Mechanics of the kinesin-based transport:
From single-molecule to multi-motor behaviors, to cell division

Wonmuk Hwang
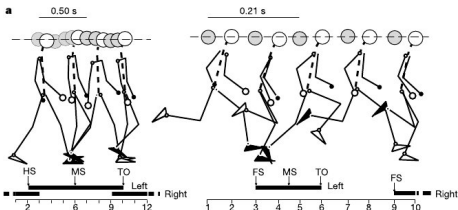hwm@tamu.edu

Departments of Biomedical Engineering
Materials Science & Engineering
Texas A&M University
College Station, TX

Korea Institute for Advanced Study
Seoul, Korea
NIH IMAG Webinar, June 24, 2013

TEXAS A&M
UNIVERSITY

Joshua Tree National Park; 2012/12/27

Motor phenomena in life: A top-down view

Biomechanics of locomotion

Bramble & Lieberman, Nature 432:345 (2004)

Intracellular transport
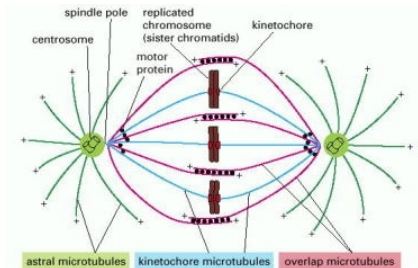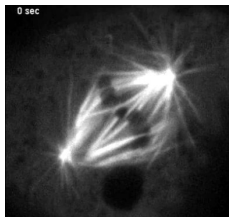

Vale, Cell 112:467 (2003)
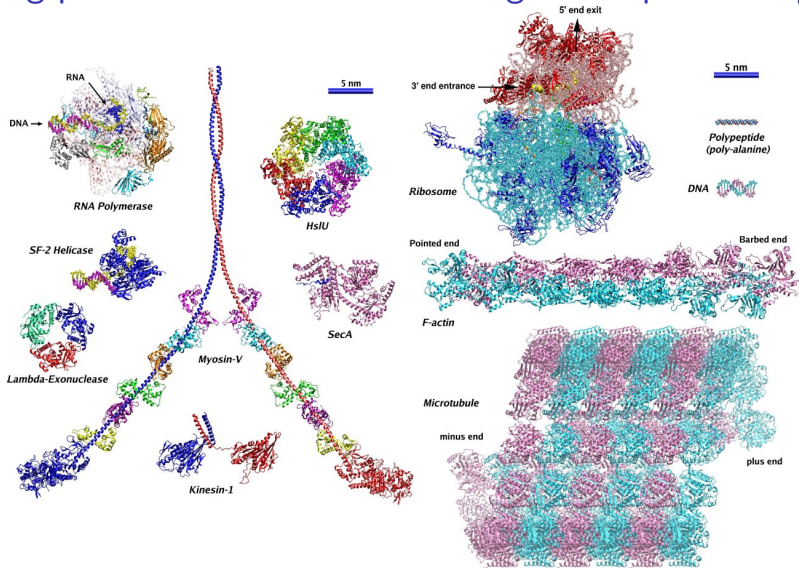
Cell division

Alberts *et al.*, Mol Biol of the Cell (4 ed, Garland)

Dynamic MT organization

Goshima, *et al.*, JCB 171:229 (2005)

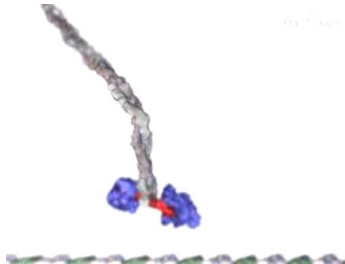# Big picture: How do translocating motor proteins operate?

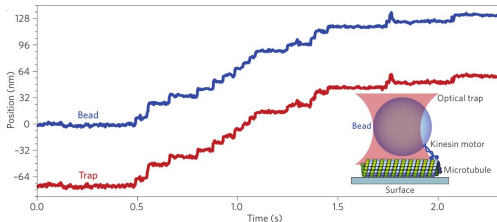*(Hwang & Lang, Cell Biochem. Biophys. 54:11 (2009))*

Emerging drug target: control traffic instead of nodes.

Need to understand from bottom-up

Kinesin-1: Semi-solo transporter

Vale & Milligan Science (2000)

Optical trap experiment resolving individual steps
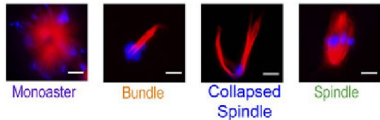

Fazal & Block, Nat Photonics 5:311 (2011)

Mechanical balance needed

Spindle architecture depends on motor processivity or force

Monoaster Bundle Collapsed Spindle Spindle
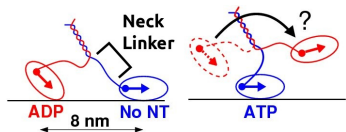
Cahu & Surrey JCS 122:1295 (2009)
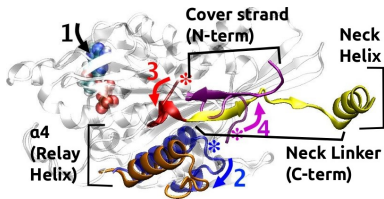
Multi-motor: cooperative or tug-of-war?
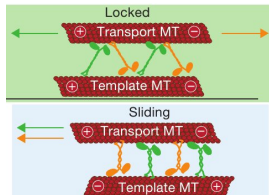
Fink *et al.*, Nat Cell Biol 11:717 (2009)
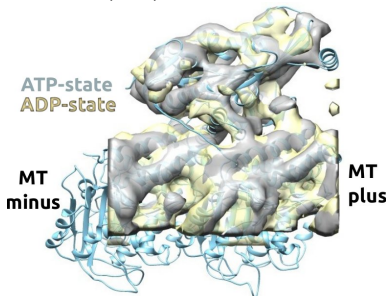
Motor ↔ Filament interaction?

Kin-1 force generation: Mechanochemical amplifier?

- 8-nm step/ATP
  Svoboda, Block et al., Nature (1993)
- ATP binding triggers a step
  Rice et al., Nature (1999)

PDB 1MKJ (ATP-like) & 1BG2 (ADP)

cryo-EM maps: Sindelar & Downing, PNAS (2010)

How does kinesin amplify small conformational changes in motor head to a large walking motion?
Use molecular dynamics (MD) simulation to find atomistic mechanism.

MD simulation in a nutshell

- Solve Newton's equation of motion for biomolecular structures in a *solvated* environment: $\vec{F} = m\vec{a} = -\vec{\nabla} U(\vec{R})$

$$U(\vec{R}) = \sum_{\text{bonds}} K_b(b - b_0)^2 + \sum_{\text{angles}} K_\theta(\theta - \theta_0)^2 + \sum_{\text{Urey-Bradley}} K_{UB}(S - S_0)^2$$

$$+ \sum_{\text{dihedrals}} K_\phi(1 + \cos(n\phi - \delta)) + \sum_{\text{impropers}} K_\omega(\omega - \omega_0)^2$$

$$+ \sum_{\text{non-bonded pairs}} \left\{ \epsilon_{ij}^{min} \left[\left(\frac{R_{ij}^{min}}{r_{ij}} \right)^{12} - 2 \left(\frac{R_{ij}^{min}}{r_{ij}} \right)^6 \right] + \frac{q_i q_j}{4\pi\epsilon r_{ij}} \right\}$$

$$+ \sum_{\text{residues}} U_{CMAP}(\phi, \psi) \qquad \text{(Brooks, \textit{et al}, J Comput Chem 30:1545 (2009))}$$
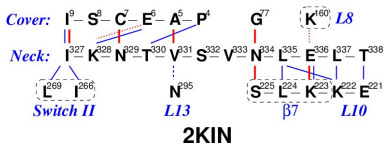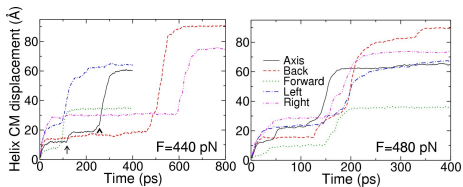
- Form of $U(\vec{R})$ and values of K_b, b_0, K_θ,...: "Force field" (*e.g.*, CHARMM).
- Provides the ultimate details (Karplus, Biopolymers (2003)).
- Issues: time scale ($\leq 10^{-6}s$), conformational sampling, water dynamics.

Probing the motor head - neck linker interaction

Multistep unbinding of the pulled neck
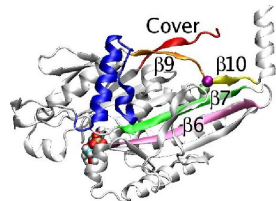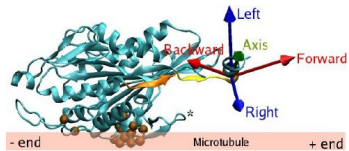
Bottom View

- N334 forms double H-bonds: 'Asparagine latch' → highly conserved.
- Little interaction between $\beta 9$ and the head → no 'zipper-like' binding of neck linker.
- What brings the neck linker forward?
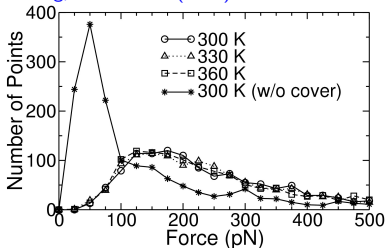 No free diffusion: Mori, Vale & Tomishige, Nature (2007), Guydosh & Block, Nature (2009).

Cover-Neck Bundle (CNB): force-generating element

- β-sheet formed between cover strand (CS) and β9 of neck linker (NL) generates forward bias. **(Video)**
- No forward bias w/o the CS. **(Video)**
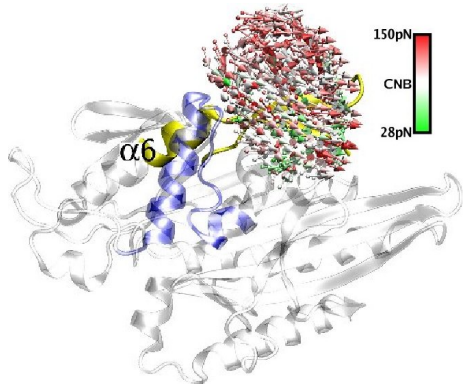
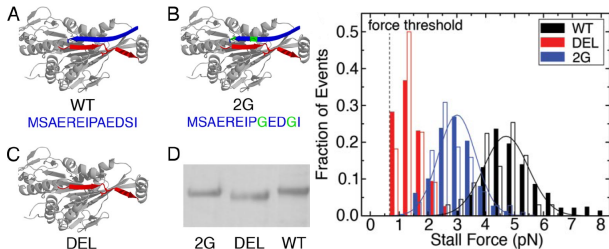Calculation of the CNB's 'force map' using *tug-of-war* sampling

Hwang, 127:175104 (2007).

Force generation by the CNB formation:

- Autonomous (no contacts needed w/ motor head)
- Temperature independent: 'Power stroke'
- Force sufficient to resist load in experiment.

Single-molecule test of the CNB mechanism

Hwang, Lang & Karplus,
Structure 16:62 (2008)

Khalil, Hwang, Lang, et al., PNAS 105:19247 (2008)

- 2G mutant: glycine makes the CNB more flexible → less force.
- DEL mutant: no cover strand → severely impaired motility.
- Kinesin mechanochemical amplifier: Force generation though disorder-to-order transition (motor head conformational change only needs to *trigger* CNB formation) ⋯ Transient formation of force-generating element.

Tug-of-war sampling: Measure conformational forces
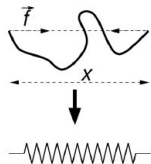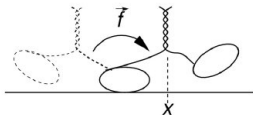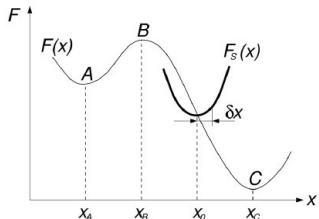
- Strategy: apply harmonic sampling potential $F_s(x) = k_s(x - x_0)^2$ (x: reaction coord), analyze fluctuation (avg and standard dev) to get free energy gradient $F'(x_0)$.

- Carry out TOWS while varying x_0 and get potential of mean force (PMF; free energy profile along reaction coordinate).

- Extendable to arbitrary dimension.

- $\vec{\nabla} F$ doesn't need to be aligned with the reaction coord.

- **"In silico force sensor"**: Conceptually similar to optical trap.

The kinesin mechanochemical cycle (Karnot cycle)
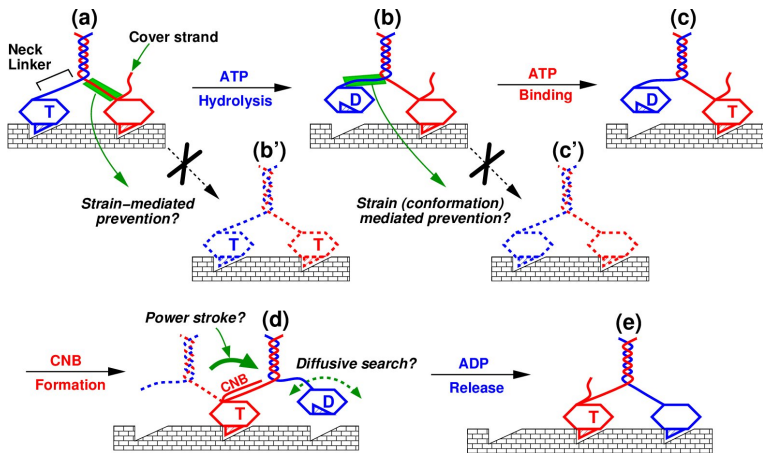
(Hwang & Lang, Cell Biochem. Biophys. 54:11 (2009))

Outstanding questions:

- How do the two motor heads keep their ATPase cycles out of synchrony?
- Mechanism for unidirectionality?
- Role of microtubule in kinesin motility?

The puzzle of Ncd (Kin-14): opposite directionality

- Kin-1: processive, MT plus-end directed (transport; semi-solo motor)
  Ncd: non-processive, MT minus-end directed (mitosis; group motor)
- Major difference in the neck domain: neck linker (Kin-1) vs. neck helix (Ncd).
- Swapping of the neck (and cover) domains between the two motors can reverse directionality (Case, Vale, et al., Cell (1997); Henningsen & Schliwa, Nature (1997); Endow & Waligora, Science (1998)).

Kin-1 (PDB 1MKJ)   Ncd (PDB 1CZ7)   Overlap
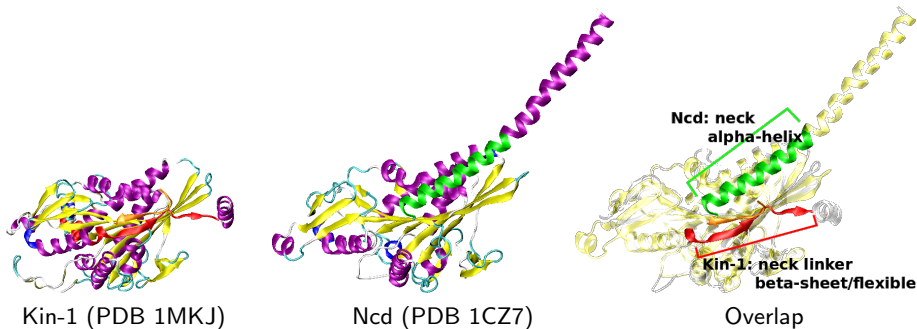
How do they achieve unidirectional motion?
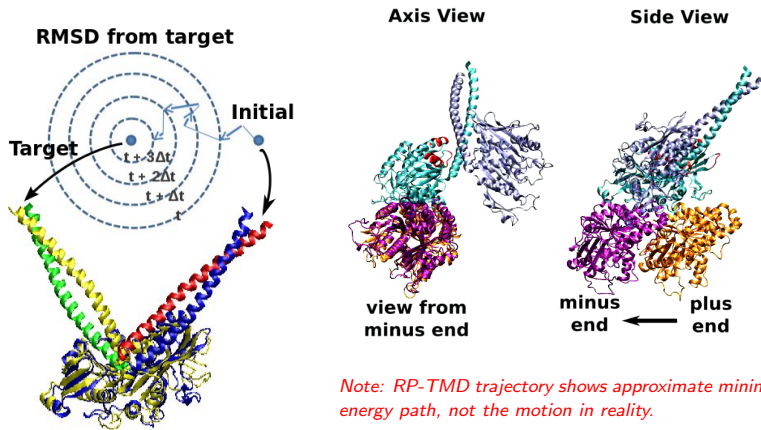
Ncd's neck: Moves like a lever-arm?
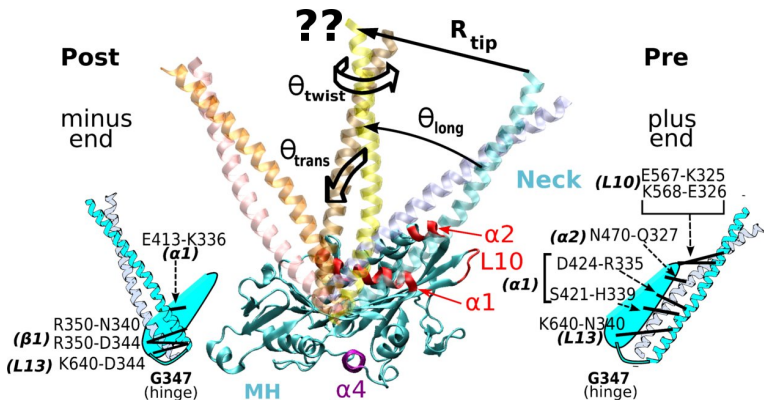
- Pre- and post-stroke structures available.
- Motion of the neck in between?
- Point mutations of residues in the head-neck contacts lead to different microtubule gliding velocities, even switching directionality
  (Sablin, Vale et al., Nature 395:813 (1998), Endow & Higuchi, Nature 406:913 (2000))

# Use RP-TMD to find the *minimum free energy path*

Restricted-Perturbation Targeted Molecular Dynamics (RP-TMD)

- TMD: Apply time-dependent holonomic constraint for the root-mean-square deviation (RMSD) between initial & target structures. (Schlitter *et al.*, Mol. Simu. 10:291 (1993))
- RP-TMD: Control magnitude and direction of constraining force (perturbation) to avoid large barrier crossings (van der Vaart & Karplus, JCP 122:114903 (2005))

RMSD from target

Target

Initial

$t + 3\Delta t$
$t + 2\Delta t$
$t + \Delta t$
t

Axis View

Side View

view from minus end

minus end ← plus end

Note: RP-TMD trajectory shows approximate minimum free energy path, not the motion in reality.

Lakkaraju & Hwang, BJ 101:1105 (2011)

Energetics of the forward motion

- Potential of Mean Force (PMF) calculated using the *tug-of-war sampling*:
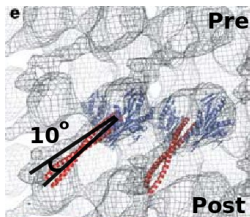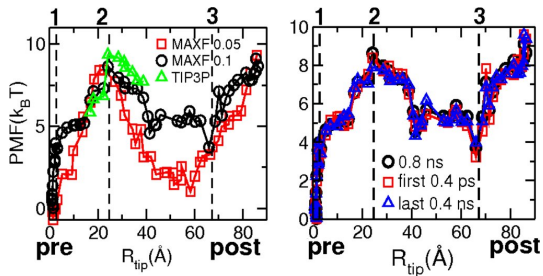
Endres *et al.*, Nature 439:875 (2006)

- Pre→**1**: Head rearrangement. Energy supplied by ATP?
- Post-stroke position higher in free energy: Neck is less visible in cryo-EM.
- Post-stroke minimum at **3**: Explains $10°$ mismatch between x-ray and cryo-EM structures (Endres *et al.*, Nature 439:875 (2006)).
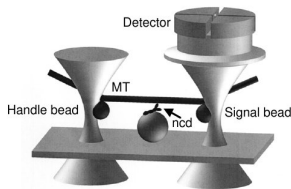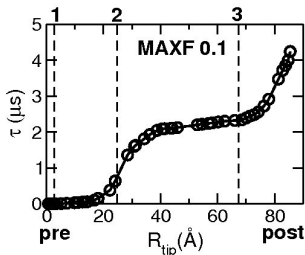- Major barrier at **2**: Mainly due to R335-D424 bond.

Lakkaraju & Hwang, BJ 101:1105 (2011)

Stepping time of the neck over the PMF?

Get first passage time to diffuse to R_{tip} (Gardiner, *Handbook of Stochastic Methods*):

$$\tau(R_{\text{tip}}) = \frac{1}{L^2 D_r} \int_0^{R_{\text{tip}}} dx \, e^{U_{PMF}(x)/k_B T} \int_0^x dy \, e^{-U_{PMF}(y)/k_B T}$$

$L = 75$ Å: Length of the neck, $D_r = 3.01 \times 10^6 \text{rad}^2/s$: rotational diffusion coeff.

de Castro *et al.*, Nat. Cell Biol. 2:724 (2000)

- For the neck rotation: $\tau \simeq 4.2 \sim 19.8$ μs
- Double-trap assay for full-length Ncd: $200 \sim 400$ ms
  (de Castro *et al.*, Nat. Cell Biol. 2:724 (2000)).
- For the head to move 4.3-μm microtubule & $2 \times$(1-μm bead): $\tau \simeq 3.6 \sim 17.0$ ms
- *c.f.*, free diffusion over 80-Å distance: 271 ns / 243 μs.

Lakkaraju & Hwang, BJ 101:1105 (2011)

Forward/reverse motions show hysteresis

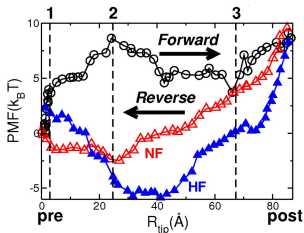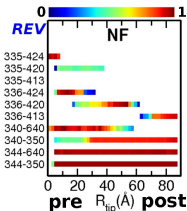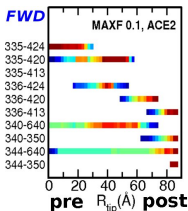Conformational relaxation causes forward and reverse motions to be different.
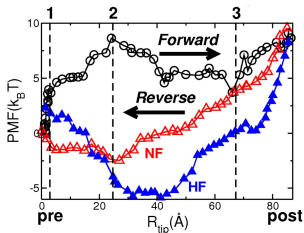
- Forward: After R335-D424 breaks at **2**, D424 relaxes.
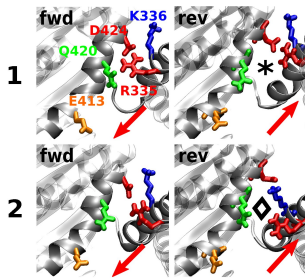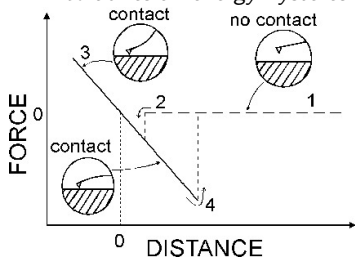- Reverse: R335-D424 can form only at **1**.
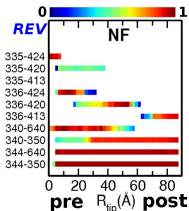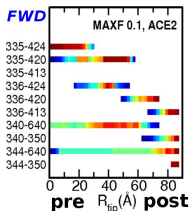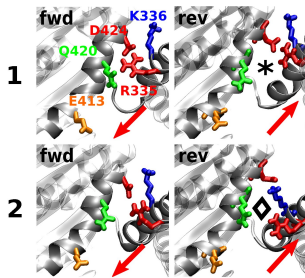
Forward/reverse motions show hysteresis

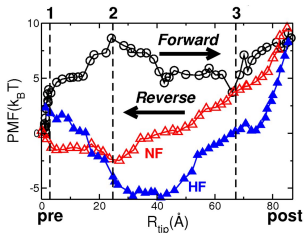Conformational relaxation causes forward and reverse motions to be different.

- Forward: After R335-D424 breaks at **2**, D424 relaxes.
- Reverse: R335-D424 can form only at **1**.
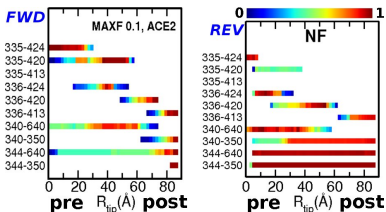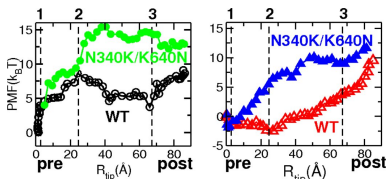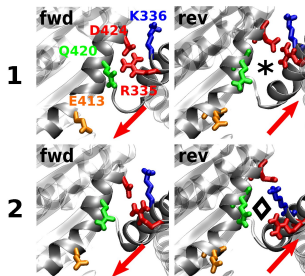- Akin to *adhesion energy hysteresis*.

Forward/reverse motions show hysteresis

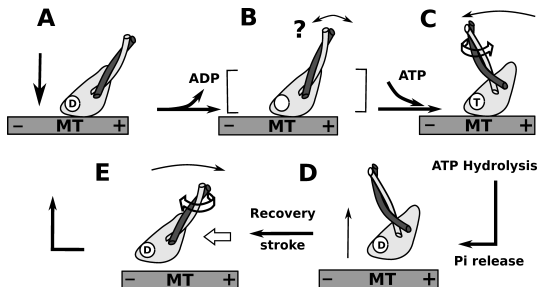Conformational relaxation causes forward and reverse motions to be different.

- Forward: After R335-D424 breaks at **2**, D424 relaxes.
- Reverse: R335-D424 can form only at **1**.

**Bidirectional N340K/K640N mutant has monotonic PMF profiles!**

Ncd mechanochemical cycle (Narnot cycle)

B→C: Guided diffusion   **D→E**: Torsional relaxation & diffusion

- Point mutations of key residues lead to greater reduction in microtubule gliding velocity (Sablin *et al.*, Nature 395:813 (1998), Endow & Higuchi, Nature 406:913 (2000)).
- Occasional (∼30%) plus-end directed stepping: slower & smaller step (Butterfield *et al.*, BJ 99:3905 (2010)): Asymmetry in forward & reverse motions.
- Diffusion guided by intermediate contacts: More tolerant to load?
- Recovery stroke: Needs less load-tolerance.
- Hysteresis: Good for directional motion out-of-equilibrium?

Lakkaraju & Hwang, BJ 101:1105 (2011)

Mixing autonomous force generation & diffusion in Kin-5
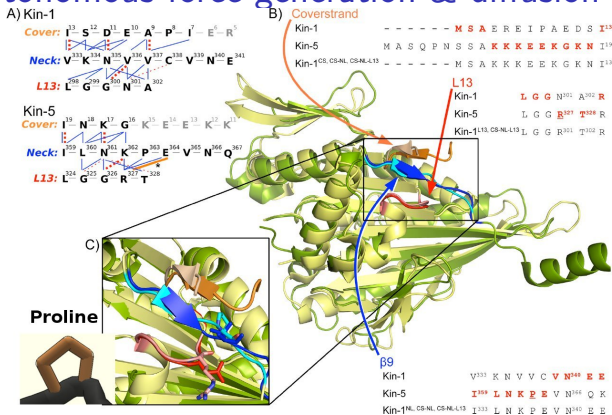
- Mix & match different domains and study motility of the chimeric motors via single-molecule exp & MD: **Measure-make-model strategy**
- Kin-5: Force generation over a short distance & quick release: Suitable for a group of motor (spindle dynamics; MT plus-end directed).
- Chimeric proteins & cover strand antibodies designed based on simulation → experimentally tested.

Issues of scale

- Vibration of covalent bonds: 1–20 fs (1 fs$= 10^{-15}$ s)
- Water rotation, hydrogen bond lifetime: 1–10 ps (1 ps$= 10^{-12}$ s)
- Protein domain motion: 1–100 ns (1 ns$= 10^{-9}$ s)
- Kinesin mechanical transition: $<30\mu$s (time resolution of optical trap's detector). . . Upper (lower) limit for all-atom MD (single-molec exp)
- Kinesin chemical transition (ATP hydrolysis, *etc.*): $\mathcal{O}(1$ ms$)$
- Transport on microtubule: $\mathcal{O}(1$ s$)$
- Cell division (spindle dynamics): $\mathcal{O}(1$ min$)$

Coarse-graining: For *predictive power*, stochastic rules of the model should be faithfully based on atomic properties.

- Kinesin-microtubule interactions: Hydrated interface.
- Microtubule mechanics: Kinesin not only bends MT, but twists it.
- Collective organization of microtubules: Filament network.
- Analysis of imaging data.

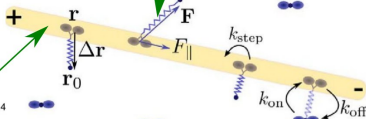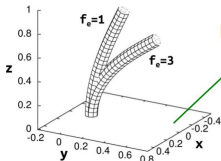# Coarse-graining (MSM) strategy I: Embrace heterogeneity

Tropomyosin local flexibility map

Lakkaraju & Hwang
PRL 102:118102 (2009)
CAMB 2:57 (2009)
Teng & Hwang (in prep)
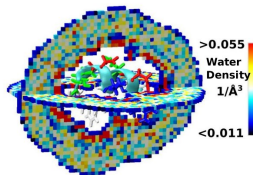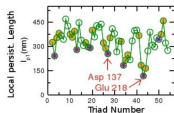
Kinesin stalk mechanics

MT mechanics

>0.055 Water Density 1/Å³
<0.011

Hydration map (density, diff coeff, etc in `voxels')
Ravikumar & Hwang, JACS 133:11766 (2011)
Hwang (in prep.)

Kin-MT interaction
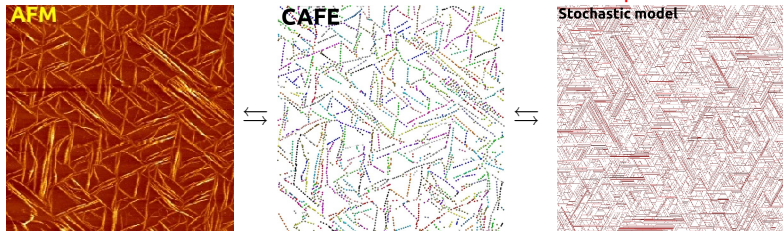
MT Gliding on kin-coated surface
Li et al., PLoS ONE (2012)

Hwang,
in "MSM in Biomech & Mechanobiol"
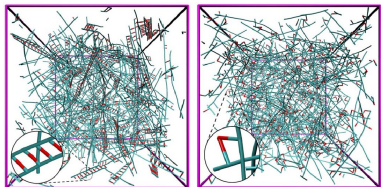De, Hwang & Kuhl, eds (2013, submitted)

MSM strategy II: Analyze imaging data

Computer-Aided Feature Extraction (CAFE)

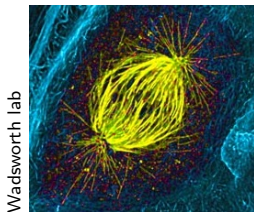Construct *in silico* model of filament network based on experimental data

Epitaxial assembly of collagen on mica. *Leow & Hwang, Langmuir 27:10907 (2011) & In preparation.*
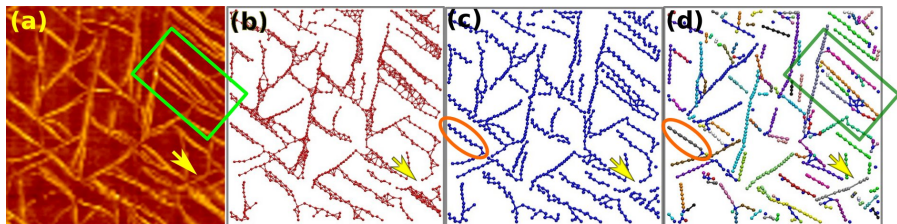

Brownian dynamics sim of crosslinked actin network

Kim, Hwang, Lee & Kamm, PLoS Comp Biol 5:1000439 (2009)

Wadsworth lab

Build exp-based, minimal in silico model of mitotic spindle

CAFE in action

Advanced recognition of features in imaging data

Acknowledgments

Matthew Lang
(Vanderbilt)

Martin Karplus
(Harvard)

Ryoma (Puck) Ohi
(Vanderbilt)