Signalling through biological membranes

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PHASE SEPARATION IN BIOLOGICAL MEMBRANES : Lipid Rafts



in vitro experiments reveal large "rafts"

in vivo the rafts are much smaller

- Consensus is 25nm (or less) ~100nm
- e.g. 26 +/- 13 nm (Pralle *et al.* JCB 2000)

Would be almost invisibly small here



Baumgart et al. Nature 2003

Equilibrium: dilute, circular rafts

$$G/k_{\rm B}T = \sum_{n=1}^{\infty} c_n (\log c_n + \sigma \sqrt{n} - \mu n)$$

$$c_n = e^{-(\sigma\sqrt{n} - \mu n)}$$

critical

$$\Phi_c = \sum nc_n \to \sum ne^{-\sigma\sqrt{n}} \approx 1/\sigma^4 \ll 1$$

Large tensions $(\sigma > 1)$

When $\Phi > \Phi_c$ an area $\Phi - \Phi_c$ phase separates into "infinite" raft

On adding material...



Crossed phase boundary into 2-phase region

So, how do intermediate sized rafts persist on cells ?

CELL MEMBRANES ARE ALIVE !



'Short' timescale (~minutes): exchange between various membrane compartments

Longer timescale (>1/2 hour): Synthesis of membrane material

Non-equilibrium: recycling



Raft dynamics



Mean field master equation

$$\frac{dc_n}{dt} = J(n) + \sum_{m=1}^{\infty} k_{n,m} c_{n+m} - k'_{n,m} c_n c_m + \frac{1}{2} \sum_{m=1}^{n-1} k'_{m,n-m} c_{n-m} c_m - k_{m,n-m} c_n$$

 c_n is number density of rafts of area n(nc_n is area fraction of rafts of size n)



Kinetics

- Simplified diffusive collisions $D \neq D(n)$
 - k' = 1

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- defines timescale in terms of a microscopic diffusion time $b^2/D \sim 10^{-4} {
 m s}$
- At equilibrium (no recycling)

$$c_{\rm eq} = e^{-E(n) + \mu n}$$
 eg
$$c_{\rm eq} = e^{-\sigma \sqrt{n} + \mu n}$$

– Detailed balance then *requires*

$$k_{n,m} = e^{-\sigma(\sqrt{n} + \sqrt{m} - \sqrt{n+m})} / k'$$

Use this out-of-equilibrium (rafts have no long "memory")

Growth from pure monomers (no recycling)

 $n c_n$

QuickTime[™] and a Animation decompressor are needed to see this picture.



$$\Phi = 2\%; \quad \sigma = 8$$

Recycling

$$\dot{c}_n = J(n) + \dots$$

"Whole raft" recycling

$$J(n) = j_{\rm on}\delta_{n1} - j_{\rm off}c_n$$

"Monomeric" recycling from rafts

$$J(n) = j_{\rm on}\delta_{n1} - j_{\rm off}(c_n - c_{n+1})$$

can suggest many other schemes

Turning whole raft recycling on (initially at equilibrium)

 $n c_n$

QuickTime[™] and a Animation decompressor are needed to see this picture.

n

$$j_{\text{off}} = 10^{-2}; \quad \Phi = 2\%; \quad \sigma = 8$$

Steady state

no scission (large σ); whole raft recycling

$$\frac{dc_n}{dt} = 0 = j_{\text{on}}\delta_{n,1} - (j_{\text{off}} + N)c_n + \frac{1}{2}\sum_{m=1}^{n-1} c_{n-m}c_m$$

$$c_{1} = j_{\text{on}} / (j_{\text{off}} + N)$$

$$c_{2} = \frac{1}{2} j_{\text{on}}^{2} / (j_{\text{off}} + N)^{3}$$

$$\vdots$$

$$c_{n} = A_{n} j_{\text{on}}^{n} / (j_{\text{off}} + N)^{2n-1} \qquad A_{n} = \frac{(2n-2)!}{2^{n-1} n! (n-1)!}$$

Steady state

no scission (large σ); whole raft recycling

Not Ostwald ripening !



$$j_{\text{off}} = 10^{-2}; \quad \Phi = 2\%; \quad \sigma = 8$$

Asymptotics

no scission (large σ); whole raft recycling



The variation of the mean raft size \bar{n} with $\phi/j_{\text{off}} \sim$ the residence time of a typical raft (solid line) when the raft scission rate is negligible. The asymptotic variation for large $\phi/j_{\text{off}} \gg 1$ is shown as $\bar{n} = \sqrt{\phi/(2j_{\text{off}})}$ (dashed line).

Results

- Raft size as non-equilibrium phenomenon
- For typical resulting raft sizes: e.g. whole raft recycling & no scission
 - For 1 min residence time steady state raft radius=30 nm
 - For 30 mins residence time → steady state raft radius=60 nm
 [b=3nm D=0.1µm²/sec]
- Can handle *perturbations*, e.g.
 - up- or down- regulate lipid recycling
 - add raft material
 - Biologically important and *testable*

Signalling II Membrane tension sensitivity



The cell would like to avoid this !

Safety valve for the cell: Mechano-sensitive channels

Function: osmotic regulation



Patch clamp measurements

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

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Frans Maathuis, York

- Open channel area controls conductivity
- 'Structural' energy barrier $\Delta G_{channel}$ estimated from gating properties



A new mechanism: gating-by-tilt



Physical model for fluid membranes should include:



 ΔA area increase

For a fluid membrane

$$H = \frac{1}{2} \int dA \left[\kappa (\nabla^2 u)^2 + \gamma (\nabla u)^2 \right]$$



$$\begin{array}{ll} \textbf{Model}\\ & & & & \\ H = \frac{1}{2} \int d^2 r \left[\kappa (\nabla^2 u)^2 + \gamma (\nabla u)^2 \right] & 1/k = \text{length} = \sqrt{\kappa/\gamma} \approx 50 \text{nm}\\ & u = \alpha K_0(kr) + \beta \log kr & u = \theta K_0(kr)/(k \; K_1(ka)) & \text{for } r \geq a \end{array}$$

$$\Delta G = \pi \kappa k a \theta^2 \frac{K_0(ka)}{K_1(ka)}$$

Compute θ required for (i) 100% and (ii) 10% of experimental estimate of ΔG_{dilate}

Data

MS Channel	$p_{1/2} (\mathrm{mmHg})$	$1/\alpha (mmHg)$	$\Delta G_{0}\left(kT\right)$	Conductance (nS)	$Diameter_{pore}({\rm \AA})$	References
MscL	75	4.4–5	14–19	3.3–3.8	35	Häse et al. 1995 Sukharev et al. 1999 Kloda and Martinac 2001 <i>c</i> , 2001 <i>d</i>
MscS	36	5	7	0.97 (+ve) 0.65 (-ve)	18	Martinac et al. 1987 Martinac (unpublished) Kloda and Martinac 2001 <i>d</i>
MscA1	34	2.3	15	0.38 (+ve) 0.68 (-ve)	11	LeDain et al. 1998
MscA2	43	1.5	29	0.85 (+ve) 0.49 (-ve)	17	LeDain et al. 1998
MscMJ	57	11	5	0.27	9	Kloda and Martinac 2001a
MscMJLR	29	1.7	17	2.2 (+ve) 1.7 (-ve)	27	Kloda and Martinac 2001b
MscTA	78	2.4	35	2.8		Kloda and Martinac 2001c, 2001d

Corresponding tilt angles

θ

Channel	θ_{100}	θ_{10}
	[100% of gating ΔG_{dilate}]	[10% of gating ΔG_{dilate}]
MscL	39°	12°
MscS	24°	8°
MscA1	21°	7°
MscA2	31 [°]	10°
MscMJ	16°	5°
MscMJR	35°	11°

Formally the *Gaussian curvature* also enters the Hamiltonian



$$H = \int dA \left(\frac{\kappa}{2}C^2 + \frac{\bar{\kappa}}{R_1R_2}\right)$$

(neglecting tension) Mean curvature: $C = 1/R_1 + 1/R_2$

This now contains *all* terms up to quadratic order in curvature

Principle curvatures can have different signs

 $C_2 = 1/R_2 < 0$

 $C_1 = 1/R_1 > 0$

QuickTime[™] and a TIFF (Uncompressed) decompressor are needed to see this picture.

Gauss-Bonnet theorem

$$n_c = 1$$
 $n_c = 1$ $n_c = 1$ $n_c = 1$ $n_c = 1$
 $n_h = 0$ $n_h = 1$ $n_h = 2$ $n_h = 5$



Remember, this term appeared in the Hamiltonian

Finite Gaussian rigidity $\bar{\kappa}$

• Membrane channel gating (structural) energy modified by $\Delta \bar{G} = \pi \theta^2 \bar{\kappa}$



No tension dependence - so doesn't contribute to mechanosensitivity

Conclusions II

- Gating-by-tilt a viable candidate for mechanical sensitivity
 - Energetically
 - Structurally
- Mechanosensitivity is independent of $\bar{\kappa}$
- Assymmetric addition of lipids facilititates gating

[E. Perozo et al. Nat. Struct. Biol. 2002]

