Ordering of living membranes - how the cell maintains lipid 'rafts'

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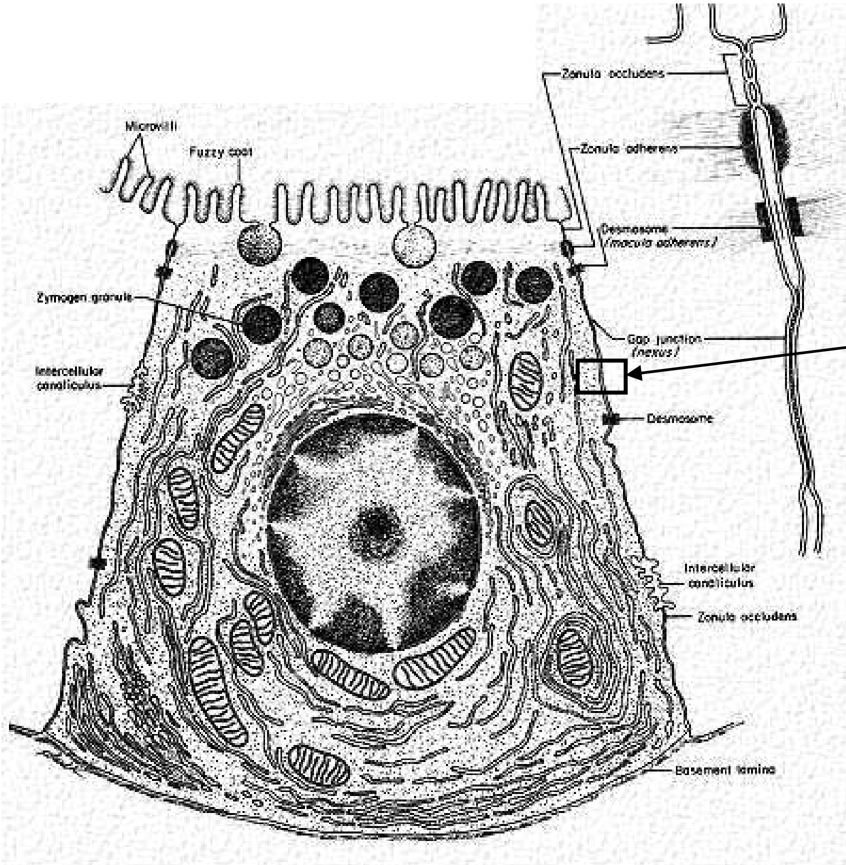
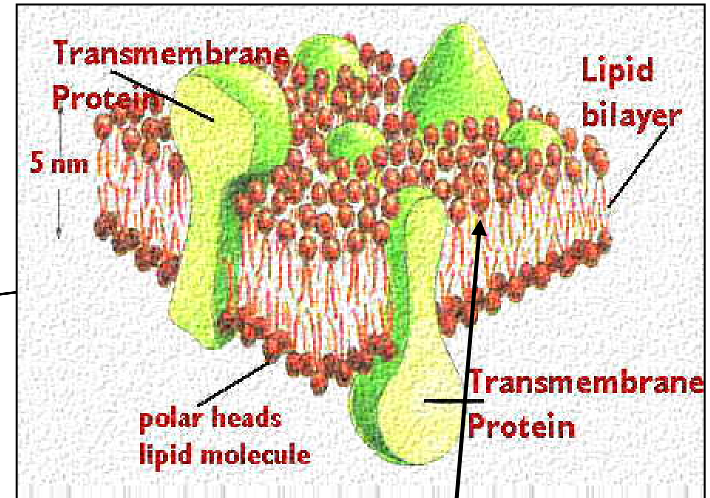
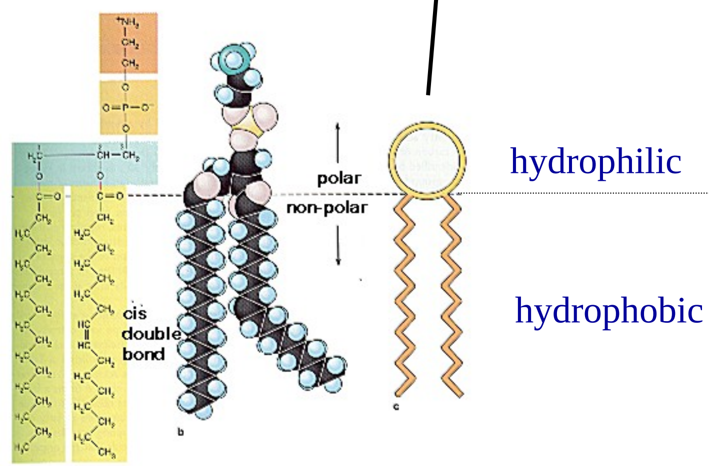
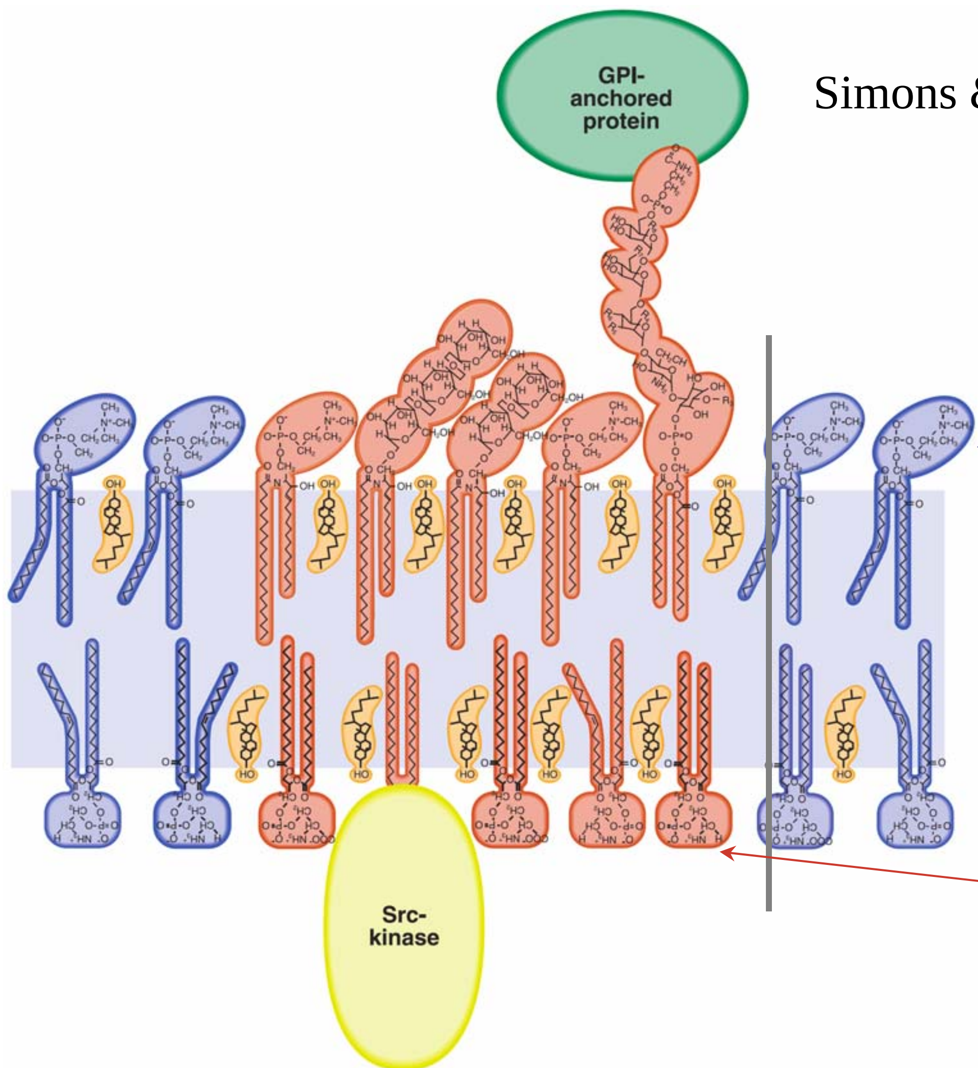

Microvilli
Fuzzy coat
Zymogen granule
Intercellular canaliculus
Zonula occludens
Zonula adherens
Desmosome (macula adherens)
Gap junction (nexus)
Desmosome
Intercellular canaliculus
Zonula occludens
Basement lamina


Transmembrane Protein
5 nm
Lipid bilayer
polar heads lipid molecule
Transmembrane Protein


cis double bond
polar
non-polar
hydrophilic
hydrophobic

PHASE SEPARATION IN BIOLOGICAL MEMBRANES : *Lipid Rafts*

Simons & Ikonen *Science* **290** 1721 (2000)



Liquid Disordered

unsaturated tails

low cholesterol content

Liquid Ordered

Saturated tails

High cholesterol content

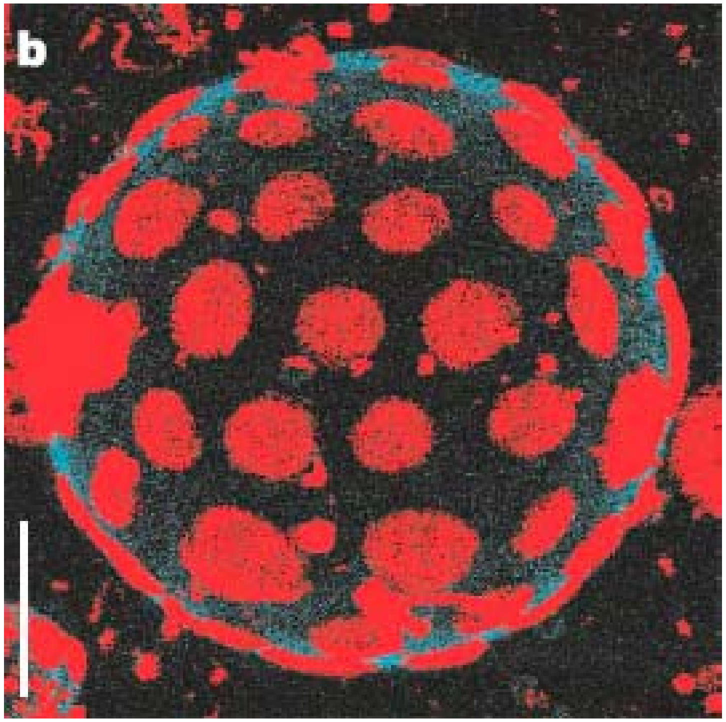
in vitro experiments reveal large “rafts”

***in vivo* the rafts are much smaller**

- Consensus is **tens of nm - 100nm**
- e.g. 26 +/- 13 nm (Pralle *et al.* JCB 2000)

Would be almost invisibly small here

5μm



Baumgart *et al.* Nature 2003

Equilibrium: dilute, circular rafts

$$G/k_{\mathrm{B}}T = \sum_{n=1}^{\infty} c_n \left(\log c_n/e + \gamma\sqrt{n} - \mu n\right)$$

$$\frac{\partial G}{\partial c_n} = 0 \Rightarrow \quad c_{\mathrm{eq}} = e^{-\gamma\sqrt{n}+\mu n}$$

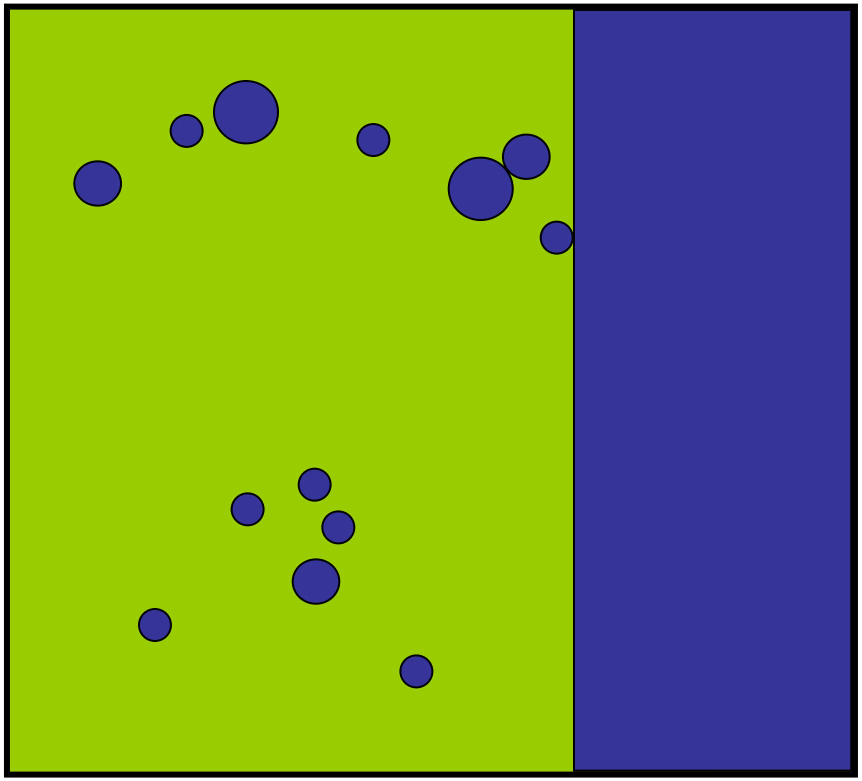
$$\phi = \sum n c_n \xrightarrow[\text{adding mass}]{} \phi_c = \sum n e^{-\gamma\sqrt{n}} \approx e^{-\gamma}$$

When $\phi > \phi_c$ an area $\phi - \phi_c$

phase separates into "**infinite**" raft

Highly bimodal distribution: ~monomers + *very large* raft(s)

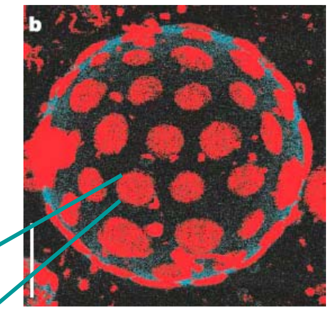
On adding material…



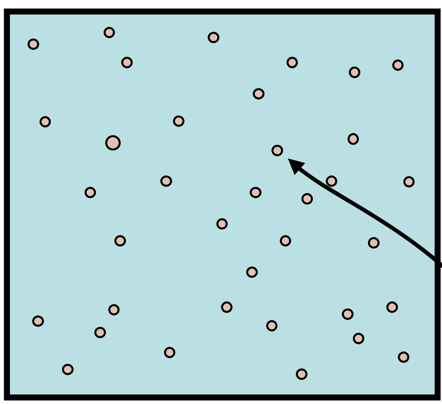
Crossed phase boundary into 2-phase region

$$\phi > \phi_c$$

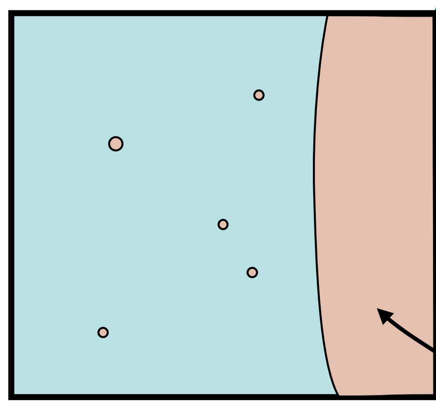
Equilibrium thermodynamics of two component membranes



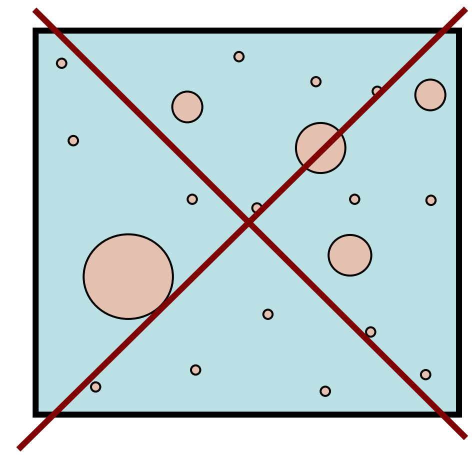
Baumgart *et al.* Nature 2003



Low LO/LD line tension γ
Small domains
~1 protein & lipid skirt



High line tension:
Small domains &
"phase separated"
very large domain(s)



Intermediate-
sized
domains are rare
at <u>equilibrium</u>

Typical (ternary) phase diagram

e.g. Sparr *et al.* Biophys J. 2002

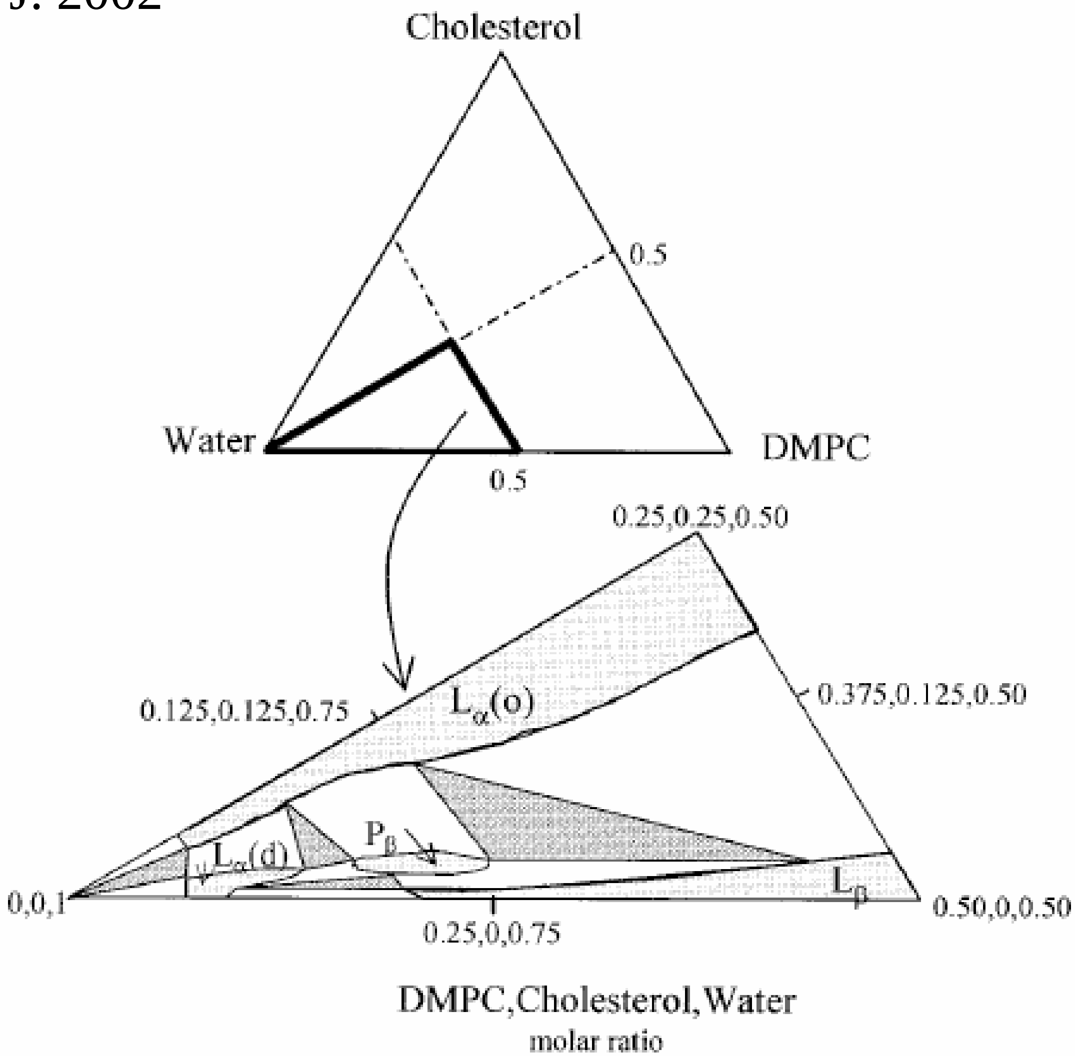
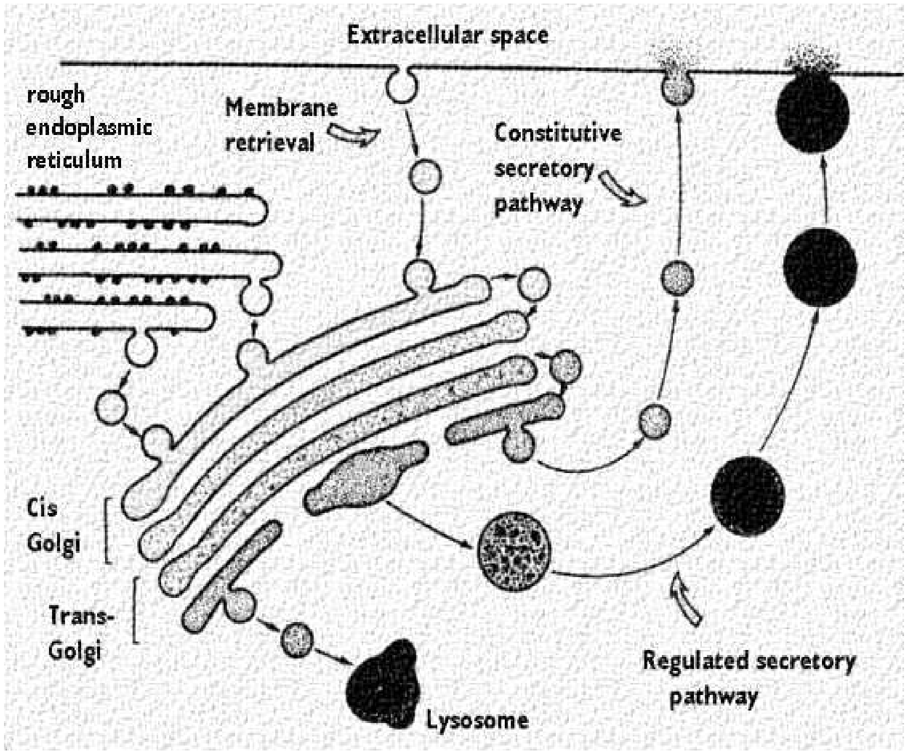


FIGURE 5 Schematic partial ternary composition phase diagram of the water-rich corner for DMPC-cholesterol-water at 27°C. The phase diagram is outlined from the microcalorimetric data and the ^{2}H-NMR spectra. The phase diagram contains four three-phase triangles (*dark gray*), four one-phase regions (*light gray*), and several two-phase regions (*white*). The exact positions of triangle corners and the phase boundaries are not determined, and the figure only provides the main features of phase diagram.

But… cell membranes are alive !



'Short' timescale (~minutes):

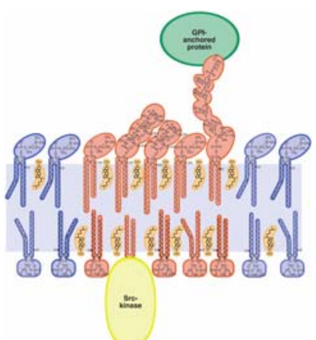
exchange between various membrane compartments

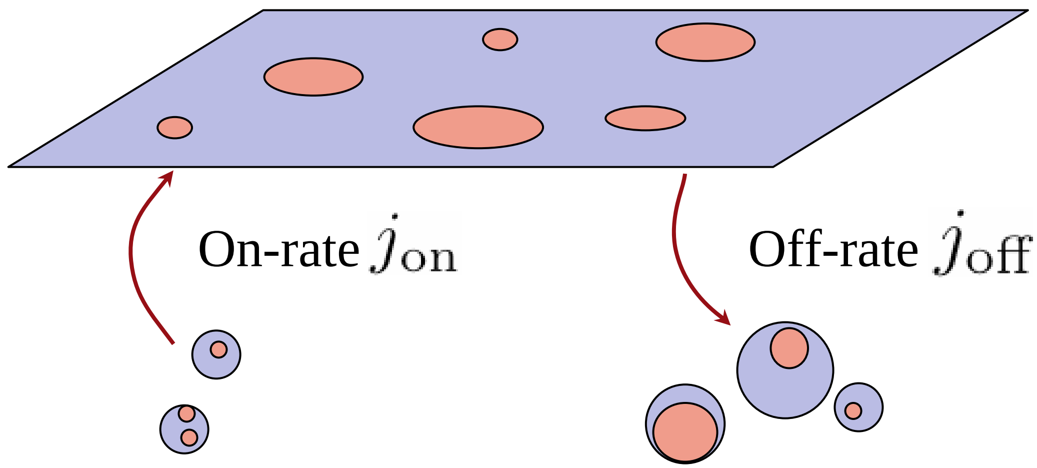
Longer timescale (>1/2 hour):

Synthesis of membrane material

Non-equilibrium: recycling

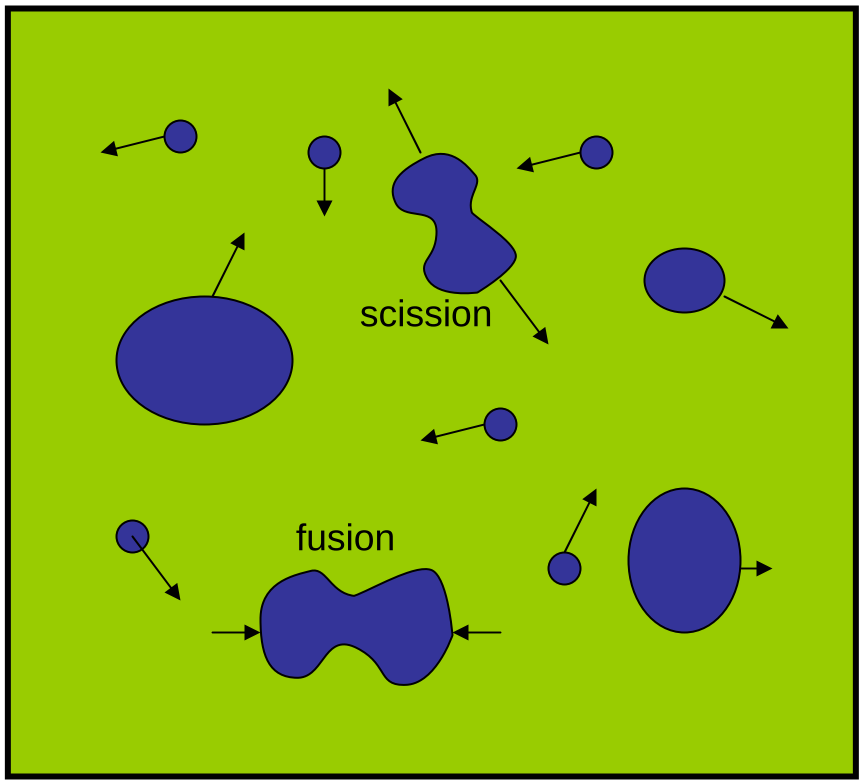
e.g. scheme #1: monomer deposition / raft removal





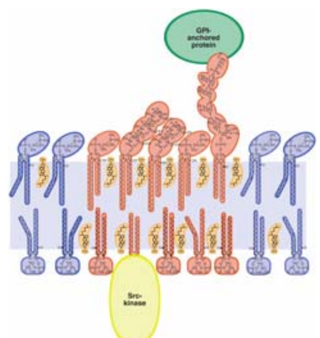
Monomeric rafts **Rafts of all sizes**

Raft dynamics



Discrete model

Introduce a ‘monomeric’ raft

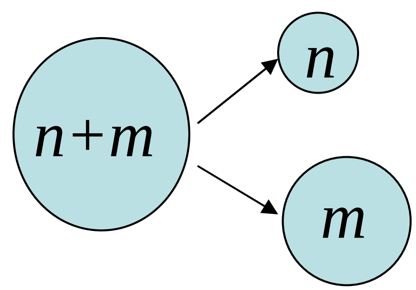


One raft-resident protein and its lipid ‘skirt’

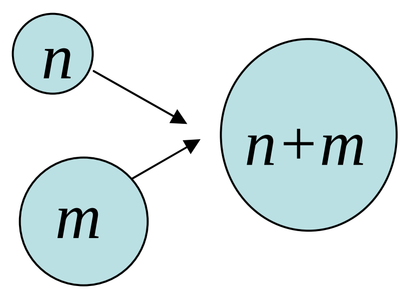
Mean field master equation

$$\dot{c}_n = \sigma(n) + \sum_{m=1}^{\infty} k_{n,m}\, c_{n+m} - k'_{n,m}\, c_n c_m$$
$$+\frac{1}{2} \sum_{m=1}^{n-1} k'_{m,n-m}\, c_{n-m} c_m - k_{m,n-m}\, c_n$$

$k_{n,m}$ is *scission* rate



$k'_{n,m}$ is *fusion* rate



See also *Turner & Cates '90* for a similar treatment of wormlike micellar systems

Raft kinetics

- Simplified diffusive collisions $D \neq D(n)$
  - Can set k′=1
    - defines timescale in terms of a microscopic time $\tau_{\mathrm{D}} = b^2/D >\sim 10^{-5}\ s$

Diameter of a monomeric raft

  - At equilibrium (no recycling)

$$c_{\mathrm{eq}} = e^{-E(n)+\mu n}$$

    - eg

$$c_{\mathrm{eq}} = e^{-\gamma\sqrt{n}+\mu n}$$

Detailed balance then *requires* that all the microscopic rates balance exactly

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

Use this out-of-equilibrium (rafts have no long “memory” of collisions)

Growth from pure monomers

(no recycling)

$n \; c_n$

QuickTime™ and a
MPEG-4 Video decompressor
are needed to see this picture.

n

$\phi = 10\%$

$\gamma = 8 \quad (\rightarrow \sim 0.5 k_{\mathrm{B}} T / nm)$

Raft recycling

$$\dot{c}_n = \boxed{\sigma(n)} + \ldots$$

1. Monomer deposition / raft removal

$$\sigma(n) = j_{\text{on}} \delta_{n1} - j_{\text{off}} c_n$$

2. Monomer deposition / monomer removal
(loss of monomers from rafts ~ radioactive decay)

$$\sigma(n) = j_{\text{on}} \delta_{n1} - j_{\text{off}} (n\, c_n - (n+1)\, c_{n+1})$$

These are the two most extreme examples in a class of *scale-free* recycling schemes

(can suggest many other schemes)

Growth from pure monomers

with recycling

(monomer deposition / raft removal)

$n\ c_n$

QuickTime™ and a
MPEG-4 Video decompressor
are needed to see this picture.

n

$\phi = 10\% \qquad j_{\mathrm{off}}\tau_{\mathrm{D}} = 10^{-2} \qquad \gamma = 8 \quad (\rightarrow \sim 0.5 k_{\mathrm{B}} T / nm)$

Turning recycling on

starting from equilibrium

(monomer deposition / raft removal)

$n \, c_n$

QuickTime™ and a
MPEG-4 Video decompressor
are needed to see this picture.

n

$$\phi = 10\% \qquad j_{\mathrm{off}} \tau_{\mathrm{D}} = 10^{-2} \qquad \gamma = 8 \quad (\rightarrow \sim 0.5 k_{\mathrm{B}} T / nm)$$

Steady state

no scission (large γ); monomer deposition / raft removal

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

$$c_1 = j_{\rm on}/(j_{\rm off} + N)$$

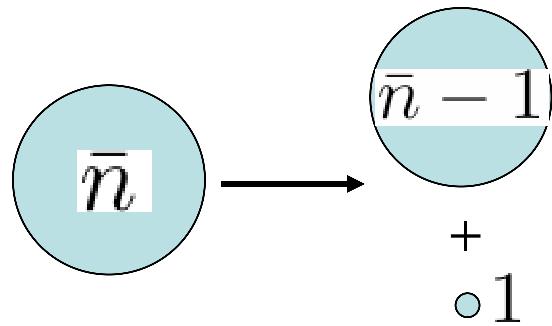
$$c_2 = \frac{1}{2} j_{\rm on}^2/(j_{\rm off} + N)^3$$

$$\vdots$$

$$c_n = A_n j_{\rm on}^n/(j_{\rm off} + N)^{2n-1} \qquad A_n = \frac{(2n-2)!}{2^{n-1} n!(n-1)!}$$

No scission: self-consistency

Fastest scission process involves *shedding monomers:*



From detailed balance $k_{n,m} = e^{-\gamma(\sqrt{n}+\sqrt{m}-\sqrt{n+m})}/k'$

$$k_{1,\bar{n}-1} \approx e^{-\gamma} k'$$ exponentially slow

rate lifetime

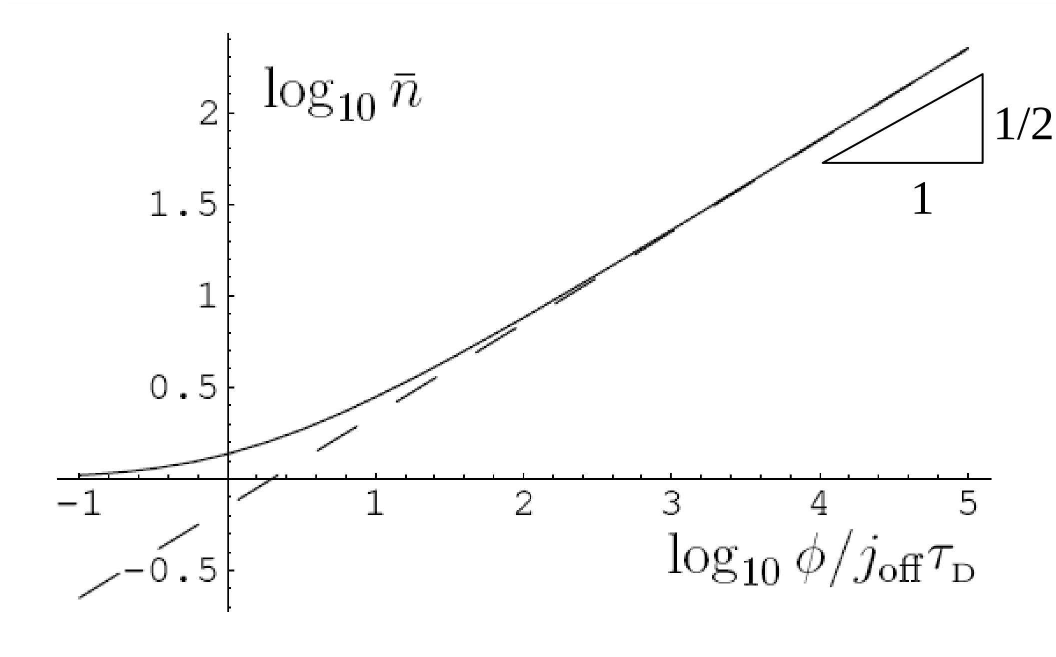
$$k_{1,\bar{n}-1} \frac{1}{j_{\rm off}} \ll \bar{n} \quad \Longrightarrow \quad \gamma \gg \boxed{\gamma^\star = \frac{1}{2} \log \frac{2}{\phi j_{\rm off} \tau_{\rm D}}}$$

Values

ϕ	j_{on} s^{-1}	j_{off} s^{-1}	τ_{D} s	$\bar{n}$ $(\gamma \gg \gamma^{\star})$	b nm	$\gamma^{\star}$ $k_{\mathrm{B}}T/\mathrm{nm}$	R nm
0.1	10^{-3}	10^{-2}	10^{-5}	700	5	0.6	66
					1	3.0	13

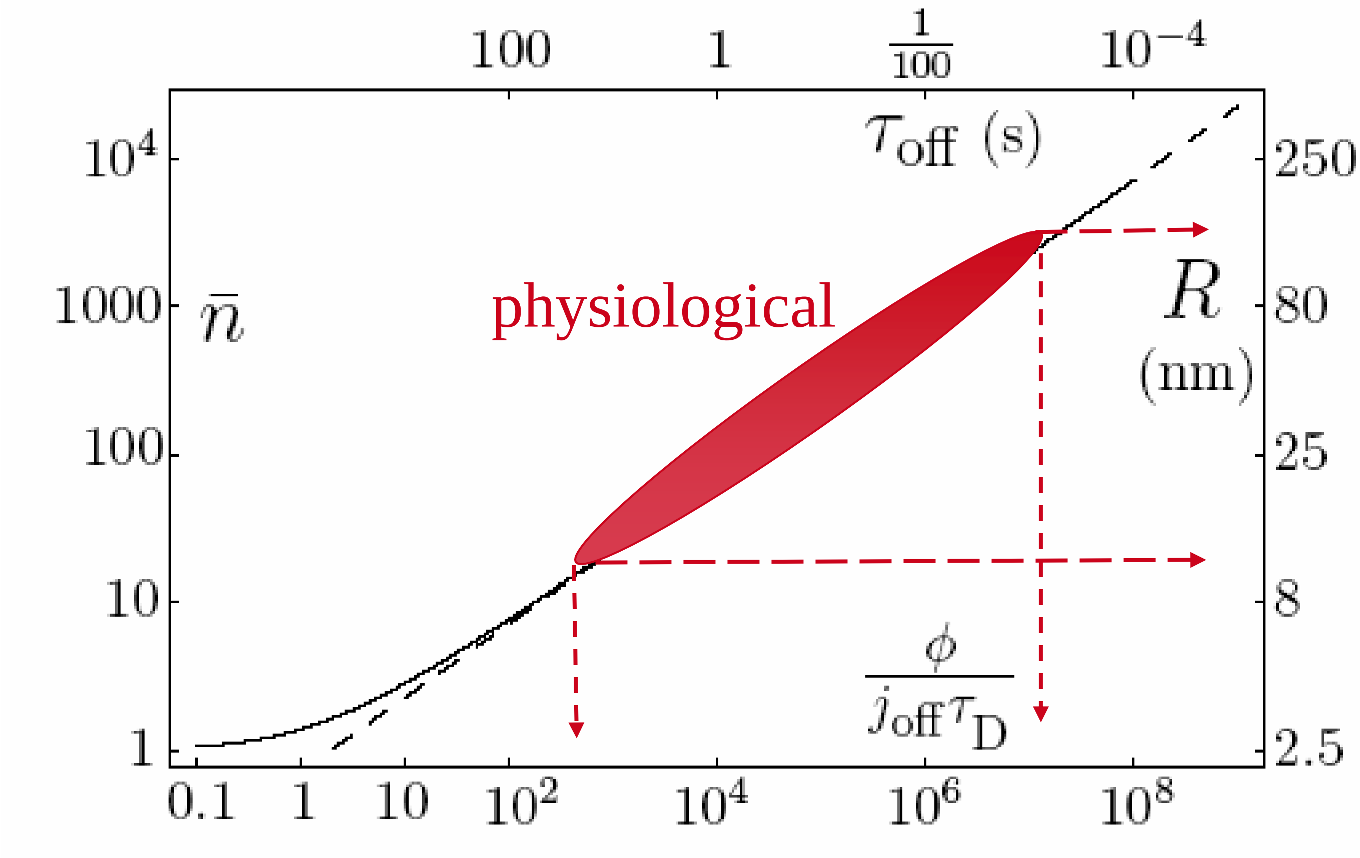
Asymptotic solution for c_n

$\Rightarrow$ mean raft size



no scission; monomer deposition / raft removal

Monomer deposition / raft removal; no scission



The steady state mean raft size is *intermediate* - tens of nm

Now…

analytical result (no-scission)

↑

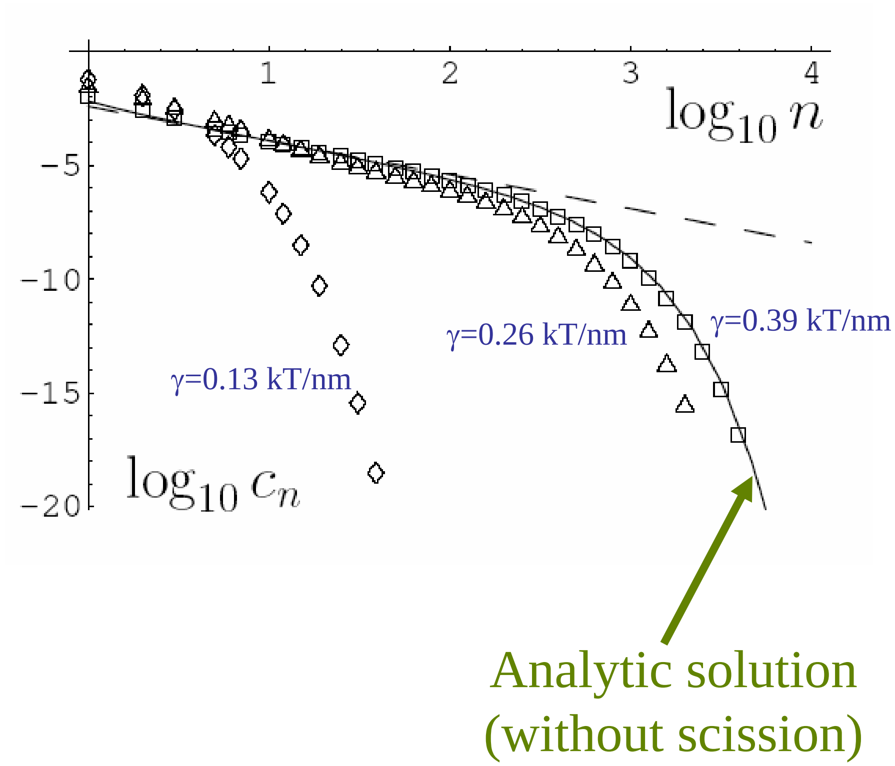
compare

↓

full numerical solution (with scission)

Numerical solution

Monomer deposition / raft removal



$$\phi = 10\% \qquad j_{\rm off}\tau_{\rm D} = 10^{-3}$$

- Power-law regime
  - extends $\Delta n \sim \tau_{\rm off} = 1/j_{\rm off}$

$$c_n \approx \sqrt{\phi j_{\rm off}\tau_{\rm D}/(2\pi)}\,\frac{1}{n^{3/2}}$$

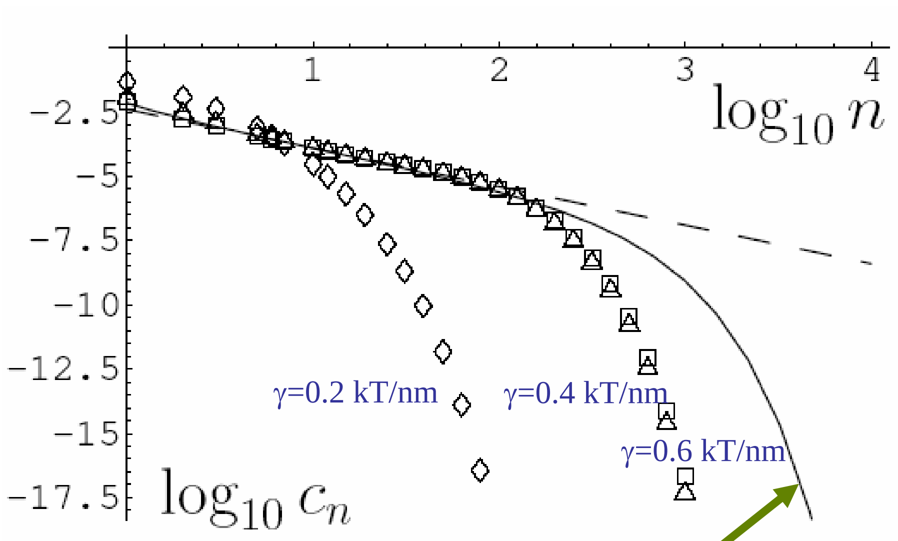
$$\text{for} \quad 1 \ll n \ll \bar{n}^2$$

- Analytic (asymptotic) solution holds for large line tensions

- Broad distribution of raft sizes

Numerical solution

Monomer deposition / monomer removal

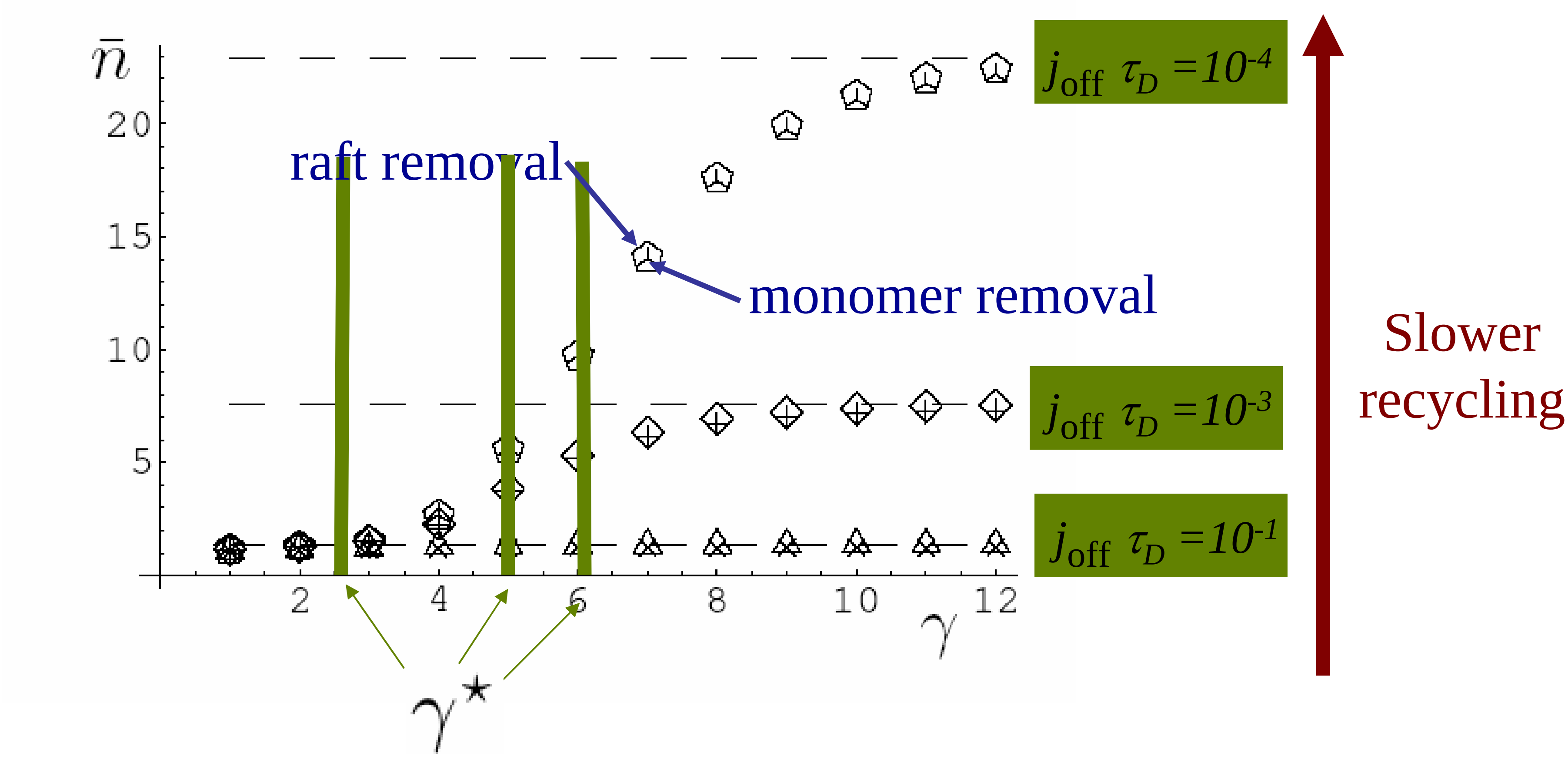


Analytic solution
(Monomer deposition / raft removal
without scission)

$$\phi = 10\% \qquad j_{\mathrm{off}}\tau_{\mathrm{D}} = 10^{-3}$$

- Power-law regime still appears
- Solutions don’t converge to analytic solution for large line tensions
  - No reason why they should !

The mean raft size is the same for the two extreme *scale-free* recycling schemes !



Propose: mean raft size is independent of recycling scheme for *all* scale-free processes

Perturbing the recycling

- add material externally
- up-/down-regulate recycling or synthesis pathways

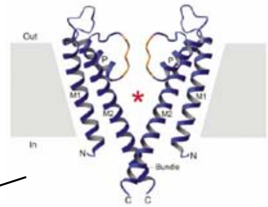
How does the membrane respond ?

$$\tau \quad \& \quad \phi \quad \Rightarrow \quad j_{\text{off}}$$

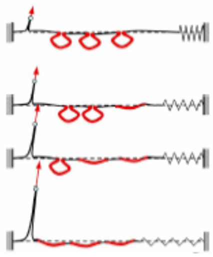
Summary

- Rafts are inherently non-equilibrium
- Recover sizes ~10-100 nm for physiological recycling rates
  - But may need to consider finite (cell) size effects
- Propose that all scale-free recycling yields the same mean raft size
- Can handle dynamic *perturbations* to the recycling
  - Biologically important and *testable*
- A connection between signaling and membrane traffic ?
- Chemical analogues with non equilibrium domains ?

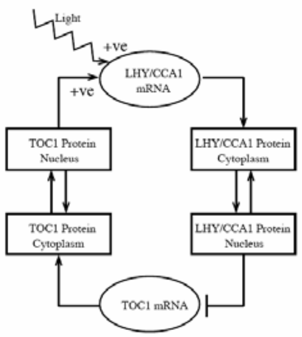
I'd also be happy to discuss…



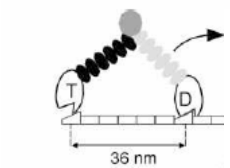
- Membranes
  - Mechanosensitive channels
  - Buffering of cell tension by buds



- Genetic networks
  - Circadian clocks



- Molecular motors
  - Towards a complete model of Myosin V



- Sickle hemoglobin fibers