Intracellular transport of cargos: tug-of-war, anomalous diffusion, and lattice deformation

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Plan - Intra-cellular Transport

- Dynamics of cargo-motors complexes
  - Tug-of-war: Mean-Field model
  - Explicit Position Based model
  - Anomalous diffusion
  - External control

- Interplay between transport and lattice dynamics
  - Impact of lattice dynamics on collective cargo transport
  - Lattice deformation driven by active cross-linkers
Intra-cellular transport

- Need for transport


From [Judith Stoffer, NIGMS]
Intra-cellular transport

Need for transport


From [Judith Stoffer, NIGMS]
Intra-cellular transport

Particular case: the axon
- up to 1 m in human beings, a few microns for the diameter
- crowded environment

Link with neurodegenerative diseases

Shemesh et al., Traffic 9, 458 (2008)

Intracellular transport

Microtubules are polarized

Motors can attach and detach

From [Alberts et al, Molecular Biology of the Cell, 5th ed. (2008)]

[Modified from www.ulyssse.u-bordeaux.fr/atelier/ikramer/biocell_diffusion]

[Modified from a wikipedia image by Kebes]
Cargo-motor complexes

Teams of motors
- Can apply stronger forces
- Increases processivity

Tug-of-war

Endosome inside Dictyostelium cells.

[Soppina et al (2009) PNAS]
Tug-of-war

Endosome inside Dictyostelium cells.

[Soppina et al (2009) PNAS]
Cargo-motor complexes

Teams of motors

- Dynamics of cargo-motors complexes
- Comparison with experimental data
- Consequences in terms of transport properties

Dynamics of cargo-motors complexes
- Tug-of-war: Mean-Field model
- Explicit Position Based model
- Anomalous diffusion
- External control

Interplay between transport and lattice dynamics
- Impact of lattice dynamics on collective cargo transport
- Lattice deformation driven by active cross-linkers
Tug-of-war, symmetric motors

[Müller, Klumpp, and Lipovsky (2008) PNAS]

Variables
Number of attached motors of each type

Mean field model
- Equal sharing of the force among attached motors of one given type

Motor Dynamics:
- detachment rate $\omega = \omega(F_i)$
- motor velocity $v = v(F_i)$
Tug-of-war, symmetric motors

- If motors stop walking before detaching

- If motors detach before stall

- Intermediate case

MF prediction
Symmetric bimodal/trimodal distributions of the velocity
Plan - Intra-cellular Transport

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Explicit Position Based Model

EPB-model

- Motor positions are explicitly taken into account
- Motors are linked by springs to the cargo

See Kunwar et al; Bouzat et al;

Variables

Position of each attached motor

Position $\Rightarrow$ Force
Explicit Position Based Model

Stochastic Motor Dynamics:
- attachment rate $\tilde{\omega}$
- stepping rate $p = p(F_i)$
- detachment rate $\omega = \omega(F_i)$

Cargo dynamics

$$m \frac{\partial^2 x_C(t)}{\partial t^2} = -\beta \frac{\partial x_C(t)}{\partial t} + F(x_C, \{x_i\})$$
For the same motors

Tug-of-war, symmetric motors
$N_+, N_-$ motors attached to the cargo, among which $n_+, n_-$ attached to the filament

Asymmetric teams
Kinesins and dyneins behave differently
Tug-of-war, asymmetric motors

$N_+, N_-$ motors attached to the cargo, among which $n_+, n_-$ attached to the filament

Stochastic motor dynamics

Detachment rate

From Kunwar et al (2011) PNAS

C. Appert-Rolland (LPT)
Tug-of-war, asymmetric motors

\(N_+, N_-\) motors attached to the cargo, among which \(n_+, n_-\) attached to the filament

Stochastic motor dynamics

- Stepping rate (for \(F_i\) below stall force):
  \[
  s(|F_i|, [ATP]) = \frac{k_{\text{cat}}(|F_i|)[ATP]}{[ATP] + k_{\text{cat}}(|F_i|)k_b(|F_i|)^{-1}},
  \]

Michaelis-Menten kinetics

- Stepping rate (for \(F_i\) above stall force):
  \[s_b = \frac{v_b}{d}\]
Tug-of-war, asymmetric motors

$N_+, N_-$ motors attached to the cargo, among which $n_+, n_-$ attached to the filament

Stochastic motor dynamics

[ATP] and force dependence

Comparison for kinesin


Tug-of-war, asymmetric motors

$N_+, N_-$ motors attached to the cargo, among which $n_+, n_-$ attached to the filament

How does this cargo-motors complex behave?
Plan - Intra-cellular Transport

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  - Anomalous diffusion
  - External control
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Anomalous diffusion

Preliminary remarks:

Mean Square Displacement

\[ \text{MSD} \equiv \langle (X(t + \Delta t) - X(t))^2 \rangle \]

- Ballistic: \( \text{MSD} \sim \Delta t^2 \)
- Purely diffusive without bias: \( \text{MSD} \sim \Delta t \)
- Anomalous diffusion: \( \text{MSD} \sim \Delta t^\gamma \) with \( \gamma < 1 \) or \( 1 < \gamma < 2 \).

\[ \langle \rangle = \text{average over } t. \]
Anomalous diffusion

Preliminary remarks:

**Mean Square Displacement**

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- Ballistic: MSD \( \sim \Delta t^2 \)
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At finite time:

- Apparent superdiffusion (\( \gamma > 1 \))
  - biased but uncorrelated motion?
  - positive temporal correlations of the displacements?
Anomalous diffusion

Preliminary remarks:

Mean Square Displacement

\[ \text{MSD} \equiv \langle (X(t + \Delta t) - X(t))^2 \rangle \]

Variance

\[ \text{Var} = \langle (X(t + \Delta t) - X(t))^2 \rangle - \langle (X(t + \Delta t) - X(t))^2 \rangle^2. \]

\[ \langle \rangle = \text{average over } t. \]

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At finite time:

Apparent superdiffusion (\( \gamma > 1 \))

- biased but uncorrelated motion?
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Anomalous diffusion

[Caspi et al, PRE (2002) 22, 011916]
Engulfed 2 and 3 $\mu$m beads, in living cells, driven by microtubule-associated motors

- Enhanced diffusion scaling as $t^{3/2}$ at short times
- Ordinary (small spheres) or subdiffusive (large) scaling at long times

black line: 3 $\mu$m beads
Anomalous diffusion

[Caspi et al, PRE (2002) 22, 011916]
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(see also in grey: subdiffusive motion of nondriven lipid spheres granules naturally appearing in these cells)
Anomalous diffusion


*In vitro* experiment with egg extract; 3 \( \mu \)m beads coated with motors and moving along MTs

\[
MSD \sim \Delta t^\gamma
\]

<table>
<thead>
<tr>
<th>Type of filament</th>
<th>( \gamma )</th>
<th>Diffusion type</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT + actin</td>
<td>( \gamma \approx 3/2 )</td>
<td>superdiffusive</td>
</tr>
<tr>
<td>MT (no actin)</td>
<td>( \gamma \approx 3/2 )</td>
<td>superdiffusive</td>
</tr>
<tr>
<td>Actin (no MT)</td>
<td>( \gamma \approx 3/4 )</td>
<td>subdiffusive</td>
</tr>
<tr>
<td>no MT, no actin</td>
<td>( \gamma \approx 1 )</td>
<td>diffusive</td>
</tr>
</tbody>
</table>

“the \( t^{3/2} \) behavior comes about due to a hindrance to ballistic motion”
Anomalous diffusion


Peroxisome trajectories in Drosophila S2 cells

- At short time scales (below 30ms): subdiffusion
- At intermediate times: superdiffusion
- At longer time scales: diffusion or subdiffusion.

$\alpha = 0.59 \pm 0.28$ and $\beta = 1.62 \pm 0.29$
Anomalous diffusion


Peroxisome trajectories in Drosophila S2 cells

\[\alpha = 0.59 \pm 0.28 \text{ and } \beta = 1.62 \pm 0.29\]

“an exponent close to 1.5, an observation challenging the simple motor-hauling-a-cargo and random motor switching model and indicating the movements of microtubules”
On a branched network, purely ballistic motion also shows
\[ \text{Var}[X] \sim \Delta t^\gamma \] with \( 1 < \gamma < 2 \)
depending on the turning angle distribution

[Shaebani et al (2014) PRE 90, 030701(R)]
Anomalous diffusion

Cargo dynamics

\[ \frac{m}{\partial t^2} \frac{\partial^2 x_C(t)}{\partial t^2} = -\beta \frac{\partial x_C(t)}{\partial t} + F(x_C(t), \{x_i\}) \]

\[ \gamma \]

\[ k_a [s^{-1}] \]

\[ \text{variance [\mu m^2]} \]

\[ t^{1.5} \]

\[ 10^{-2} \quad 10^{-1} \quad 1 \quad 10 \]

\[ 1 \quad 10 \quad 10^2 \quad 10^3 \quad 10^4 \quad 10^5 \]

\[ \text{Time [s]} \]

[Klein et al, EPL (2014)]
Anomalous diffusion

Cargo dynamics

\[ m \frac{\partial^2 x_C(t)}{\partial t^2} = -\beta \frac{\partial x_C(t)}{\partial t} + F(x_C(t), \{x_i\}) \]

[Klein et al, EPL (2014)]

No need for further ingredient

Superdiffusion can be explained by cargo-motors dynamics
Anomalous diffusion

Cargo dynamics with thermal noise

$$m \frac{\partial^2 x_C(t)}{\partial t^2} = -\beta \frac{\partial x_C(t)}{\partial t} + F(x_C(t), \{x_i\}) + \sqrt{2k_B T \beta} \xi(t)$$

$\nu_f = 1000 \text{ nm/s}$

$d = 8 \text{ nm}$

Crossover time $\simeq$ delay between steps

$\text{variance [nm}^2\text{]}$ vs $\Delta t [\text{ms}]$
These velocity relaxation events indicated the presence of an elastic component in the system and suggested that bent and buckled microtubules could influence peroxisome transport.

Relaxation phenomenon

“These velocity relaxation events indicated the presence of an elastic component in the system and suggested that bent and buckled microtubules could influence peroxisome transport”


Time (ms)
Relaxation phenomenon


EPB Model
Elastic energy can be stored and released when a motor detaches
Dynamics of cargo-motors complexes along a single filament

- The EPB model allows to reproduce some experimental observations:
  - anomalous diffusion
  - elastic relaxation events
- Why is it interesting for the cell to have these cargo-motors complexes?
Active transport versus diffusion

Intracellular transport

(a) $\eta^* = \eta$
(b) $\eta^* = 10\eta$
(c) $\eta^* = 100\eta$

[Klein et al, EPJST (2014)]
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Control by fuel supply

Stall force ATP dependence

- Kinesin: constant $F_s = 2.6 \text{ pN}$
- Dynein: $F_s$ varies linearly from 0.3 pN at vanishing [ATP] to 1.2 pN for saturating [ATP]

\[ N_+ = N_- = 5 \]

From [Klein et al (2014) EPL]

ATP dependence

- More energy (ATP) can slow down the cargo
- It can also reverse cargo velocity
Control by External Force

Effective viscosity dependence

Viscosity dependence

- Increase of viscosity can speed up the cargo
- It can also reverse cargo velocity

Advantage

Easy control of the cargo-motors complex by a single external parameter

\[ N_+ = N_- = 5 \]

From [Klein et al (2014) EPL]
Conclusion

- Many experimental observations can already be explained by the stochastic motion of cargo-motors complexes along a single microtubule.
- Highly controllable system.
- Our predictions for [ATP] dependence or external force control could be tested experimentally.

Challenges

- Need of well controlled experiments to check tug-of-war scenarios
- In vitro / in vivo differences?
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Collective effects

Cellular automata models with one type of motors

\[ \alpha \quad \omega_a \quad \omega_d \quad \beta \]

\[ 1 \quad 2 \quad L-1 \quad L \]

In vitro

- well suited for motility assays, predicts the experimentally observed bulk localization of high and low density domains

In vivo

- Crowded environment
- No infinite diffusion

References:

[Lipowsky, Klumpp, & Nieuwenhuizen, P.R.L. (2001)]
[Parmeggiani, Franosch, & Frey, P.R.L. (2003)]
[J. Tailleur, M. Evans, & Y. Kafri, P.R.L. (2009)]

[Nishinari, Okada, Schadschneider, & Chowdhury, P.R.L. (2005)]

[by Tim Vickers]
Collective effects in bidirectional intracellular transport

**Ingredients**
- Two types of complexes going in opposite directions
- Confined diffusion in the surrounding cytoplasm

**Jamming**
- No transport in thermodynamic limit
- Offering multiple filaments enhances cluster formation.

MTs exhibit stochastic switching between a shrinking and a growing state

Microtubules seen by fluorescence in S. pombe (yeast)

Scale bar = 5 \( \mu \text{m} \)

[Shemesh, Erez, Ginzburg, Spira. Traffic (2008)]

1s (video) = 120s (real time)
Scale bar = 10 \( \mu \text{m} \)


Dynamics of the lattice

\[ \omega_a, \omega_d, \omega, k, p, k_d, k_p \]

Reservoir

Filament

\[ \text{static filament, dynamic filament} \]

[Ebbinghaus, Appert, Santen, PRE 82 (2010) 040901]
Drugs modifying the dynamics of the microtubules induce jams

- video 1: microtubule dynamics with and without drugs (Paclitaxel)

Drugs modifying the dynamics of the microtubules induce jams

- video 2: microtubule dynamics and pinocytotic vesicles transport without drugs

Drugs modifying the dynamics of the microtubules induce jams

- video 3: microtubule dynamics and pinocytotic vesicles transport with drugs

Challenges

- A full picture of the axonal MT network is still missing
- Understanding the mechanisms of transport breakdown in neurodegenerative diseases
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MT deformation: Observations

**In vivo**

- [Brangwynne et al, PNAS (2007)]

**In vitro**

- [Brangwynne et al, PRL (2008)]
  - MT embedded in acto-myosin gel

**In vitro measurements**

- Persistence length of a free MT = a few mm

**In vivo measurements**

- Persistence length of MTs = 30 µm

MT-MT interactions mediated by conventional kinesin (fungus Ustilago maydis)

[Straube et al, Molec. Biol. of the Cell (2006)]
Model definition

[Ines Weber, Cécile Appert-Rolland, Grégory Schehr, and Ludger Santen, "Non-equilibrium fluctuations of a semi-flexible filament driven by active cross-linkers" (2017) submitted]

Model

- Semi-flexible filament $E = k \int_0^L \left( \frac{\partial \theta}{\partial s} \right)^2 ds$
- Connected to a background network by active cross-linkers
- Stochastic dynamics for the cross-linkers
- Cross-linkers feel a force $F_{SFF}$ only for elongated chain

$k =$ bending rigidity
Model definition

Active Cross-linkers

- bind at available sites with rate $\omega_a$
- Polarity of the background filament chosen at each attachment event
- unbind with rate $\omega_d = \omega_{d0} \exp \left( \frac{|F_{SFF}|}{F_d} \right)$
- step with rate $p(F_{SFF})$

$F_s =$ stall force.
Model definition

### Coupling SFF / linkers

- \( \{x_i\} = \) positions of pulling linkers (elongated chains)
- Between \( x_i \) and \( x_{i+1} \):
  \[
  E_i = k \int_{x_i}^{x_{i+1}} \left[ \frac{\partial^2 u_i(x)}{\partial x^2} \right]^2 \, dx
  \]
  and
  \[
  F \sim \frac{\partial^4 u_i(x)}{\partial x^4}
  \]
- Equilibrium shape
  \[
  F = 0 \quad \Rightarrow \quad u_i(x) = a_i(x - x_i)^3 + b_i(x - x_i)^2 + c_i(x - x_i) + d_i
  \]
Model definition

Coupling SFF / linkers

- We put end-to-end these segments with boundary conditions:

\[ u_i(x_i) = z_i, \quad u_i(x_{i+1}) = z_{i+1} \]

\[ \partial_x u_i(x_i) = v_i, \quad \partial_x u_i(x_{i+1}) = v_{i+1} \]

- \( z_i \) = vertical displacement of the SFF imposed at position \( x_i \)
- \( v_i \) = the local slope \( \Rightarrow \) differentiability of the global polynomial
Model definition

For each set of positions \( \{x_i\} \), we find
- the global equilibrium shape of the SFF
- the local force exerted at each attachment point:

\[
F_k = \frac{\partial E}{\partial z_k} = 24k \left( \frac{\Delta z_{k-1}}{\Delta x_{k-1}^3} - \frac{\Delta z_k}{\Delta x_k^3} \right) - 12k \left\{ v_k \left( \frac{1}{\Delta x_{k-1}^2} - \frac{1}{\Delta x_k^2} \right) \right\} - 12k \left\{ \frac{v_{k-1}}{\Delta x_{k-1}^2} - \frac{v_{k+1}}{\Delta x_k^2} \right\}
\]
Update scheme

- We update the system of linkers with a tower sampling algorithm and perform stochastic events until the occurrence of an event that modifies the force exerted on the SFF.
- Then the new equilibrium shape of the SFF is calculated.
- The new forces exerted on the linkers are obtained and the value of force-dependent rates is calculated for each linker.

- Can model cascades of detachment
- Understanding how deformations originate from microscopic discrete forcing
Non-equilibrium fluctuations of a SFF driven by active cross-linkers

Results

- Obtain deformation spectrum
- Obtain persistence length
- Investigate the role of parameters (rigidity, mesh size, etc)
- Compare thermal and active linkers to identify geometrical effects
Deformation spectrum

Decomposition into cosine modes

\[ \theta(s) = \sqrt{\frac{2}{L}} \sum_{n=0}^{\infty} a(q) \cos(qs) \]

with the wavenumber \( q = \left( \frac{n\pi}{L} \right) \)

Thermal fluctuations in 2D

The variance of cosine modes’ amplitudes is known to vary with \( q \) as

\[ \text{Var}(a(q)) \equiv \langle a(q)^2 \rangle = \frac{1}{L_p} \frac{1}{q^2} \quad \text{with} \quad L_p^{\text{thermal}} = \frac{2k}{k_B T}. \]

Non-thermal fluctuations

No reason to have \( q^{-2} \) dependence
In vivo

Deformation spectrum

In silico

Red squares: parameters inspired by biological system. The $q^{-2}$ behavior (orange line) for small $q$ can be associated with a persistence length of 26 $\mu$m.

Generic: no clear $q^{-2}$ behavior.

[Brangwynne et al, PNAS (2007)]

[I. Weber et al, submitted (2017)]
Persistence length

For two dimensional fluctuations

Auto-correlation of the tangent angle $\theta$

$$\langle \cos (\theta(s) - \theta(s')) \rangle = \exp \left( -\frac{|s - s'|}{2L_p} \right)$$

$$L_p^{\text{thermal}} = \frac{2k}{k_B T}$$
Persistence length

$\textbf{Rigidity dependence}$

- For small $k$, deformations are limited by the mesh size.
- For $k \geq 1$, super-linear increase of the persistence length up to, and beyond the SFF length.

$k = 1$ for MTs

Straight line = the linear increase expected for purely thermal fluctuations
**Persistence length**

$k = 1$ for MTs

Straight line = the linear increase expected for purely thermal fluctuations

**Mesh size dependence (fixed number of linkers)**

- For small $k$, the persistence length slightly decreases with $d_{\text{mesh}}$ (This dependence is linear in $d_{\text{mesh}}$)

- For large $k$, it is the contrary! When $d_{\text{mesh}}$ decreases, the curvature induced by a single linker step is more pronounced. At high $k$, this results into strong load forces, which most likely the linker will not be able to sustain. Therefore, it is difficult to deform the stiff SFF at all, if the density of cross-linkers is too high.
Non-equilibrium fluctuations of a SFF driven by active cross-linkers

- Model allowing to couple the dynamics of a SFF with the stochastic dynamics of active linkers.
  - Deformation spectrum
  - Investigate the role of parameters (rigidity, mesh size, etc)
- Can be generalized to other types of cross-linkers.
- We could have expected that one type of motors could pull off the other. This is not the case.
  - cf Tug-of-war

Challenges

- In vitro experiments in simplified geometry?
For more details:
http://www.th.u-psud.fr/page_perso/Appert/

Thank-you