NUMERICAL MODELING OF F-ACTIN BUNDLES INTERACTING WITH CELL MEMBRANES

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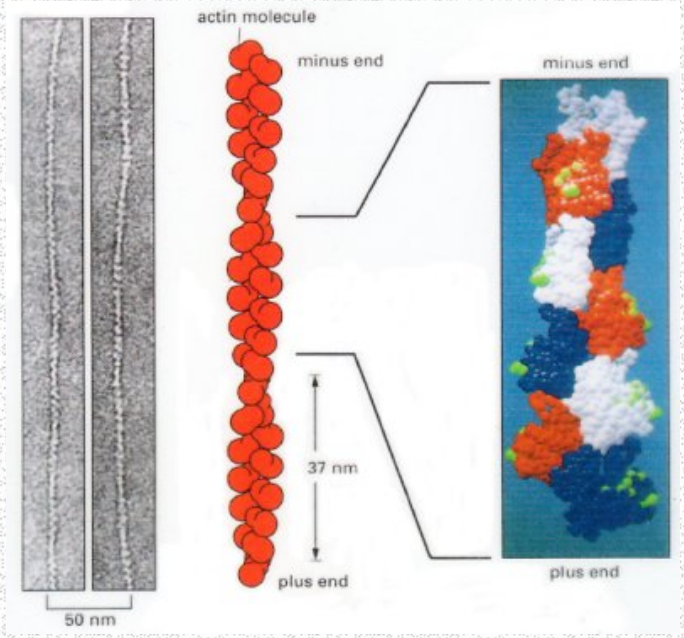
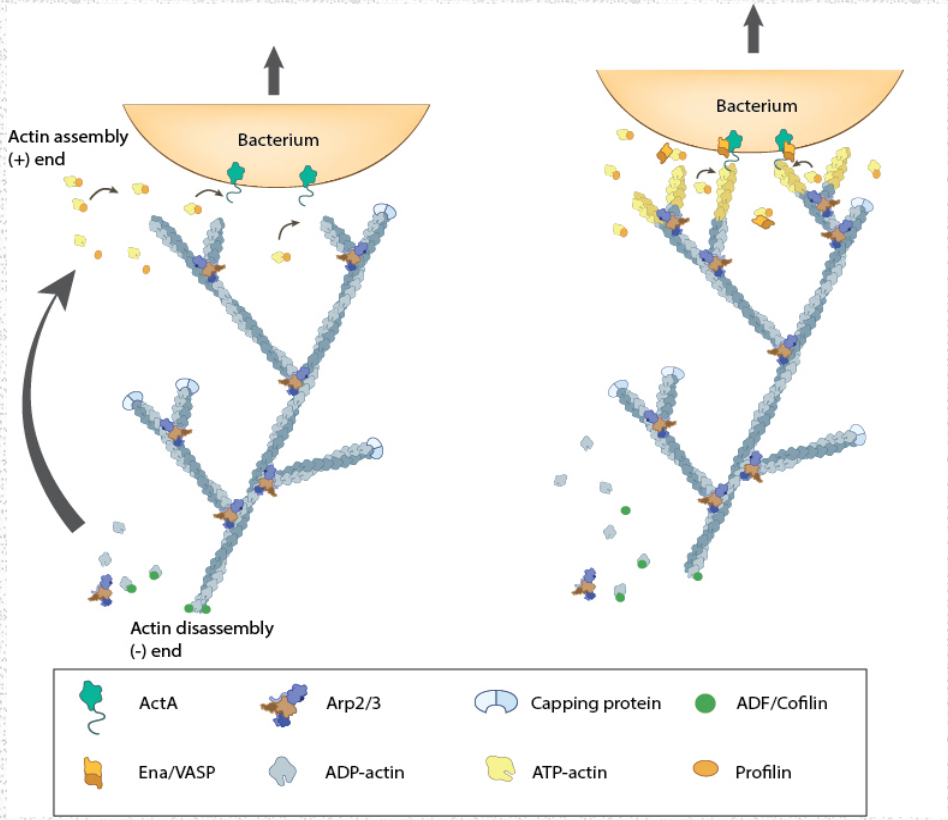
Introduction: Actin

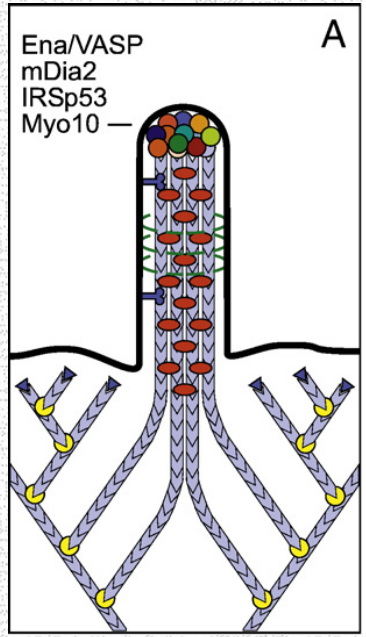
- Actin is one of the most abundant and strongly conserved of all eukaryotic proteins.
- It is one of the main constituents of the cytoskeleton.
- G-actin: monomeric state, globular multi-functional protein.
- F-actin: filamentous state, two protofilaments that form a double helical microfilament.
- Cellular functions (e.g. cell motility, cell division and cytokinesis, vesicle and organelle movement, cell signaling, the establishment and maintenance of cell junctions and cell shape).
- Different types of structures: dendritic network, lamellipodium, *filopodium*.

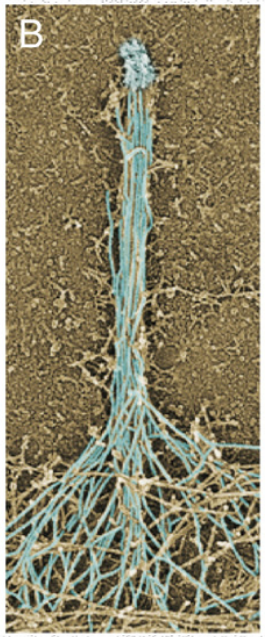


F-Actin bundles: polymerization force

- Self-organization to create polarized arrays and fairly rigid bundles
- Polymerizing networks or bundles of actin filaments are capable of exerting significant mechanical forces, used by cells to change shape, to move or to push obstacles.







F-Actin bundles growing against an external load

1. Velocity-load relationship: velocity at which an obstacle advances under the combined action of the polymerization force and an external load, as a function of the external load.

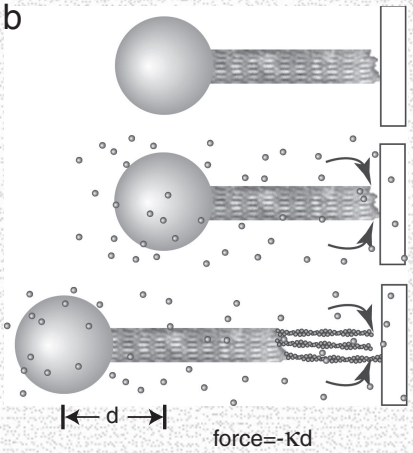
2. What's the amount of force that a filament or a bundle can sustain before they stop growing? (Stalling force)

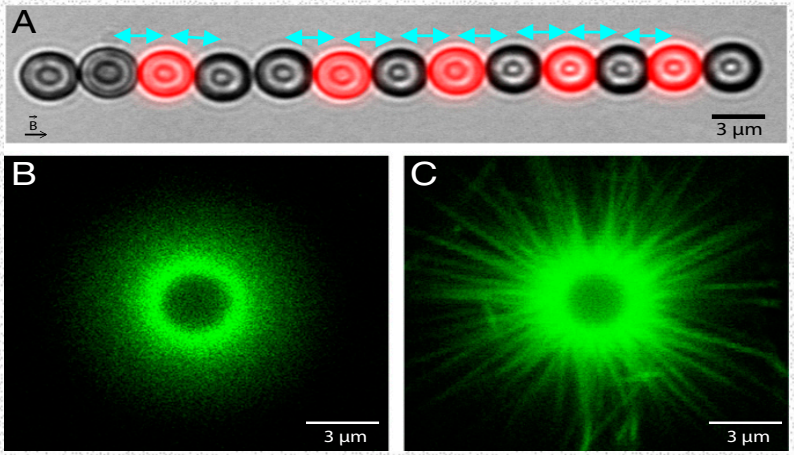
3. How does the actin machinery tune the number of filaments at the leading edge of the bundle to work in an optimal regime of force production? ("Perfect work sharing" – "no work sharing" condition)

4. Dynamic interplay between the cell membrane and F-actin?

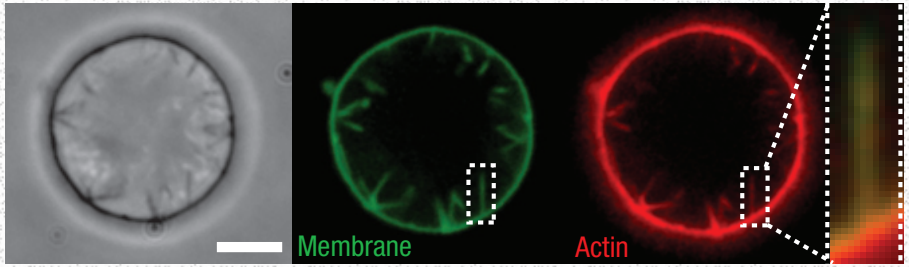
F-Actin bundles: Experiments

- Bundles subject to external forces:
  - ✓ Stalling force measurement in optical trap (Footer et al., 2007)
  - ✓ Velocity-load relationship (Démoulin et al., 2014)





- Actin network grown on Giant Unilamellar Vesicles (GUV)
  - ✓ Membrane-induced bundling of actin filaments (Liu et al., 2008)

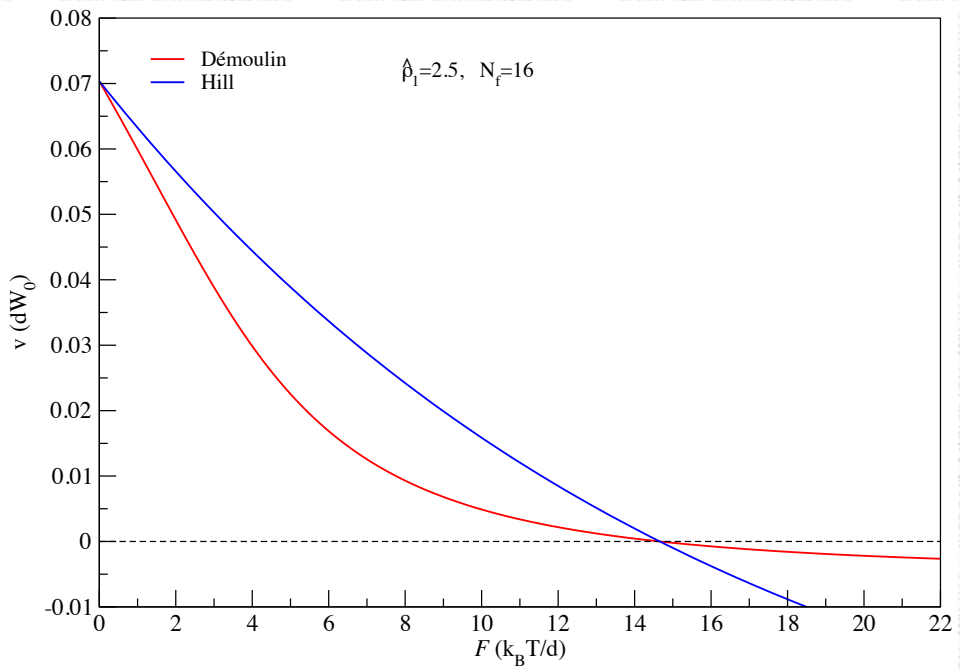


F-Actin bundles: Models

- F-actin: large persistence length $\ell_p \simeq 5370d$
- Models for rigid filaments $\ell_p = \infty$:
  - ✓Hill model (1981): *mean-field theory*
  - ✓Peskin (1993), Mogilner and Oster (1996), Démoulin (2014)…: *brownian ratchet model*

➢ Different *velocity-load relationships*

➢ Same *stalling force*



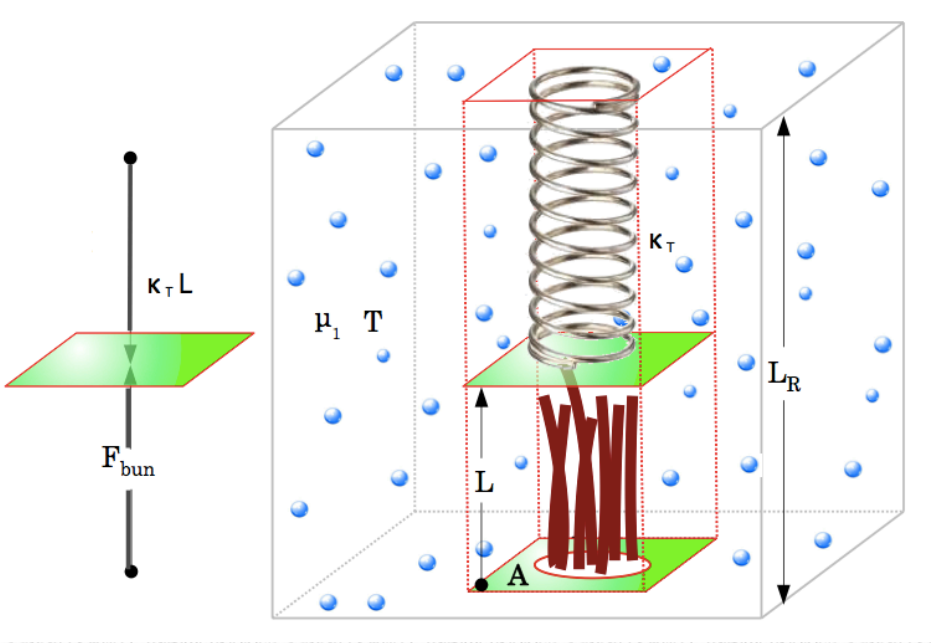
$$F_{stall} = N_f \frac{k_B T}{d} \ln \hat{\rho}_1$$

Free monomers reduced density

Past and Present work: Flexible F-actin Model

- ✧ Introduction of *flexibility* in the filament model
- ✧ Bundle in the *optical trap set-up:*

  - ✓ Filaments: discrete Worm-Like Chains (WLC) - *Filament force: Frey[1] compression law*



- ✓ $\ell_p =$ persistence length
- ✓ N_f grafted filaments, growing against the wall at position L
- ✓ $j_n =$ number of monomers in filament n
- ✓ $\kappa_T =$ trap strength
- ✓ Load force: $F_L = \kappa_T L$

1. A. Gholami, J. Wilhelm, and E. Frey, *Phys. Rev. E* **74**, 041803 (2006)

Past and Present work: Dynamical Model

- Sample space: $\{j_1, \ldots, j_{N_f}, L\}$
- Time evolution of the joint probability $P_{j_1,\ldots,j_{N_f}}(L,t)$: space and time continuous/discrete Fokker-Planck equation

$$\frac{\partial P_{j_1,\ldots,j_{N_f}}(L,t)}{\partial t} = D\frac{\partial}{\partial L}\left[\frac{\partial P_{j_1,\ldots,j_{N_f}}(L,t)}{\partial L} + \frac{1}{k_B T}\left(\sum_{n=1}^{N_f}\frac{\partial w_{j_n}(L)}{\partial L} + \kappa_T L\right) P_{j_1,\ldots,j_{N_f}}(L,t)\right]$$

$$+\widetilde{U}_0\left[\sum_{n=1}^{N_f}\frac{\alpha(j_n,L)}{\alpha(j_n-1,L)}(1-\delta_{2,j_n})P_{j_1,\ldots,j_n-1,\ldots,j_{N_f}}(L,t) - \sum_{n=1}^{N_f}\frac{\alpha(j_n+1,L)}{\alpha(j_n,L)}P_{j_1,\ldots,j_n,\ldots,j_{N_f}}(L,t)\right]$$

$$+\widetilde{W}_0\left[\sum_{n=1}^{N_f}P_{j_1,\ldots,j_n+1,\ldots,j_{N_f}}(L,t) - \sum_{n=1}^{N_f}(1-\delta_{2,j_n})P_{j_1,\ldots,j_n,\ldots,j_{N_f}}(L,t)\right]$$

Bulk polymerization rate

Bulk depolymerization rate

potential of mean force of a grafted WLC against a hard wall (Frey)

$$\alpha(j_n, L) = \exp\left(-\beta w_{j_n}(L)\right)$$

Past and Present work: Discretized Dynamical Model

- Spatially continuous stochastic process approximated by spatially discrete jump process

$\longrightarrow$ Discretization of the wall motion over a grid of step $\delta = d/M$

$$L_k(t) = k(t)\delta$$

- Forward Equation for the probability $\mathcal{P}(t) \equiv \mathcal{P}_{j_1,\ldots,j_{N_f},k}(t)$:

$$\frac{d\mathcal{P}}{dt} = \mathcal{P}\mathbf{Q}$$

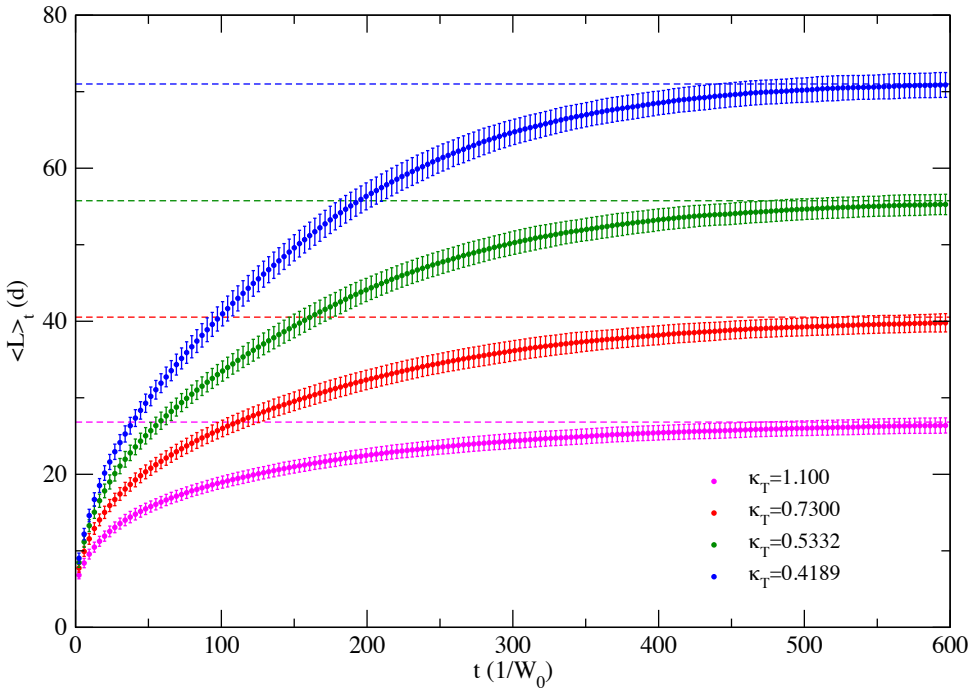
$\longrightarrow$ *Continuous Time Markov Chain with generator matrix* $\mathbf{Q}$ *for which numerical realizations can be constructed*

Past and Present work: Stochastic Dynamics Simulations

- Gillespie algorithm[1]
- Random number generation to determine the jump to occur (*one* filament single (de)polymerization *or* wall forward or backward jump) and the time elapsed from the last one
- Independent trajectories (realizations)

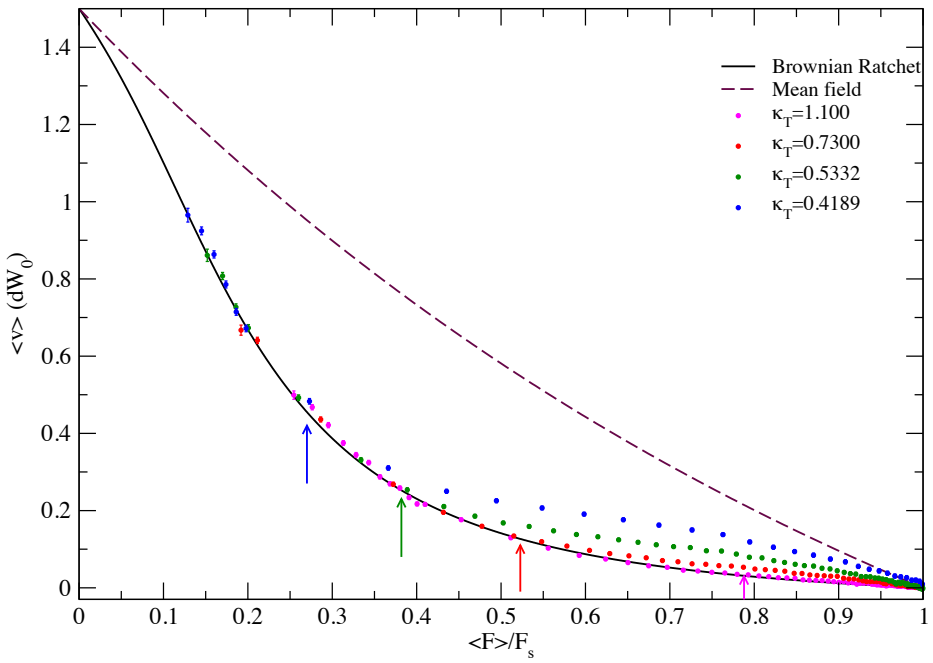
$$\begin{cases} \langle L(t) \rangle \\ \langle v(t) \rangle = \frac{d\langle L(t) \rangle}{dt} \\ \langle F_L(t) \rangle = \kappa_T \langle L(t) \rangle \end{cases}$$

$$\langle v(t) \rangle \; vs \; \langle F_L(t) \rangle$$



1. D. T. Gillespie, *The Journal of Physical Chemistry* **8**, 2340 (1977).

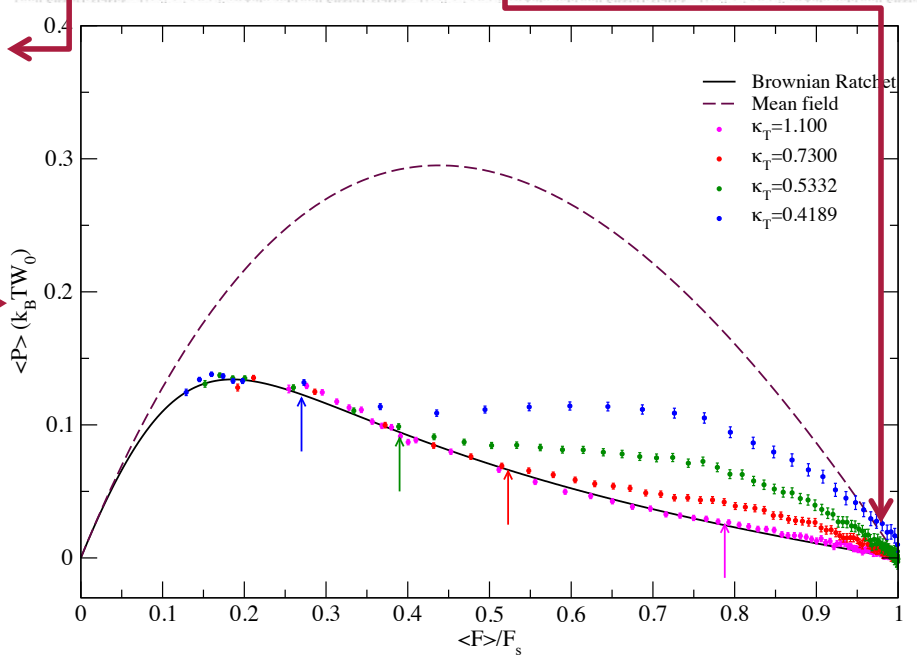
Velocity-load and Power-load relationships



Velocity vs load for various trap strengths

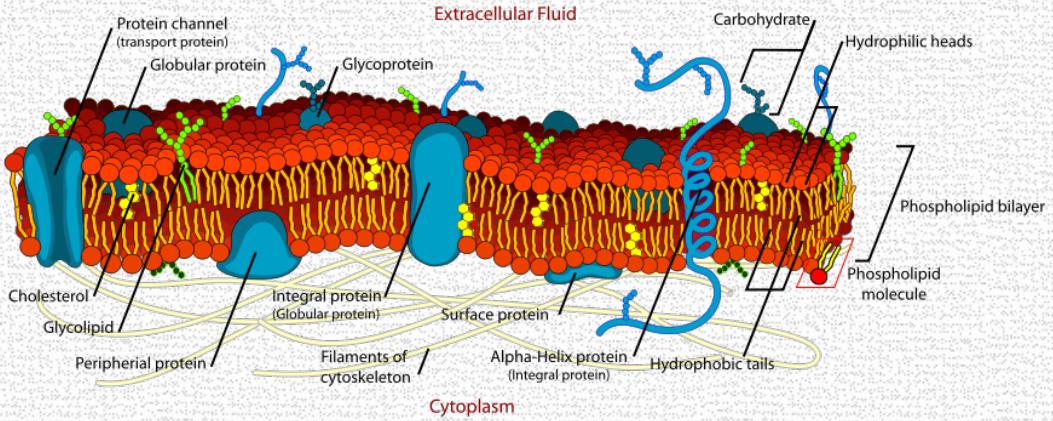
Stalling force: same as previous models

Power vs load for various trap strengths



Future work: Bundle interacting with cell membrane

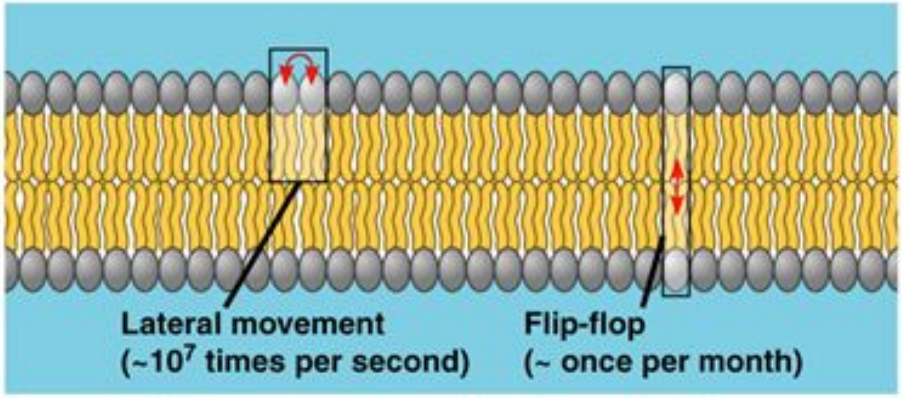
- Cell (or plasma) membrane
  - ✓ Biological membrane which separates the interior of all cells from the outside environment
  - ✓ Phospholipid bilayer with embedded proteins
  - ✓ Soft with respect to the cytoskeleton
  - ✓ Highly deformable to adapt to cell shape variations (endocytosis, exocytosis, tubules formation…)



Dynamic interplay between the plasma membrane and the cytoskeleton in the tubules formation still not fully understood

Future work: Bundle interacting with cell membrane

- Fluidity: Phospholipids and proteins can drift laterally along the membrane



- Bilayer thickness: small compared to the length scales describing its shape and its undulations

→ *Zero-thickness limit*

- Compressibility modulus: usually rather large

→ *Incompressible limit – fixed membrane area*

Future work: Bundle interacting with cell membrane

Continuum model for thin, incompressible membranes (Canham 1970, Helfrich 1973):

- Only contribution to the configurational energy: *bending energy*

$$\mathcal{H}_{el} = \int dS \left[\frac{\kappa}{2} (H - H_0)^2 + \bar{\kappa} K \right]$$

H = mean curvature

H_0 = spontaneous curvature

K = Gaussian (intrinsic) curvature

κ = bending (rigidity) coefficient

$\bar{\kappa}$ = Gaussian bending coefficient

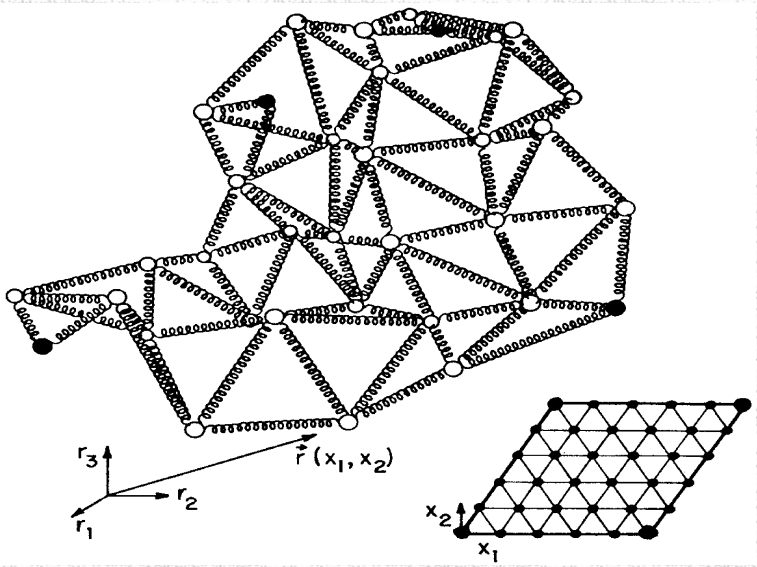
Randomly-triangulated-surface model for fluid membranes

- Bending energy -- Discretization of the surface into hard spheres tethered into a triangular network embedded in 3-dimensional space (*triangulated surface*)

$$E_{bend} = \frac{1}{2}\lambda_b \sum_{\langle ij \rangle} |\mathbf{n}_i - \mathbf{n}_j|^2$$

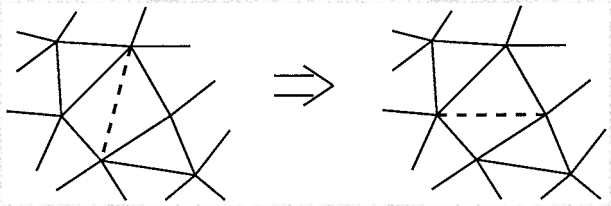
Sum over all pairs of neighbor triangles

Normal versor to triangle *i*



- Fluidity -- Network model must allow for the diffusion of vertices along the membrane

Connectivity = dynamic variable by cutting and reattaching tethers

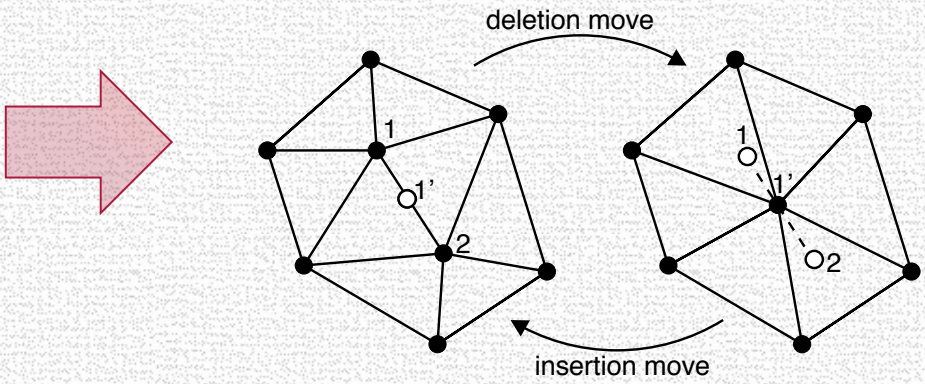


Randomly-triangulated-surface model for fluid membranes

- Fluctuations -- *In-vitro* experiments: μm-length membrane tubes protruding from the surface of a large vesicle

  - ✓ it is considered as a fixed reference frame and reservoir of lipids
  - ✓ it fixes the *surface tension*

Grandcanonical Montecarlo:
MC moves attempting to change the number of vertices in the triangulated surface



J. Weichsel and P.L. Geissler, PLoS Comput Biol **12**(7), e1004982 (2016)

Future work: Algorithm implementation

- Algorithm for numerical simulations:
  - ✓ Living filaments:
    1. 3D coarse grained particle-based model where each particle represents a monomer
    2. Bonding, bending and excluded volume interactions between monomers inside a filament
    3. Langevin dynamics for the monomers with a second-order-accuracy numerical integration scheme
    4. (De)polymerization events with fixed attempted rates through a Monte Carlo scheme
  - ✓ Membrane:
    1. Dynamically triangulated surface with variable connectivity of vertices
    2. Membrane fluctuations reproduced by a grandcanonical Monte Carlo scheme
    3. Langevin dynamics for the membrane particles with a second-order-accuracy numerical integration scheme
  - ✓ Solvent:
    1. Langevin approach for actin and membrane units
    2. Hydrodynamic interactions: *neglected*

Future work: Investigated quantities

- *Approach:* numerically reproduce the phenomenon of membrane tubule formation induced by the bundle polymerization force
- *Measured quantities:* forces, tubule length and number of involved filaments, for which experimental data are available
- *Purpose:* clarify the mutual role of actin filaments and cell membrane in the tubule formation
  1. Relationship between protrusion elongation and membrane surface tension
  2. Is actin alone sufficient to induce the filopodium growth or are other accessory proteins needed to drive cellular shape change?
  3. Is filopodium elongation limited by G-actin diffusion?
  4. …

Thanks for your attention!