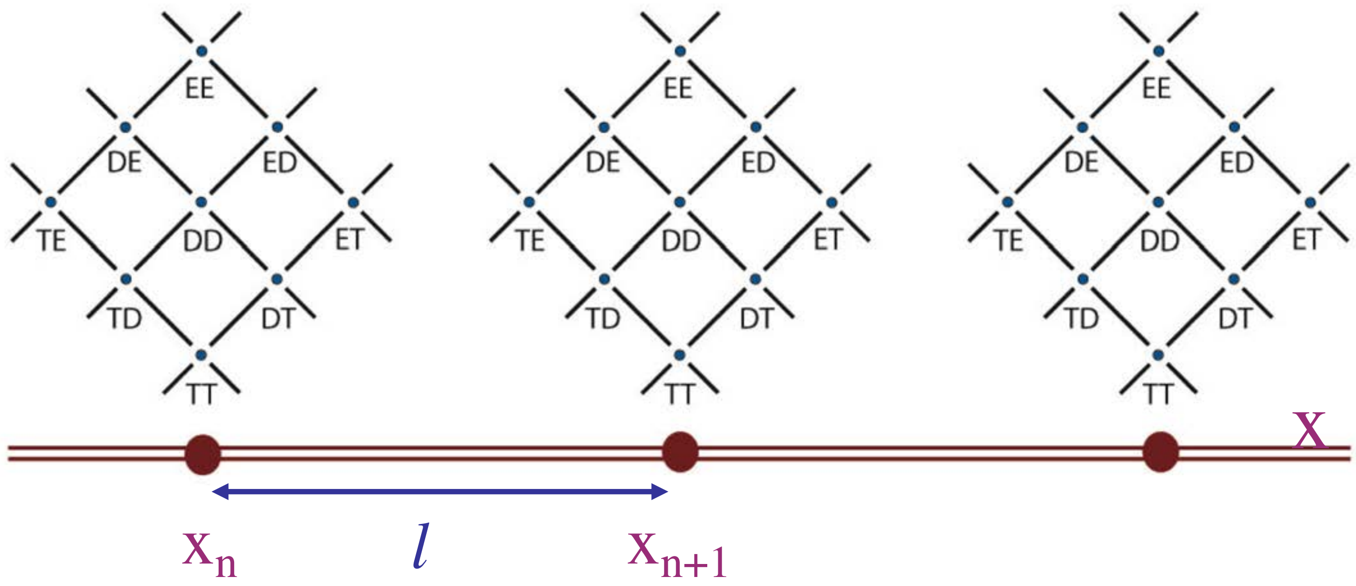
- Two heads can attain 3x3 = 9 states with 2 x 18 = 36 transitions



- States + transitions define chemical network with many cycles

Mechanical Transitions

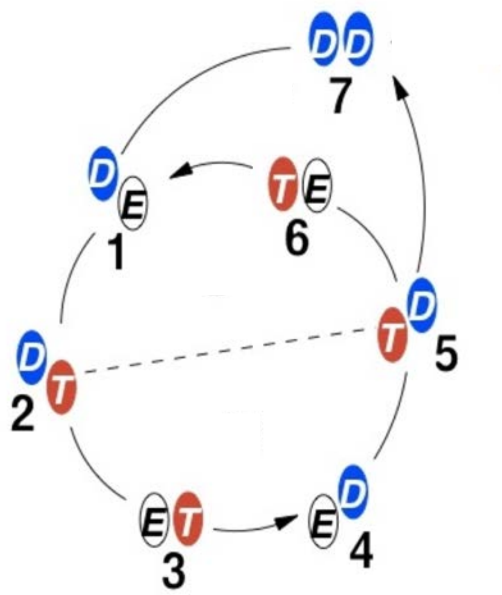
- Mechanical transitions = Spatial displacement along filament
- Discrete step size l defines lattice of motor positions:



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

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

Kinesin: Several Motor Cycles



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

Three chemomechanical motor cycles

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

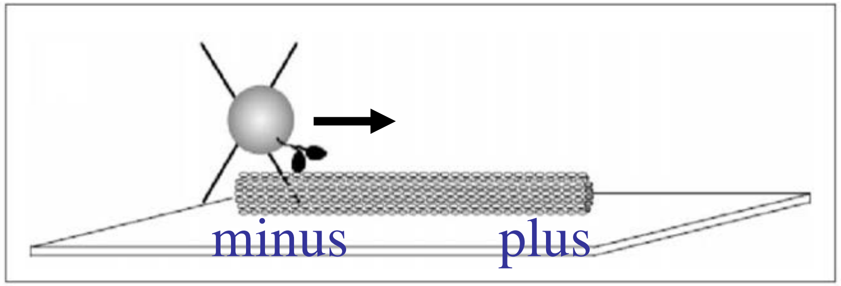
- Small ADP and P, small load force: dicycle |25612>
- Small ADP and P, large load force: dicycle |52345>
- Large ADP, small load force: dicycle |25712>

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

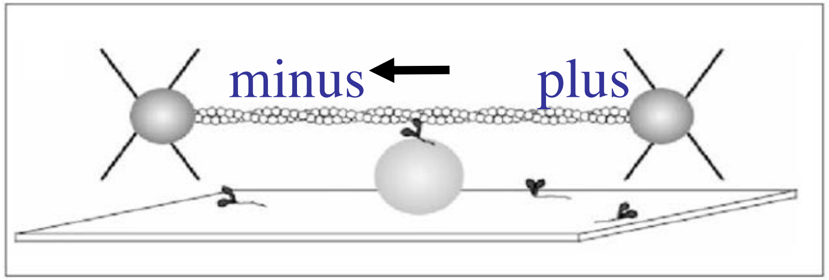
Single Motor Forces

- Motors attached to beads, force F applied to beads via laser traps

- Bead assay:



- Gliding assay:

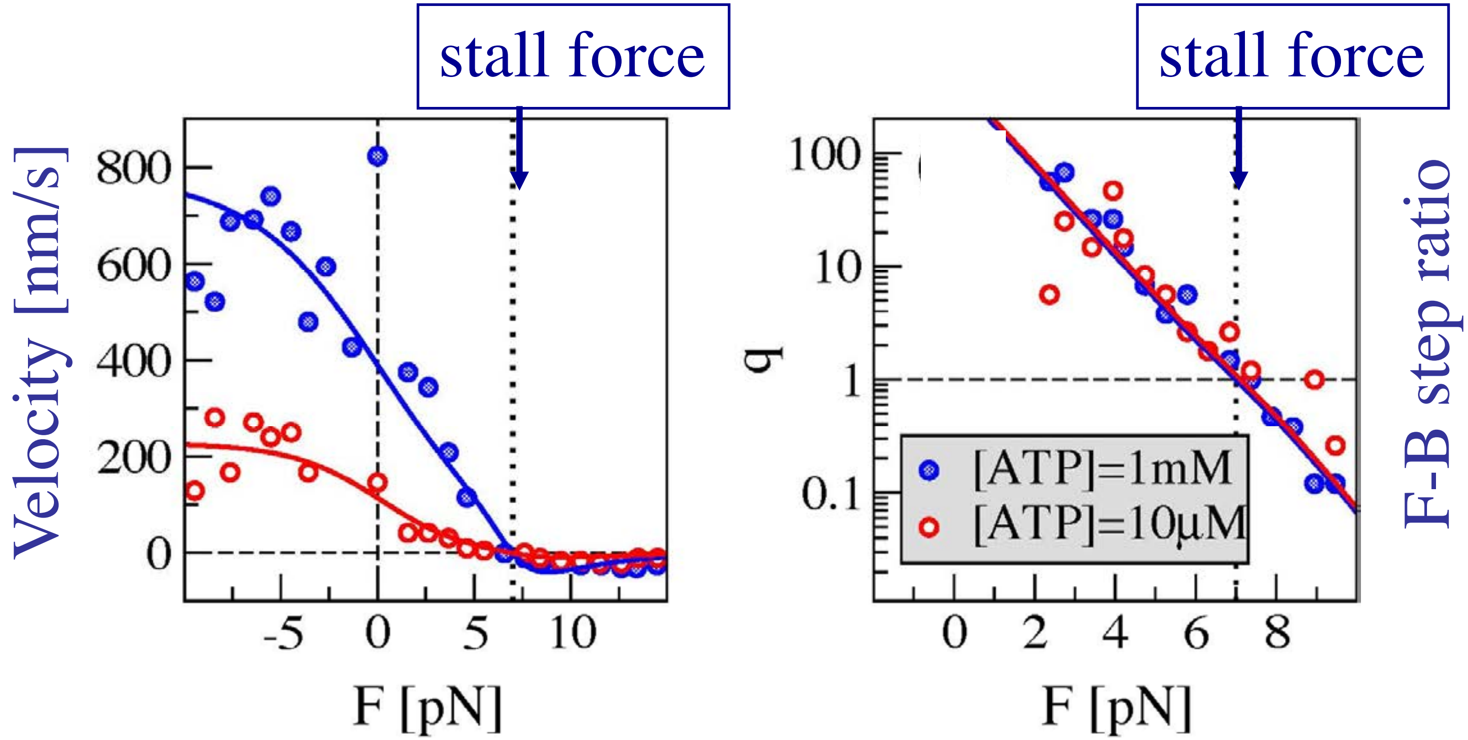


- Both mechanical and chemical transition rates depend on single motor force F : $\omega_{ij} = \omega_{ij}(F)$

- Convention about sign of F : Resisting forces are positive,
  assisting forces are negative

Stall Force

- Motor velocity *v* decreases with increasing force *F*
- Velocity vanishes at stall force F_s



- Single motor can generate force up to stall force F_s

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

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

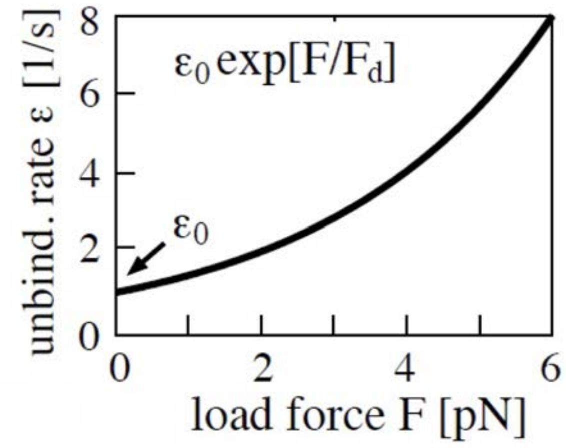
Detachment Force

- Thermal noise leads to unbinding of single motor from filament

- Unbinding rate ε is F-dependent:

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

Detachment force F_d



- Single motor can sustain force up to detachment force F_d

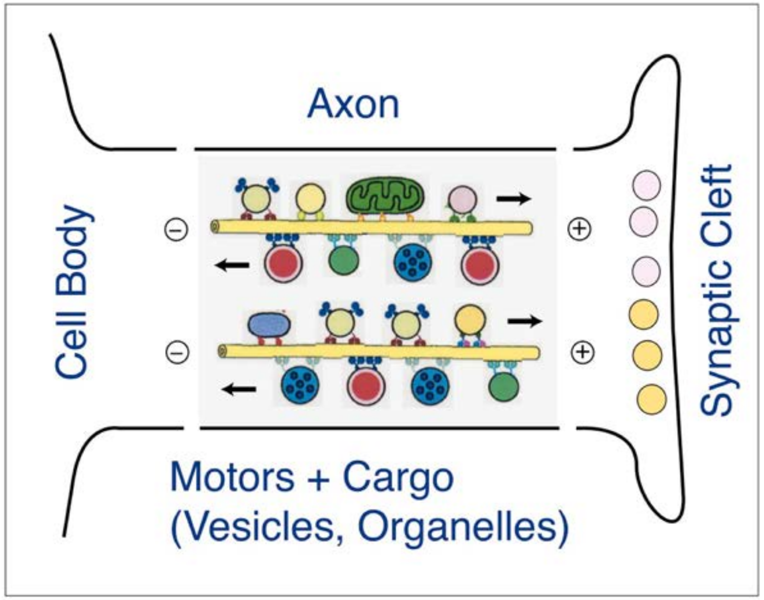
- Multiscale Motility
- Single motor properties

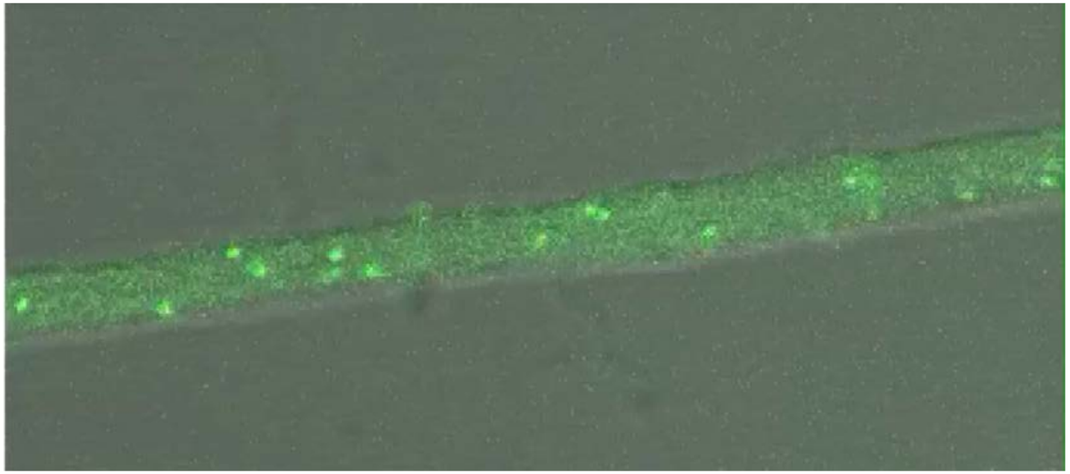
- Cooperative cargo transport
- Tug-of-war with elastic coupling

- Outlook: Motor traffic

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

Cargo Transport by Motor Teams

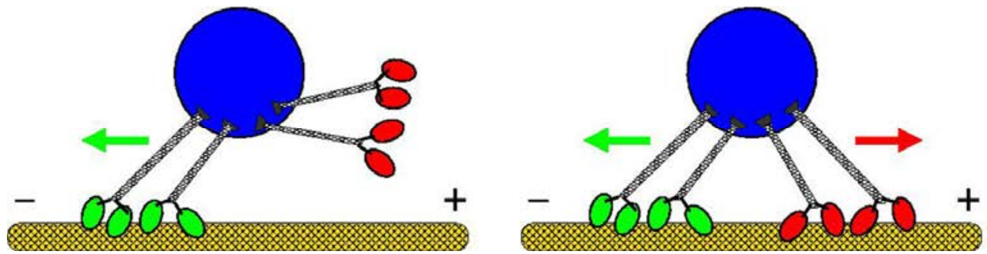
- Transport by N ≥ 2 identical motors

Klumpp, RL, *PNAS* (2005)

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

Müller, Klumpp, RL, *PNAS* (2008)

MKL model



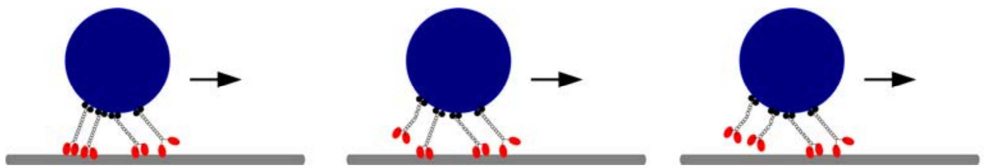
- Number of actively pulling motors varies with time:
  stochastic motor unbinding and rebinding

- Force balance between different motor-cargo forces

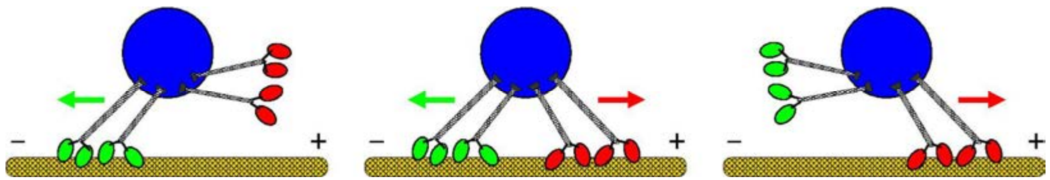
Basic Terminology

- Cargo attached to N_- minus and N_+ plus motors: $[N_- + N_+]$ system
- Examples:

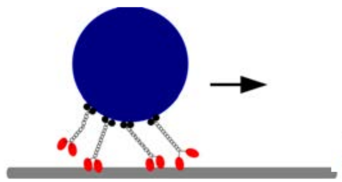
[4+0] system



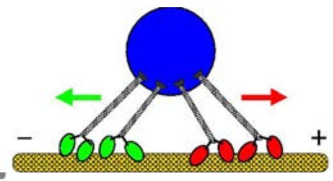
[2+2] system



- Define activity states by # of actively pulling motors = motors attached to filament
- Activity state (n_-, n_+) corresponds to $n_- \leq N_-$ actively pulling minus motors and $n_+ \leq N_+$ actively pulling plus motors



(0,3)



(2,2)

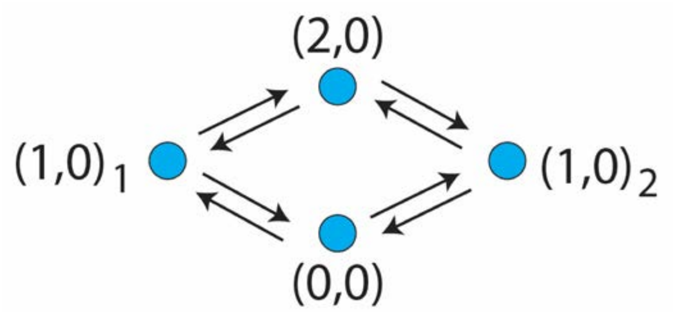
Activity States - Examples

- Motors identical but distinguishable
- Distinguish identical motors by some label
- Activity state (n_-, n_+) corresponds to a certain subset of motors

- Two identical motors: [2+0] or [0+2]

  Activity states of [2+0]-system:

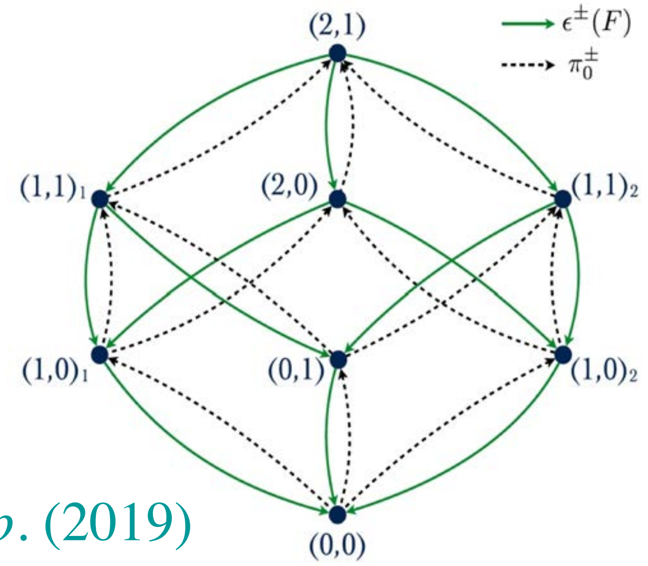
  $(0,0)$ $(1,0)_1$ $(1,0)_2$ $(2,0)$



- Two identical motors plus one antagonistic motor: [2+1] or [1+2]
- Activity states of [2+1] system

  $(0,0)$ $(1,0)_1$ $(1,0)_2$ $(0,1)$

  $(1,1)_1$ $(1,1)_2$ $(2,0)$ $(2,1)$



Ucar, RL, *Scientific Rep*. (2019)

Motor Dynamics – Original Models

- State space = network of activity states
- Motor dynamics via Markov process on state space
- Simplification: Coarse grain over discrete motor steps
- Assume equal force sharing between identical motors
- Impose force balance (Newton's 3rd law)
- Controversial: Effects of force fluctuations?
- Simplification: Ignore elastic motor-cargo linkers
- Controversial: Unequal force sharing from elastic forces?

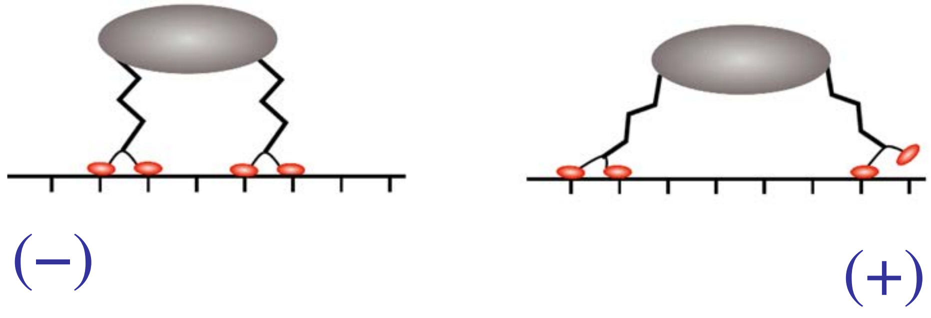
=> Improve theory by including both discrete motor steps and elastic motor-cargo coupling

Discrete Steps + Elastic Coupling

- Two identical motors:

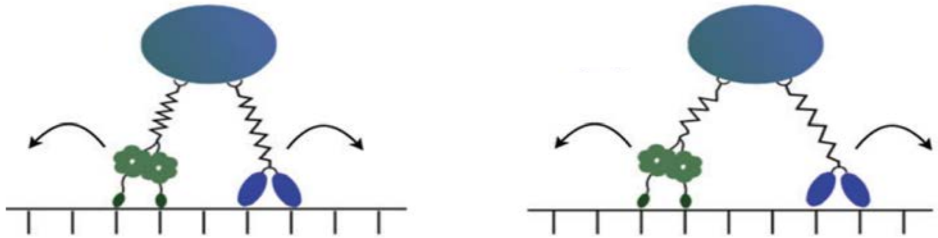
Berger et al, *Phys. Rev. Lett.* (2012)

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



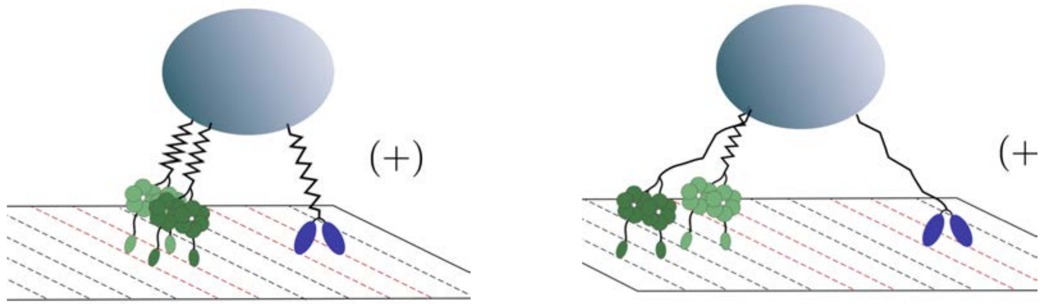
- 1 dynein + 1 kinesin

Ucar, RL, *Soft Matter* (2017)



- 2 dyneins + 1 kinesin

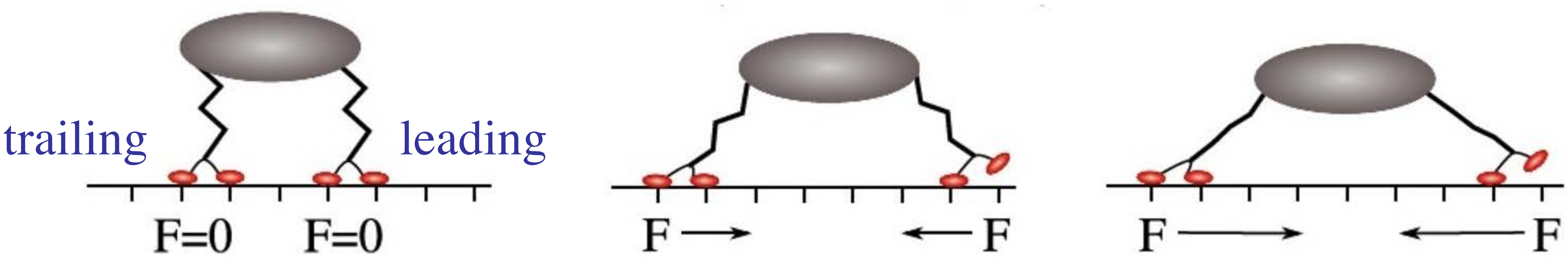
Ucar, RL, *Scientific Rep.* (2019)



Two Identical Motors

Berger, Klumpp, RL, *Phys. Rev. Lett.* (2012)

- Two identical motors 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

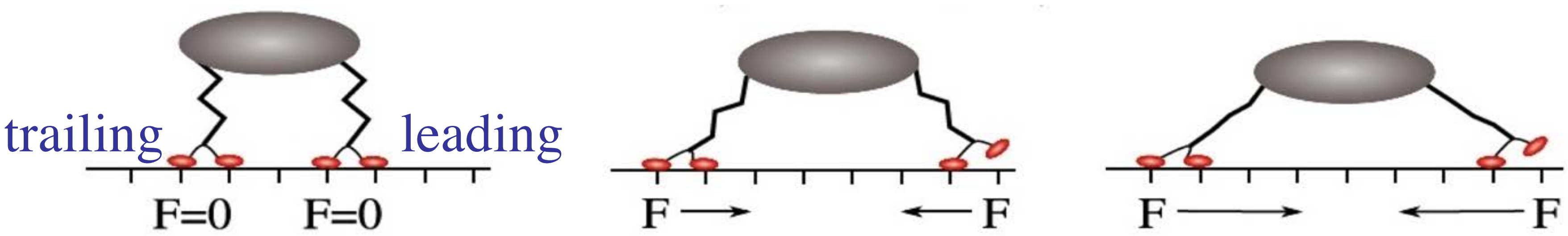
several steps by leading motor

- Effective spring with spring constant K

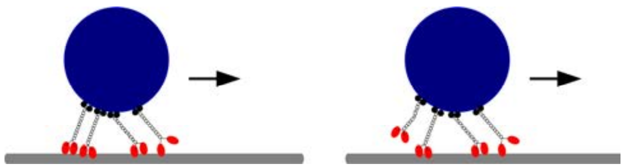
  Extension ΔL leads to mutual elastic force $F = K\,\Delta L$

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

Different Transport Regimes



- Reduced state space with coordinate ΔL only
- Single step leads to elastic 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

Elastic Motor-Cargo Forces

- Focus on certain activity state with $n_- + n_+ \geq 2$
- Subset of actively pulling motors labeled by j
- All active motors are elastically coupled to cargo
- Motor j exerts motor-cargo force $F_{j,ca}$ onto cargo
- Force balance (Newton's 3rd law): $\Sigma_\mathrm{j} F_{j,ca} = 0$
- Single motor forces:

$$F_j = -F_{j,ca} \quad \text{for minus motors}$$

$$F_j = +F_{j,ca} \quad \text{for plus motors}$$

- Force balance: $\Sigma_\mathrm{minus} F_j = \Sigma_\mathrm{plus} F_j$

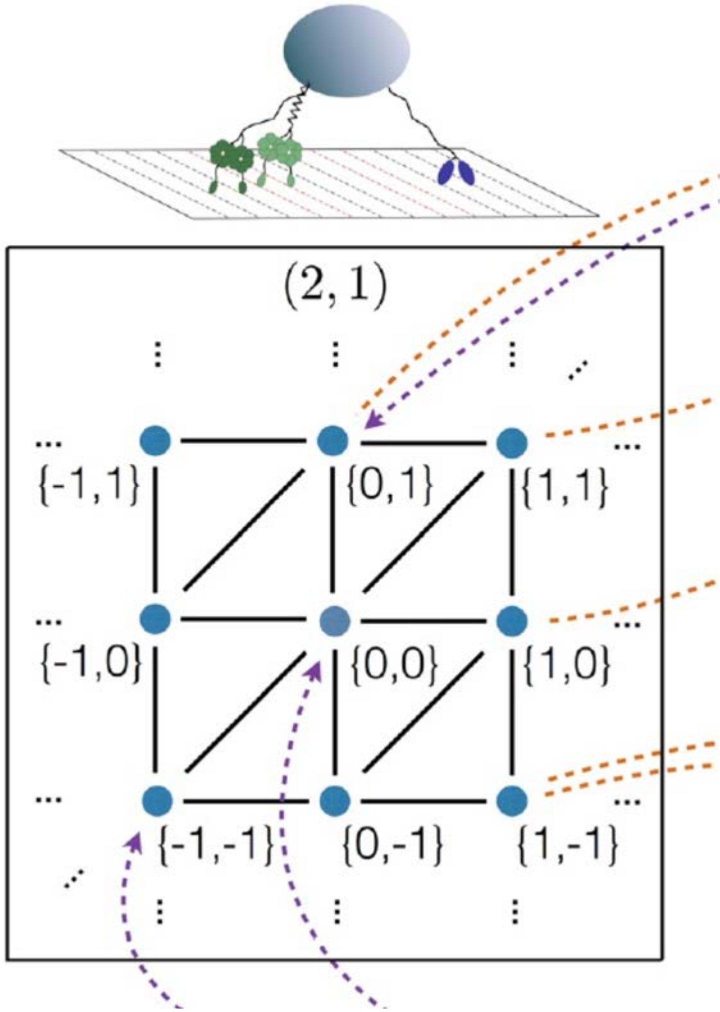
Elastic Substates

- Focus on certain activity state with $n_- + n_+ \geq 2$
- Mechanical forward or backward step of any motor:

  Motor-cargo forces change =>
  Single motor forces change =>
  Force-dependent transition rates change

- Starting from relaxed elastic couplings, each mechanical step leads to new elastic substate
- Naive expectation: Elastic substates of $n_- + n_+$ motors form $(n_- + n_+)$-dimensional lattice
- Force balance: Constraint that reduces dimensionality to $(n_- + n_+ - 1)$-dimensional lattice

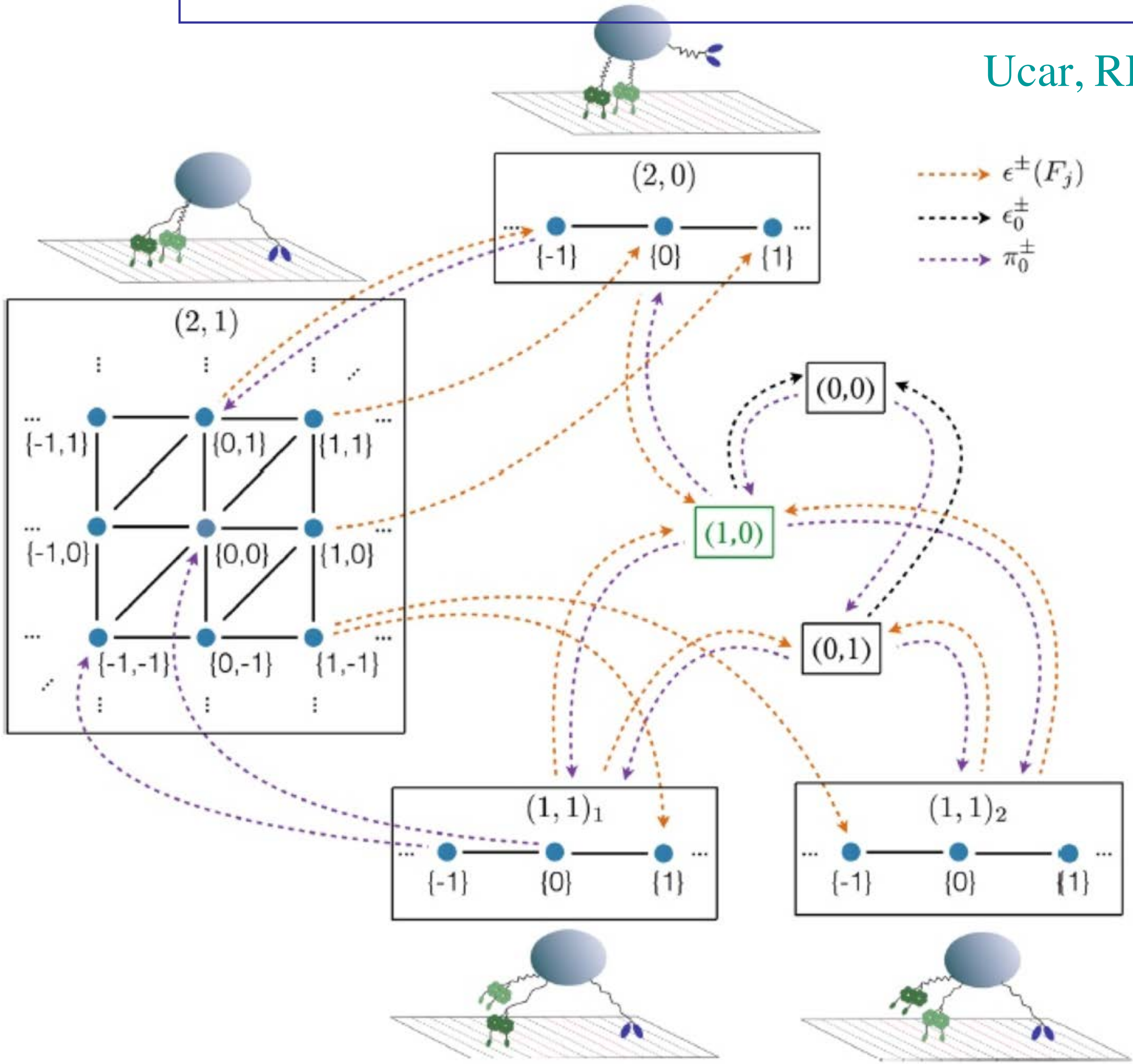
Elastic Substates for Activity State (2,1)



- $n_{-} + n_{+} - 1 = 2$
- Elastic substates form 2-dimensional lattice
- x-coordinate = 'separation' of dynein 1 from kinesin
- y-coordinate = 'separation' of dynein 2 from kinesin
- 'Separation' = # of discrete steps from relaxed state

Hierachical State Space

Ucar, RL, *Scientific Rep*. (2019)



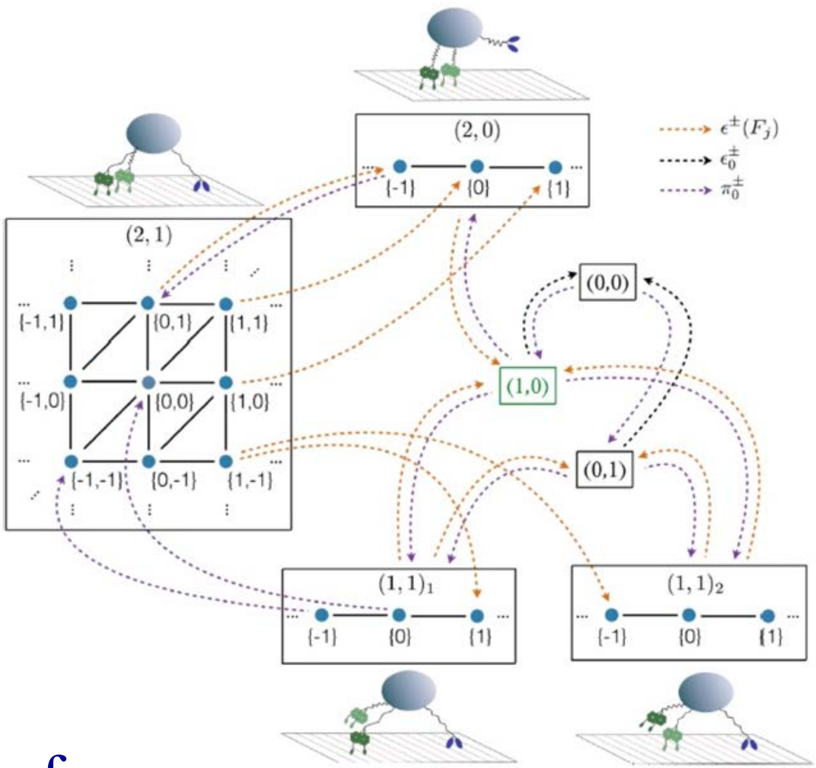
- Kinesin unbinding: (2,1) -> (2,0)
- Kinesin rebinding: (2,0) -> (2,1)
- Dynein_2 unbinding: (2,1) -> $(1,1)_1$
- Dynein_2 rebinding: $(1,1)_1$ -> (2,1)
- Dynein_1 rebinding: …

Dynamics of Motor System

- Continuous time Markov process on discrete state space, master equations
- Many transitions and transition rates
- But all rates can be deduced from single motor rates
- Steady state probability distributions
- Force distributions for each motor

  Strong force fluctuations reduce average forces by force-induced unbinding ...

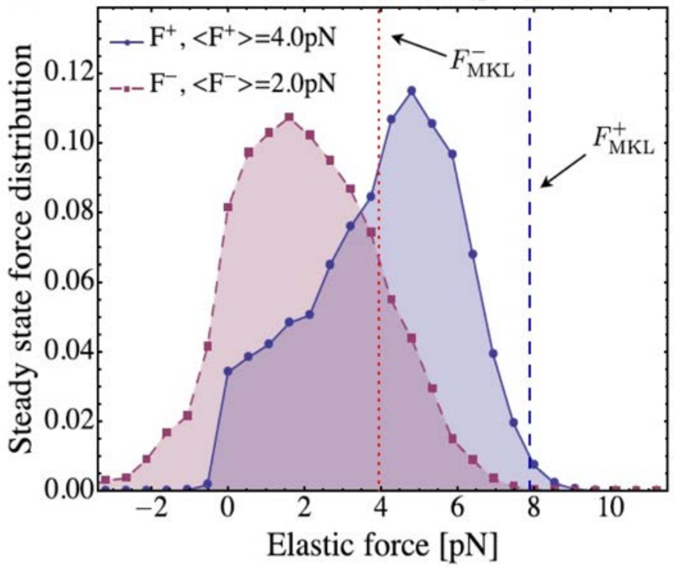
  ... but equal force sharing
- Time-dependent evolution of distributions



Strong Force Fluctuations

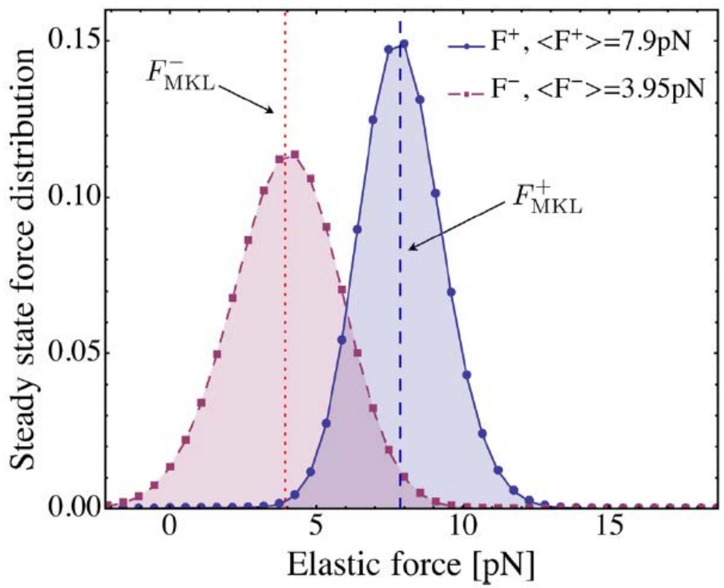
- Example: Strong dynein against kinesin-1
- Activity state (2,1) with two dyneins plus one kinesin
- Distributions of single motor forces
- Strong dependence of average force on unbinding rates:

Realistic unbinding rates, 1/s



Compared to MKL: Significant reduction of average force

Small unbinding rates, 1/100s



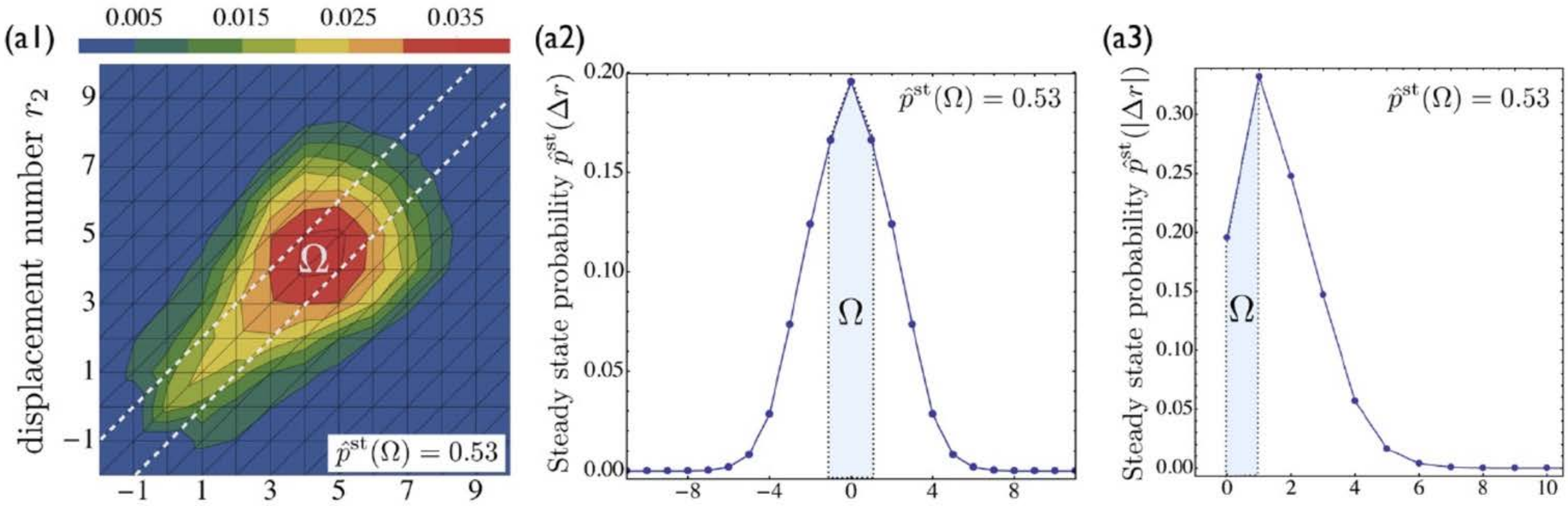
Compared to MKL: Same average force

Ucar, RL, *Scientific Rep.* (2019)

Equal Force Sharing

Ucar, RL, *Scientific Rep*. (2019)

- To address force sharing: Activity states (2,1) or (1,2)
- Example: Two strong dyneins and one kinesin-1
- Steady state distributions:



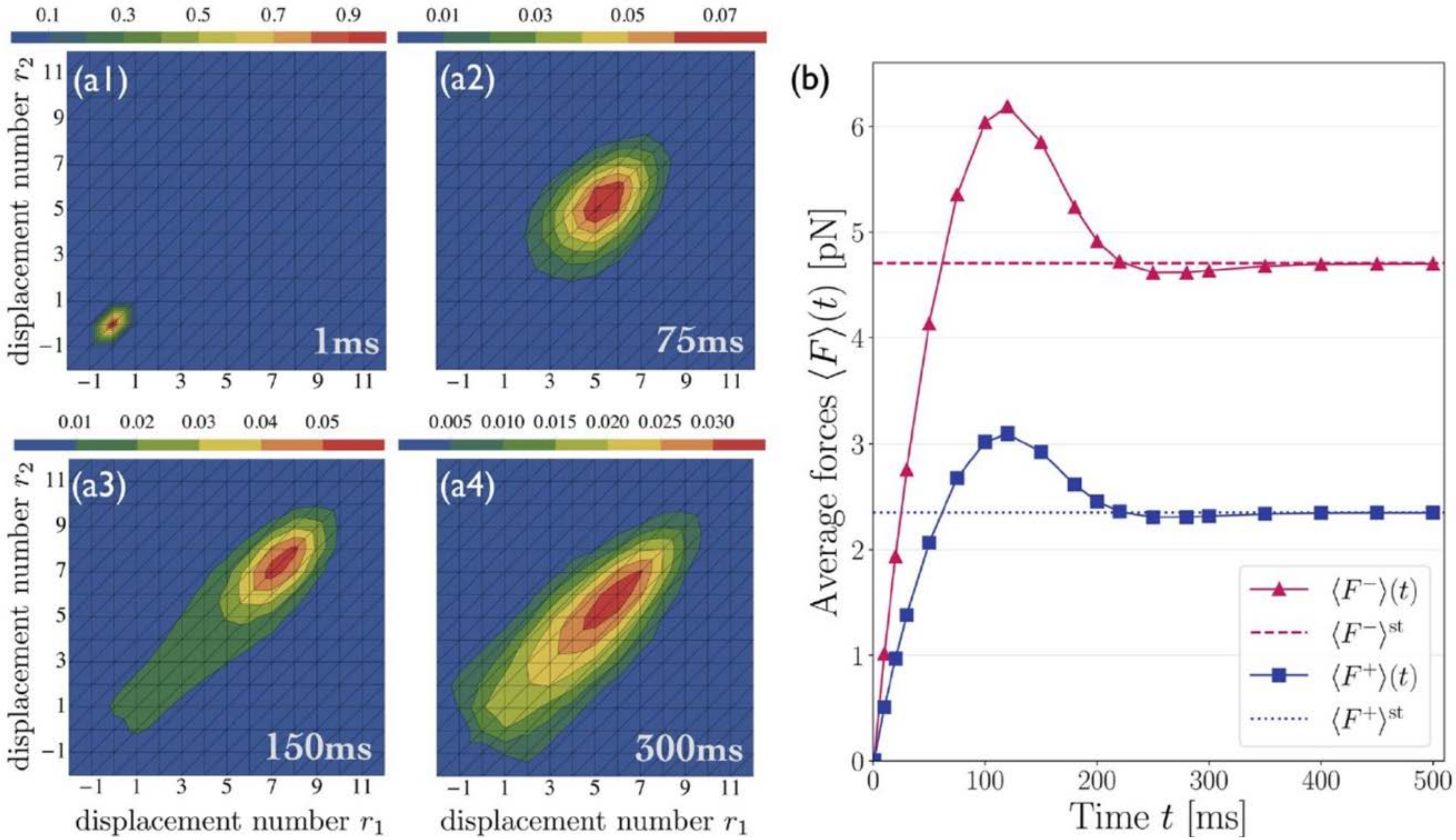
Probability distribution for elastic substates: single maximum along diagonal

Distributions for spatial separation *r* and absolute value |*r*| of two dyneins

Build-Up of Elastic Forces

Ucar, RL, *Scientific Rep.* (2019)

- Time evolution of force distributions and average forces:



Maximum disappears for small unbinding rates

Summary: Elastic Tug-of-War

- Discrete state space = activity states plus elastic substates
- Activity state with n_- minus and n_+ plus motors: elastic substates form a $(n_- + n_+ - 1)$-dimensional lattice
- Many transitions and transition rates but all rates are based on single motor rates
- Strong force fluctuations + equal force sharing
- For realistic values of the unbinding rates: significant reduction of single motor forces
- Limit of small unbinding rates: average forces approach average force of MKL model

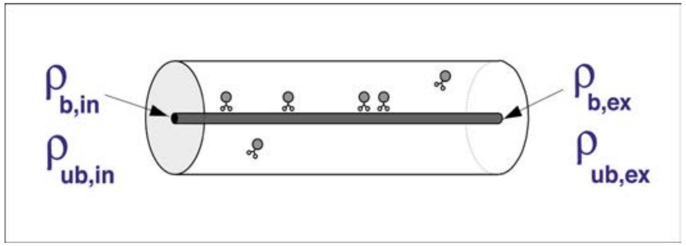
=> Elastic tug-of-war improves MKL model and allows to determine additional force-dependent properties

Outlook on Motor Traffic: Patterns and Phase Transitions

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

- Tube with two open boundaries:

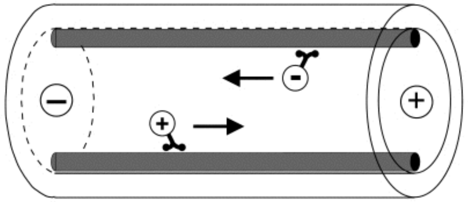
  MT transitions related to ASEP phases



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

- Traffic of two motor species in tubes:

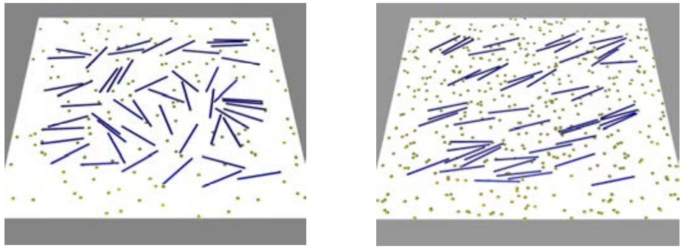
  Symmetry breaking MT transition



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

- Traffic of filaments along substrates:

  Isotropic-nematic MT transition



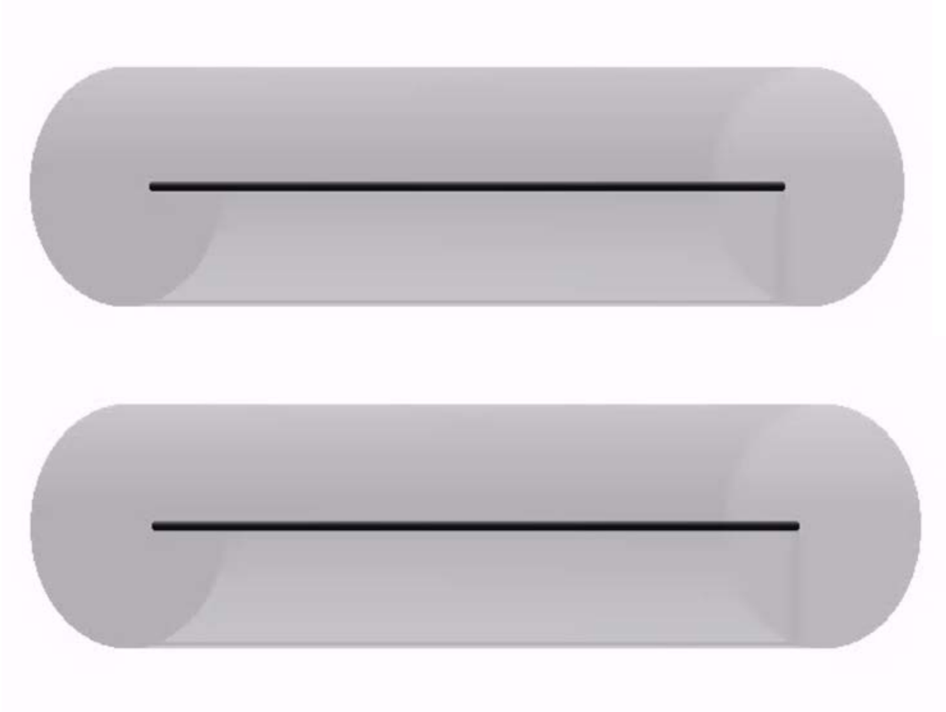
Traffic in a half open tube

MKL, 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

Mehmet Ucar

- **Membranes**

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  Tom Robinson
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  Tripta Bhatia
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  Jan Steinkühler

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