WORKSHOP ON DRIVEN STATES IN SOFT AND BIOLOGICAL MATTER
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Active Thermodynamics of Cell Membranes

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Workshop on Driven States in Soft and Biological Matter
ICTP, April 18-28, 2006
Introduction: Microvilli

Living epithelial cell (A6 cell line)

J. Gorelik et. al.; PNAS 100, 5819–5822 (2003)
Introduction: Microvilli

human blood lymphocyte

S. Majstoravich et. al.;

J. Gorelik et. al.;
Introduction: Microvilli

Actin bundles:

Dynamic:

J. Gorelik et. al. ; PNAS 100, 5819–5822 (2003)
Microvilli

Schematic picture:

Actin filament break-up at base

Actin monomer diffusion to tip

Tip complex

R
Actin polymerization driven motion

1. Extracellular stimuli

2. Produce active GTPases & PIP2

3. Activate WASp/Scar

4. Activate Arp2/3 complex to initiate new filaments

5. Barbed ends elongate

6. Growing filaments push membrane forward

7. Capping protein terminates elongation

8. Aging

9. ADF/cofilin severs & depolymerizes ADP-filaments

10. Pool of ATP-actin bound to profilin

11. Pool of ATP-actin bound to profilin

12. LIM-kinase inhibits ADF/cofilin

PAK

Actin treadmilling

10. Profilin catalyzes exchange of ADP for ATP
Our model: link actin polymerization with membrane curvature

Dynamics of Membranes Driven by Actin Polymerization

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Filopodia: Membrane proteins at the tip

Phospho-tyrosine (PY), Cdc42, Src etc.

Do these active proteins have a positive spontaneous curvature?
Filopodial tip complex

- Also important are bundling proteins such as Fascin.
- Tip contains formins, protecting actin from capping.

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Restoring force: saturation

\[ h_c \propto L \]

\[ F_0 \approx \kappa / R \]

"Long cylinder" limit

Equation of motion

\[
\frac{\partial h}{\partial t} = -\omega h + An
\]

Where:

\[\omega \propto \frac{F_0}{h_c}\]

\[v_0 = A\langle n \rangle\]

Restoring force

Growth velocity

And a noise term:

\[\langle (n - \langle n \rangle)^2 \rangle \neq 0\]
Microvilli: Height distribution

Observations:

Microvilli: Height probability distribution

From the equation of motion

→ Fokker-Planck equ.:

\[
\frac{\partial P}{\partial t} = \frac{\partial}{\partial h} \left( \omega h P \right) + \frac{1}{2} \frac{\partial^2 P}{\partial h^2} \frac{A}{\omega} \left\langle n^2 \right\rangle - \frac{\partial P}{\partial h} A \left\langle n \right\rangle
\]

At steady-state we get: \( \frac{\partial P}{\partial t} = 0 \)

\[\Rightarrow P(h) = R_n e^{2hv_0/D_h} e^{-h^2\omega/D_h} \]

where:

\[ D_h = A^2 \left\langle n^2 \right\rangle / \omega = (\Delta n/n_0)^2 v_0^2 / \omega \]

Describes the distribution of tip activity
Fluctuations in the size of the MV tip

Aggregation driven by spontaneous curvature:

\[ n \propto R^2 \]

\[ \langle n^2 \rangle = \frac{k_B T}{\partial^2 E_n / \partial n^2} \approx n_0^2 \frac{k_B T}{\kappa} \Rightarrow \left( \frac{\Delta n}{n_0} \right)^2 \approx \frac{k_B T}{\kappa} \]

Typically \( \kappa \sim 5-20 \ k_B T \)

Thermal noise!
Microvilli: Height probability distribution

Why narrow distribution in ridges?
Microvilli: Spatial distribution/patterns


Reduced membrane curvature energy between the MV:

\[ E_{\text{curv}} \approx \frac{\kappa h}{R} \]

Increased curvature energy of flattened tip:

\[ E_{\text{bend}} \approx \frac{\pi \kappa}{8} \]

Min. height for ridge:

\[ E_{\text{curv}} \geq E_{\text{bend}} \Rightarrow h_{\text{ridge}} \approx \frac{\pi}{8} R \]
Microvilli: ridges & height distribution

MV of unequal heights attract each other less than MV of equal heights

Additional restoring force: $-\omega(h - h_{nn})$
MV in ridges: Height prob. distribution

Ridge height equation of motion:

\[
\frac{\partial h}{\partial t} = -\omega h + A n_0 - \omega (h - h_{nn})
\]

Mean-field:

\[ h_{nn} \approx \langle h \rangle \]
Exponential tails: due to force saturation

human blood lymphocyte

Long Microvilli: Force saturation

\[ \langle h \rangle \approx A \langle n \rangle / \omega < h_c \]

\[ P(h) = \begin{cases} 
R_n e^{2hn_0/D_h} e^{-h^2 \omega / D_h} & h < h_c \\
R'_n e^{2h(-\omega h_c + A \langle n \rangle) / D_h} & h > h_c 
\end{cases} \]

\[ \frac{\partial h}{\partial t} = \begin{cases} 
-\omega h + An & h < h_c \\
-\omega h_c + An & h > h_c 
\end{cases} \]
Long MV: Height probability distribution
Microvilli: Spatial distribution/patterns


Microvilli: Spatial distribution/patterns

- Dynamic $\rightarrow T_{\text{eff}}$
- “Thermodynamic” phase diagram
Microvilli: Spatial distribution/patterns

- Linear aggregates due to positive spontaneous curvature of tip complex

T. Tlusty & S. Safran; Science 290 (2000) 1328

2D dipolar fluid, network of worm-like micelles etc.
Microvilli: Spatial distribution/patterns

- Assume single height of MV: $<h>$
- Excluded volume interactions
- Defects: free ends and 3-fold junctions

Based on:
Microvilli: Energy of defects

\[ E_{\text{end}} \approx \frac{2\pi \kappa \hbar}{R} - \frac{\pi \kappa}{8} \]

Increases with \( h \)

\[ E_{\text{junct}} \approx \frac{\pi \kappa \hbar}{6R} + \frac{2\pi \kappa}{15} \]

Increases more slowly with \( h \)
Formation of networks

If the MV height increases, junctions multiply over ends:

Phase transition to a connected network:

$$\frac{\partial^2 F}{\partial \phi^2} = 0$$

Spinodal
Free energy of gas of defects

\[
F(\phi)/k_BT = (1 - \phi) \ln (1 - \phi) + \phi_e (\ln \phi_e - 1) + \phi_j (\ln \phi_j - 1) \\
+ \phi_e \epsilon_e + \phi_j \epsilon_j - \frac{1}{2} \phi_e \ln \phi - \frac{3}{2} \phi_j \ln \phi
\]

\(\phi\) is the area fraction of the MV
\(\phi_e\) & \(\phi_j\) is the area fraction of the ends and 3-fold junctions respectively

Minimize with respect to independent defects’ concentrations:

\[
\phi_j = \phi^{3/2} e^{-\epsilon_j} \\
\phi_e = \phi^{1/2} e^{-\epsilon_e}
\]
Using: $\kappa = 10 \ k_B T$, $h = 400$nm

Microvilli: Spatial distribution/patterns

Percolation line

Coexistence region

"Gas"

Network "liquid"
Note: Large “effective” temperature

Using: $\kappa = 10 \, k_B T$, $\Phi = 0.05$

$h_{ridge} \approx \frac{\pi}{8} R$

$\frac{\partial^2 F}{\partial \phi^2} = 0$
Using a fixed $T_{\text{eff}}$
Does it really behave as “active”-thermodynamics?

Predictions of thermodynamic theory:

\[ P(l) \sim e^{l/\bar{l}}, \text{ where } \bar{l} = \frac{\phi}{\phi_e / 2 + 3\phi_j / 2} \]
- Phase separation?
- What changes between cells?
- Equilibrium between systems in contact?
Conclusions

- **Spontaneous curvature of membrane proteins** that activate actin polymerization drives Microvilli dynamics and morphology.

- **Coupling of active and thermal fluctuations** on different length and time scales: “Active-Thermodynamics”
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Multi-scale Modeling

Thermal & actin-driven aggregation → Tip formation

~100nm

Thermal & actin-driven aggregation of MV → Network formation

~1µm

Cell-wide morphology and shape

~10µm
Experimental challenge:

We need to characterize the physical parameters of the protein aggregates at the membrane:

- What is the spontaneous curvature?