UNITED NATIONS EDUCATIONAL, SCIENTIFIC AND CULTURAL ORGANIZATION
INTERNATIONAL ATOMIC ENERGY AGENCY
INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS
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SMR.961 - 8

WORKSHOP ON:
PROTEINS, MEMBRANES and their INTERACTIONS

22 JULY - 2 AUGUST 1996

"Mechanics and statistical mechanics of fluid lipid-bilayer structures"

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Mechanics + Statistical Mechanics of Fluid-Phase Lipid-Bilayer Structures in Aqueous Solution

with Biological Applications!

Michael Wortis, Simon Fraser University, CANADA

At "Workshop on Proteins, Membranes, + Their Interactions"
Trieste, I.C.T.P, July 22 - 24, 1996

D. Beal (SFU)
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B. Fourcade
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E. Tüzücher

L. Mir
H-G. Döbereiner
M. Mierlić
+ Helfrich
Sackmann
Lipowsky
(many others) ...

1. Introduction

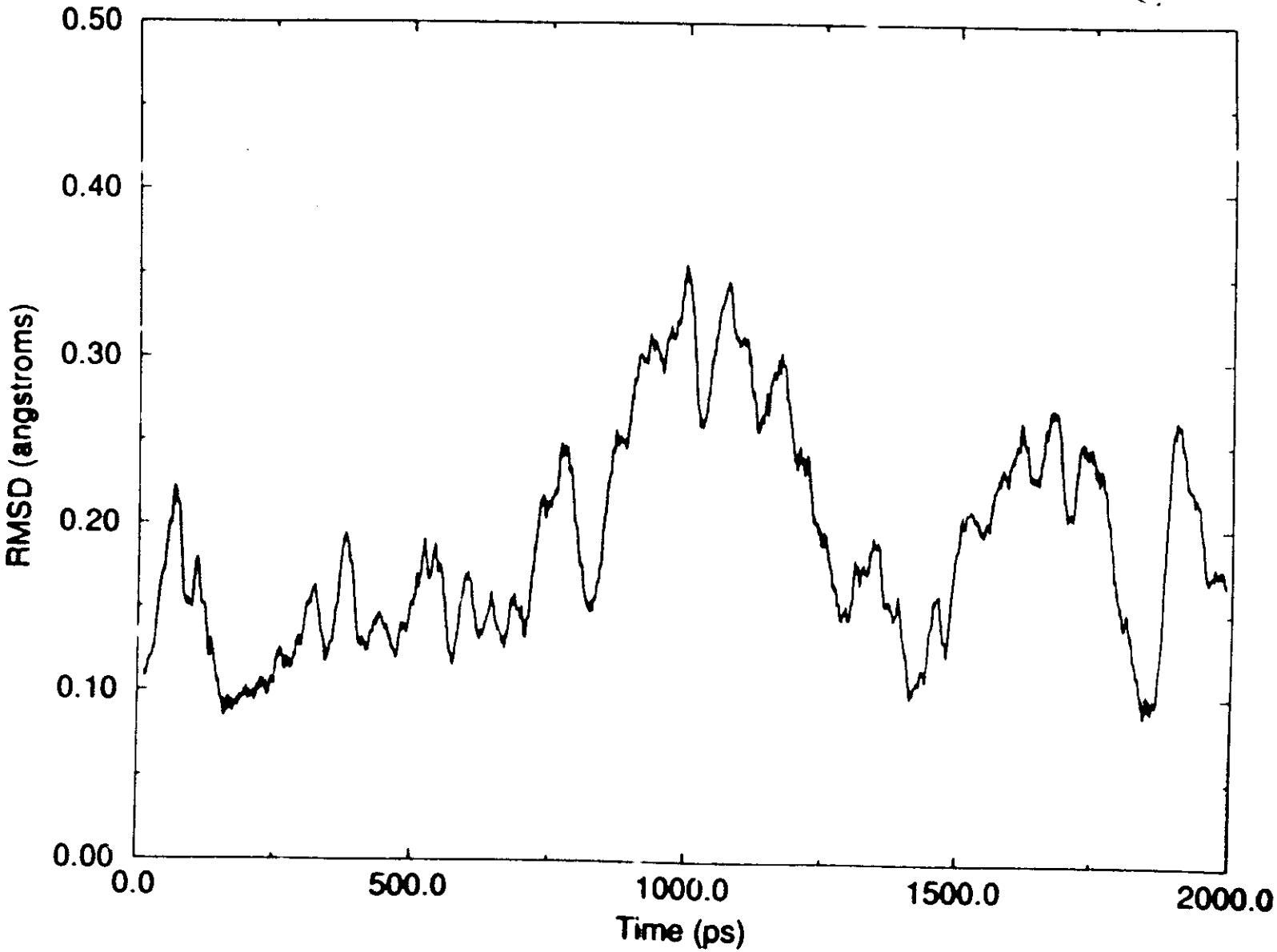
2. Aggregation Phenomena

3. Mesoscopic Description of Bilayer Shape Mechanics
   - Hamil[illegible] (Landau)
   - Persistence length
   - Bilayer Phases (revisited)
   - Fluid vs tethered Membranes (Gompper)

4. Vesicle Shapes + Shape Fluctuations
   - Shapes
   - Phase Diagram
   - Stability
   - Shape Fluctuations
   - Budding.

   ⇒ Dynamics (Seifert)

Fluctuations of optimized CHDLFC (300°K)



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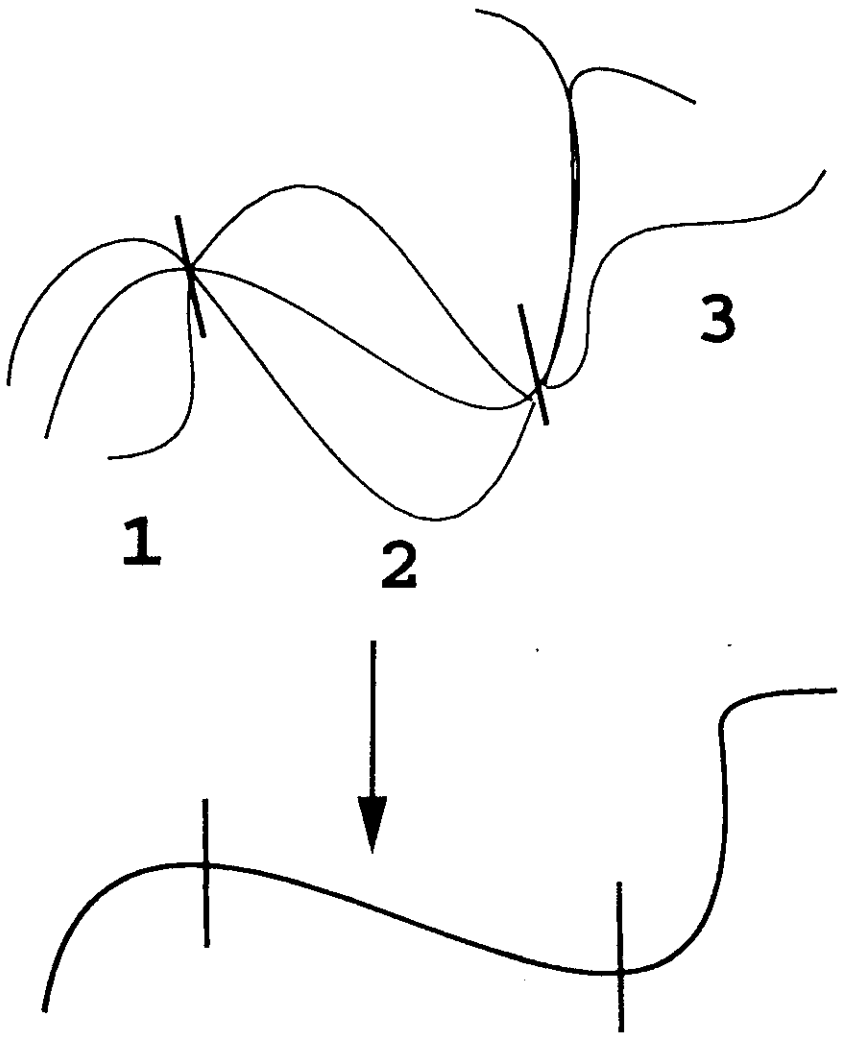
LES: Locally Enhanced Sampling to smooth energy surface and to obtain more sampling



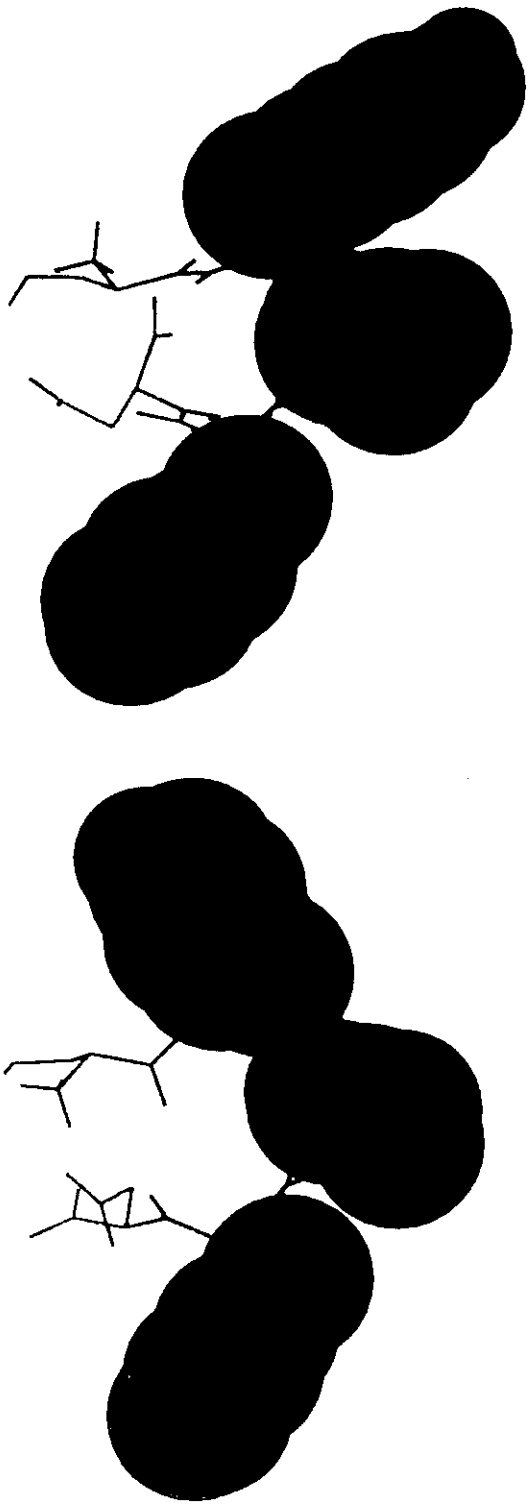
SYPFDV:

Electrostatic interactions between the C and N terminals induce the turn strcuture.

Hydrophobic interactions between the side chains come later. They are much weaker than the first and require considerably more gentle simulation conditions and the success rate is not that high as for the backbone

Final Structures from Short Annealing **Runs, superposed on NMR structure** using backbone **atoms of TYR-PRO-PHE**

Final Structures from Long Annealing Runs, superposed on NMR structure
using backbone atoms of TYR PRO PHE



Apply Energy Entropy to Membrane (Fluid)

4) Can membrane remain aligned at $T > 0$? Yes (as it does for tethered)

$$E = \frac{\kappa_b}{2}\int dS\,(c_1+c_2)^2$$

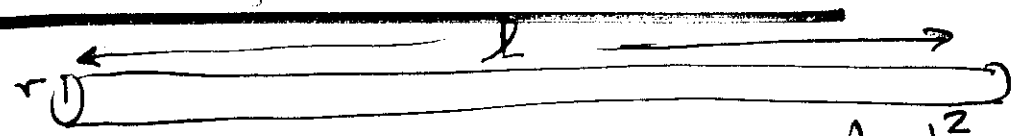
single crease of radius R

$$E^* = \frac{\kappa_b}{2} L R \left(\frac{1}{R}\right)^2 = \frac{\kappa_b}{2}\frac{L}{R}$$

$$g(E^*) \cong L^x \quad \Rightarrow \quad F = \frac{\kappa_b}{2}\frac{L}{R} - kT(\,)\ln L$$

c.f. $F = 0$ at $T = 0$

5) Can membrane lower its free energy by taking on linear shape?



$$A = L^2 = 2\pi r l$$

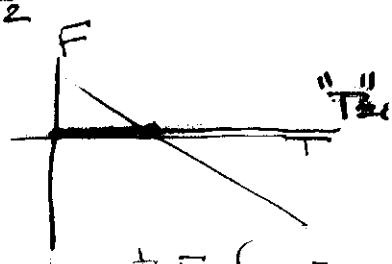
$$E = \frac{\kappa_b}{2} A \left(\frac{1}{r}\right)^2 \quad \text{(Energy cost)}$$

$$g(E) = q^{l/r} \qquad (q = \#\text{directions/bend})$$

$$S = k\ln g(E) = k\frac{l}{r}\ln q = \frac{kA}{2\pi}\frac{\ln q}{r^2}$$

$$F = \frac{1}{r^2}\left[\kappa_b - (\,)\,kT\ln q\right]$$

$\Rightarrow$ (branched) polymer for $k_B T > \kappa_b$

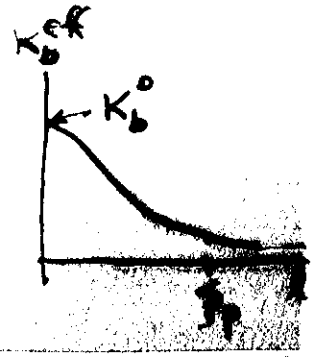


Lowest F for $r \to \infty$

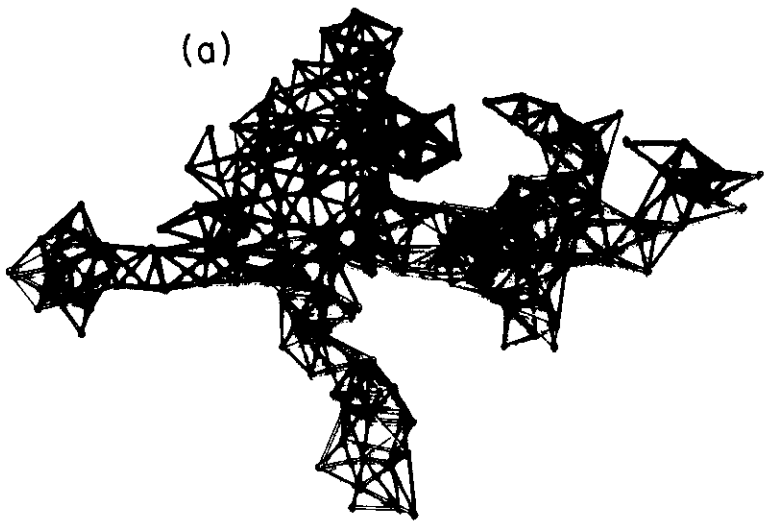
Not quite right!

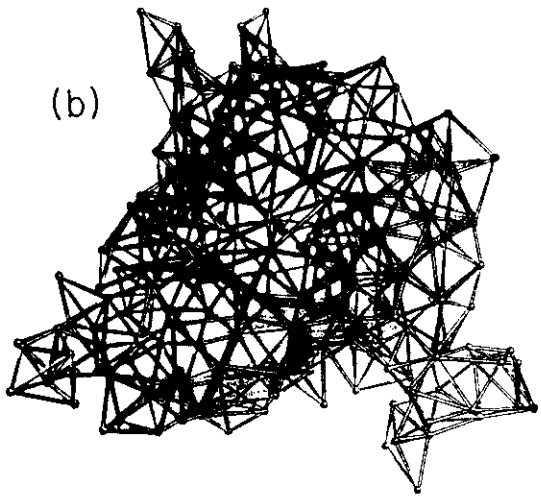
1. Renormalization of bending rigidity

$$\kappa_b^{eff}(\,) = \kappa_b^0\left[1 - \gamma\frac{kT}{4\pi\kappa_b^0}\ln(\,/a) + \ldots\right]$$



2. Microscopic length scale cuts off at ξ_p





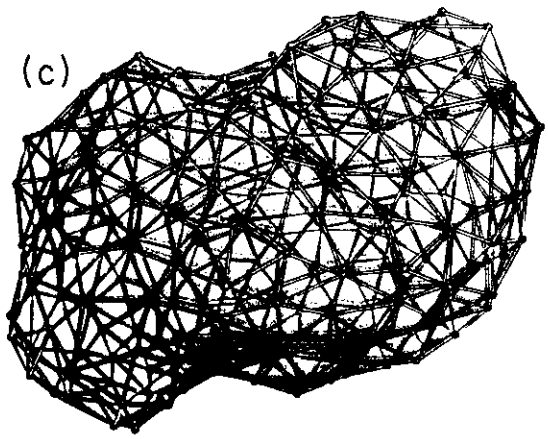
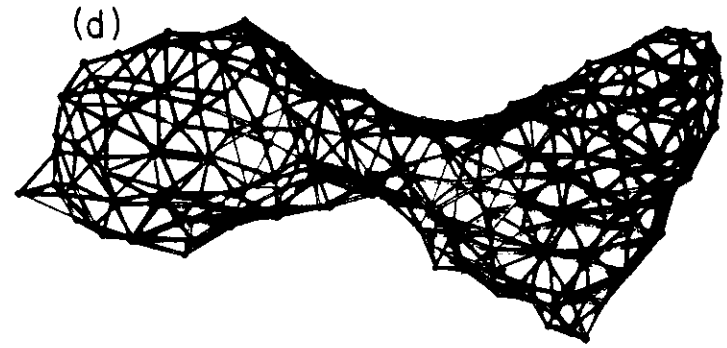


FIG. 2. Typical configurations of vesicles with $N = 247$ monomers. (a) $\lambda = 0.35$, $p = 0.125$ (branched polymer), (b) $\lambda = 0.35$, $p = 0.125$ (inflated), (c) $\lambda = 2.0$, $p = -0.05$ (prolate), (d) $\lambda = 2.4$, $p = -0.19$ (dumbbell), (e) $\lambda = 2.4$, $p = -0.35$ (branched polymer), (f) $\lambda = 3.0$, $p = -0.27$ (stomatocyte), (g) $\lambda = 3.8$, $p = -0.35$ (discocyte).



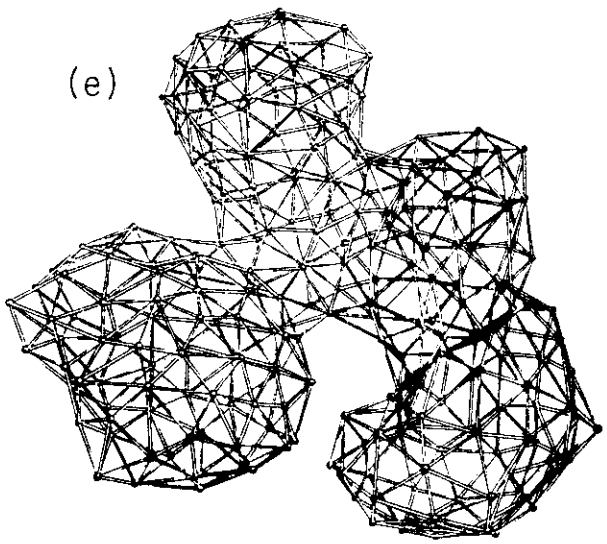
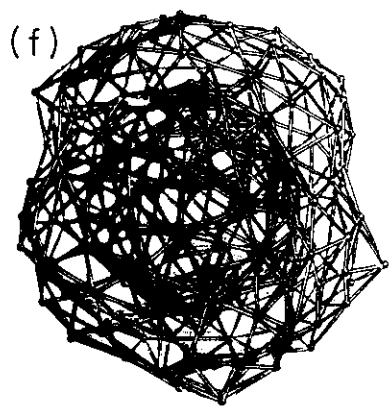
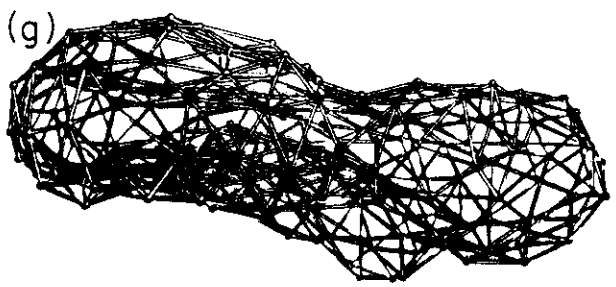


FIG. 2 *(Continued).*





What is the Hamiltonian $\mathcal{H}[S]$ for describing closed micron-scale phospholipid vesicles?

$$\mathcal{H}[S] = \gamma_0 \int dS + \boxed{\frac{1}{2}\kappa_b \int dS (C_1 + C_2 - C_0)^2} + \kappa_g \int dS\, C_1 C_2$$

topology fixed

area fixed

$$A_0 \simeq \left(\frac{N_1 + N_2}{2}\right) a_0(T)$$

+ higher order in d/R + λ_{edge} edge

$\frac{d}{R} \sim 10^{-3}$

large enough to close

Plus one additional term + subject to constraints!

Overall stretching energy is large $\Rightarrow$ incompressibility $a(\vec{r}) \simeq a_0(T)$

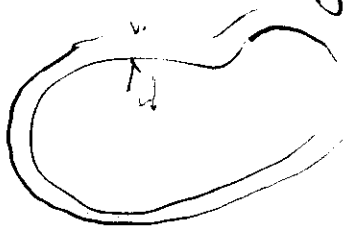
$$E_{str} = \frac{K}{2} \int dS \left(\frac{a(\vec{r}) - a_0}{a_0}\right)^2 \sim K R^2 \left(\frac{\Delta a}{a_0}\right)^2$$

i.e. $\frac{\Delta a}{a_0} \sim \frac{d}{R} \sim 10^{-3}$

BUT It is not generally possible to have $a = a_0$ in both leaves of the bilayer simultaneously!

$$\Delta A = \int dS\, d(C_1 + C_2)$$

depends on shape



check for sphere:

$$\Delta A = d \cdot 4\pi R^2 \cdot \frac{2}{R} = 4\pi R d \quad \checkmark$$

while $\boxed{\Delta A_0 = (N_1 - N_2) a_0}$ fixed at closure.

Comment: We might apply this as constraint on shape S.

But, better, simply allow individual monolayers to accomodate by making

$a_{in}, a_{out} \gtrsim a_0$ COSTS ELASTIC ENERGY

Compare (estimate) elastic stretching cost w. bending:

$\frac{K_s}{R^2}$: $2 \cdot \frac{1}{2} \cdot \frac{K}{2} \cdot \left(\frac{\Delta t - \Delta t_c}{t_c}\right)^2 \sim \frac{(Kd^2)}{R^2}$ same as bending energy density

$\frac{1}{2} K_b (c_1 + c_2)^2$ $(d/R)^2$

$$H[S] = \frac{K_b}{2} \int dS \left(c_1 + c_2 - c_0\right)^2 + \frac{\bar{K}}{2} \frac{\pi}{A_0 d^2} \left(\Delta A[S] - \Delta A_0\right)^2$$

i.e. $K \left(\frac{\Delta t - \Delta t_0}{A_c}\right)^2 \times A_c \times \frac{d^2}{d^2}$

Where a_{in} + a_{out} same over entire leaf (fluidity)
but not quite equal to a_0 (or to each other)

$\bar{K}$ called "renormed" bending modulus

$\bar{K}/K_b \equiv \alpha \sim 1$ ($\alpha_{SCPC} \approx 1.4$; $\alpha_{DMPC} \approx 1.1$)
material parameters

—H→

Expect laboratory shapes to be LOCAL minima of $H[S]$ importance of metastable since $\Delta E \sim K_b \gg k_B T$

Subject to constraints of

$V[S]$ = constant (in practice determined by osmotic)

$\Delta_c[S]$ = constant determined by $N_1 + N_2$ (T)

A_c = constant

Note V, t_c, and Δt_0 do change with T,

since, e.g., $A_0 = \frac{1}{2}(N_1 + N_2)\, a_0(T)$

$\Delta t_0 = (N_1 - N_2)\, a_0(T)$

Finding the local-minimum shapes:

1. Numerical: (Seifert et al.)

   Triangulated surface S
   Discrete representation of $H, V, A[S]$
   Track energy to local minimum
   Start again

   conceptually simple
   works for all kinds of shapes

   But very time consuming
   not very accurate
   { unreliable for singular shapes, near phase boundaries

2. Quasi-analytic:

   Form variational functional to incorporate constraints

   $$\Phi[S] = H[S] + \sigma A[S] - pV[S]$$

   Derive Euler equations: $\frac{\delta \Phi}{\delta S} = 0$ (Shape Equations)

highly nonlinear → Solve shape equations (numerical/analytic)
multiple solns

   Find parameters σ, p

   (Check energy minima!)

   only practical for axisymmetric shapes
   (but, for high volumes, most important shape branches are axisym!)

   BUT, very accurate.

Results:

"Shapes" (shape classes)
"Phase Diagram" (lowest energy vs parameters)
Stability (Regions where each shape class is local minimum)
(Stability Boundaries)

FIG. 1. — *Formes réversibles du globule rouge.* A : Stomatocyte II ; B : Stomatocyte III correspondant aux cellules 3 et 5 de la figure 3 ; C : Echinocyte II ; D : Echinocyte III correspondant aux cellules 2 et 4 (gross. : × 8 500).

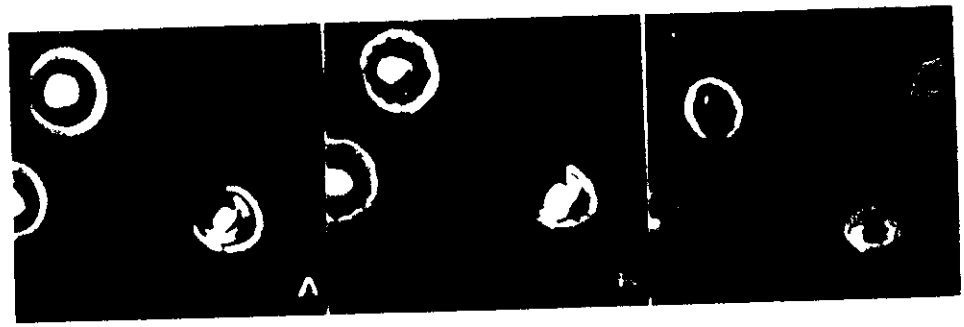


FIG. 2. — *Transformation directe d'un stomatocyte* (A) *en un stomatocyte crénelé* (B-C), observée au microscope à contraste de phases, par effet d'une micro-injection de salicylate de Na. Cinq secondes séparent la première image de la dernière.

what physical variables are changed? (to modify shape)

RÉSULTATS

Concentrations faibles

Les globules rouges lavés et transformés (fig. 1) par les faibles concentrations, indiquées plus haut, [illegible] sont capables de reprendre la forme discocyte s'ils sont ensuite mis en suspension dans du plasma frais, ou s'ils sont lavés dans une solution de NaCl. Il faut noter que, dans le cas de la chlorpromazine et de la prométhazine, deux ou trois lavages peuvent être nécessaires, suggérant une fixation de ces substances au niveau de la membrane.

Le mélange de deux de ces substances, aux effets opposés, n'a aucune action sur la morphologie des globules rouges, permettant de croire à l'antagonisme de ces agents.

Les cellules, une fois induites en échinocytes, peuvent être changées en stomatocytes par l'action de la chlorpromazine. Au cours de la transformation, les cellules peuvent repasser par le stade discocyte, mais de manière fugace. Lorsque la substance stomatocytaire est ajoutée tout d'abord, on obtient le phénomène inverse.

Parfois, pourtant, il semble que, dès que l'échinocyte a retrouvé sa concavité (*Echinocyte I*), la cellule prend la forme en coupe sans reprendre totalement l'état discocyte. On observe aussi, de temps en temps, une transformation directe d'un stomatocyte en un stomatocyte crénelé (cette cellule conserve la capacité de reprendre la forme discocyte) (fig. 2). Ceci suggère que ces deux types d'agents, bien qu'antagonistes, n'agissent pas aux mêmes sites de la membrane et paraissent être des inhibiteurs non compétitifs. La séquence des transformations morphologiques induites par les faibles concentrations chimiques est représentée sur la figure 3, de *1* à 5.

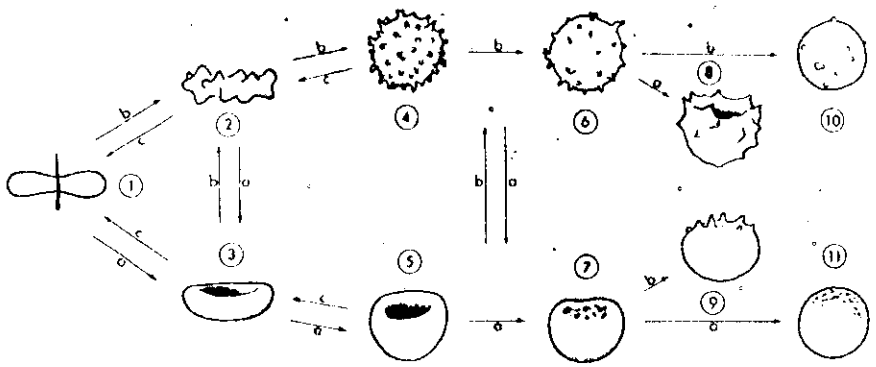


Fig. 3. — *Le rapport et la convertibilité des formes discocytaires, échinocytaires et stomatocytaires du globule rouge.* La direction *a* indique un changement stomatocytaire; la direction *b* un changement échinocytaire; la direction *c* indique un effet permettant au globule rouge de reprendre la forme discocytaire. Les cellules *10* et *11* évoluent vers l'hémolyse.

Symmetry

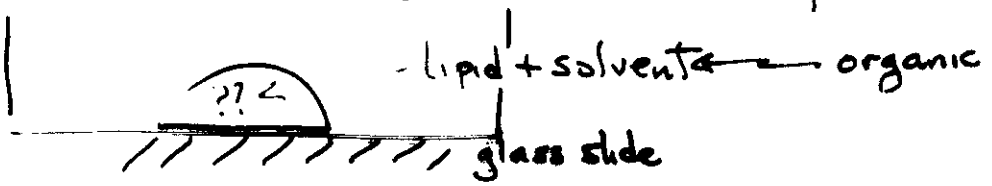
what is being manipulated in these transformations?

Do you see similar shapes and shape transitions for artificial vesicles?

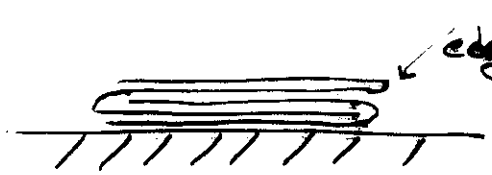
YES

Expt: Evans, UBC)

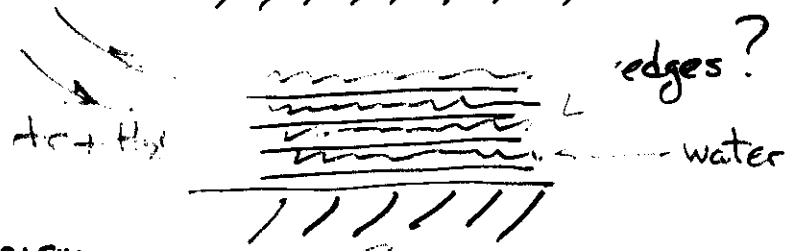
Produces SOPC vesicles ~ 20 μm.

1. 

2. Evaporate solvent ⇒ dry lipid

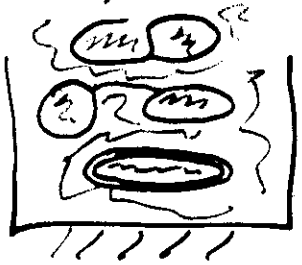


3. Careful swelling of layers



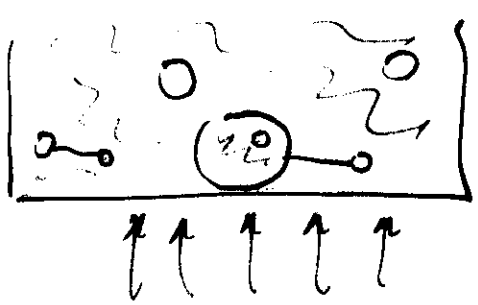
Closure mechanism is not known.

4. Rehydration



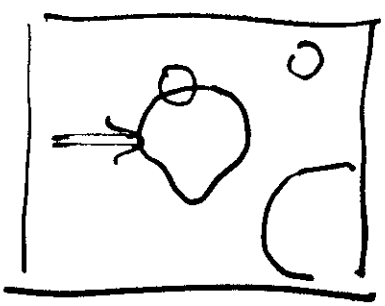
double layers etc.

5. transfer (micropipette manipulation)



inside/outside asym.

6. Phase Constrast Video Microscopy through bottom of cell (gravity)

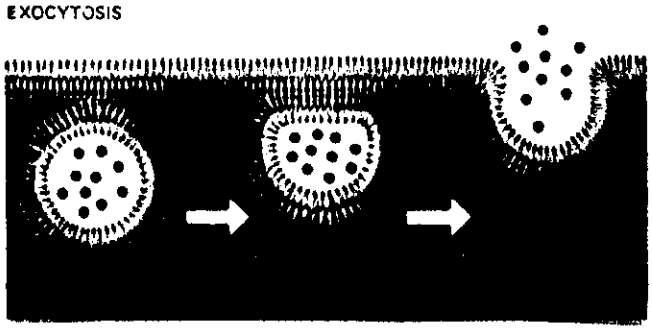


EXOCYTOSIS & ENDOCYTOSIS

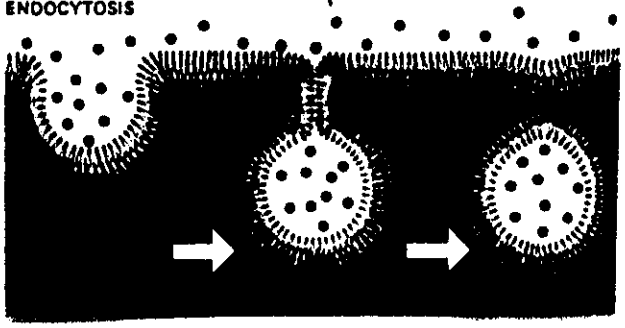
from Alberts et al., Molecular Biology of The Cell

biological importance of shape transformations

EXOCYTOSIS



ENDOCYTOSIS



SOPC

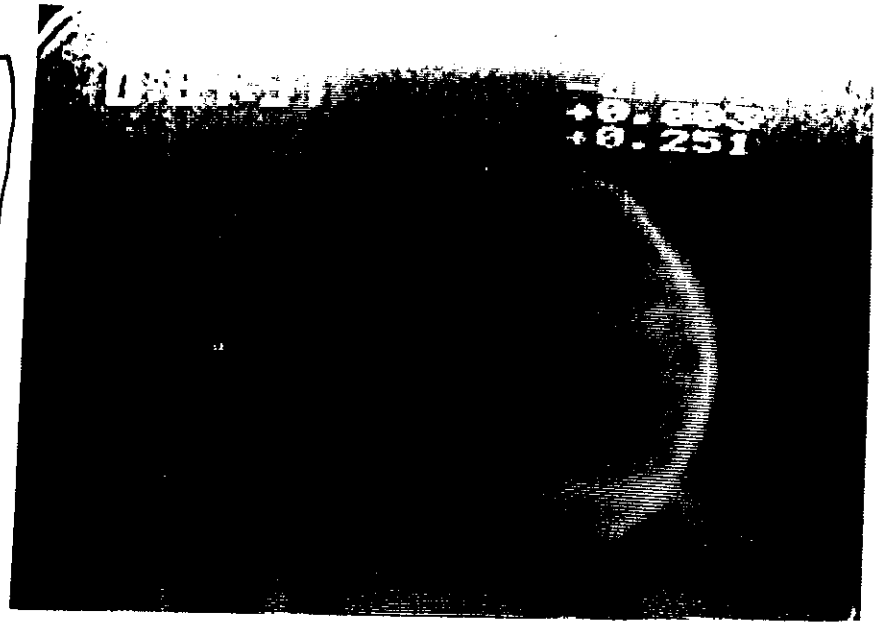
T↑

Lab of E. Evans.

Data: HG D.

~ 5 μm

E. Evans
W. Rawicz
A. Yeung



20 μ

lecithin (~OPC)
+
~6%
neg. charged lipid

differential phase-contrast microscopy

Sequence

volume ~ Const.
Area ↑

Morphology change at ~ 5% excess area (ΔA)

REVERSIBLE!

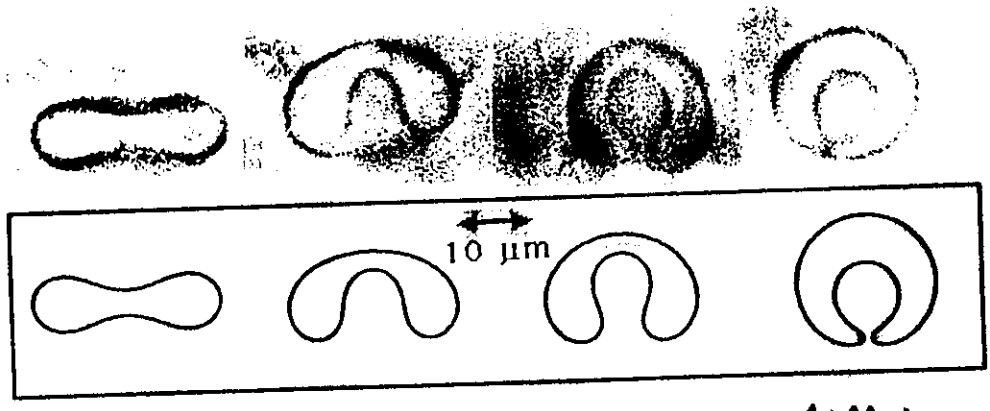
focus on "ball" not tubule?

explanation

Berndl, Käs, Lipowsky, Sackmann, Seifert
DMPC (lecithin) artificial vesicles

SAVE 11 +13a

The discocyte - stomatocyte - transition. (4)

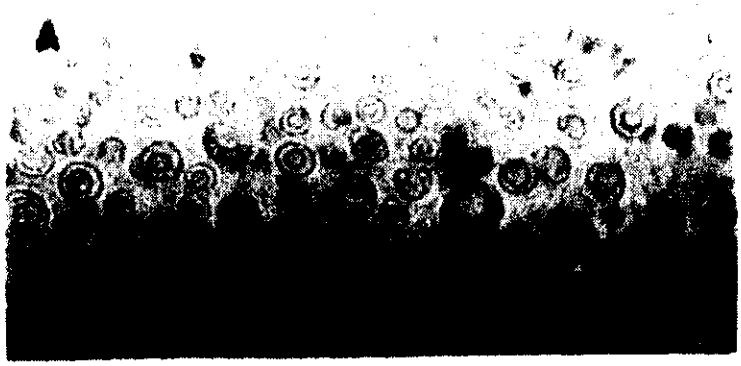


T= (°C) 43.8 43.9 44.0 44.1

Note: Changing T is modifying constraints: $A = A(T)$

$T\uparrow$, $A\uparrow$

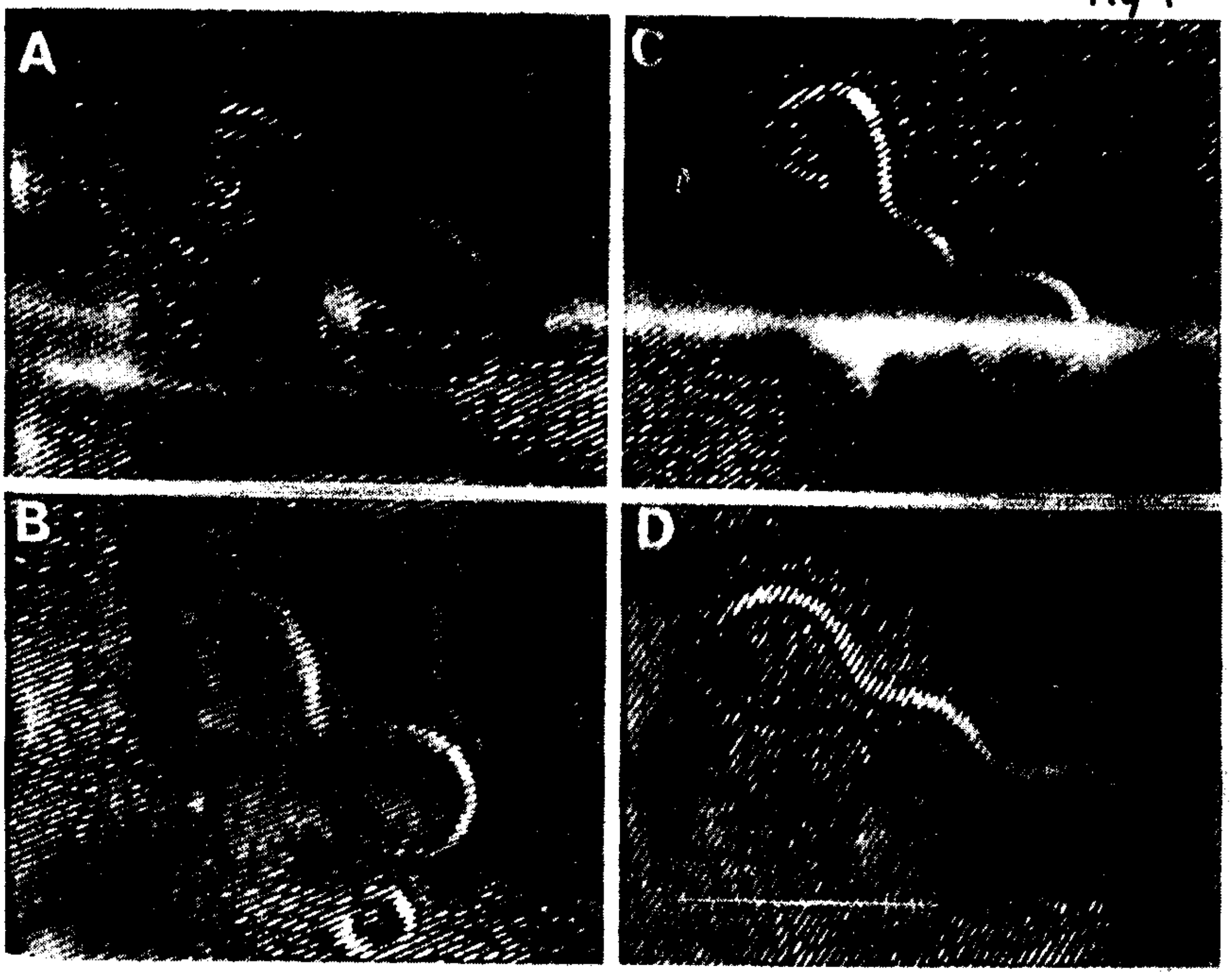
Model = bilayer-couple (ΔA-model)
$\Delta H(T)$ need small differential
Thermal expansion



stomatocytes

Farge + Devaux '92

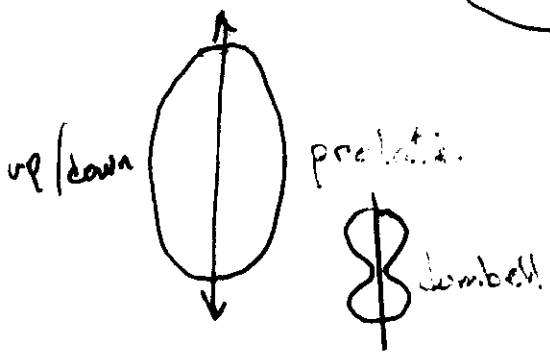
Fig 1

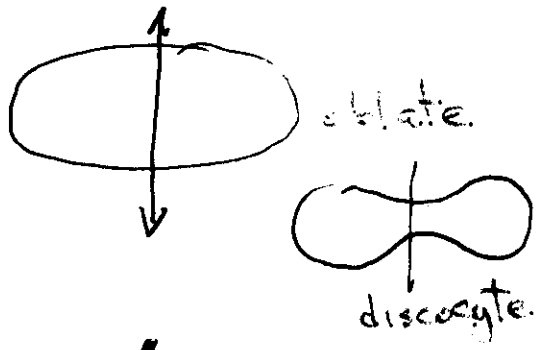


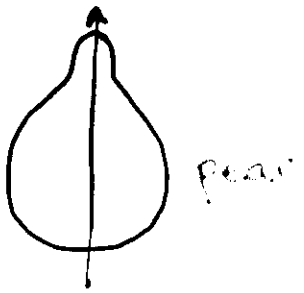
[illegible] (10:1)

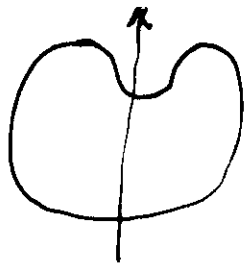
Xfer to high pH @ A ⇒ more EP (→ outside)

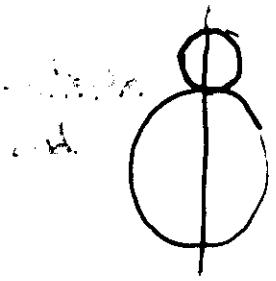
Sequence is 82 s.

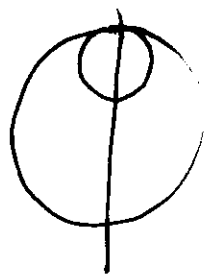


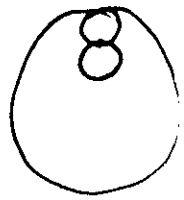








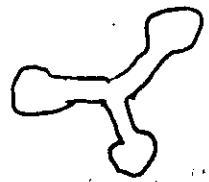




Comment on narrow neck energetics

SHAPES (NAS)

3 inequiv axes



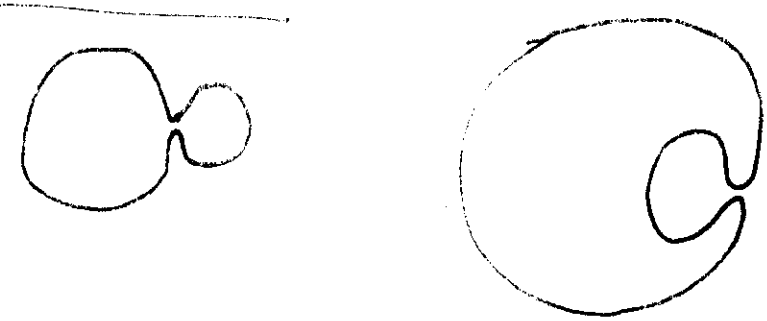
Seifert + Dibereiner

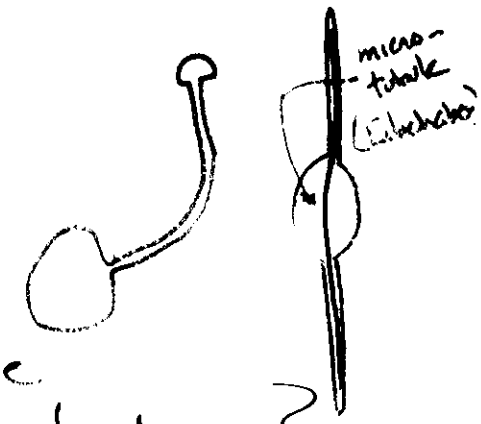
ALL these are seen to lab !!

Also other topologies:
eg. tori
(Seifert)

but not echinocyte

Narrow necks:





- neck remains open
- easily drawn into microtube

pico Newton force balance

- how can these be low-energy structures?

$$\frac{K_b}{2}\left(\frac{1}{R_1}+\frac{1}{R_2}-C_0\right)^2$$

$1/R_1 > 0$, $1/R_2 < 0$

$\frac{1}{R_1}+\frac{1}{R_2}$ small (needs $d=3$)

- locally the neck region

$\sim H=0$ "zero mean curvature"

- Contribution to bending energy (radius a)

$$\Delta E = \text{Const.} \times a - \text{Const.}\, a^2 \ln a + \dots$$

- "Kissing" Condition: $\frac{1}{R_1}+\frac{1}{R_2}=C_0$

Control Parameters: Fixed V, A_0

$$H = \frac{\kappa_b}{2}\left[\int_S dS\,(c_1+c_2-c_0)^2 + \frac{\alpha\pi}{A_0 d^2}\left(d\int_S dS\,(c_1+c_2) - \Delta A_0\right)^2\right]$$

κ_b irrelevant at T = 0

$\nu = \bar{\kappa}/\kappa_b$ } assume $\nu < 0$

Phase Diagram { A_0, V } visible; { ΔA_c, c_c } "invisible"

Overall length scale: Dilation "Reduced" variables

Let $A_0 = 4\pi R_A^2$

$$c_0 \to R_A c_0$$

$$V \to v \equiv \frac{V}{\frac{4\pi}{3}R_A^3}$$

$$\Delta A_0 \to \Delta a_0 \equiv \frac{\Delta A_0}{8\pi d R_A}$$

Biology controls shape via biochemical adjustment of c_0, Δa_0

$\Delta a_0 < 0$ $V_{in} > V_{out}$ ○

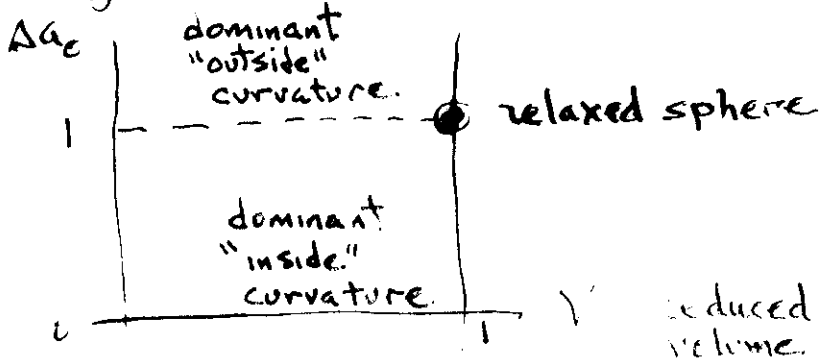
$\Delta a_0 > 0$ $V_{in} < V_{out}$ 8

Under variation:

$$c_0 \to c_c = c_0 - \frac{\alpha\pi}{2\nu}\left(\int dS - \Delta A_0\right)$$

effectively combines c_0 and Δa_0 (self-consistency)

Shape) Phase Diagram:



reduced area difference

Comment on Control Parameters

One overall length scales out of energy
("geometric" integrals)

Let: $A \equiv 4\pi R_A^2$:

$$c_0 \rightarrow c_0 \equiv R_A C_0$$

"reduced volume" $\quad v \rightarrow v \equiv \dfrac{V}{\frac{4\pi}{3} R_A^3}$ $\quad v = 1$ sphere (only)
$0 \le v \le 1$

"reduced area difference" $\quad \Delta A_0 \rightarrow \Delta a_0 \equiv \dfrac{\Delta A_0}{8\pi D R_A}$ $\quad \Delta a_0 = 1$ for relaxed spherical vesicle.

$\Delta a_0 < 0 \quad N_{inner} > N_{outer}$ 

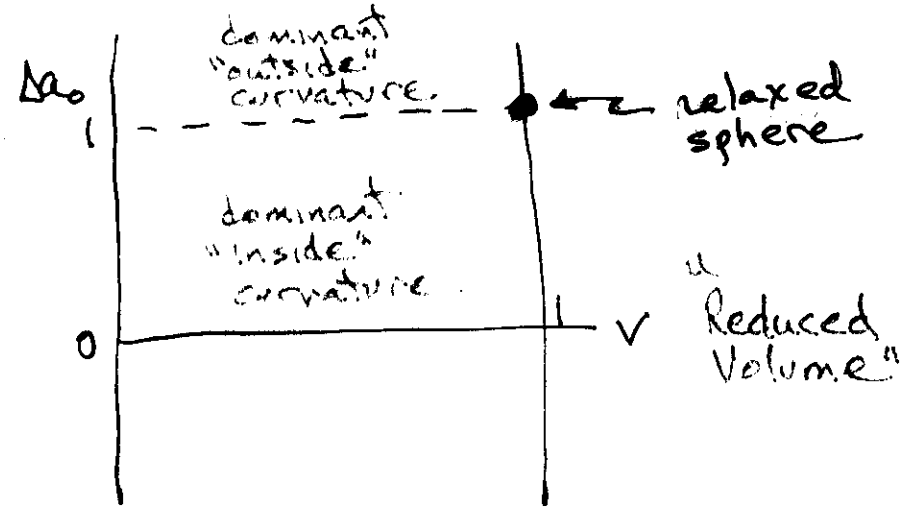
$\Delta a_0 > 0 \quad N_{inner} < N_{outer}$ 

Biology can "control" shape by using biochemical mechanisms to adjust the control parameters α, c_0, v, Δa_0

But does it?

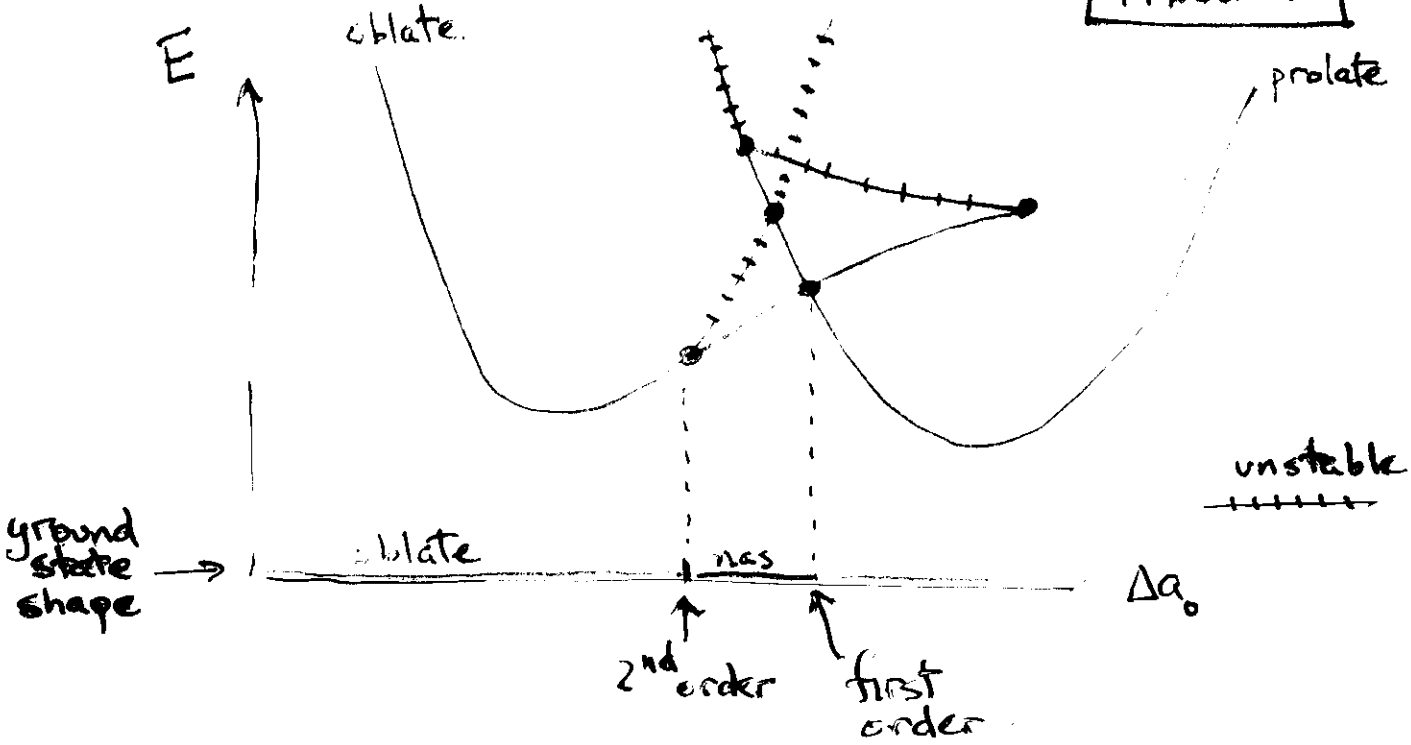
(Shape) Phase Diagram:

for Two shapes
(c_0, α fixed)



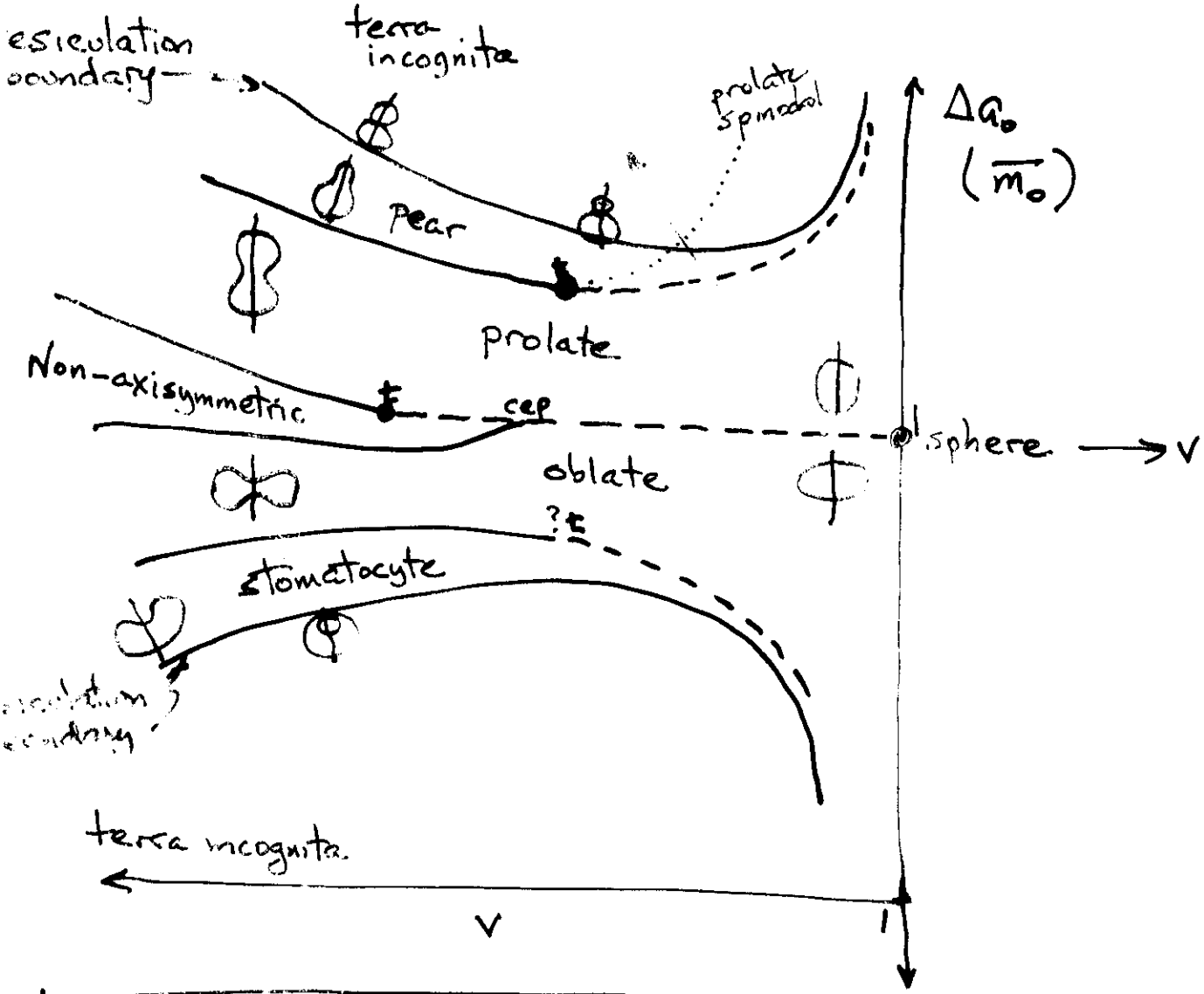
Stationary – Shape – Energy Diagrams

fixed V



Phase Diagram at $\frac{\bar{K}}{K_b} \sim 1$

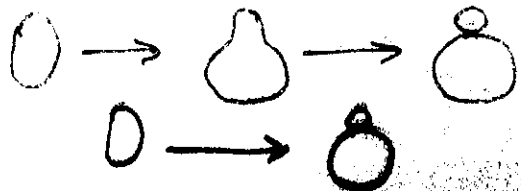
incl. data of V. Heinrich + Wang



Key
- —— continuous (2^{nd} order)
- ---- discontinuous (1^{st} order)
- t tricritical

α-dependence of phase diagram

trajectories



metastability

prolate → pear → vesiculated transition

$\Delta c_0 = \frac{\bar{m}_0}{4\pi}$

$\chi = 1.4$ (DMPC)

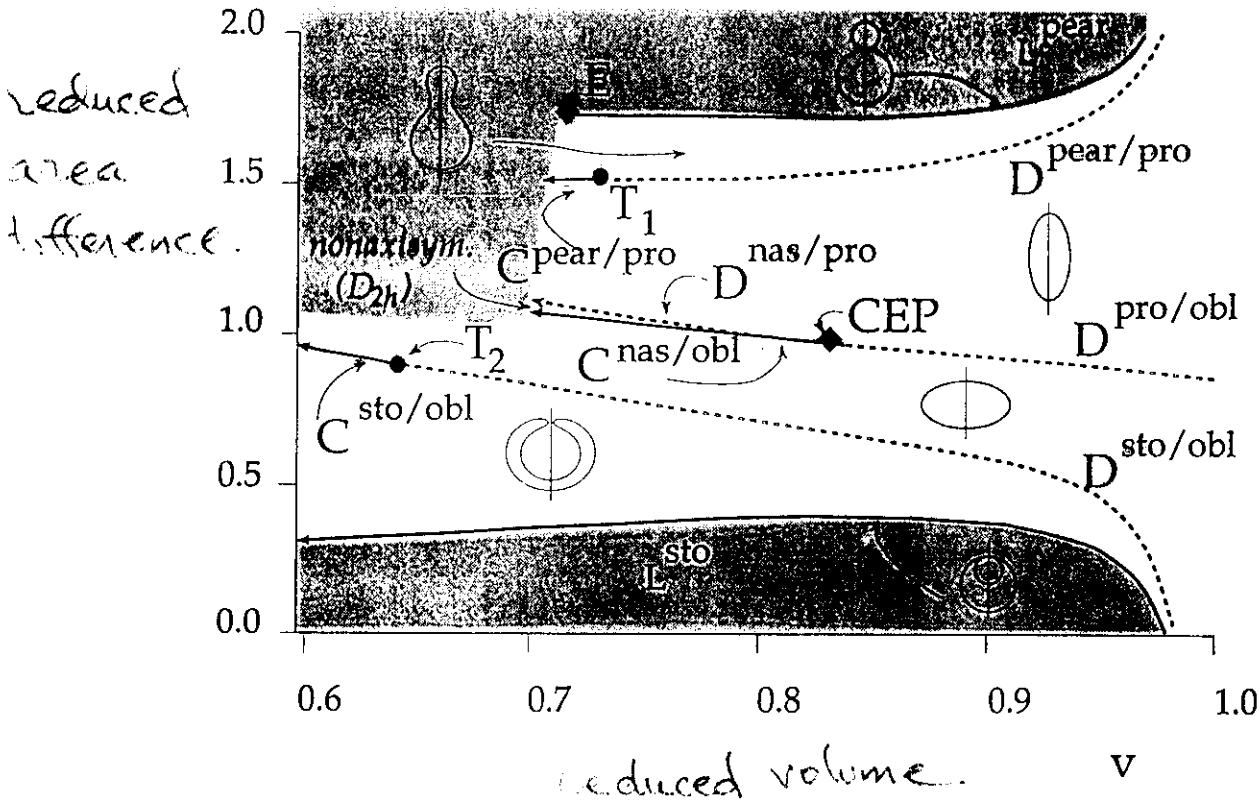


Fig. 2 (Nikolic

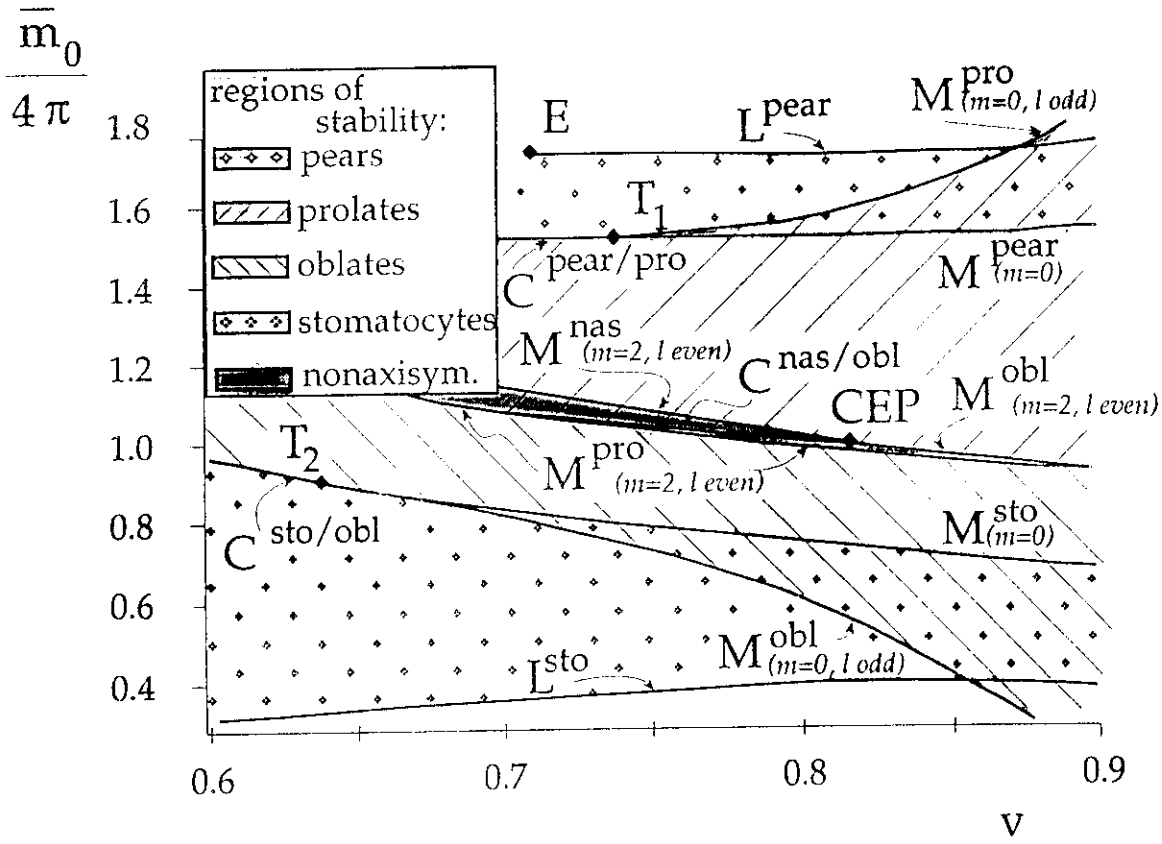
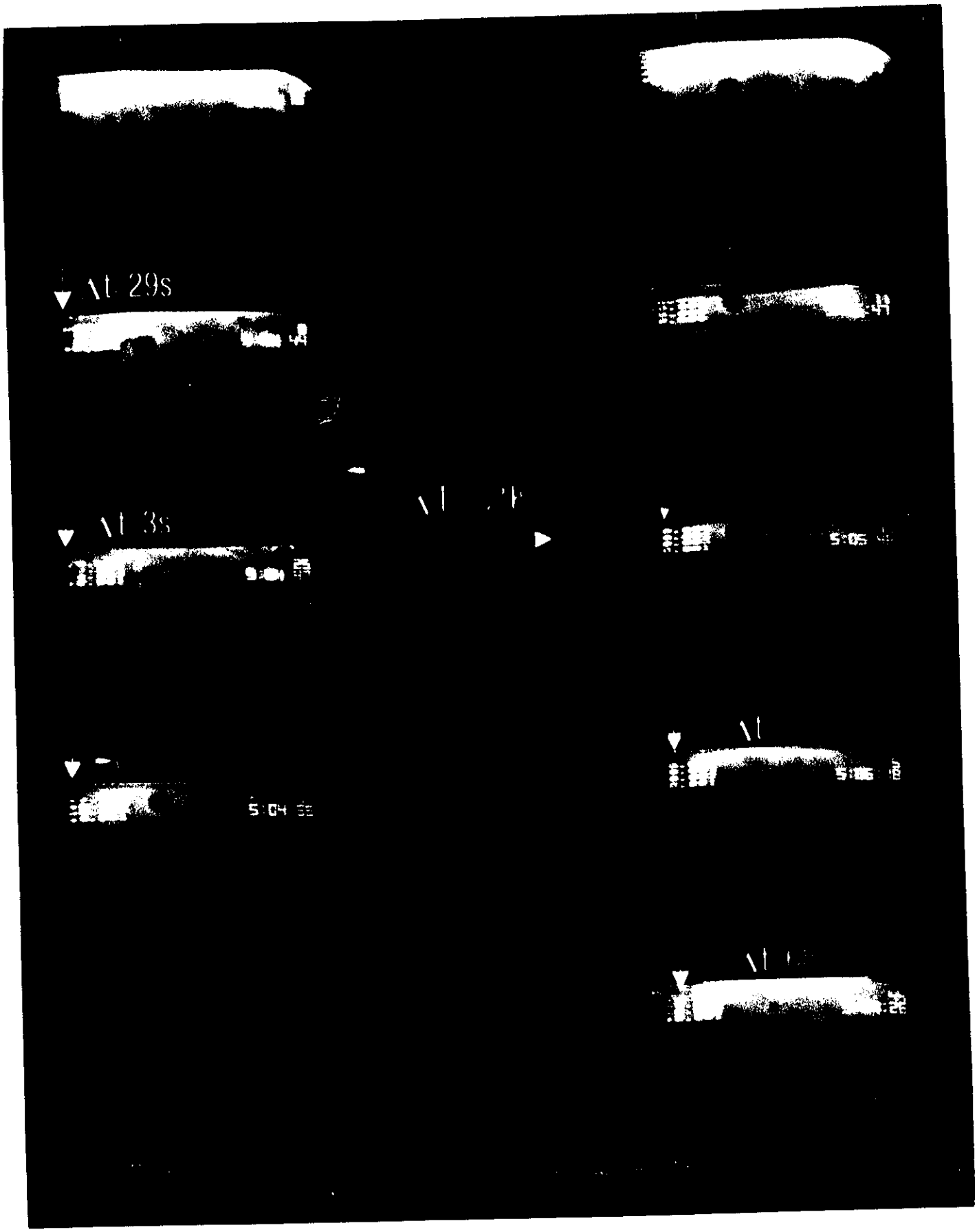


Fig. 3 (Nikolic et al


Δt 29s
Δt 3s

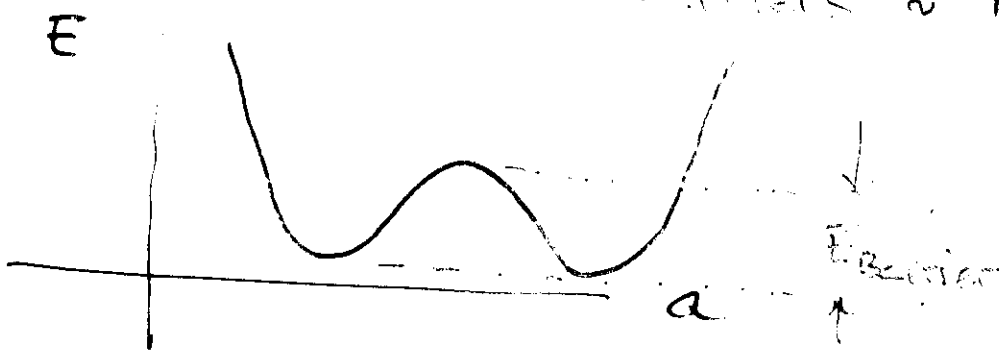
Shape Fluctuations, Metastability, & "Spinodal" Behavior

thermally driven shape fluctuations are observed & can be important in lab.

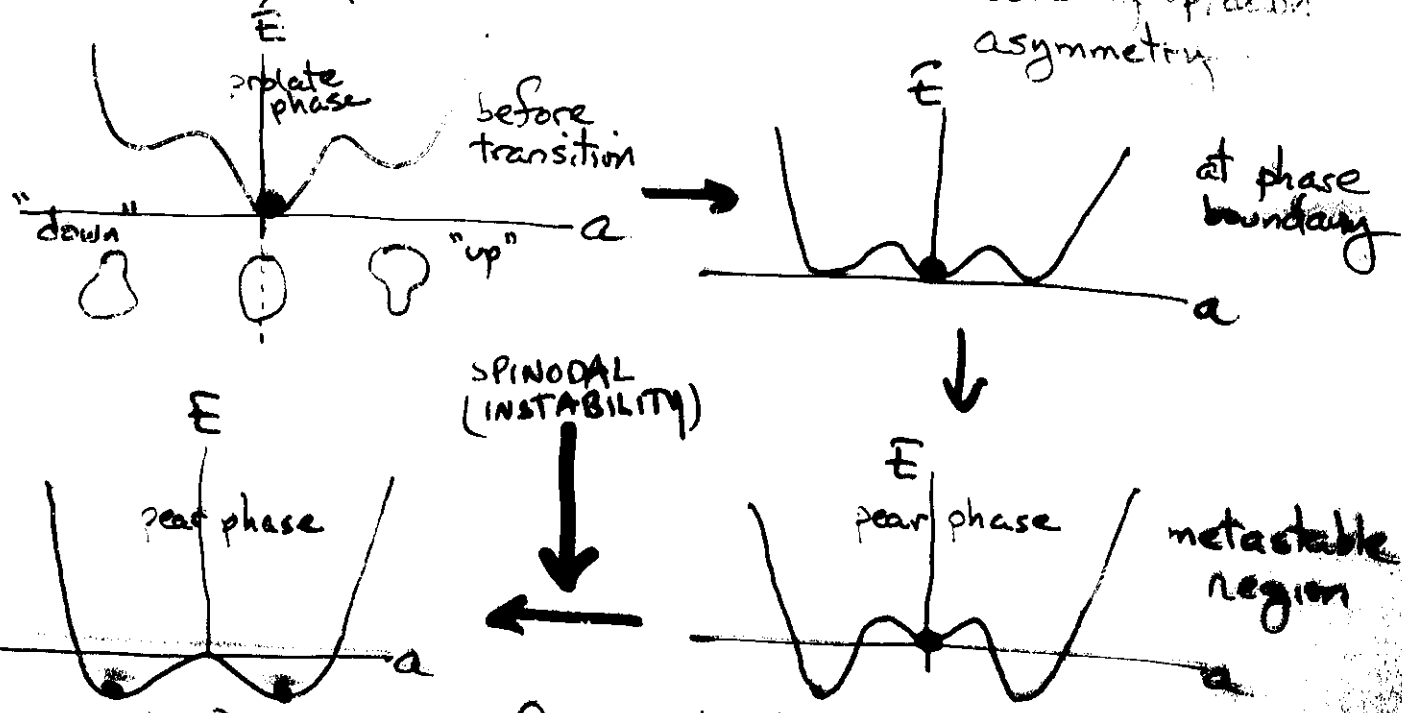
slide

for any $T > 0$ "phase" boundaries are (in principle) fuzzy

but we profit with barriers $\sim k_B T$:



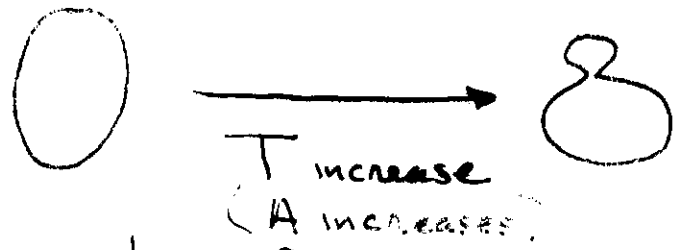
(First-order) Spinodal behavior: u = measure of up/down asymmetry



(in lab) ⟹ large fluctuations + slow timescales near "spinodal"

Observation of "The budding instability"

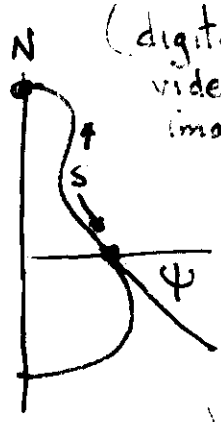
Evans
Sachmann
Käs
Döberereiner



(not reversible)
HYSTERESIS

- large shape fluctuations just before transition
- soft mode "quasi-critical fluctuations"
- slow dynamics "quasi-critical slowing down"

spinodal instability *



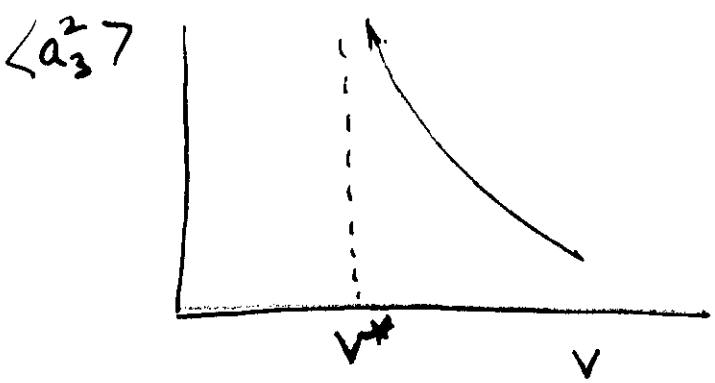
(digitized video images)

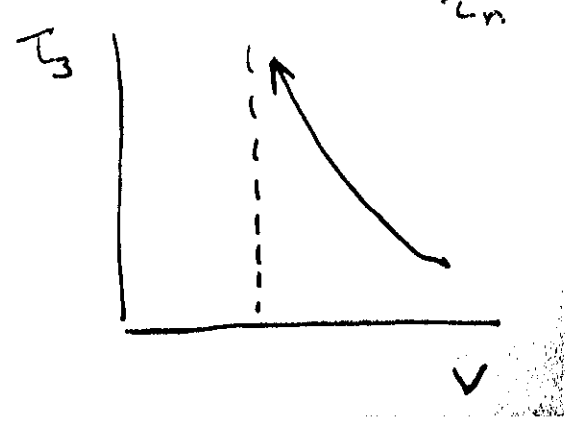
mode analysis of shape fluctuations:

$$\psi(s) = \underbrace{\pi \frac{s}{s^*}}_{\text{circle}} + \sum_{n=1}^{\infty} a_n \sin\left(n\pi \frac{s}{s^*}\right)$$

$$\langle (a_n(t) - \langle a_n \rangle)(a_n(0) - \langle a_n \rangle) \rangle \equiv C_n(t)$$

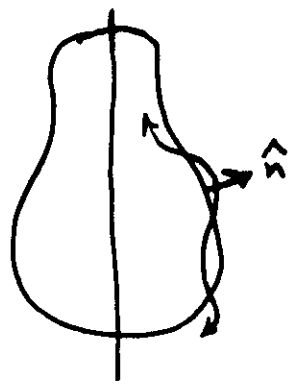
dominant exponential decay τ_n





Fluctuations about $T=0$ Shapes (axisymmetric)

subject to "hard" constraints



$$[s] \equiv \vec{R}(s_1, s_2)$$

$$= R_0(s_1, s_2) + \varepsilon(s_1, s_2)\,\hat{n}(s_1, s_2)$$

$$\varepsilon(s_1, s_2) = \sum_{\ell, m} u_{\ell m}\, Y_{\ell m}\left(\pi \frac{s}{s^*}, \varphi\right)$$

- Constraints $\quad V[s] = V[s_0] \equiv V_0$

  $A[s] = A[s_0] \equiv A_0$

- Removal of Euclidean modes

  Projection of Constraints

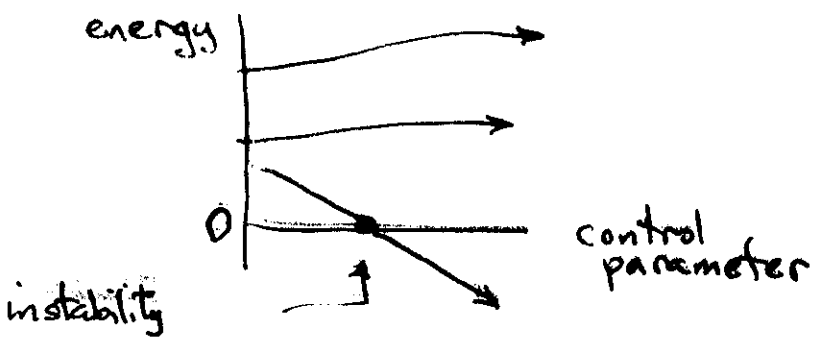
  $\Rightarrow$ spectrum of physical energy eigenmodes of fluctuation.

Sectors : $|m|$ (axisymmetry)

ℓ-even/odd (up/down)

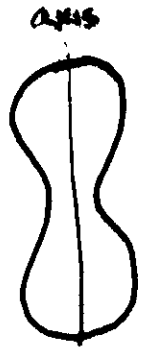
Fluctuation modes $\quad \omega_n, \varphi_n$

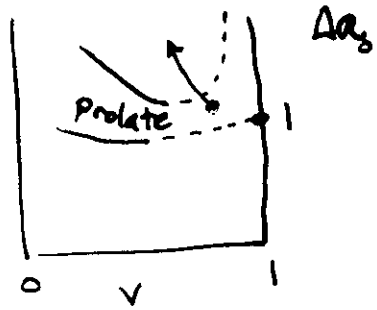
Soft modes near instability:

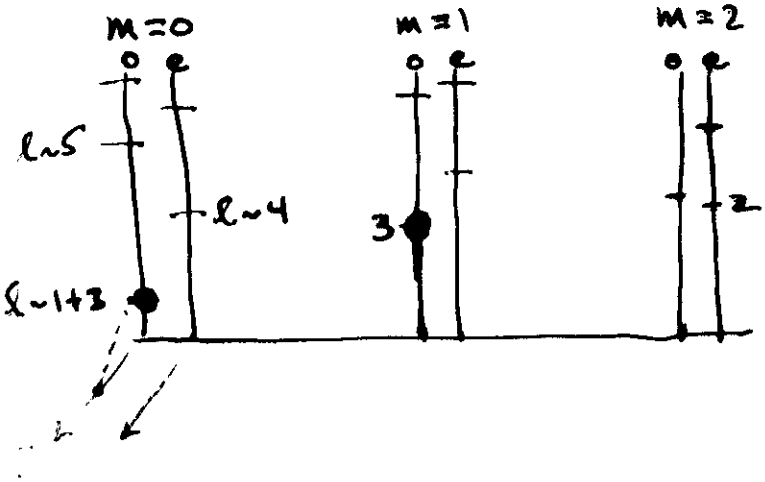


Example:

Fluctuations of up/down symmetric prolate shape





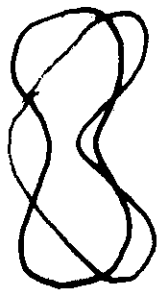


m=0
ℓ~1+3

"pear" fluctuation

(SOFT at bidding spinodal)

m=1
ℓ~3



"banana" fluctuation

Does biology "use" these shape mechanics?

Every case is different.

A. Red blood-cell shapes:
Yes (?). But, structure is complicated.
and mechanisms of control of A, V, C_0
incompletely understood.

B. Budding processes: PM
Golgi

- Often controlled by proteins (clathrins)
(not always??)
- May have played more role
early in evolution (??)
- Energy scale is right!

C. Other budding mechanisms:

Real membranes have many lipids (10^3!)

Domain-induced budding (Lipowsky)