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INTERNATIONAL ATOMIC ENERGY AGENCY
INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS
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SMR.961 - 7

WORKSHOP ON:
PROTEINS, MEMBRANES and their INTERACTIONS

22 JULY - 2 AUGUST 1996

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# "Molecular dynamics studies of thermodynamics and kinetics"

## PART II

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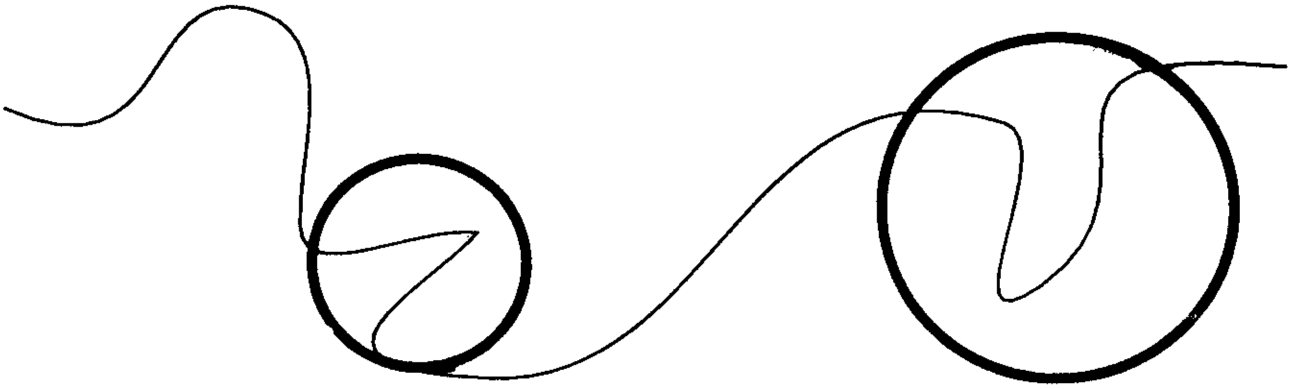
# EARLY events in protein folding

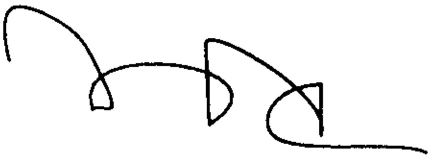
(nucleation sites)



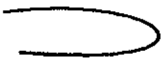
(a) What are the nucleation sites?

Secondry structure elements?

helices?

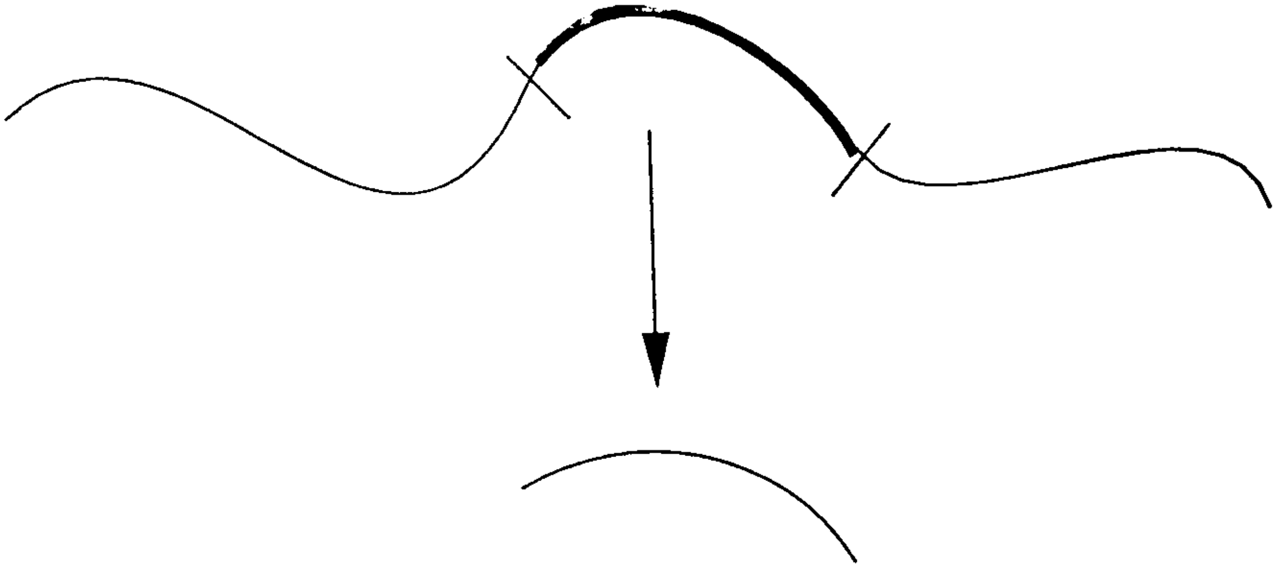


β turns?



Hydrophobic clusters?

Take a protein fragment
in water



Does it fold?

Does it have *some* tendency or
preference to a given structure?

Role of hydrogen bonding,
hydrophobic forces. structured
waters as a driving force to structure

# Initiation of folding

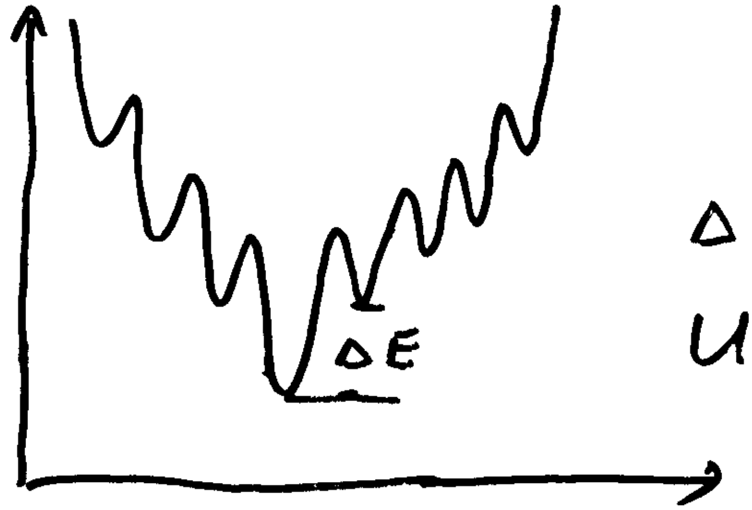
* What are the forces at the begining?

* Hydrogen bonds? Hydrophobicity?

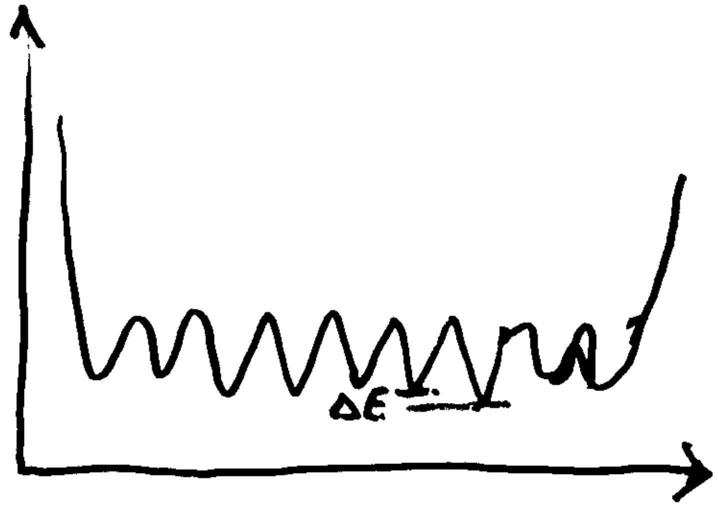
*Structured water molecules around the peptide?

*Can we suggest separation of time scales between solvent/side chains/back bone dynamics?

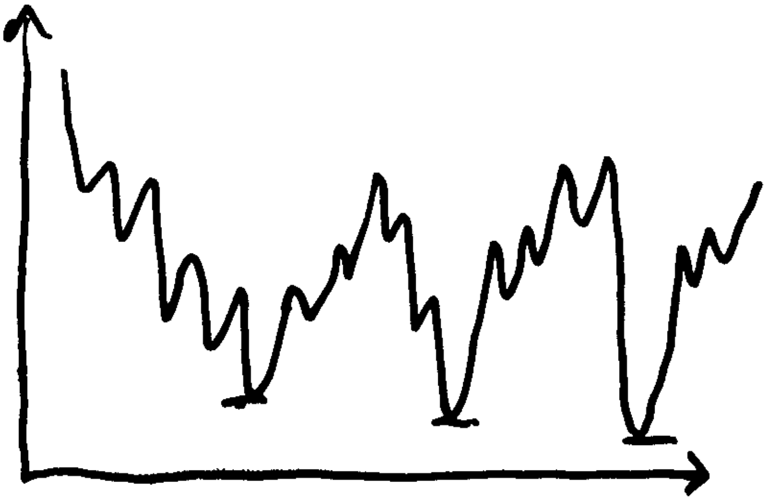
# Possible shapes of the energy surface



$\Delta E > k_B T$
unique structure



$\Delta E < k_B T$
many structures



a few structures

# Examples: Four peptides

CAAAAC – reference

CHDLFC – analog to peptides that block channels

RVEW – Experiment suggests some structure – Bundi et al., Eur. J. Biochem. 91,201(78)

SYPFDV – Experiment by J. Yao et al. JMB 243,736(1994) – D. Mohanty

## (*) Roitberg and Elber – LES

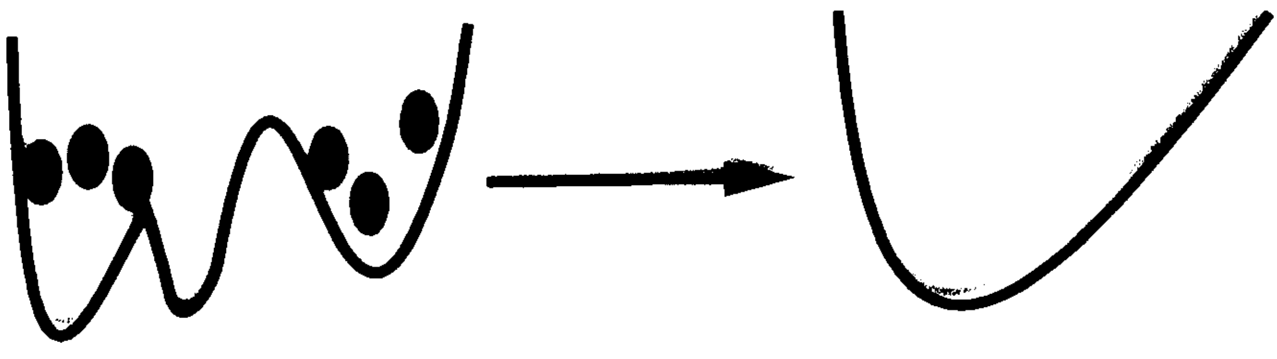
Use a mean field approx. for the density, represent the density by a sum trajectories, e.g.

$$\rho(R_1,R_2) \approx \rho(R_1)\rho(R_2)$$

$$\rho(R_i)=\Sigma w_k \delta(R_i - R_{ik}(t))$$

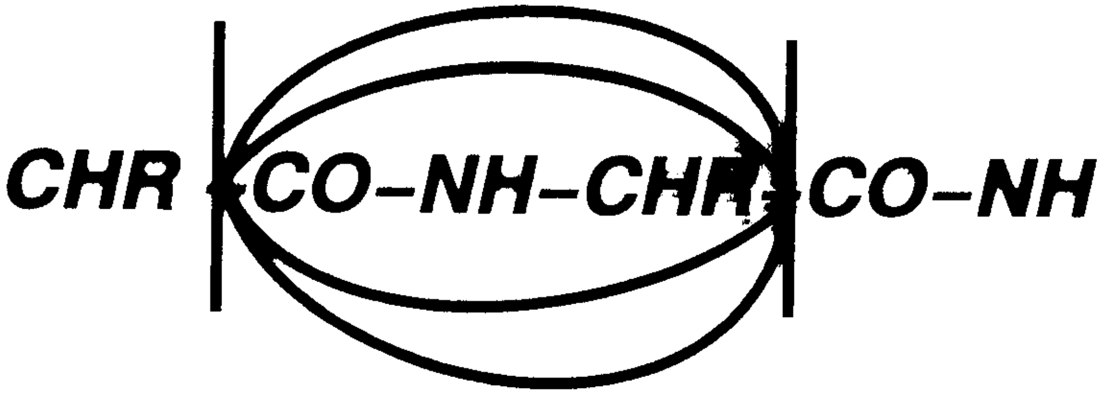
Perform annealing.

$$Md^2R_{ik}(t)/dt^2 = \Sigma w_{k'} dU/dR_{ik}(t)$$

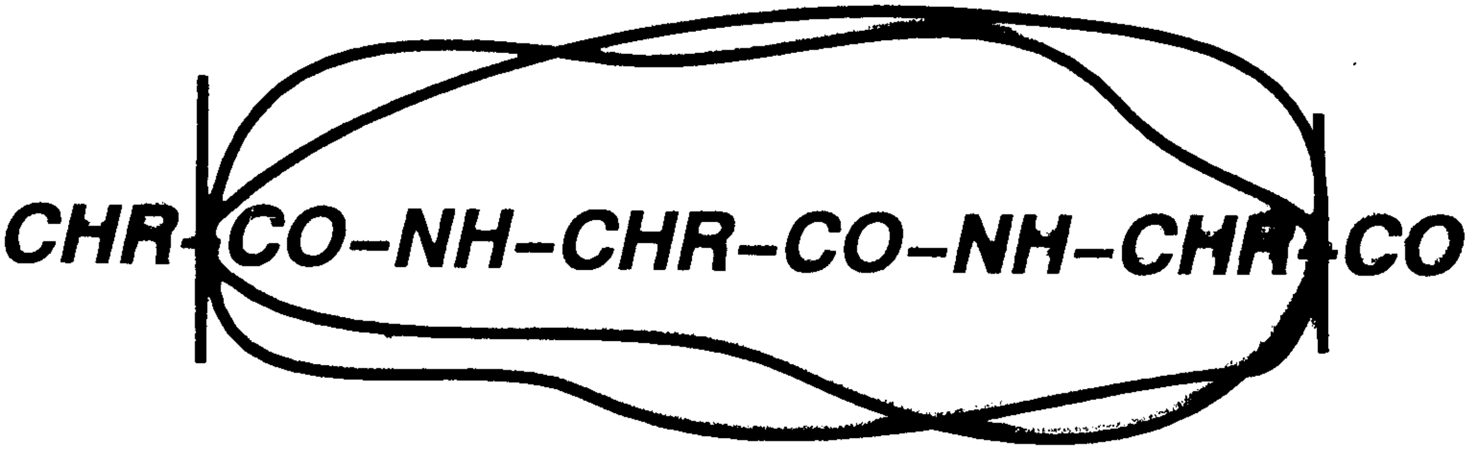


# *What to "multiply"? (4 copies)*

**CO–NH–CHR–CO–NH**



**CHR–CO–NH–CHR–CO–NH**



**CHR–CO–NH–CHR–CO–NH–CHR–CO**

***The last option works best***

# Examples: Four peptides

CAAAAC - reference

CHDLFC - suggested by lattice studies to have a strong tendency to structure (E. Shakhnovich) analog to peptides that block channels

RVEW - experiment suggests some structure, 1DNMR Bundi et al. Eur.J. Biochem 91, 201(78)

Simmerling & Elber

SYPFDV - Experiment by the Dyson group: Yao et al, JMB 243,736

Mohanty & Elber

CA4C

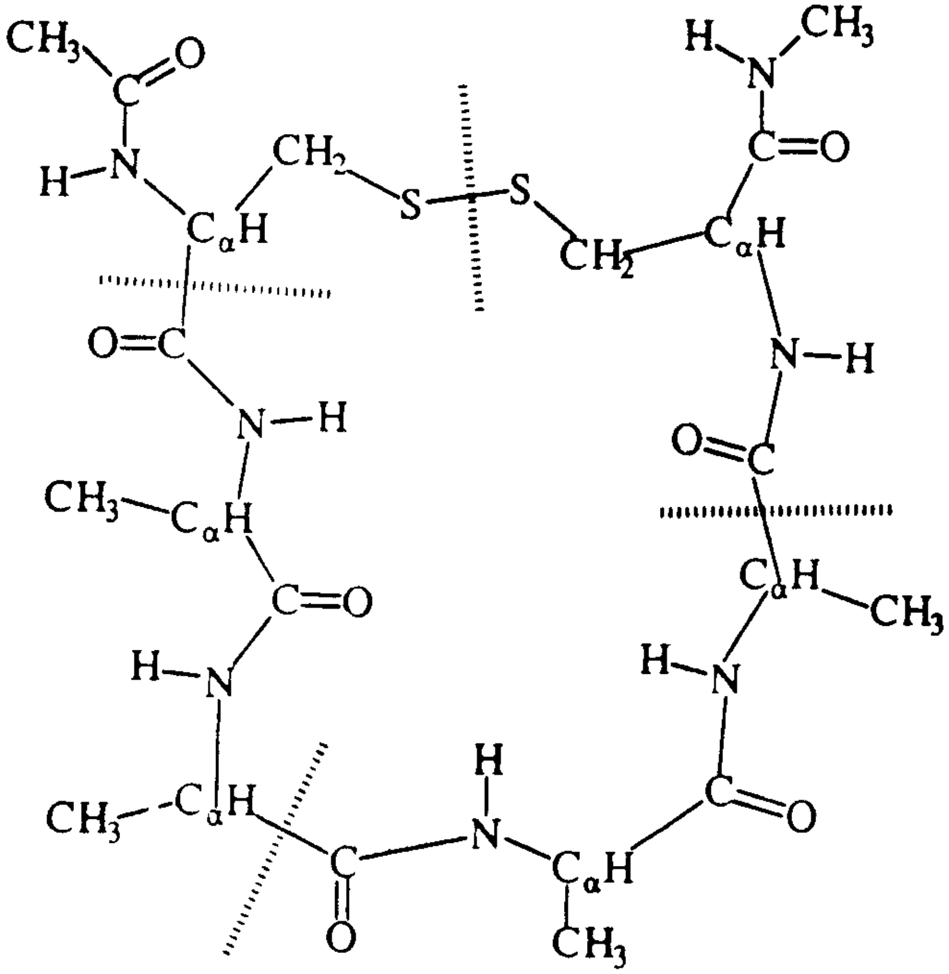
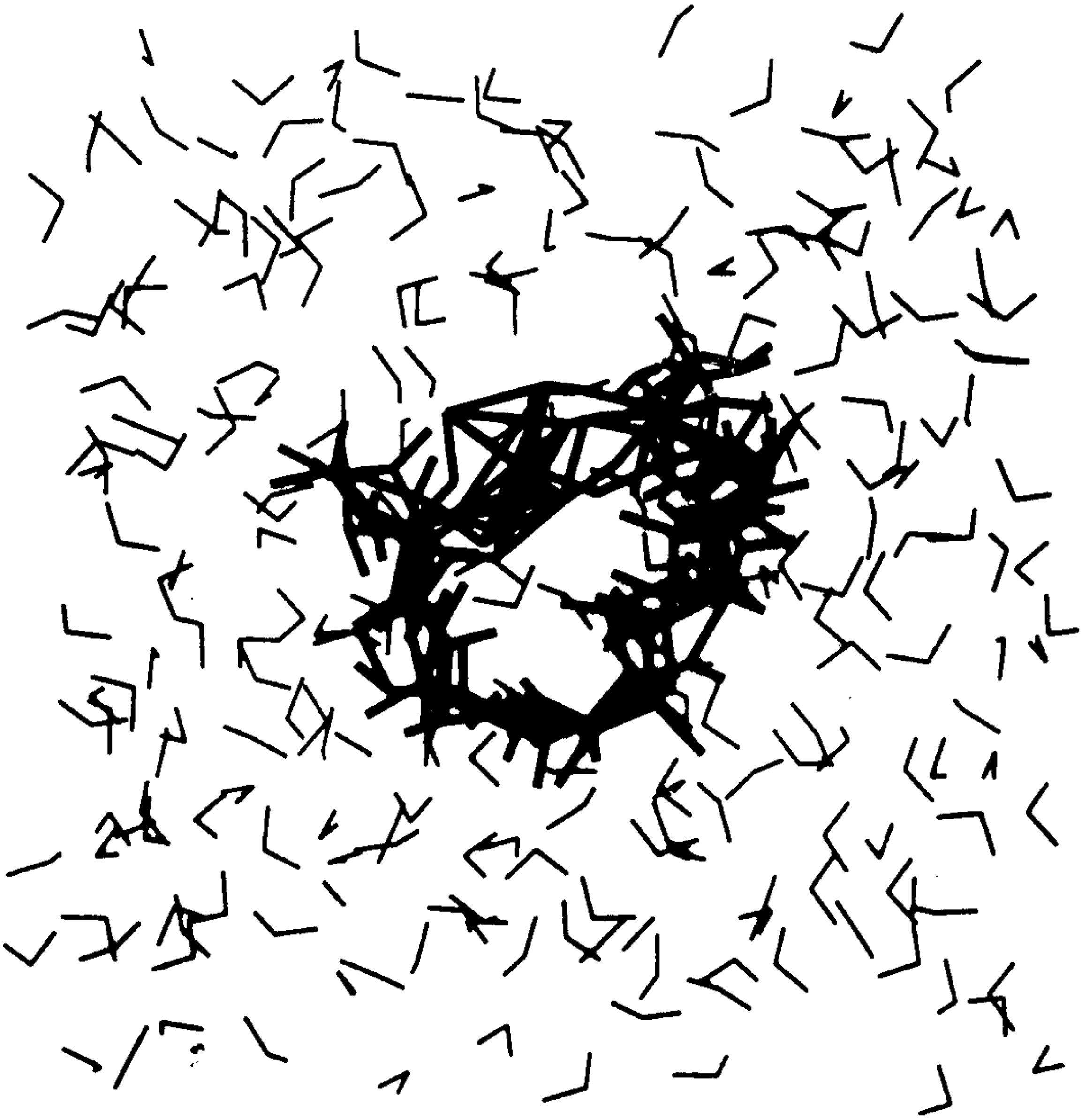


Figure 18. The atomic structure of the peptide CAAAAC. The dashed lines indicate the position of the cutting point between LES fragments.

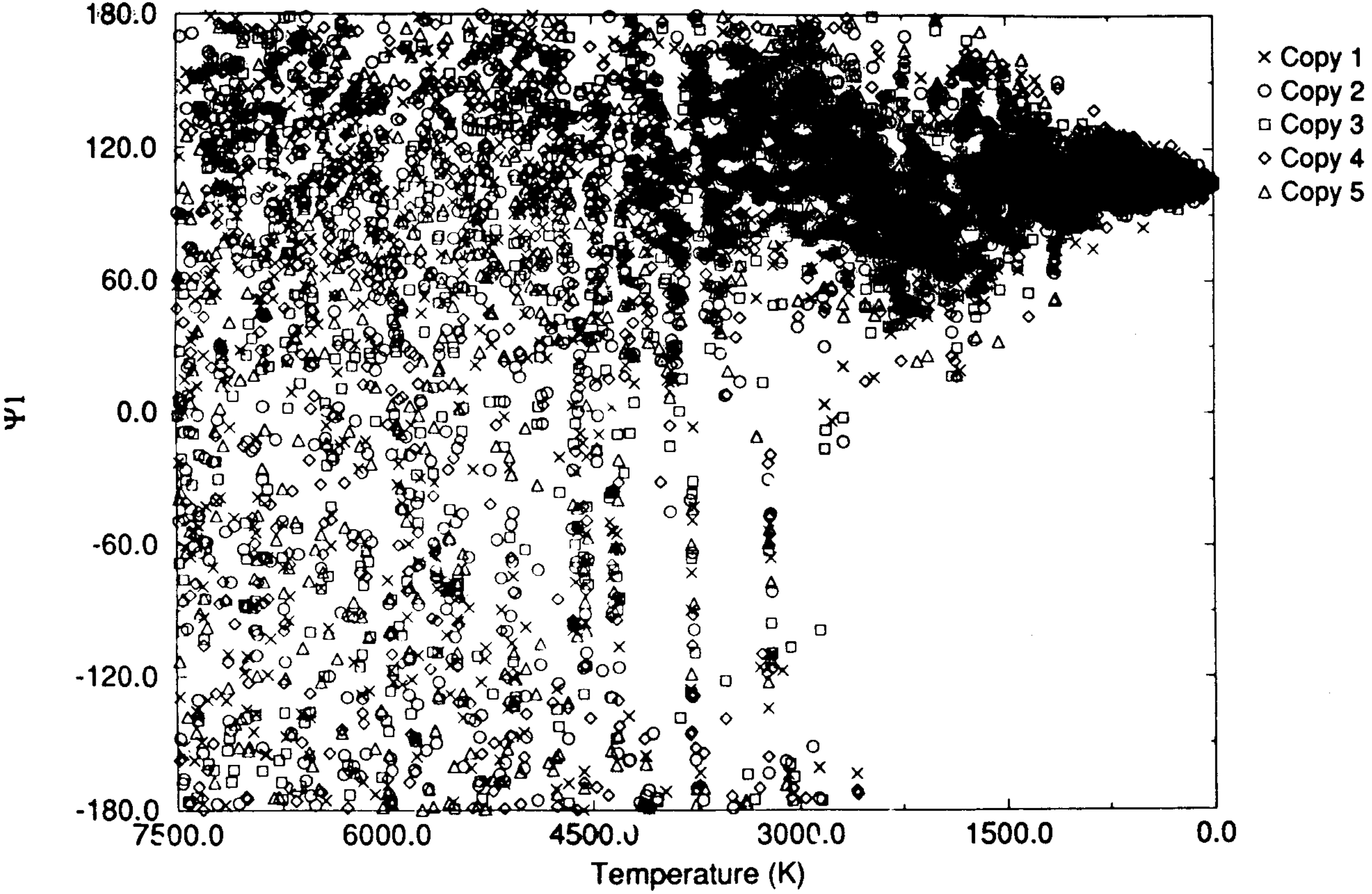
High temperature structure of CAAAAC in water



21Å Cubic box, periodic boundary conditions

fig. 16

Annealing history of cA$_4$C in water



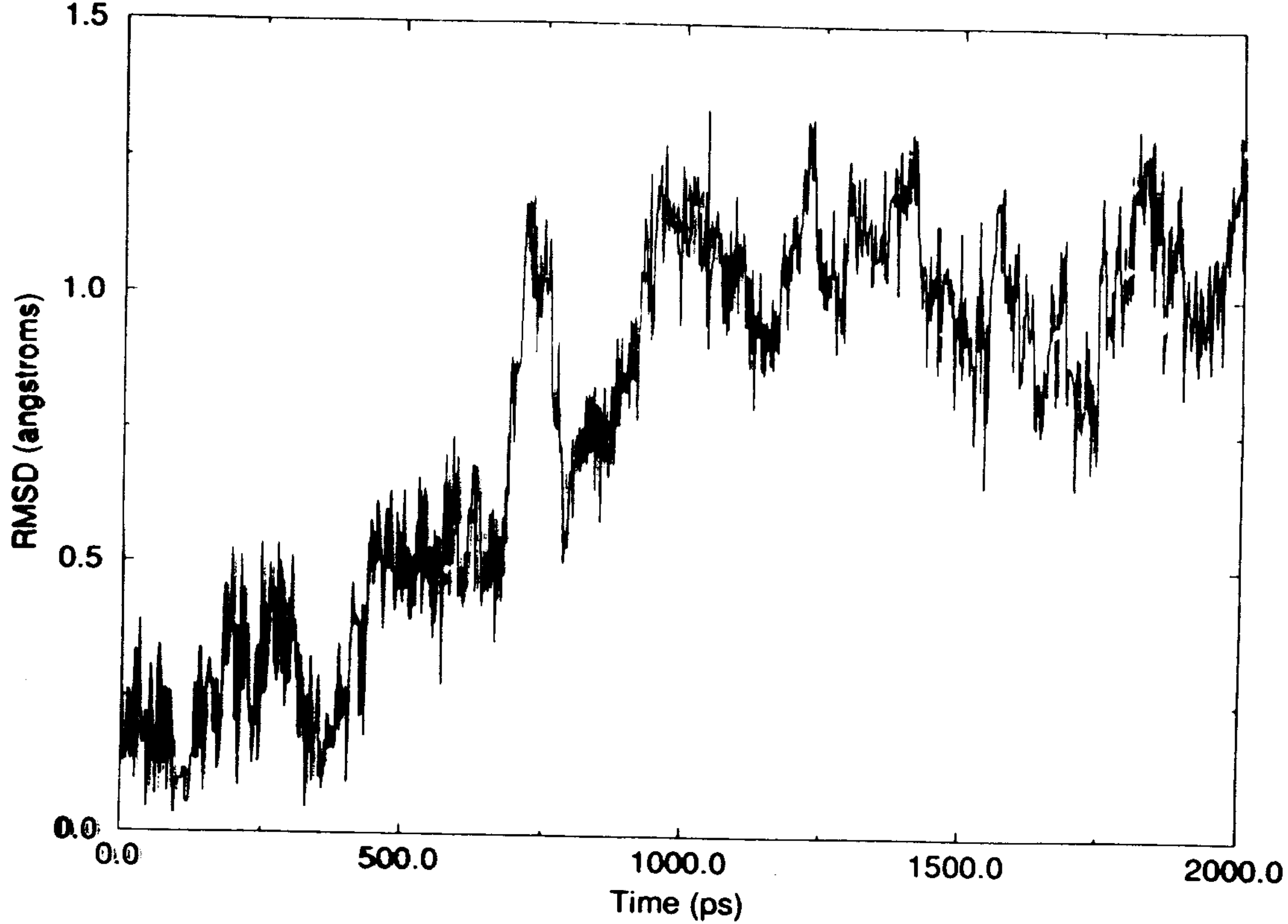
## TABLE VI

## RESULTS FROM LES ANNEALING SIMULATIONS OF CAAAAC IN SOLUTION

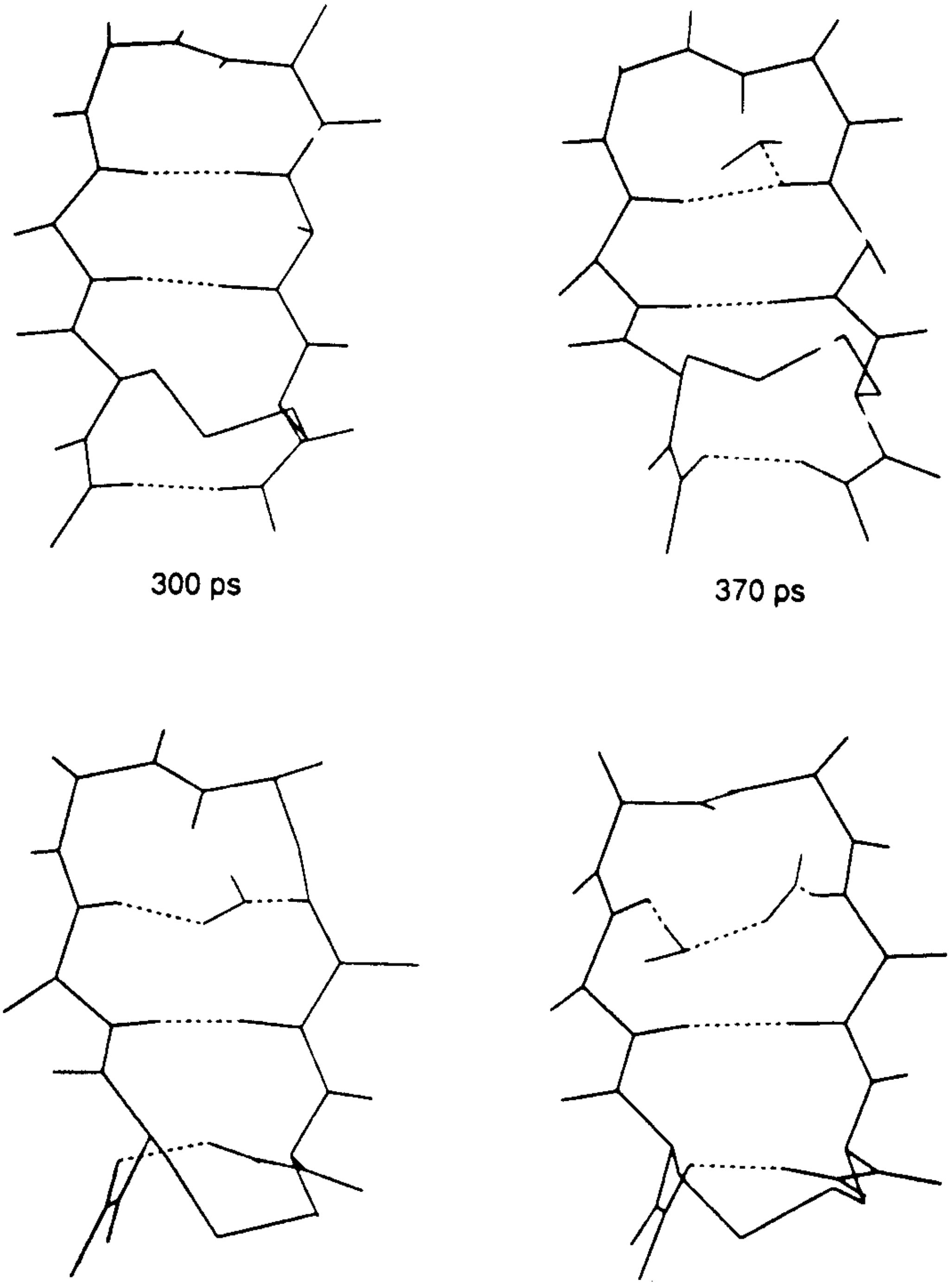
| Simulation Number | Simulation Length (ns) | Initial Temperature | RMSD* to Structure #12 (Å) |
|---|---|---|---|
| 1 | 0.25 | 7500K | 1.9 |
| 2 | 0.25 | 7500K | 2.3 |
| 3 | 1.0 | 7500K | 2.0 |
| 4 | 1.0 | 7500K | 2.0 |
| 5 | 1.0 | 7500K | 1.4 |
| 6 | 1.0 | 7500K | 1.1 |
| 7 | 1.0 | 7500K | 1.9 |
| 8 | 1.0 | 7500K | 1.8 |
| 9 | 1.0 | 7500K | 2.2 |
| 10 | 2.0 | 7500K | 0.42 |
| 11 | 2.0 | 7500K | 2.0 |
| 12 | 4.0 | 7500K | 0.0 |

* Root Mean Square Deviation of $C_\alpha$ atoms

[illegible] fluctuations of "[illegible]" [illegible]



"Unfolding" of $CA_4C$



300 ps

370 ps

440 ps

500 ps

Figure 32. Snapshots in time from a simulation of CAAAAC in solution, with the initial peptide coordinates obtained through annealing in vacuum. Water molecules insert into intra-peptide hydogen bonds and lead to unfolding of the structure. For clarity, not all water molecules are shown.

# SUMMARY OF CAAAAC IN WATER

*No unique structure

*Hydrogen bonds replaced by many alternative hydrogen bonds to water molecules

*No "structured" waters

*"Unfolding" of vacuum structure by water insertion

Results of 2 annealing runs:

CHDLFC/$H_2O$

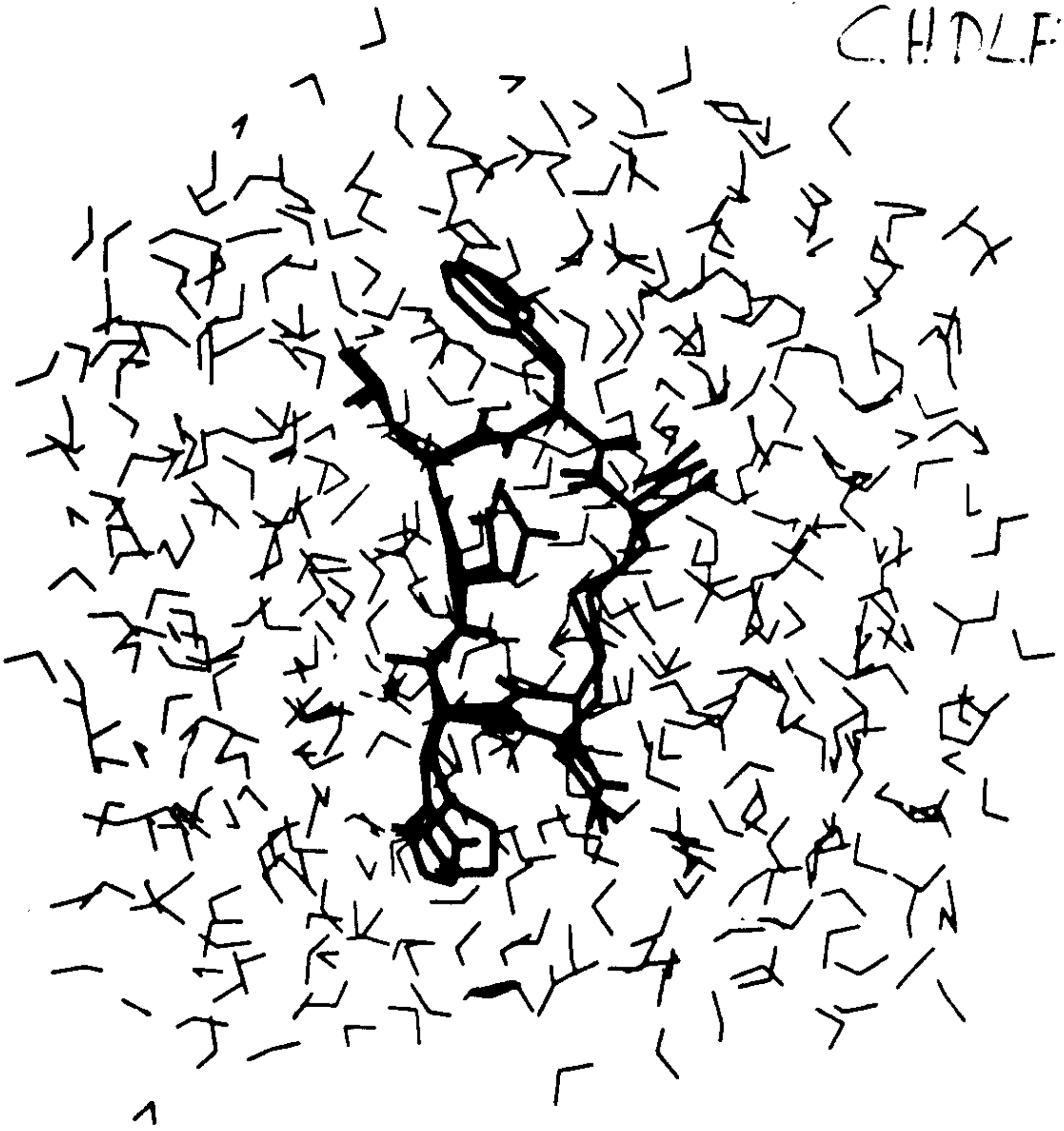


Figure 47. Overlap of two final annealed LES structures for CHDLFC in solution. Only a single set of water molecules is shown. The structures are very similar.

## TABLE VIII

## RESULTS FROM LES ANNEALING SIMULATIONS OF CHDLFC IN SOLUTION

| # | Trajectory Length (ns) | Box Size (Å) | Initial T (K) | Phe-Leu Distance[a] (Å) | RMSD[b] to #15(Å) |
|---|---|---|---|---|---|
| 1 | 0.75 | 26 | 7500 | 7.4 | 0.46 |
| 2 | 0.75 | 26 | 7500 | 7.4 | 0.16 |
| 3 | 0.75 | 26 | 7500 | 4.4 | 0.40 |
| 4 | 1.0 | 26 | 7500 | 6.9 | 0.24 |
| 5 | 1.0 | 26 | 7500 | 4.3 | 0.28 |
| 6 | 1.0 | 26 | 7500 | 6.7 | 0.26 |
| 7 | 1.0 | 21 | 7500 | 10.5 | 0.17 |
| 8 | 1.0 | 21 | 7500 | 9.6 | 0.27 |
| 9[c] | 1.5 | 26 | 7500 | 4.4 | 0.16 |
| 10 | 1.5 | 21 | 7500 | 4.8 | 0.13 |
| 11 | 1.5 | 21 | 7500 | 4.3 | 0.29 |
| 12[c] | 2.0 | 21 | 7500 | 5.2 | 0.40 |
| 13[e] | 2.0 | 21 | 7500 | 4.2 | 0.19 |
| 14 | 2.0 | 21 | 7500 | 4.4 | 0.25 |
| 15 | 2.0 | 21 | 7500 | 4.6 | 0.0 |
| 16 | 2.0 | 22[d] | 7500 | 4.4 | 0.30 |
| 17[e] | 2.0 | 22[d] | 6500 | 4.3 | 0.12 |
| 18[e] | 2.0 | 22[d] | 7500 | 4.6 | 0.30 |
| 19 | 2.8 | 26 | 7500 | 4.5 | 0.20 |
| 20 | 2.8 | 26 | 7500 | 4.0 | 0.27 |
| 21[c] | 4.0 | 21 | 7500 | 4.2 | 0.18 |
| 22 | 4.0 | 21 | 7500 | 4.8 | 0.37 |

[a] Distance between side chains: from the $C_\gamma$ of Leu to the center of mass of Phe ring

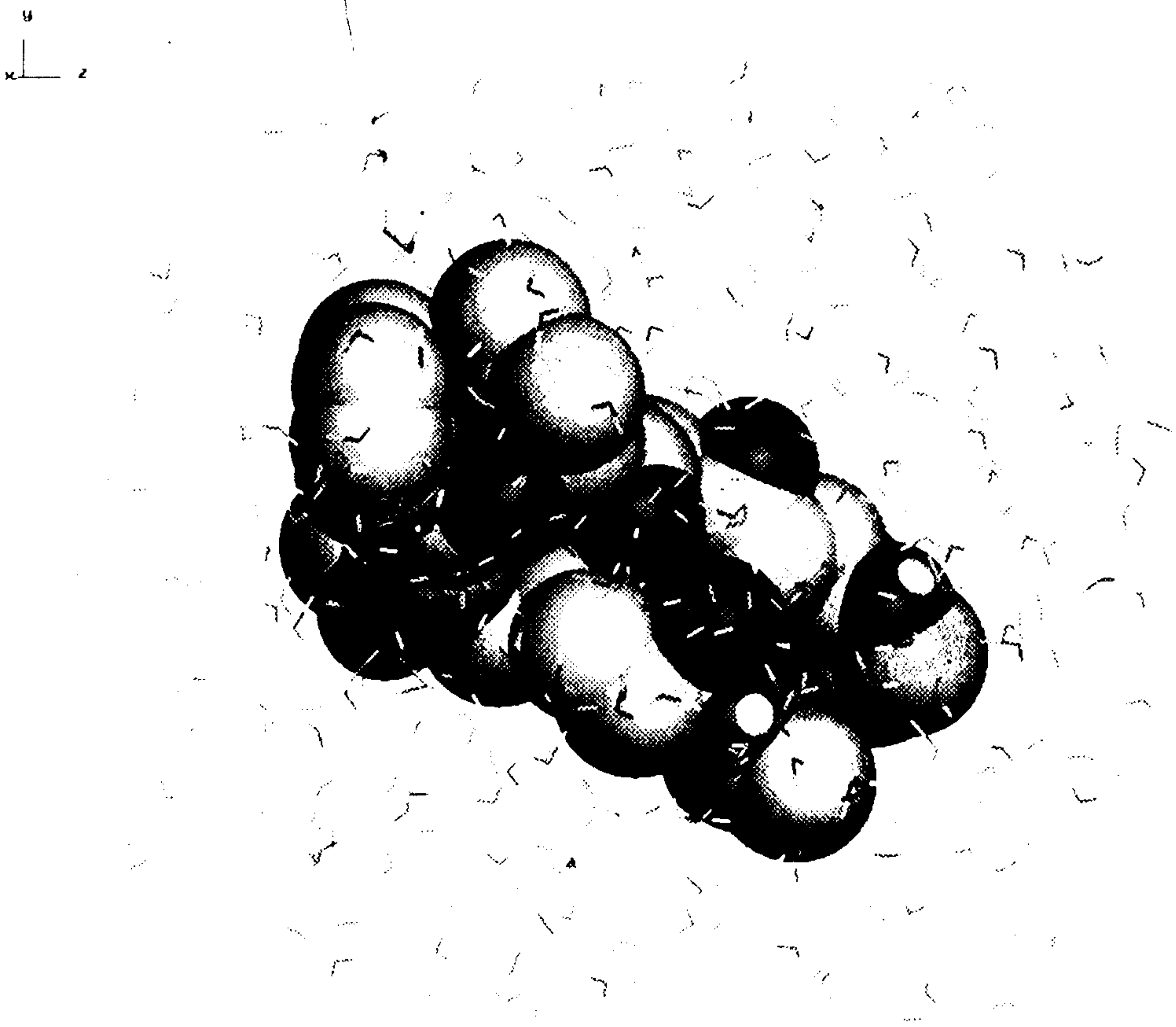
[b] Root Mean Square Deviation of $C_\alpha$ atoms in HDLF residues

[c] Sodium counterion used

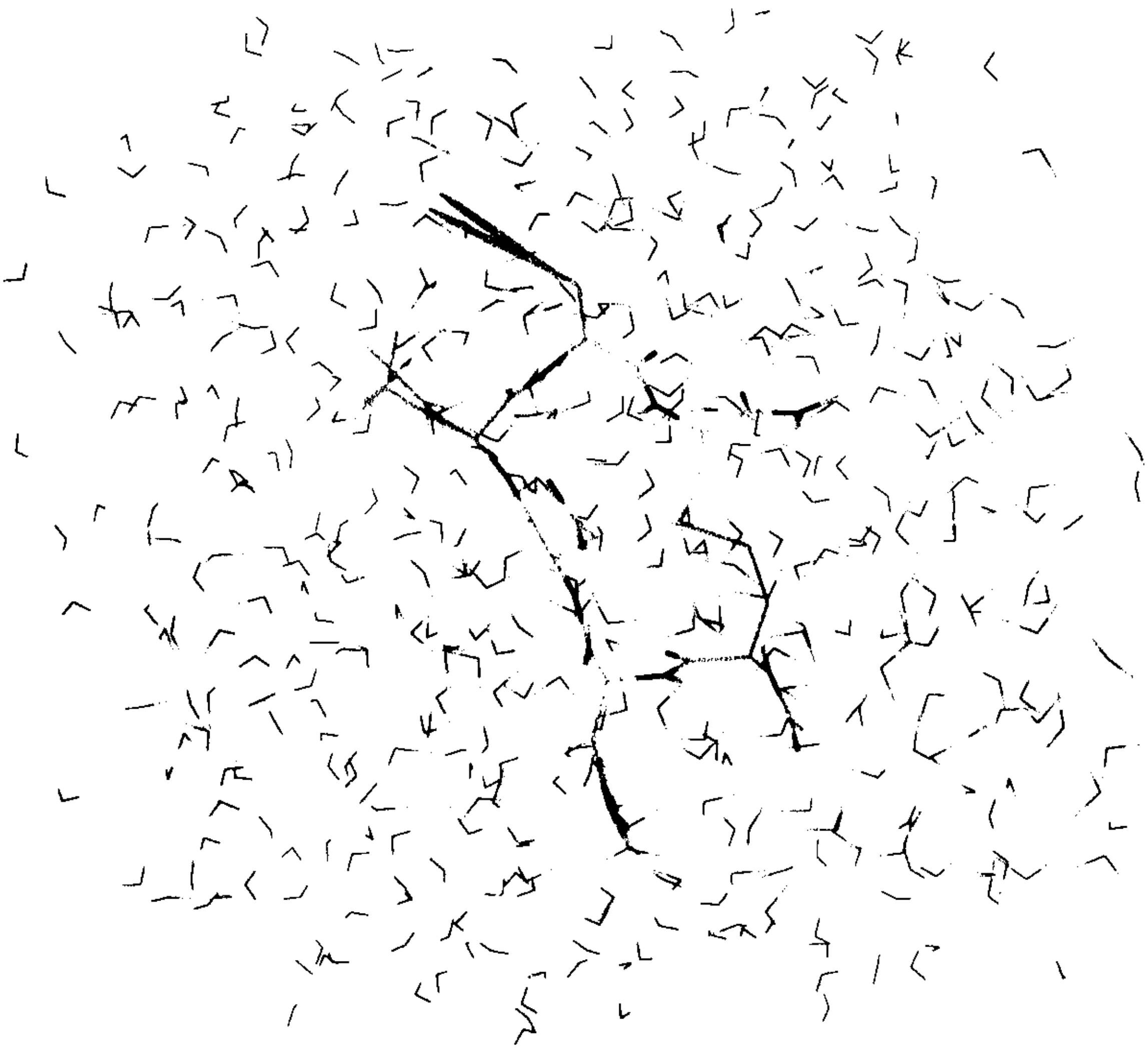
[d] Variable box size used, initial size was 25 Å, final size was 22 Å

[e] All atom OPLS/AMBER used for His and Phe side chain aromatic rings

Hydrophobic pair



Annealed structure of CHDLFC



CHDLFC in solution
5 copies, annealed

CHDAAC . CHDLFC

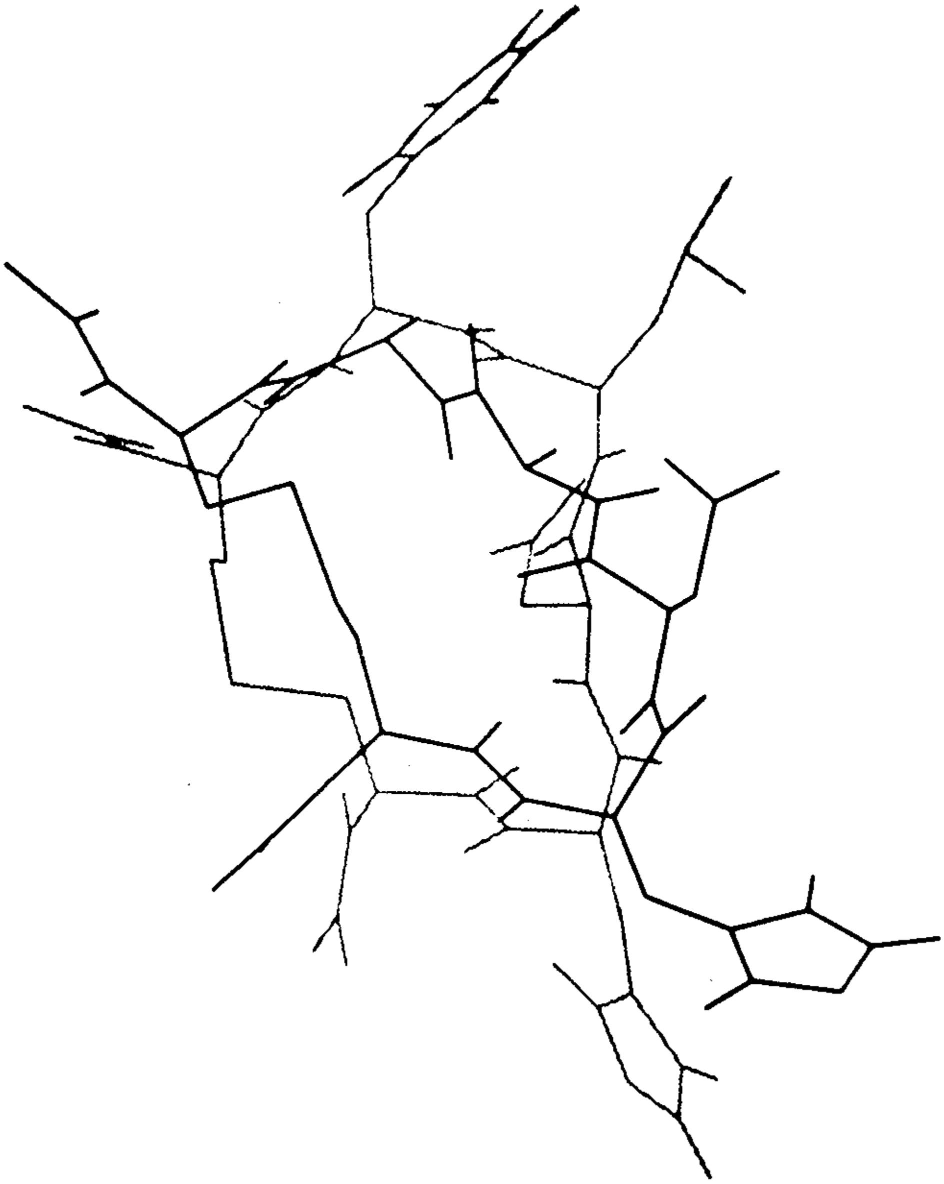
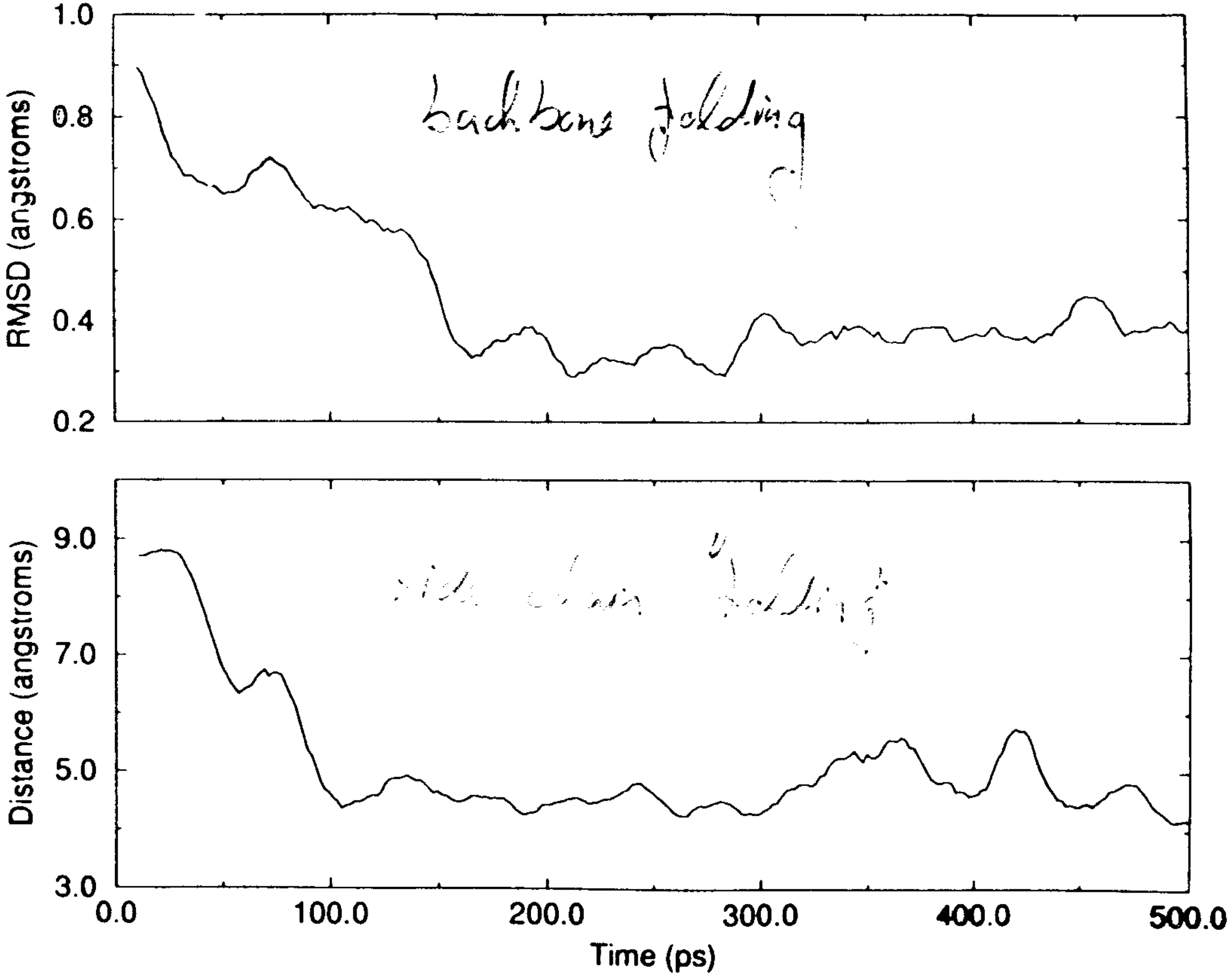


Figure 58. A stick figure showing the best overlap of the optimized structure for CHDAAC in solution, with the solution structure of CHDLFC.

Simulation of peptide folding using mean field (300°K)



# SUMMARY OF CHDLFC IN WATER

*Unique structure. Induced by hydrophobic pair: L/F

*No strong hydrogen bonding

*Time scale for side chain motions shorter than backbone

RVEW

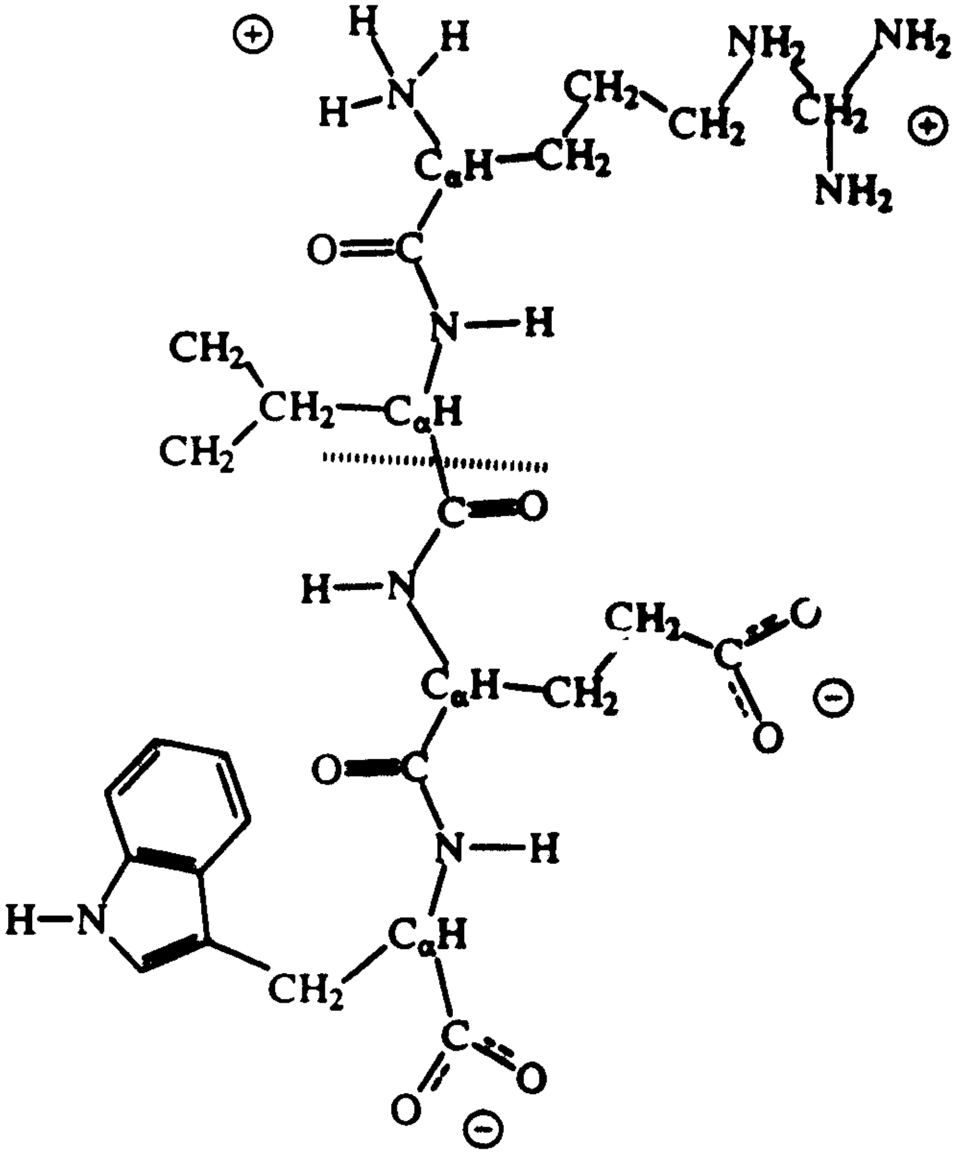
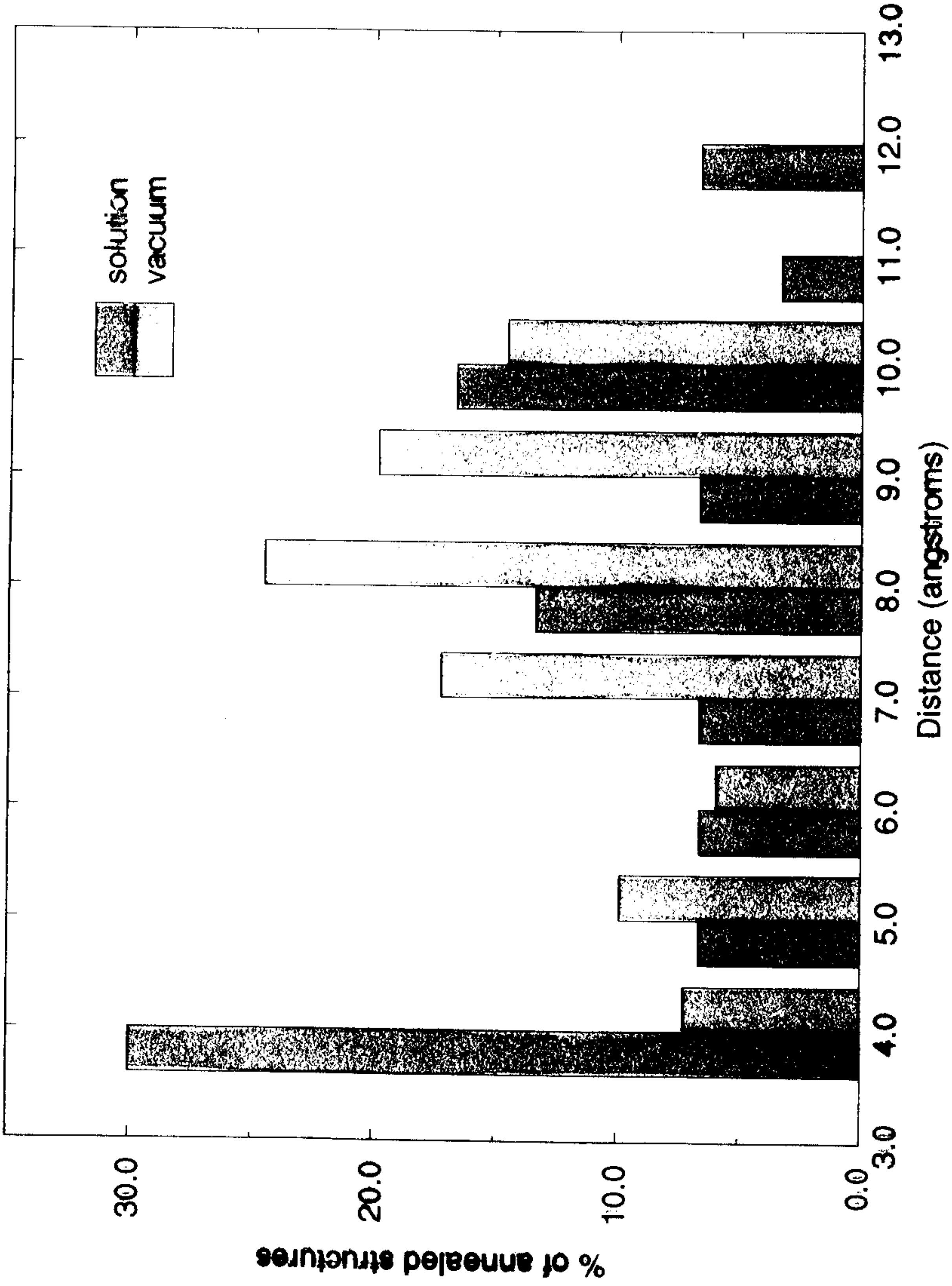


Figure 79. The atomic structure of the peptide RVEW. Dotted lines denote the division between LES fragments.

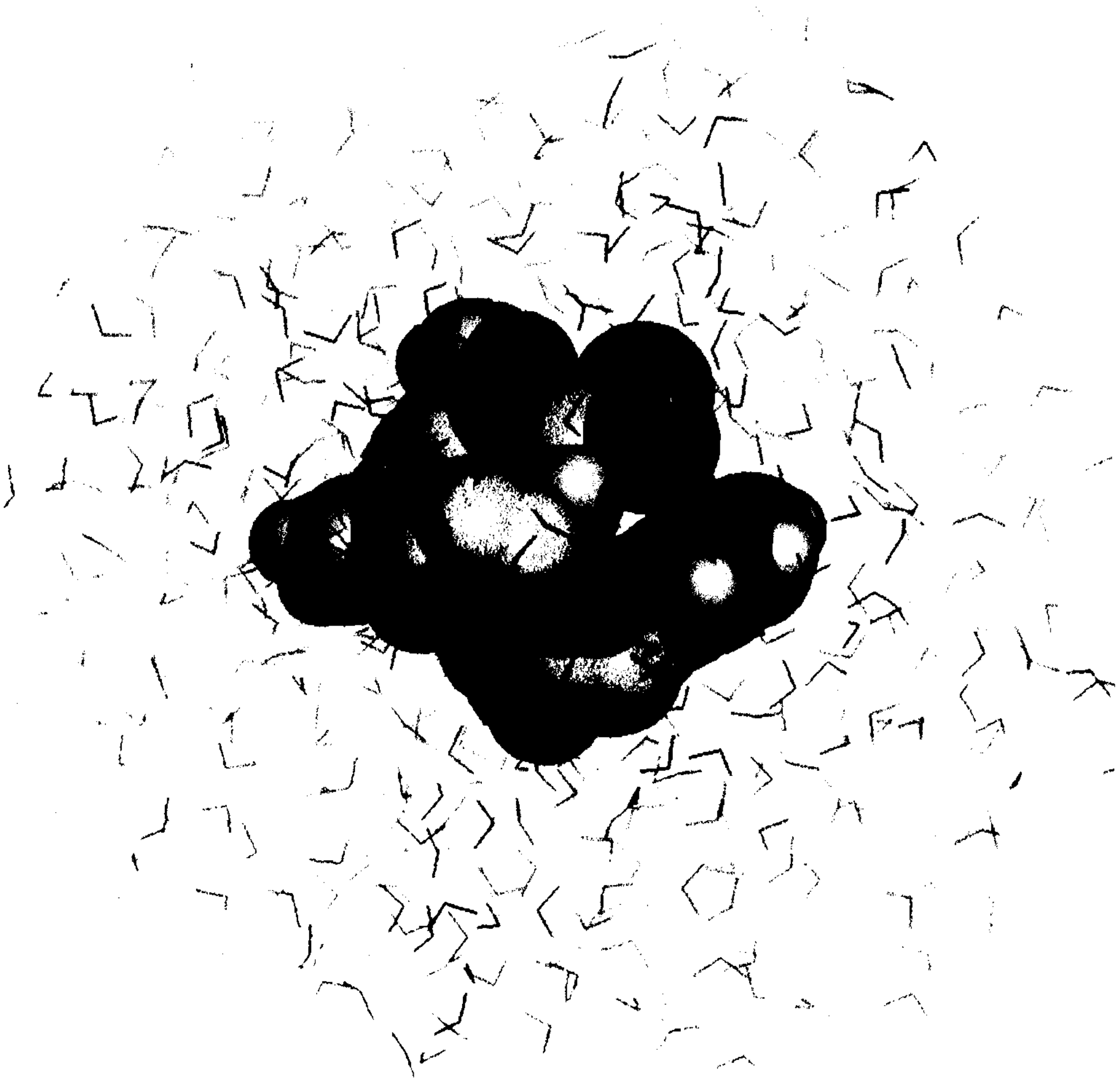
# RVEW

distance between Trp and Val side chains



30 percent of the RVEW structures have a hydrophobic contact pair:

V/W

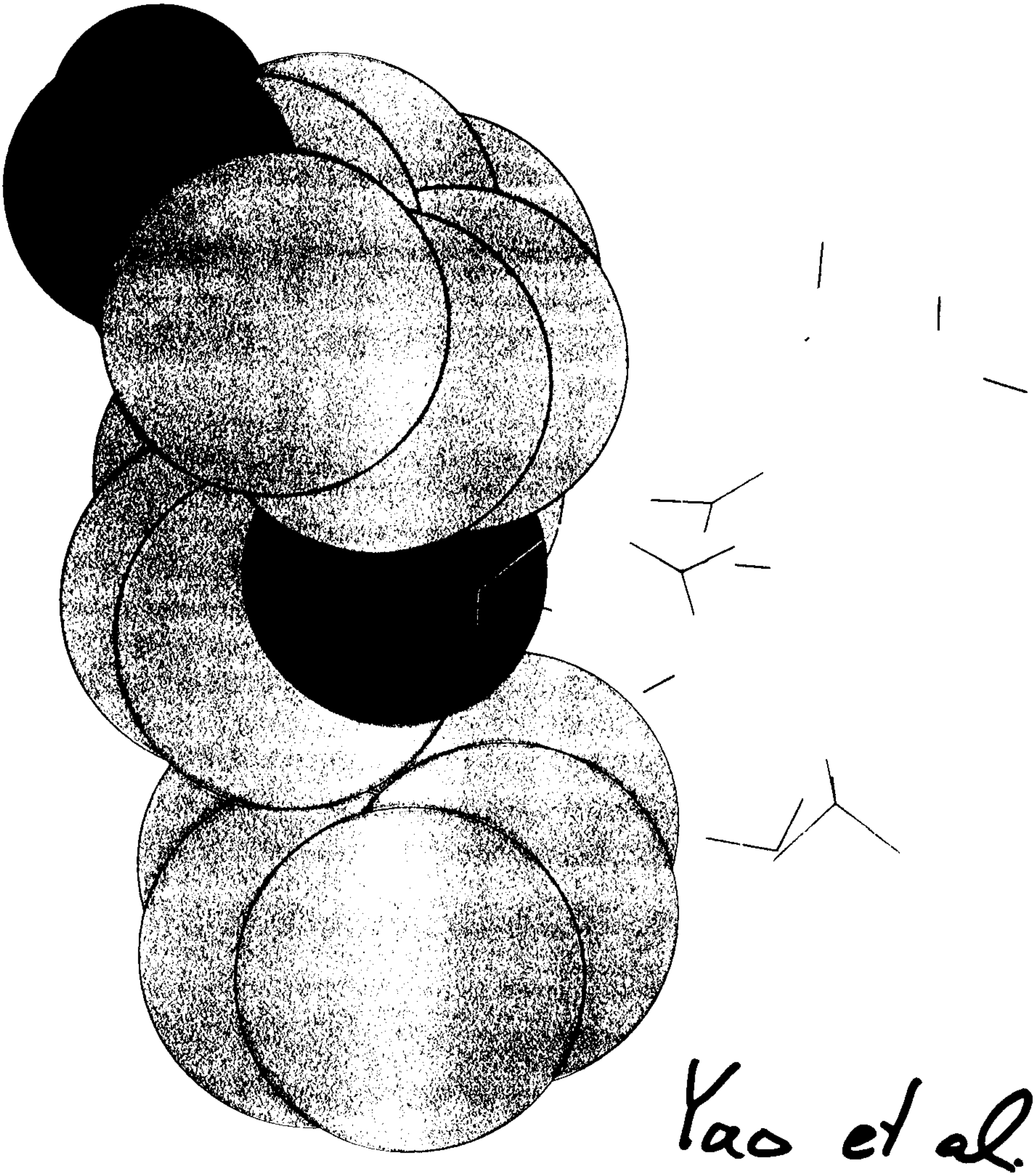


RVEW

SYPFDV - Type VI turn and a hydrophobic core suggested by experiment (YPF)
Yao et al. JMB 143,736

(*) Are hydrophobic forces drive the structure?

(*) If not what drive it to structure?



Yao et al.

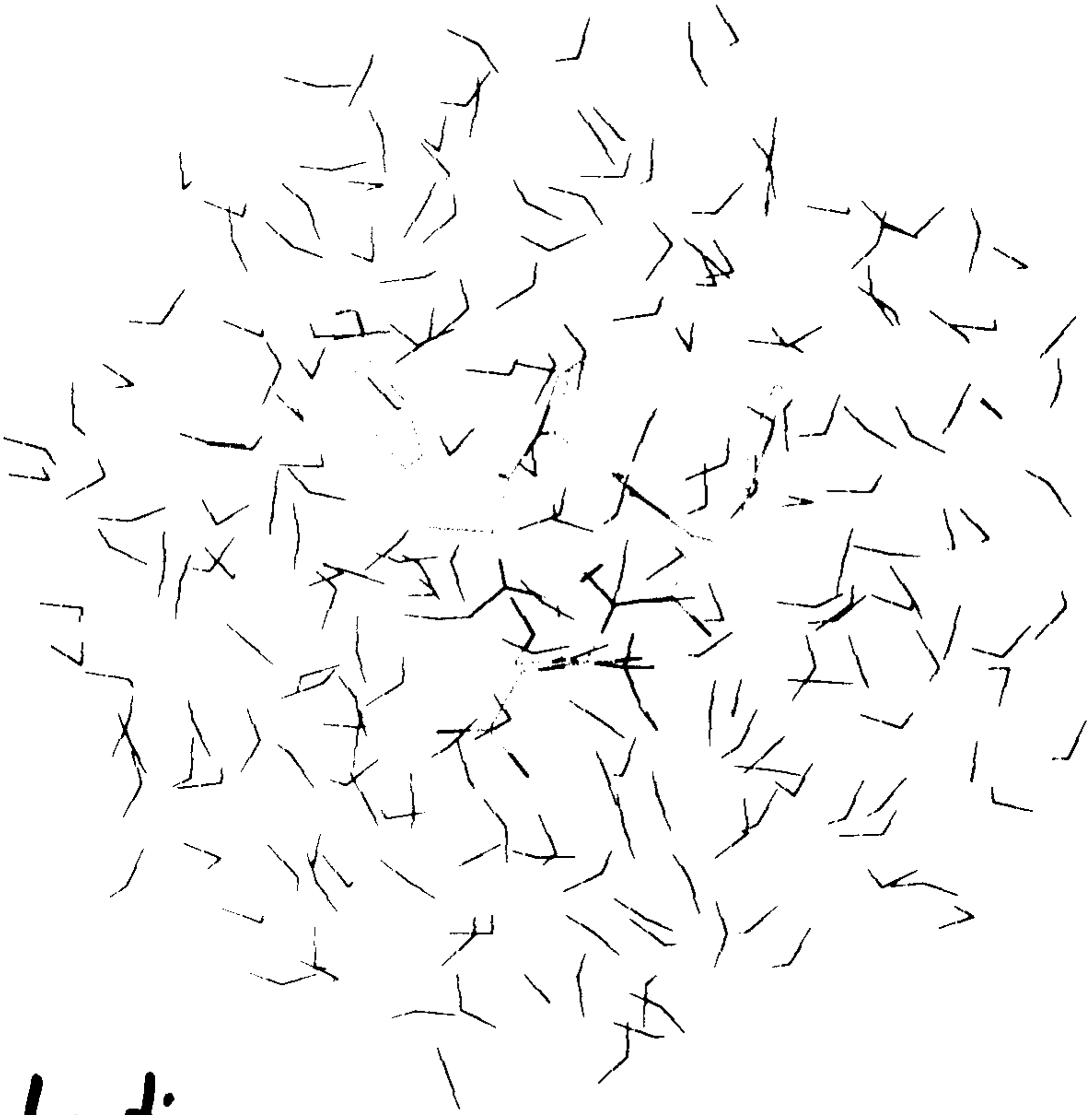
NMR structure

use now the

NMR Structure

# NMR structure

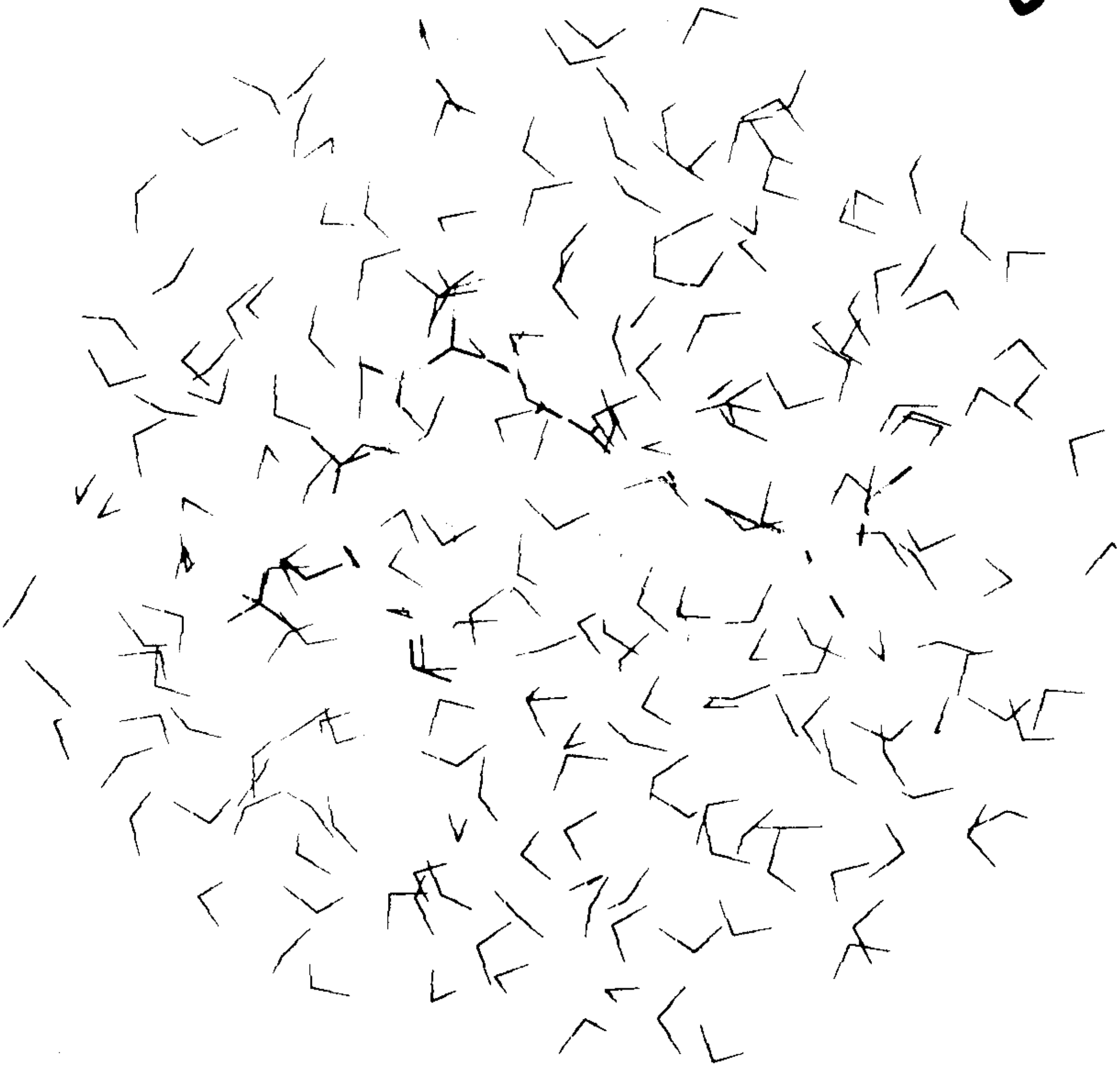
$\chi^1_{PHE} = g^-$
$\chi^1_{TYR} = t$



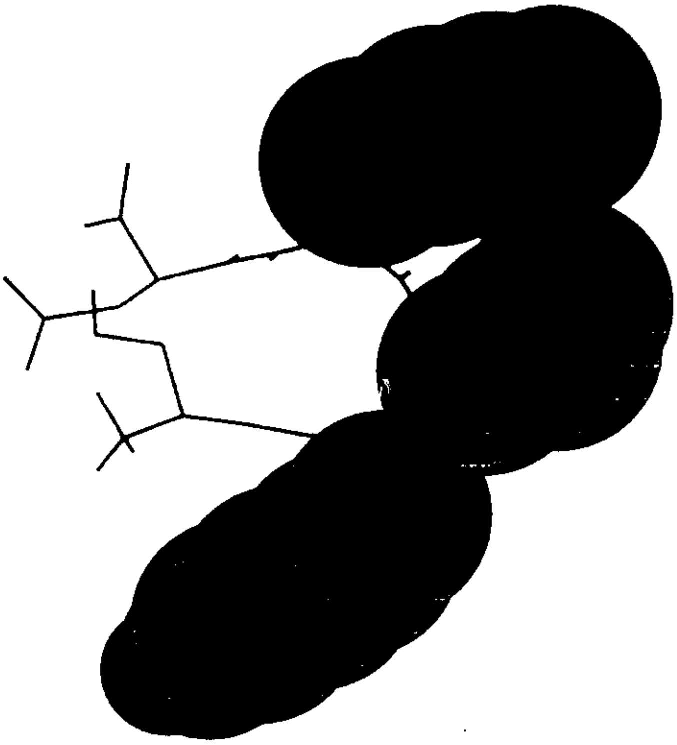
Stochastic
Solvent boundary
(B. Roux)

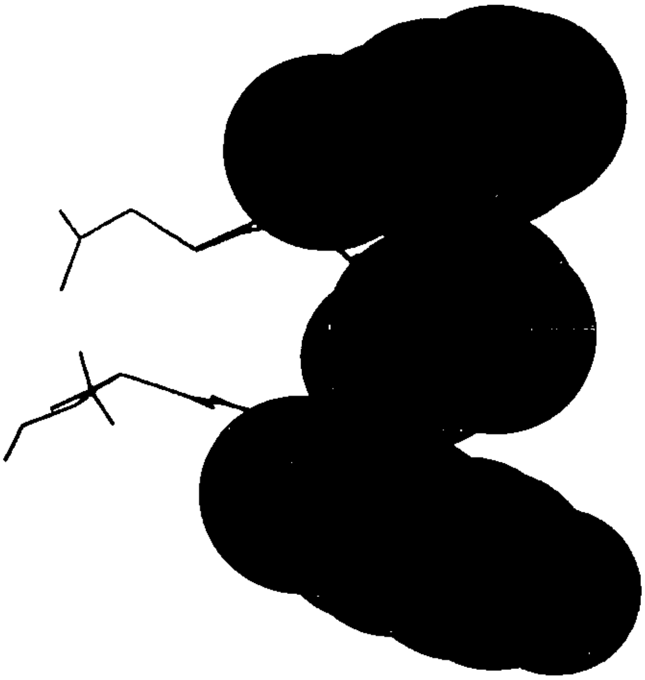
One of the typical
starting str. for
annealing.

# Typical starting structure for LES annealing



With Dr. D. Mohanty

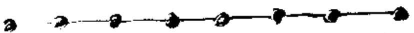




Apply to polymer:

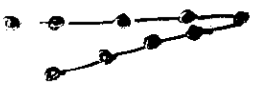
1 Can polymer remain aligned at $T>0$? **No!**

$$E = K\sum_{i=1}^{N}(1-\cos\theta_i) \Rightarrow 0 \quad \text{at } T=0$$



Consider single completely misaligned bend

Suffices to misalign

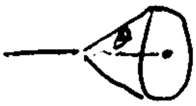


$E^* = 2K$ (energy cost)

$\#$ places $= N \Rightarrow g(E^*) = N$

$$\Rightarrow F = E^* - kT\, g(E^*) = \underbrace{2K - kT\ln N}_{\text{always lowers free energy!}}$$

3, What is typical misalignment bend? $\theta$



$$\int_0^\theta d\hat{\Omega} = 2\pi\int_0^\theta d\theta \sin\theta$$

$$E \sim NK(1-\cos\theta)$$

$$S \sim k\ln\left[\int_0^\theta (d\hat{\Omega})\right]^N = kN\ln\left[2\pi(1-\cos\theta)\right]$$

$$\Rightarrow F(\theta) \sim N\left[K(1-\cos\theta) - kT\ln 2\pi(1-\cos\theta)\right]$$

Minimize $\frac{\partial F}{\partial\theta} = 0 \Rightarrow \boxed{\frac{kT}{K} = 1-\cos\theta} \sim \frac{\theta^2}{2}$ at low $T$

Connection to persistence length:

Misalignment in one step: $\Delta\theta \sim \sqrt{\frac{kT}{K}}$

Misalignment in $n$ steps: $\sqrt{n}\,\Delta\theta$

How many steps to lose persistence?

$$\sqrt{n_p}\sqrt{\frac{kT}{K}} \simeq 1 \rightarrow n_p \sim \frac{K}{k_BT}$$

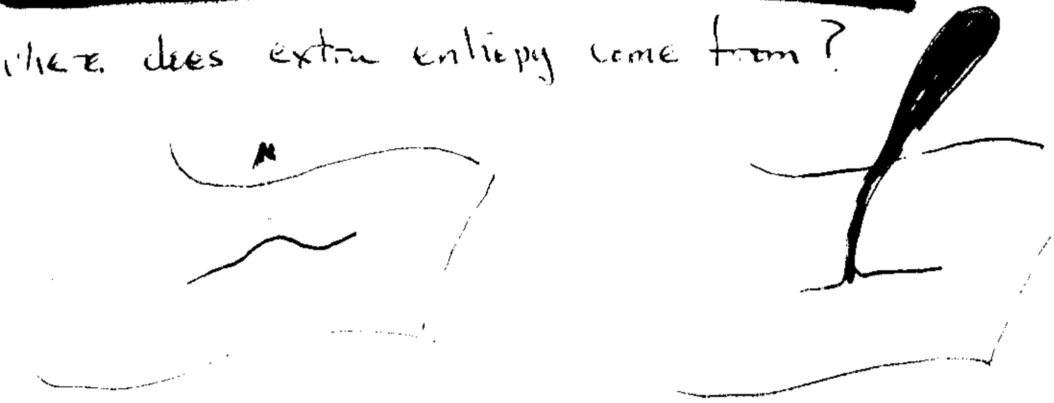
$$\Rightarrow \boxed{\xi_p \sim a_0\frac{K}{k_BT}}$$ c.f. [illegible]

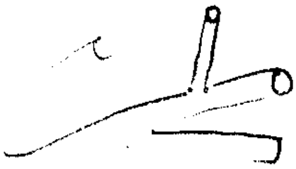
## 2. Fluid Membranes: Completely Different: ③

1': Fluid membrane is entropy dominated at long wavelength for all $T > 0$.

$$E = \int dS \frac{\kappa}{2}(c_1+c_2)^2 \quad \text{(SAME)}$$

where does extra entropy come from?





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Fact: Fluid surface always looks like branched polymer at long length scales $R \gg \xi_p$

"The branched polymer instability"

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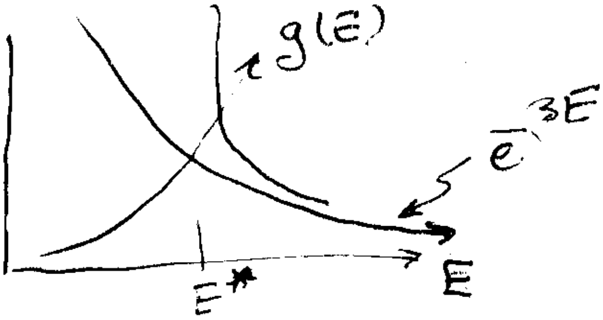
Energy-Entropy Arguments:

$$Z = \mathrm{Tr}\, e^{-\beta H} \quad (\text{e.g., } \mathrm{Tr} = \int \prod_i d\vec{r}_i \text{ for polymer})$$

$$= \int dE\, g(E)\, e^{-\beta E}$$

$$\sim \underbrace{g(E^*)\, e^{-\beta E^*}}_{\max} \cdot (\text{width})$$



$$\Rightarrow F = -kT \ln Z$$

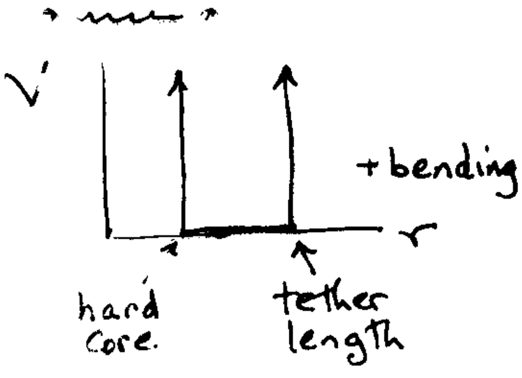
$$= E^* - \underbrace{kT \ln g(E^*)}_{TS}$$

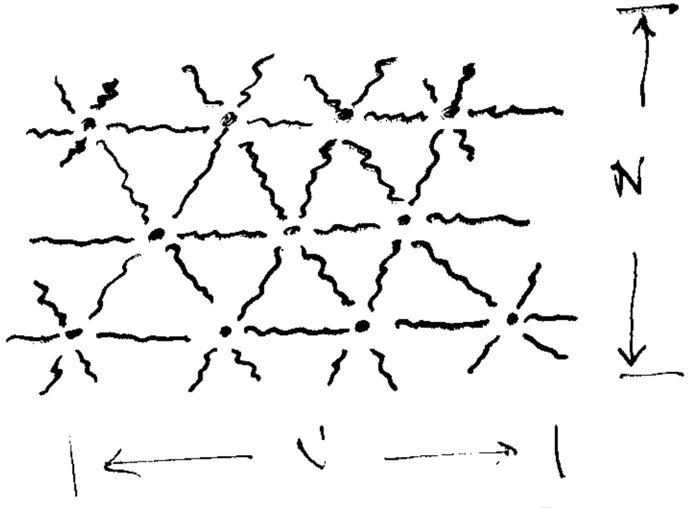
Note $E^*$ minimizes $F$! (since it maximizes $Z$)

# embranes : All membranes are NOT The same.

## Tethered Membranes:

(T=c flat) (hexatic?)





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

No self avoidance: $\langle R^2 \rangle = a^2 \ln N \ll (N^2)^{2/3}$

self-avoidance IMPT!

---

Add Self-Avoidance:

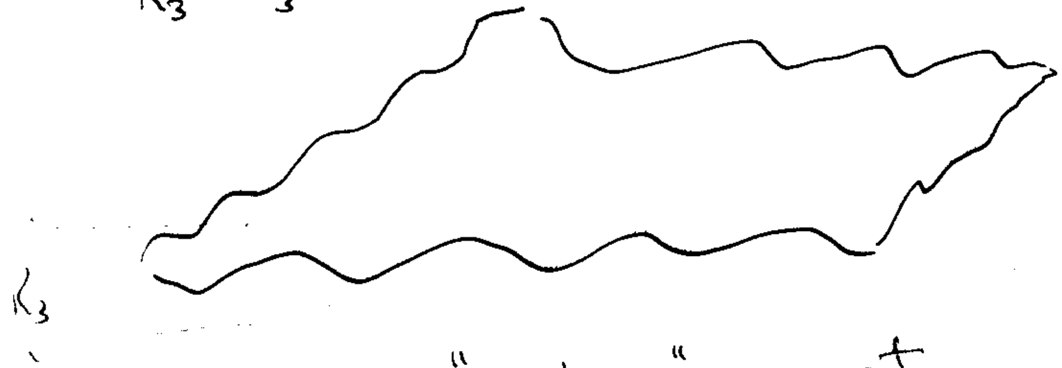
$R_1 = a_1 N^{\nu_1}$

$R_2 = a_2 N^{\nu_2}$

$R_3 = a_3 N^{\nu_3}$

$\boxed{\nu_1 = \nu_2 = 1}$ $a_1 > a_2$.

$\nu_3 = 0.8$ (?)



$\nu_3$ is "roughness" exponent

+ tethered membrane is "flat" at long distances no matter how large The bending rigidity ($T > 0$)

"Energy dominated"

Entropy is "small" for $d=2$ object in $d=3$

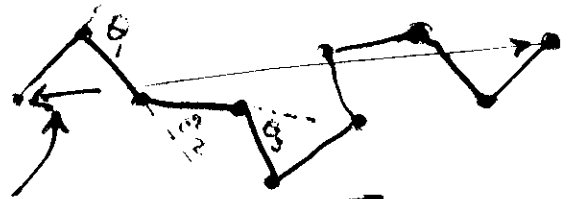
his result holds even $\kappa \to 0$ at low T ($\neq 0$).

(c.f., $d=1$ in $d=3$)

Statistical Mechanics of Membranes at large distances

# ENOMENA: $\boxed{R \gg \xi_p}$ c.f., polymers

**lymers:** All polymers are the same! One "universality class"!



N = number of segments

$a_0$ = segment length

$\theta_i$ = angle

ze = ?

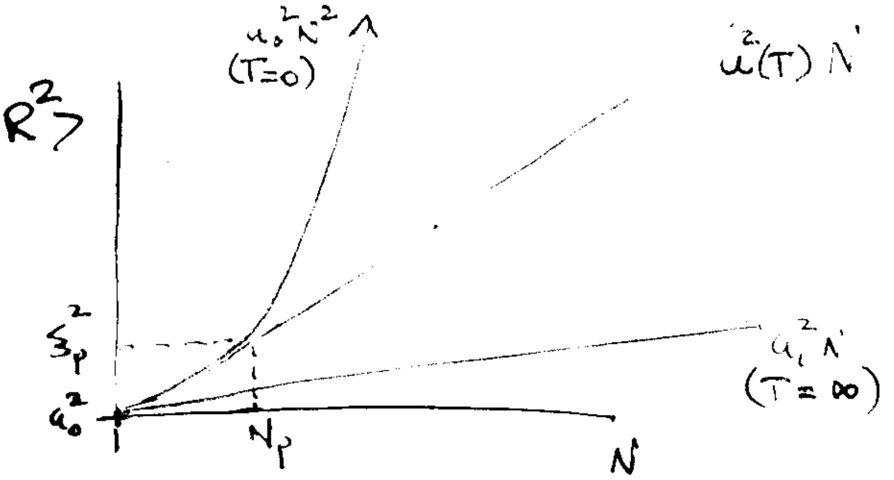
$\boxed{R = \text{end-to-end length}}$

$$E = \kappa_b \sum_i (1-\cos\theta_i) \simeq \int ds\, \frac{\kappa}{2} c^2$$

t T=0 ($\kappa = \infty$, stiff rod): $\langle R^2 \rangle = L^2 = a_0^2 N^2$

t T = ∞ ($\kappa = 0$, random walk): $\langle R^2 \rangle = a_0^2 N$

etween:



i.e., $\left(\langle R^2 \rangle\right)^{1/2} \xrightarrow[N\to\infty]{} a(T) N^{\nu_0}$, $\nu_0 = 1/2$ (no self-avoidance)

---

Now add self avoidance:

$$\left(\langle R^2 \rangle\right)^{1/2} \xrightarrow[N\to\infty]{} b(T) N^{\nu}, \quad \nu \approx 0.588 \text{ SAW)} \quad \boxed{d=3}$$

← indep of $\kappa$

(small effect)

↑ depends on energetics

Random coil:

$R_1 = a_1 N^{\nu_1}$

$R_2 = a_2 N^{\nu_2}$

$R_3 = a_3 N^{\nu_3}$

moments of inertia tensor

$a_1 > a_2 > a_3$

BUT

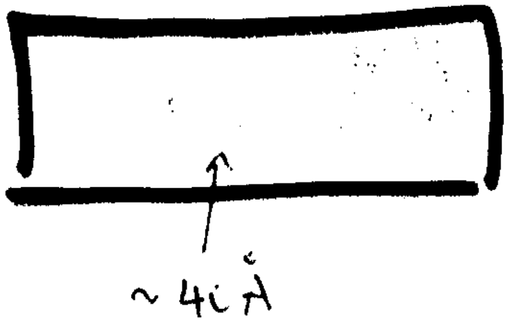
$\nu_1 = \nu_2 = \nu_3 = \nu$

"UNIVERSALITY"

Is it a sphere

No!

# III. Mechanics/Stat. of Membrane Shapes



with $\kappa_b \sim 20\, k_B T$
(common phospholipids)

A. $R \gg \xi_p$ Statistical Mechanics
(long-distance behavior)
Gompper

B. $R \ll \xi_p$ Mechanics "$(T=0)$"
Vesicle Shapes

C. Thermal Fluctuations of Vesicle Shapes
(Stat. Mech, again)

(But, soft modes!)

# Phase Diagram for Surface Phase: The effect of The Gaussian term (TOPOLOGY)

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Huse + Leibler, J. de Physique 49, 605 (1988)

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$$\mathcal{H}[S] = r_0 \int dS + \frac{\kappa_b}{2} \int dS\,(c_1+c_2)^2 + \kappa_g \underbrace{\int dS\,(c_1 c_2)}_{4\pi\kappa_g (N_p - N_h)}$$

---

Three dimensionless parameters;

$\frac{\kappa_b}{k_BT}$ $\frac{\kappa_g}{k_BT}$ $\frac{r_0 \xi_p^{2}}{k_BT}$ ← since $[r_0] = \left[\frac{\text{energy}}{\text{area}}\right]$

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To simplify: assume $\frac{\kappa_b}{k_BT} \approx 1$ otherwise no structured phases

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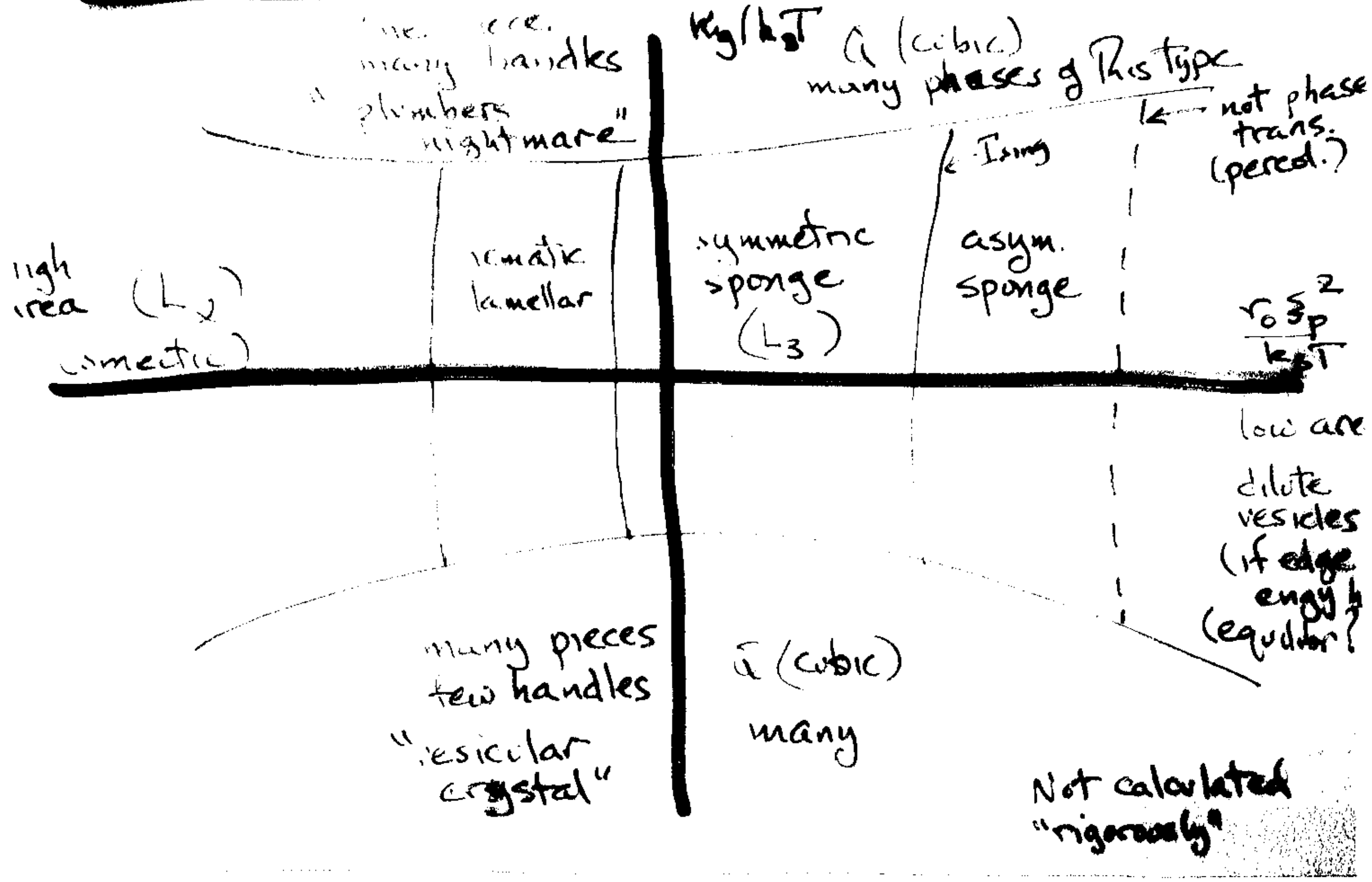
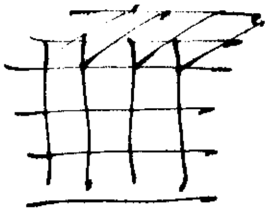
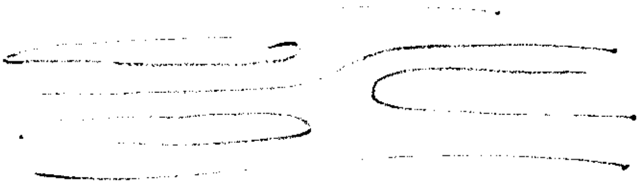


## Discussión

- $\frac{\kappa_g}{k_BT}$ axis — topological axis — # handles extensive ↕ # pieces extensive −
- "Plumber's nightmare" Imagine lattice



- $\frac{\tilde{u}\,3p^2}{k_BT}$ axis — Area axis — high area − ⟵⟶ + low area
- Smectic
- Nematic Lamellar (holes)

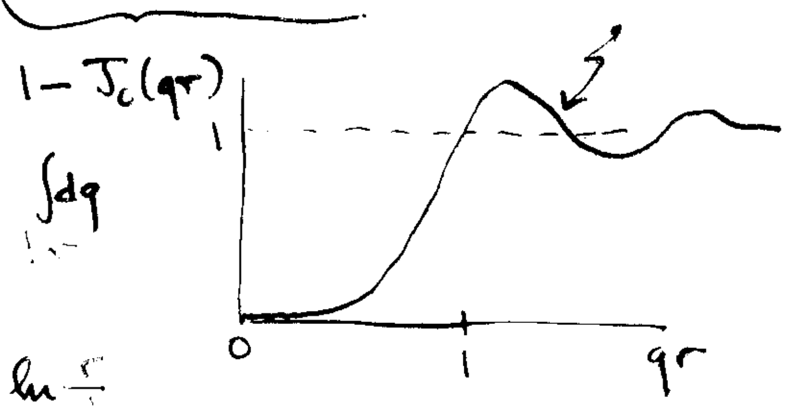


- Symmetric Sponge (outside/inside equiv.) This sym. <u>must</u> be broken to get to vesicles!
- Vesicular Phase (percolation)
- Metastability

$$\langle \hat{n}(\vec{r}) \cdot \hat{n}(\vec{0}) \rangle - 1 \qquad \text{cutoff}$$

$$= \frac{k_B T}{2\pi K_b} \int_0^{1/d} \frac{dq}{q} \left( 1 - \underbrace{\frac{1}{2\pi} \int_0^{2\pi} d\theta \cos(qr\cos\theta)} \right)$$

effect of $(1 - J_0(qr))$ is to cut off low limit $\int dq$



$$\Rightarrow \langle \hat{n}(\vec{r}) \cdot \hat{n}(\vec{0}) \rangle \simeq 1 - \frac{k_B T}{2\pi K_b} \ln \frac{r}{d}$$

which describes initial fall-off of corres.,
before membr. is too far from flat

Define $\xi_p$ as distance where $\langle \hat{n}(\vec{r}) \cdot \hat{n}(0) \rangle$ has fallen to $(1 - \alpha)$.

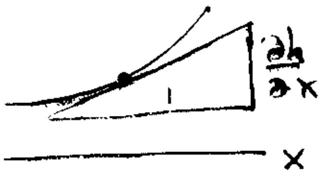
i.e.,
$$\frac{k_B T}{2\pi K_b} \ln \frac{\xi_p}{d} = \alpha$$

$$\Rightarrow \boxed{\xi_p = d \exp\left( \frac{2\pi \alpha K_b}{k_B T} \right)}$$

- Temperature dependent ( $\to \infty$ at $T \to 0$ )
- exponential form (c.f., flexible polymer)
  $\langle \hat{t}(s) \cdot \hat{t}(0) \rangle =$
- very large for phospholipid membrane ( $K_b \sim 20\, k_B T$ )
- Better calculation gives
  $$\langle \hat{n}(r) \cdot \hat{n}(0) \rangle - \langle \hat{u} \rangle^2 \sim e^{-r/\xi_p}, \quad \xi_p = d\, e^{\frac{4\pi K_b}{3 k_B T}}$$

# Calculation of the Persistence Length $\xi_p$ (30)

What is $\hat{n}$ in Monge representation?



$$\hat{n} \perp (1, 0, \frac{\partial h}{\partial x})$$

$$\hat{n} \perp (0, 1, \frac{\partial h}{\partial y})$$

$$\hat{n} \parallel (-\frac{\partial h}{\partial x}, -\frac{\partial h}{\partial y}, 1)$$

normalize: $\hat{n} = \dfrac{(-\frac{\partial h}{\partial x}, -\frac{\partial h}{\partial y}, 1)}{\sqrt{1+(\nabla h)^2}}$

expand: $\hat{n} = (-\frac{\partial h}{\partial x}, -\frac{\partial h}{\partial y}, 1-\frac{1}{2}(\nabla h)^2) + O(h^3)$

---

$$\hat{n}(\vec{0})\cdot\hat{n}(\vec{r}) = 1 - \frac{1}{2}\left[(\nabla h(0))^2 + (\nabla h(\vec{r}))^2\right] + \frac{\partial h}{\partial x}(\vec{0})\frac{\partial h}{\partial x}(\vec{r}) + \frac{\partial h}{\partial y}(\vec{0})\frac{\partial h}{\partial y}(\vec{r}) + O(h^3)$$

$$= 1 - \frac{1}{2}(\nabla h(\vec{r}) - \nabla h(\vec{0}))^2 \quad O(h^3)$$

But, $h(\vec{r}) = \int \frac{(d^2q)}{(2\pi)^2} e^{iq\cdot r} h(\vec{q}) = \int \frac{(d^2q)}{(2\pi)^2} e^{-iq\cdot r} h^*(\vec{q})$

$$\Rightarrow \langle \hat{n}(0)\cdot\hat{n}(r)\rangle = 1 - \frac{1}{2}\int\frac{(d^2q)}{(2\pi)^2}(e^{iq\cdot r}-1)(e^{-iq\cdot r}-1)\,q^2\,\langle|h_q|^2\rangle$$

$$= \int\frac{(d^2q)}{(2\pi)^2}\, 2\sin^2(\tfrac{q\cdot r}{2})\, q^2\, \frac{k_BT}{\sigma q^2 + \kappa_b q^4}$$

So, for tensionless membrane ($\sigma = 0$),

$$\langle \hat{n}(\vec{r})\cdot\hat{n}(\vec{0})\rangle = 1 - \underbrace{\int\frac{(d^2q)}{(2\pi)^2}\frac{k_BT}{\kappa_b}\frac{(1-\cos(q\cdot r))}{q^2}}_{} + \ldots$$

aligned (flat) membrane

effect of flucts
(→ 0 at $\vec{r}=0$)
estimate

# The de Gennes – Taupin "Persistence Length" for a symmetric ($c_0=0$) fluid sheet

flat at T=0

$$\mathcal{H} = \int dS \left[ r_0 + \frac{\kappa_b}{2}\underbrace{(c_1+c_2)}^{2} \right] \qquad h(x,y)$$

$$dx\,dy\sqrt{1+(\nabla h)^2}\left[ r_0 + \frac{\kappa_b}{2}\left(\frac{d^2h}{dx^2}+\frac{d^2h}{dy^2}\right)^2 \right]$$

(expand)

$$= \text{Constant} + \int_S d^2r \left[ \frac{r_0}{2}(\nabla h)^2 + \frac{\kappa_b}{2}(\nabla^2 h)^2 + \text{higher order} \right]$$

$$\longrightarrow \text{Constant} + \frac{1}{2}\int \frac{d^2q}{(2\pi)^2}\Big[ (\underset{\substack{\uparrow \\ \text{mechanical tension}}}{r_0} q^2 + \underset{\substack{\uparrow \\ \text{curvature rigidity}}}{\kappa_b} q^4)|h_q|^2 + O(h^4) \Big]$$

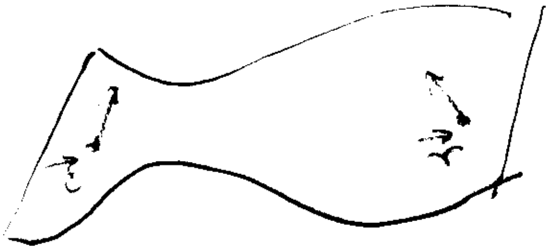
---

At T=0 this membrane is flat (planar) under combined effect of surface tension and bending rigidity.

$\langle \hat{n}(\vec{0})\cdot\hat{n}(\vec{r})\rangle = 1$

For T>0,

hermally induced undulations occur, so normals $\hat{n}(\vec{r})$ and $\hat{n}(\vec{0})$ lose correlation when $\vec{r}$ is far from $\vec{0}$.



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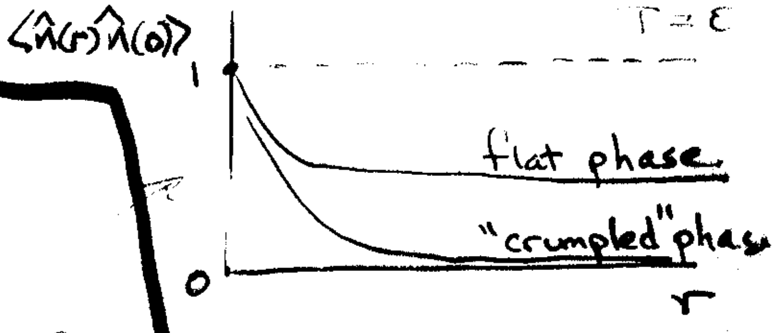
aussian fluctuations:

$$\langle |h_q|^2 \rangle = \frac{k_B T}{r_0 q^2 + \kappa_b q^4}$$

se This to calculate initial fall-off
hen membr. is not too far from planar).



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$$\mathcal{H}[S] = \sigma_0 \int dS + \frac{1}{2} \kappa_b \int dS \, (c_1 + c_2 - c_0)^2 + \kappa_g \int dS \, c_1 c_2$$

$+$ higher order in $d/R$ $+ \lambda_{edge} L_{edge}$

- when $c_0 \neq 0$ membrane has lowest energy in "bent" state, i.e., with a "spontaneous" curvature.
  If the two sides are equivalent, then $c_0 = 0$.
  (not true with [illegible]!)

- $\int dS \, c_1 c_2 = 4\pi \, (N_{pieces} - N_{handles})$ for closed topology
  " Gauss - Bonnet Theorem "
  Topological invariant.
  ( = constant for any system with fixed topology)
  But crucial if topology is variable !

- $\kappa_g \sim \kappa d^2$ hard to measure
  can be positive or negative !

- $\sigma_0$ term measures area constant if area fixed.
  But crucial if amphiphile density in solution is important variable !

- For incompressible closed membrane with given (fixed) $N_{amphi}$ and given (fixed) topology

"the Helfrich Hamiltonian"

$$\boxed{\mathcal{H}[S]_{Helfrich} = \frac{1}{2} \kappa_b \int dS \, (c_1 + c_2 - c_0)^2}$$

$+$ one additional contrib. due to elasticity ① + bilayer ④

Applications

$$c_1 = \frac{1}{R_1}, \quad c_2 = \frac{1}{R_2} \quad \Big\} \quad R_1, R_2 = \text{local principle radii of curvature}$$

Note: $c_1 + c_2 = \mathrm{tr}\, K = 2H$

curvature tensor (K) — mean curvature (H)

Local shape invariants:

$c_1, c_2$ + coeffients of all higher order terms

$c_{1,2} \sim 1/R$ $\qquad$ $(1/R^2, 1/R^3, \text{etc})$

Concentrate for the moment on $c_1, c_2$:

- Any fcn $f(c_1, c_2)$ is a Euclidean invariant
- To satisfy isotropy, we can't distinguish $c_1$ + $c_2$

What about $|c_1 - c_2|$?

linear: $(c_1 + c_2) \sim 2H = \mathrm{tr}\, K$ $\qquad \sim 1/R$

quadratic: $c_1^2 + c_2^2,\ c_1 c_2 \longleftrightarrow (c_1+c_2)^2,\ c_1 c_2$ $\qquad \sim 1/R^2$

cubic: $(c_1^3 + c_2^3)\quad c_1 c_2 (c_1 + c_2)$ $\qquad \sim 1/R^3$

• • • • •

But, membrane elastic strains $\sim \frac{d}{R}$

$\Rightarrow$ higher powers of $1/R$ alway bring $10^{-3}$ in higher powers of small parameter

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bending modulus — spontaneous curvature — gaussian modulus

$$H[S] = \int_S dS \left[ r_0 + \underbrace{\tfrac{1}{2} \kappa_b (c_1 + c_2 - c_0)^2}_{O(\kappa d^2)} + \underbrace{\kappa_g c_1 c_2}_{O(\kappa d^2)} + O\left(\frac{d}{R}\right)^3 \right]$$

$\sim (d/R) + (d/R)^2$

$r_0 \times 1$ controls membrane area

cubic? invar

What is energy functional (Hamiltonian) for $d=2$ fluid membrane ? incompressible, untilted, uncharged.

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dynamics : $\{\vec{r}(s_1, s_2)\} = [\text{Shape}]$

---

Assume Landau theory):

$$\mathcal{H}[S] = \int_S dS\, f(\vec{r})$$

local elastic deformation energy

If f depends on "shape", then it must be independent of rotations and translations. (Euclidean transformations)

- In addition, if fluid is isotropic, it cannot distinguish any special direction in the membrane.

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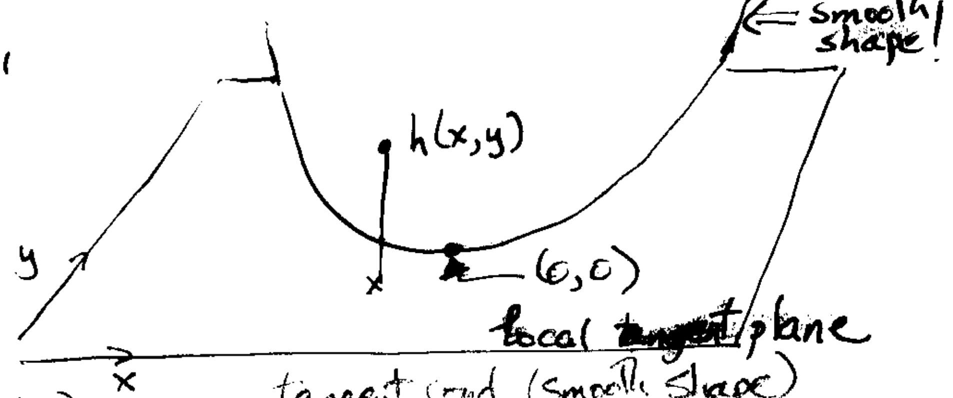
## Comment: Local Euclidean Invariants

"Monge representation"

local shape → height above local tangent plane



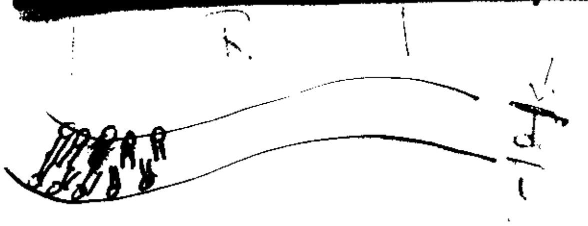
tangent cond. (Smooth Shape)

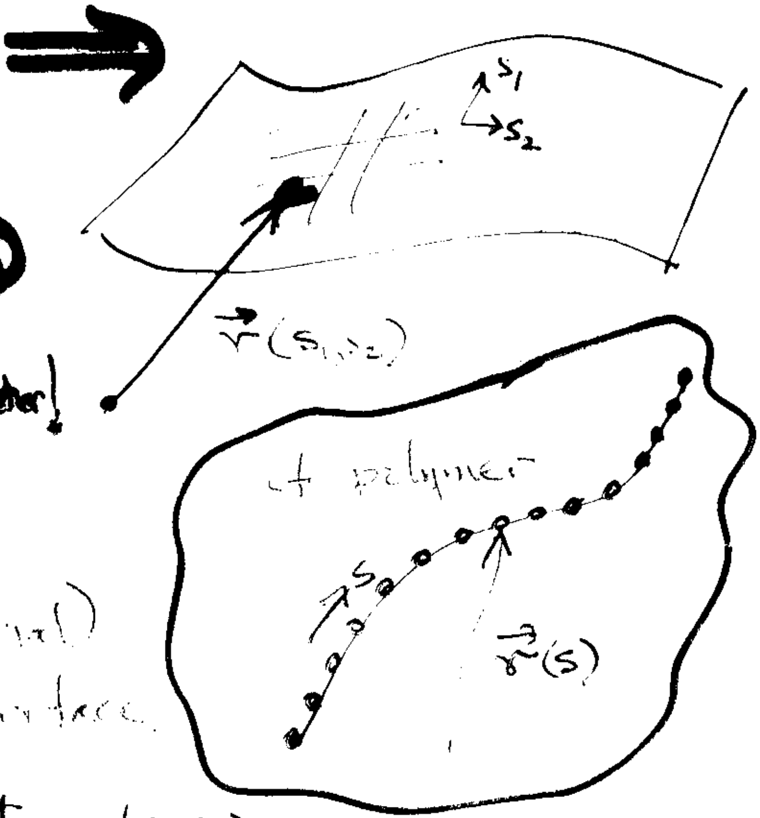
$$h(x,y) = \overset{\text{(translation)}}{\cancel{h_0}} + x \overset{0}{\cancel{\frac{dh}{dx}\Big|_0}} + y \overset{0}{\cancel{\frac{dh}{dy}\Big|_0}} \quad \text{(choice of local axes)}$$

$$+ \frac{1}{2} x^2 \frac{d^2h}{dx^2}\Big|_0 + xy \frac{d^2h}{dx\,dy}\Big|_0 + \frac{1}{2} y^2 \frac{d^2h}{dy^2}\Big|_0 + \dots$$

$$\Longrightarrow = \frac{1}{2} c_1 x^2 + \frac{1}{2} c_2 y^2 + O(x^3, x^2 y, x y^2, y^3 \dots)$$

# Mesoscopic Description [of $L_\alpha$ (fluid) Phase(s)]





$$\frac{40\text{Å}}{1\,\mu m} = 10^{-3} \qquad \boxed{\frac{d}{R} \ll 1} \Rightarrow$$

! important small parameter !

what is new here relative to polymer is that different (additional) fields can live on the surface.

1. local phospholipid density $n(s_1,s_2)$
   [or, equivalently, $a(s_1,s_2)$ = area/molecule]
2. tilt fields } for tilted or gel phases only
3. hexatic fields }
4. Plus: these fields can be different for the two leaves of the bilayer

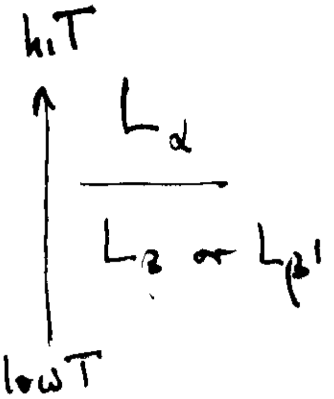
Most of these field theories are incompletely understood.

For simple "incompressible" $d=2$ untilted fluid we can neglect them all

(but, later, we will have to come back and look more closely at points ① + ④

**Comments:**

Transition:



"Main transition"
(weakly) first order

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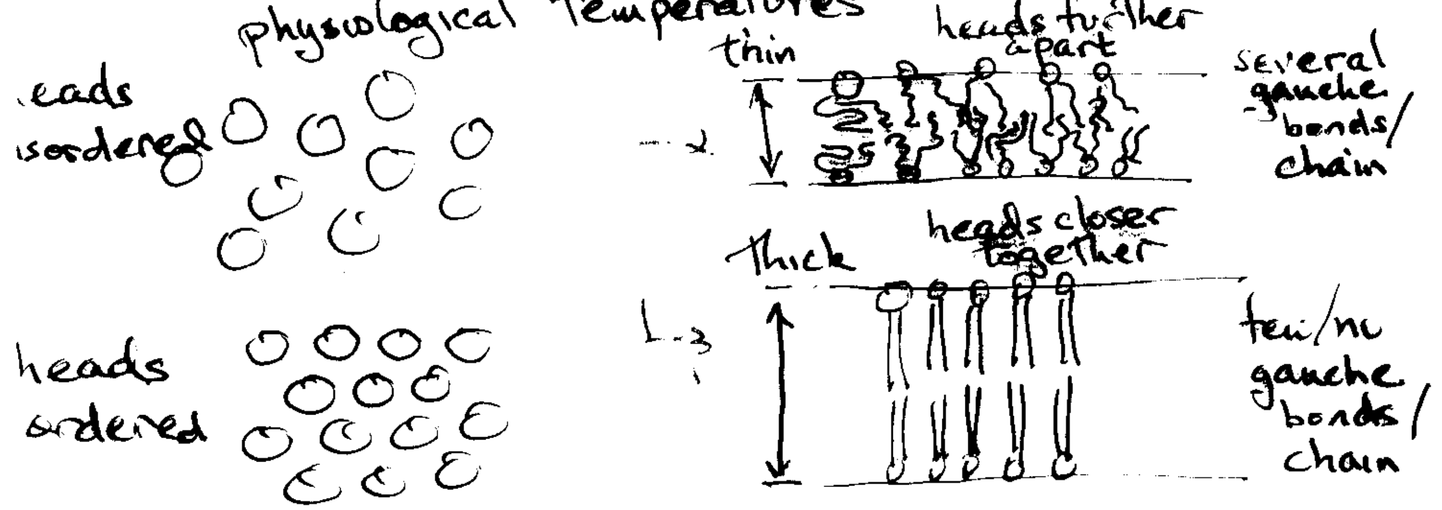
For common phospholipids takes place near physiological temperatures



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- Volume preserved to good approx) tails = "oil" layer
- Nature uses $L_\alpha$ phase (needs good mobility?)
- But likes to operate just above main transition for reasons which are not understood.
- Regulation via { cis bonds in (one) lipid chain
  { cholesterol

# Bilayer phases :

Bilayer structures can exist with many different internal states! (phases)

cf: liquid crystals : SmA no tilt; SmC (tilted)

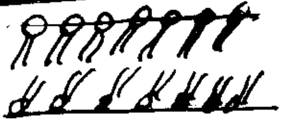
| d=2 (flat) materials: | | fluid | hexatic | quasi-solid |
|---|---|---|---|---|
| Order | translation | SR | SR | QLR |
| | orientation | SR | QLR | LR |

## Bilayer fluids:

No shear rigidity
No average mol. tilt.
Isotropic d=2 fluid
D large

Biology Uses This Phase

"liquid crystal"
α SmA fluid



No shear rigidity
+ molecular tilt
Anisotropic d=2 fluid
D large

SmC fluid

## Bilayer gel (hexatic)

headgroups

shear rigidity = ?
No average tilt
"Solid" or "gel"
(probably hexatic)
"no" diffusion

β "SmA" d=2 hexatic gel

β' "SmC" d=2 hexatic gel

DMPC

same with ave tilt
"gel"
"no" diffusion

3

shear rigid ?
periodic tilt

ripple phase

㉒

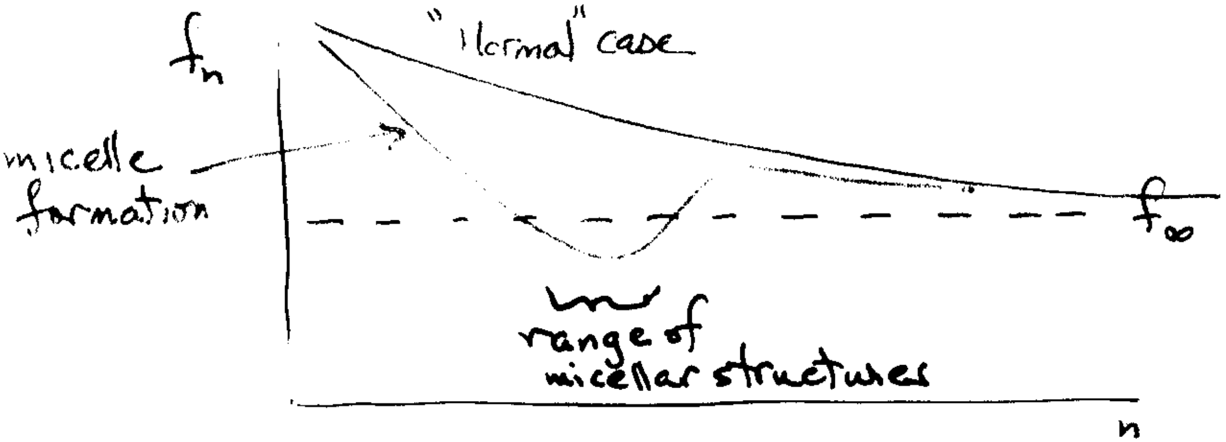
Thus,

$$\ln \Xi = V \sum_{n=1}^{\infty} \left( \frac{e^{-\beta(E_n - n\mu)}}{n!} = \frac{e^{-\beta n(\varepsilon_n - \mu)}}{n!} \right)$$

$$\langle N_n \rangle = -\frac{\partial}{\partial(\beta E_n)} \ln \Xi = V \frac{e^{-\beta n(\varepsilon_n - \mu)}}{n!}$$

$$\Rightarrow \boxed{\frac{\langle N_n \rangle}{V} = e^{-\beta n(f_n - \mu)} , \quad f_n = \varepsilon_n + kT \ln n}$$

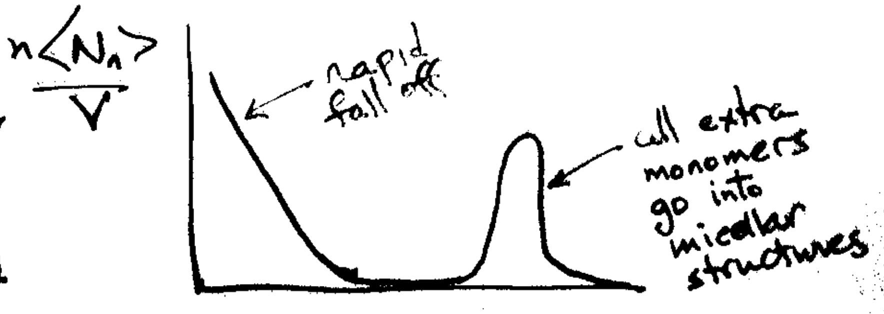
What happens as monomer density increases? — dominated by $1/n^x$
(i.e., as $\mu \uparrow$)



"Normal" case

all density remains at small n until infinite aggregate ~~forms~~

monomeric density in n-mers



"micellar case"

Total # monomers:

$$N = \sum_{n=1}^{\infty} n N_n$$

$N_1$ = # monomers
$N_2$ = # pair aggregates
...

So,

$$E_{total}(\{N_n\}) = \sum_{n=1}^{\infty}\left(E_n N_n = n\varepsilon_n N_n\right)$$

(neglects inter-aggregate interactions)
OK at low density

Number of configurations:
How many different ways can we put N particles in volume V aggregated according to $\{N_n\}$?

$$g(\{N_n\}) \simeq N! \prod_{n=1}^{\infty}\left[\frac{V^{N_n}}{(n!)^{N_n} N_n!}\right]$$

neglects excluded volume (OK low density)

---

Partition Function:

$$Z_N(T,V) = \sum_{\substack{\{N_n\} \\ N=\sum_n nN_n \text{ fixed}}} g(\{N_n\})\, e^{-\beta E_{total}(\{N_n\})}$$

---

Grand Canonical

$$\Xi(T,V,\mu) = \mathrm{Tr}\, e^{-\beta(H-\mu N)} = \sum_{N=0}^{\infty} \frac{e^{\beta\mu N}}{N!} Z_N(T,V)$$

$$= \prod_{n=1}^{\infty}\left[\sum_{N_n=0}^{\infty} \frac{e^{-\beta N_n(E_n-n\mu)}\, V^{N_n}}{N_n!\,(n!)^{N_n}}\right]$$

$$= \prod_{n=1}^{\infty} \exp\left[\frac{V e^{-\beta(E_n-n\mu)}}{n!}\right]$$

## A.3 Statistical Mechanics of Aggregation

Small- vs large-scale structures

micelles — hex, lamellae

Note: The CMC is NOT a thermodynamic phase trans.!

NOT sharp!

$\phi \ll$ CMC: No micelles present in solution. only monomer

$\phi \gtrsim$ CMC: Virtually all added amphiphile goes into aggregates

---

Consider a set of monomers controlled by a single overall chemical potential $\mu$

It costs $E_1$ to put an isolated monomer into solution

But, if we put in two, then a two-particle aggregate can form with energy $E_2 < 2E_1$

(e.g., because of hydrophobic shielding)

Generally, there can be n-particle aggregates with

$$E_n = n\,\varepsilon_n$$

$\varepsilon_n$: energy/particle for particle in n-aggregate

**Note: There may several different n-particle aggregates. We ignore this complication!**

For a large aggregate (e.g., big bilayer fragment) we expect $\lim_{n\to\infty} \varepsilon_n \to \varepsilon_\infty$ and for n large

$$E_n = n\,\varepsilon_\infty\left(1 + \frac{\alpha}{n^x}\right)$$

$\alpha$: positive

$x$: power

due to edge/surface effects

⑲

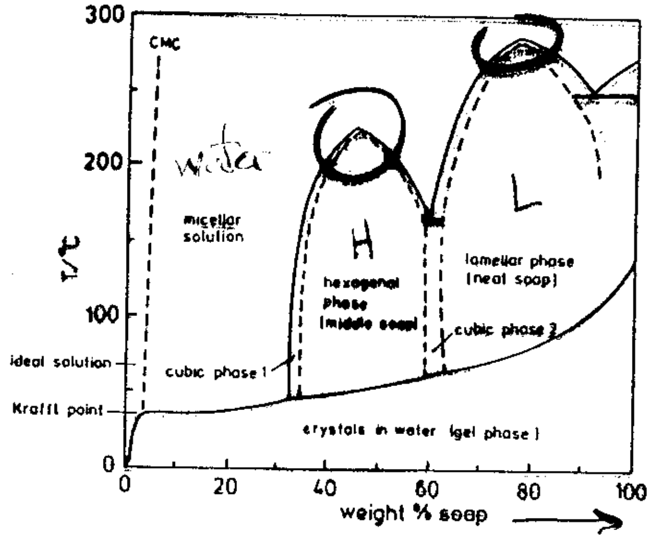
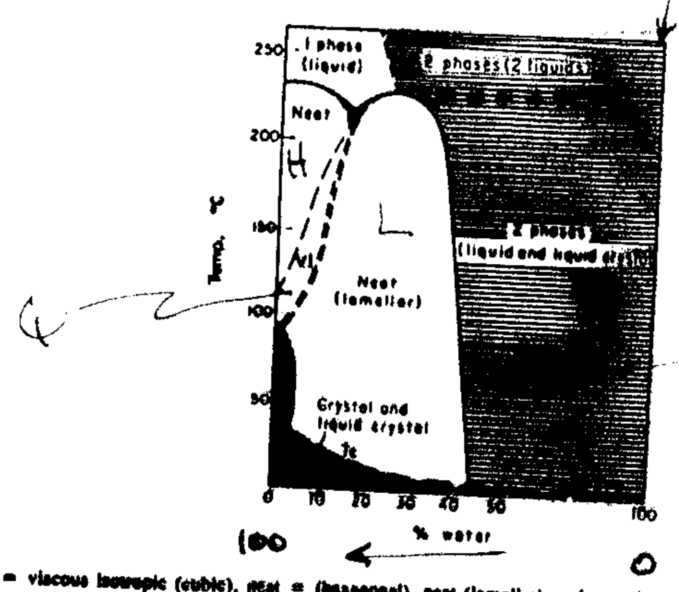


Fig. 4.6. Simplified phase diagram of a typical soap-water system

Vertogen & de Jeu
Thermotropic Liquid Crystals, p. 59

**Egg Phosphatidylcholine**

CMC structure



Marsh
Handbook of Lipid Bilayers, p. 125

W' + L

Note inversion of axis

V.I. = viscous isotropic (cubic), neat = (hexagonal), neat (lamellar) = $L_\alpha$, and I phase (liquid) = isotropic. (From Small, D. M., *J. Lipid Res.*, 8, 551, 1967. With permission.)

**PLUS**

disordered versions of The above
e.g., hex → spaghetti
lamellar cubic → sponge

inverted versions of The above
e.g., inverted hex (water inside)

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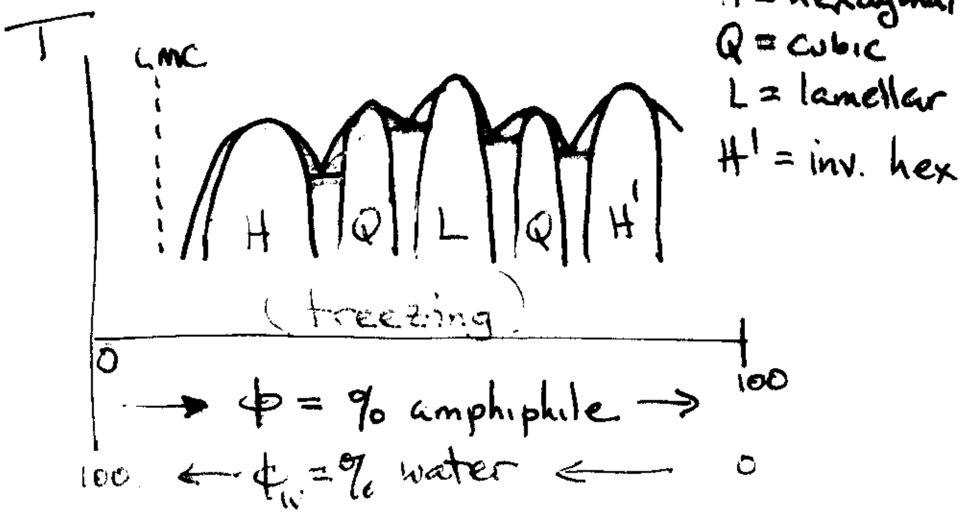
## A.2. Phase diagrams

Generic phase diagram



- CMC = "critical micelle concentration"
  where do micelles first form?
  (not a sharp line)
  $10^{-10}$ M for phospholipids.
- Usually first-order coexistence betw. phases
- Bulk amphiphile may be lamellar (so sequence is cut off)
- Phase separation of water-rich / amphiphile-rich regions
  may be large
- Known poorly at best (active area)

# A.1 Amphiphile-water structures

This is generic.
Phospholipid phase diagrams remain incompletely kno

**single molecules**

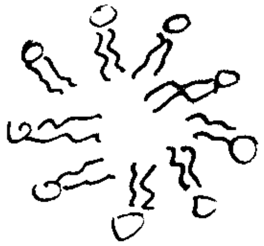
- only at low concentration ($10^{-10}$ M)

**small-n complexes (?)**

- anything which shields hydrocarbons!

**micelles**

(chain packing)

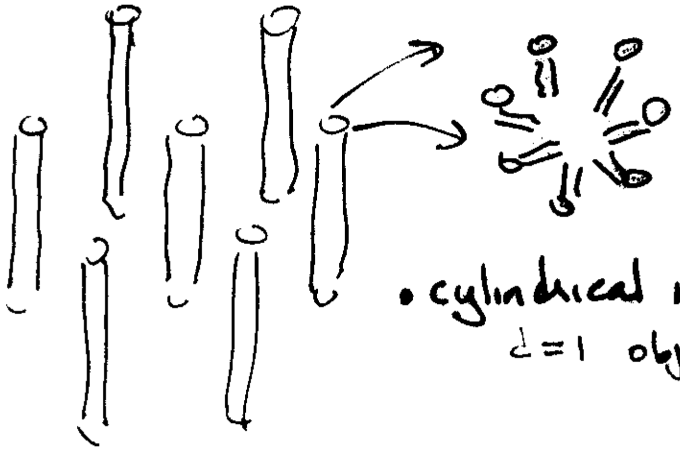


- may or may not be spheres
- relative size of head/tail
- new length scale!

**micellar colloidal**

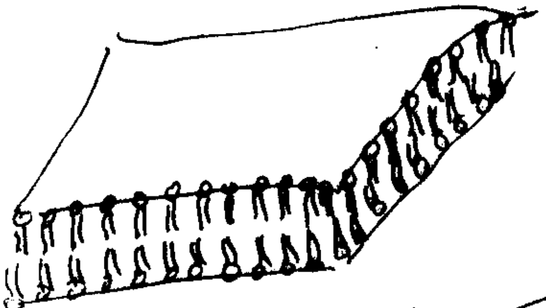
- micellar crystals
- micellar liquid crystals

**hexagonal**



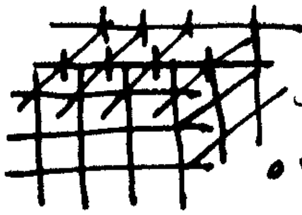
- cylindrical micelles
  d=1 objects!

**lamellar**



d=2 objects

**cubic (bilayer)**



→ hex or lamellar

d=3 crystalline structu

- plumber's nightmare

Aggregation Phenomena

- A. Phases + Phase Diagrams
- B. CMC, etc.
- C. Bilayer Phases + The "Main Transition"

Mesoscopic Shape-Energy Functional

- A. "The Helfrich Hamiltonian"
- B. deGennes - Taupin persistence Length. $\xi_p$
- C. Lamellar Phase Diagram.

Membrane Statistical Mechanics for $R \gg \xi_p$

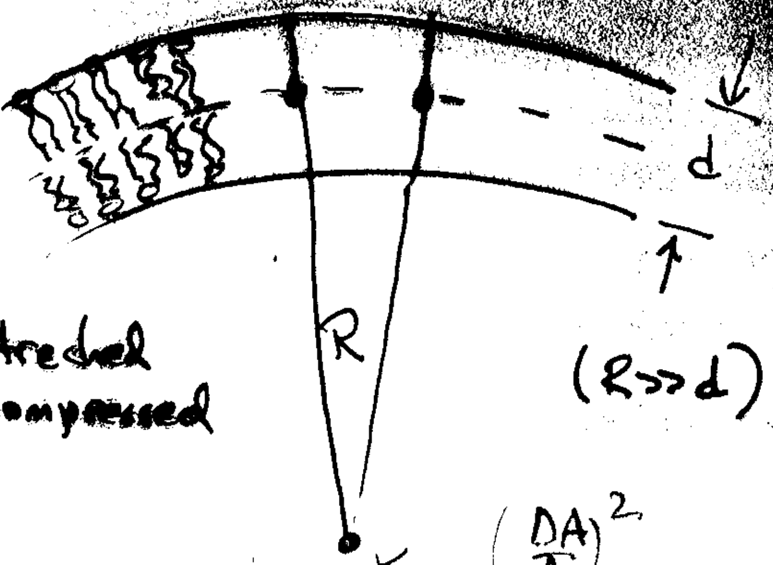
- A. Tethered Membranes
- B. Fluid Membranes

} energy/ entropy arguments.

---

# II. Shapes of micron scale fluid vesicles

# Bending modulus of bilayer $\kappa_b$



$(R \gg d)$

When membrane bends,

- outer leaf of bilayer stretched
- inner leaf of bilayer compressed

$$\text{bending energy per unit area} = \varepsilon_b = 2 \times \overset{\frac{1}{2}}{\frac{1}{2}} \overset{K_s}{\left(\frac{K}{2}\right)} \overset{\left(\frac{\Delta A}{A}\right)^2}{\left(\frac{d/2}{R}\right)^2}$$

$$= \frac{1}{2} \kappa_b \frac{1}{R^2}$$

$$\text{with} \quad \kappa_b = \frac{K d^2}{4} \sim \frac{10^2}{4} \left(40 \cdot 10^{-8}\right)^2 = 4 \times 10^{-12} \text{ ergs}$$

too large!
(where to measure area?)

$$\boxed{\kappa_b \sim 10^{-12} \text{ erg} \sim 25\, k_B T}$$ typical

Note: $\kappa_b$ is energy

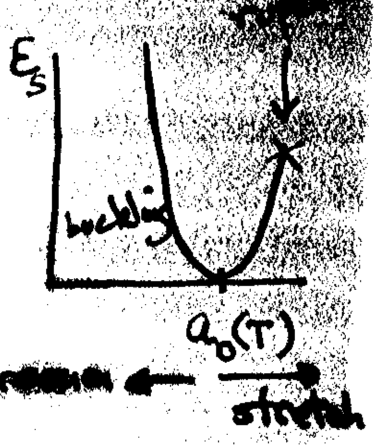
The fact that $\kappa_b \gg k_B T$ will make the vesicle-shape problem a <u>low-temperature</u> problem!

## Two more important estimates [illegible]

- Area compressiblity modulus of bilayer K

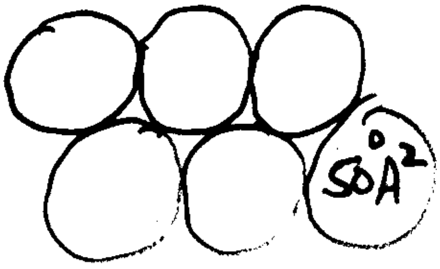
energy/area (relative to relaxed self-assembled state) $= \frac{1}{2} K \left(\frac{\Delta A}{A}\right)^2 = \varepsilon_s$

i.e. $\tau = K\left(\frac{\Delta A}{A}\right)$ (tension)



$a_0(T)$ = relaxed area/headgroup $\sim 50\,\text{Å}^2$

hypothesis: energy cost is due to hydrophobic exposure as head groups are pulled apart.



suppose pulling apart by 1Å produces one water mol. contact/head. <u>on each side</u> $(1\,\text{Å}^2)$

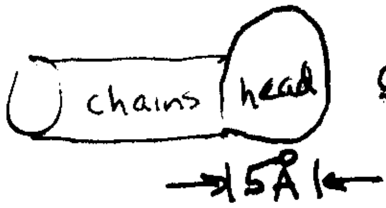
$$\frac{K}{2}\left(\frac{\Delta A}{A}\right)^2 = \frac{K}{2}\left(\frac{1}{7}\right)^2 = \frac{\overset{\swarrow}{2}\cdot\ \overset{\swarrow}{(0.07\,k_BT)}}{\underset{\nwarrow\ \text{area/head}}{50}}$$

$$\Rightarrow \boxed{K = 0.3\,k_BT/\text{Å}^2 = 10^2\ \text{erg/cm}^2}\ \text{typical}$$

Note: Fluid membrane rupture under tension typically occurs at $\Delta A/A \sim 1\text{-}2\%$ $\swarrow \left(\frac{1}{7}\right)^2$

Under tension $\tau_{\text{rupture}} = K\frac{\Delta A}{A} = 10^2 \cdot 10^{-2} \sim 1$

- Note that, once bilayer is formed, it is very difficult (unless there are edges/pores) to swith a phospholipid molecule from one side to the other:



$50\,\mathring{A}^2$ $\quad A \sim 175\,\mathring{A}^2$

$\Rightarrow \Delta E = 12\, k_BT$

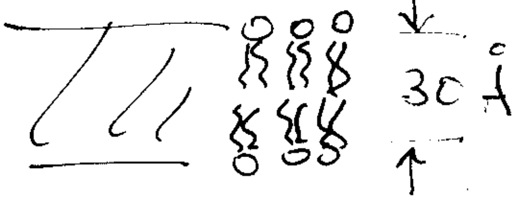
- By contrast the energy cost of pulling a single water molecule into the bilayer interior is roughly.

Covalent C– plus H–

$$4\pi(1\,\mathring{A})^2 \sim 12\,\mathring{A}^2 \Rightarrow \sim k_BT$$

so water passes readily through membr.

- Edges of bilayer cost hydrophobic energy:



$\Rightarrow$ EDGE ENERGY / length

$= 30(0.07) = 2\, k_BT/\mathring{A}$

$$\boxed{\lambda_{edge} = 8 \times 10^{-6}\ \text{erg/cm}}$$

This provides the "force" which acts to force bilayer sheets into closed ("vesicular") structures.

+ other topologies!

When water sits against another material,
This local order is reduced

$\Rightarrow$ less bonding

$\Rightarrow$ energy cost/unit area

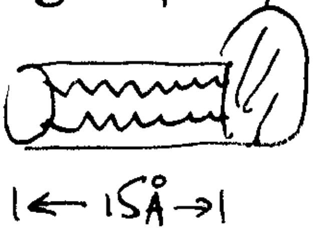
Note: Important entropic effects, too!

$\sigma_{\text{water/air}}$ (30°C) $= 70\ \text{erg/cm}^2 \sim 0.2\ k_BT/\text{Å}^2$

$$\boxed{\sigma_{\text{water/oil}} \sim 30\ \text{erg/cm}^2 \sim 0.07\ k_BT/\text{Å}^2}\ ?$$

---

- A single phospholipid molecule is <u>very insoluble</u>!



$\Rightarrow$ hydrophobic area/molecule $= 300\ \text{Å}^2$

$\Rightarrow$ energy cost $\sim \underline{20\ k_BT}$/molecule

i.e., expect factor $e^{-\Delta E/k_BT}$ is solubility

$\Rightarrow$ monomeric concentrations of order

$$e^{-20} \sim \boxed{10^{-10}\ \text{Molar}}\ /\ \text{typical phospholipid}$$

---

This forces phospholipids in water to aggregate in such a way as to sheild tails from hydrophobic exposure to water.

ONE way to do This is bilayer:

# Energetics of phospholipid self-assembly

! Spontaneous self-assembly is **NOT** driven by attraction between phospholipid molecules !

Energy units:

(physicist) (chemist)

$k_BT_{room} = 1.38\times10^{-16}(300) = 4\times10^{-14}\,\text{erg} = 4\times10^{-24}\,\text{kJ}$

so, $1\,\text{kJ/mole} = 1.04\times10^{-2}\,\text{eV/part} = 1.6\times10^{-14}\,\text{erg} = 0.4\,k_BT_{room}$

---

In these units:

covalent bonds: C–C 140 $k_BT$

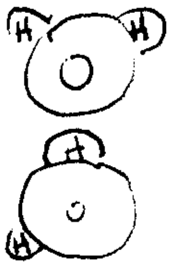
C=C 244 $k_BT$

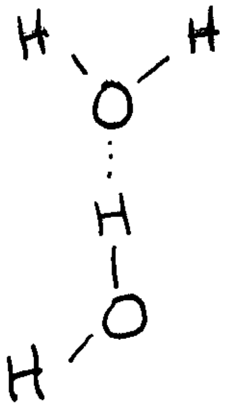
vdW bond: $\overset{|}{\underset{|}{CH_2}}$ $\overset{|}{\underset{|}{CH_2}}$ 3 $k_BT$

trans/gauche 5 $k_BT$

water–water }
water – polar head } hydrogen bond 8–11 $k_BT$

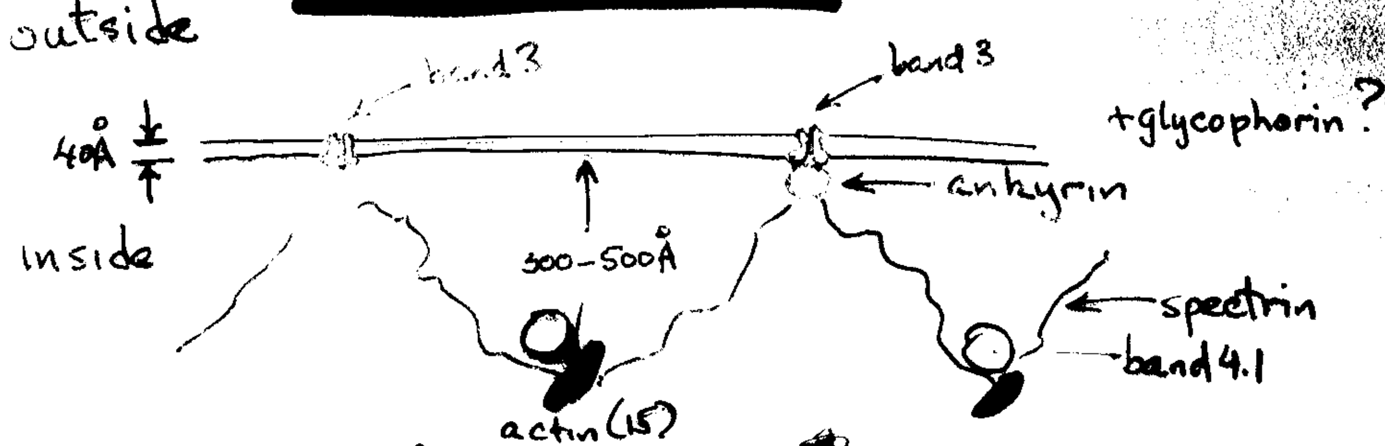
(but not water–hydrocarbon!)

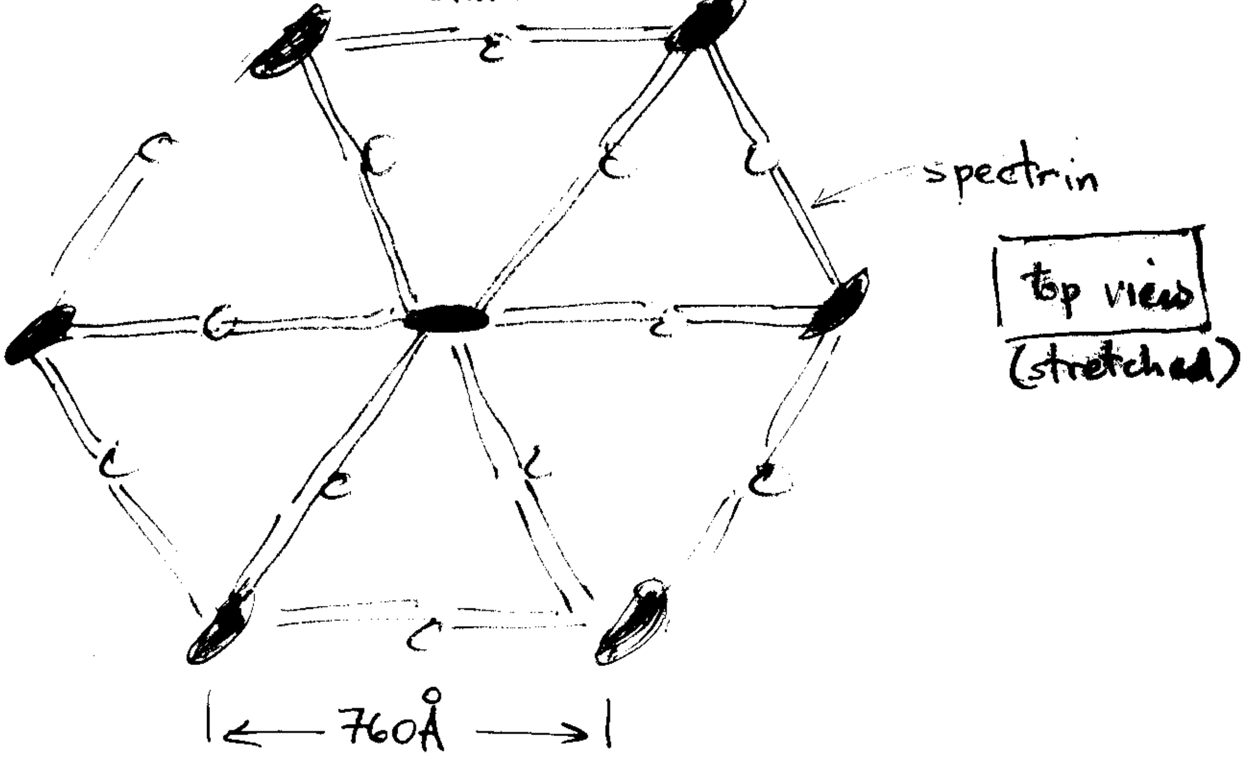




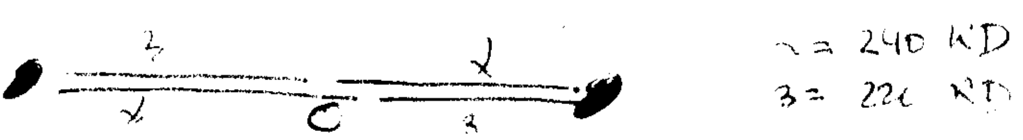
local order in liquid $H_2O$ maximizes these bonding contacts

# The Cytoskeleton (rbc only)





(2000Å contour length)



spectrin <u>tetramer</u> (twisted)

# CARTOON: Red blood cell membrane

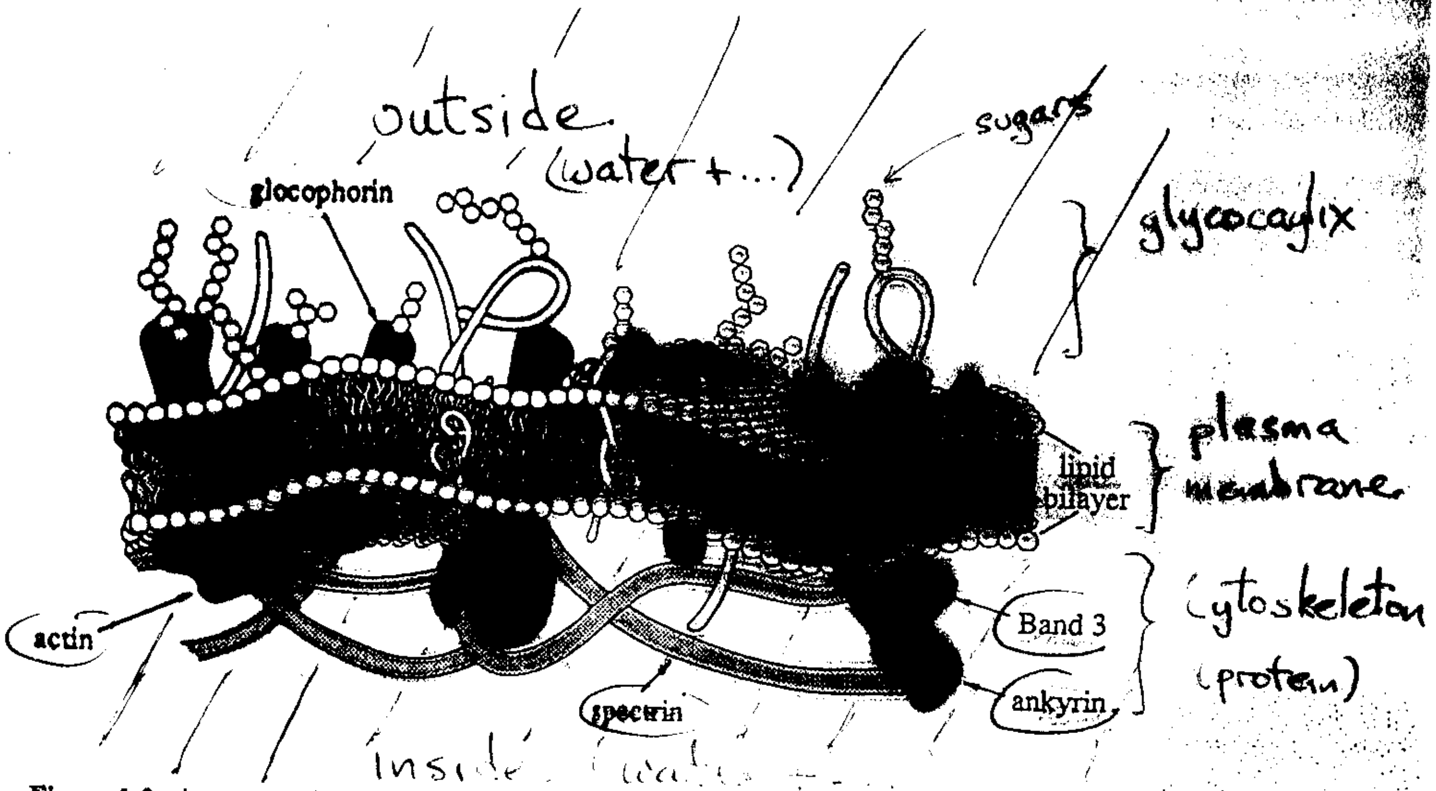


**Figure 1.2:** Anatomy of a red-cell membrane (Ref.[5]). The lipid bilayer is its main structural component. Band 3 and glycophorin are two types of intramembrane proteins. The cytoskeleton on the cytoplasmic (inner) side of the cell is formed primarily of linear spectrin molecules cross-linked via actin molecules. It is anchored to the lipid bilayer by its association with glycophorin via band 4.1 (protein), and with band 3 via ankyrin (protein).

- Composite material (fluid membrane + network)

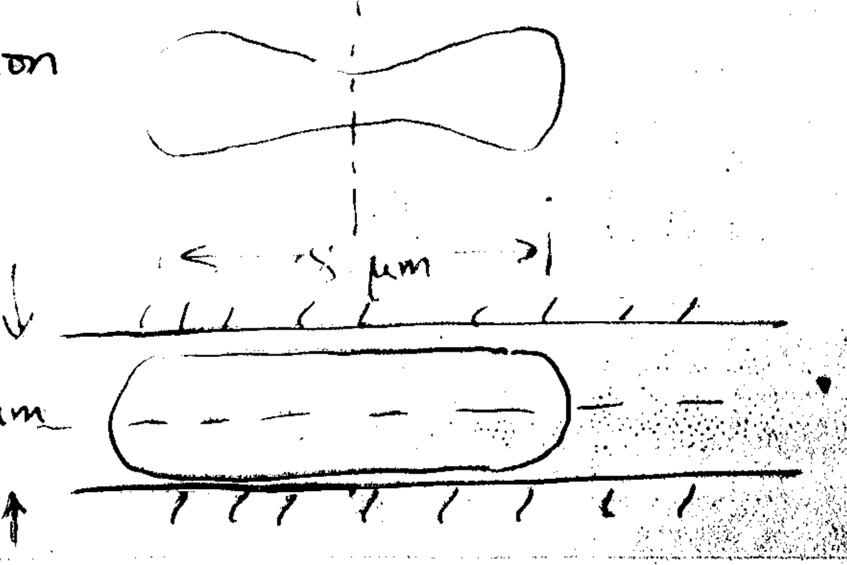
typical of nature

phospholipid bilayer soft fluid sealer (100 K)

protein network cross linked toughener (10$^6$K)

120k

- Shape / function



deforms to sausage to fit thru 2-3 μm capillaries.

Biological membranes are not Thus simple:

1. Lipid mixture: Up to $10^3$ types

   e.g. rbc membrane (human)

| PC | PE | PS | Sphinglipid | Cholesterol |
|---|---|---|---|---|
| 25% | 22% | 10% | 18% | 25% |

2. Additional Glycolipids, lipoproteins, etc

3. Cytoskeleton (protein) $\frac{\text{Lipid}}{\text{Protein}}(\text{wt}) = 0.75$

   spectrin
   Band 3
   glycophorin
   ...

   (often linked to cell interior)
   but not for rbc

4. Functional proteins in membrane (substrate)

   pumps
   adhesion molecules
   clathrins
   ....

   "BIOLOGY"

# DMPC BILAYER

Israelachvili, p 376

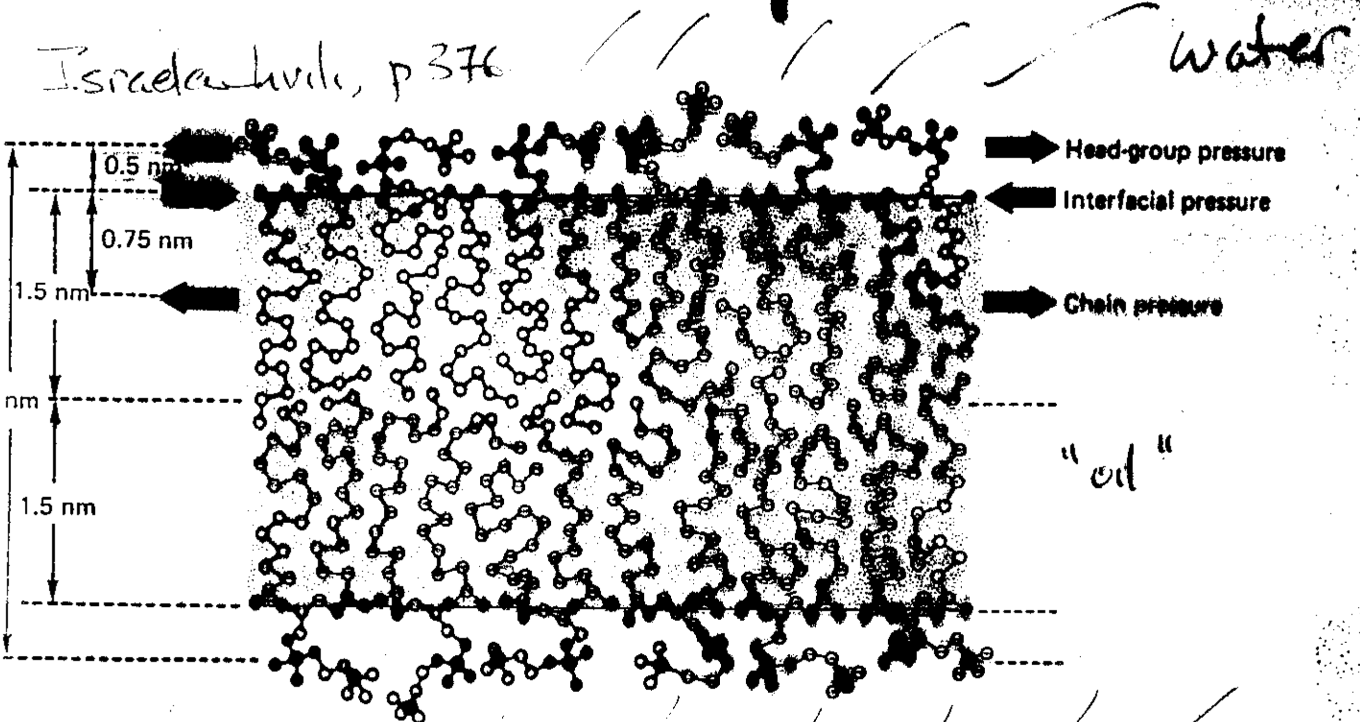


Fig. 17.4. Lecithin bilayer drawn to scale. The lipid bilayer is the basic structure of biological membranes, and most membrane lipids contain two hydrocarbon chains. The lipids diffuse rapidly in the plane of the bilayer, covering a distance of about 1 μm in 1 s. They also cross the bilayer from one side to the other ('flip-flop'), as well as exchange with lipids in the solution, but at much slower rates, of the order of hours (cf. the lifetime of single-chained surfactants in micelles of $10^{-5}$ to $10^{-3}$ s). (From Israelachvili *et al.*, 1980a.)

In fluid phase there are several gauche bonds/chain

In gel phase chains are all-trans.

# DMPC
# MOLECULE

Jennis, p. 38

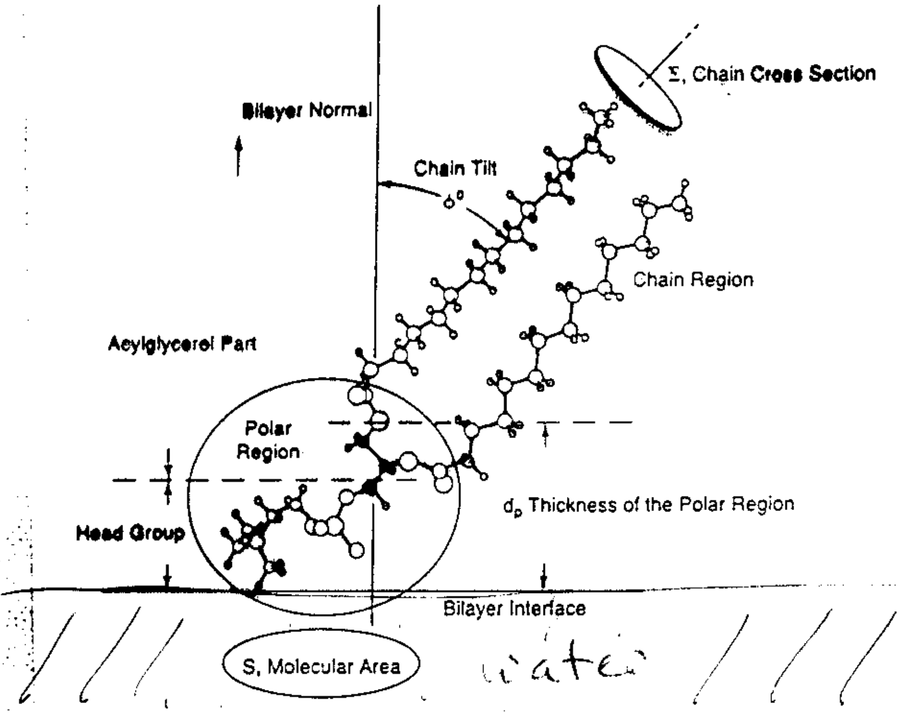
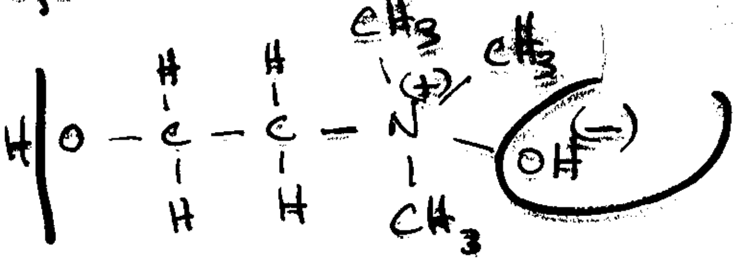


**Figure 2.2.** Schematic of a phosphatidylcholine molecule showing the structural notation defining various regions. Note that the cross-sectional area of the hydrocarbon chain, Σ, is taken perpendicular to the chain axis. The cross-sectional area parallel to the plane of the membrane will be, in this case, significantly larger due to the tilt of the chains. Adapted from ref. 604.

Chain Tilt: (DMPC)
$\phi = 0$ in fluid (LC, $L_\alpha$) phase (Sm A)
$\phi = 12^\circ$ in gel phase (Sm C)

Common headgroups:

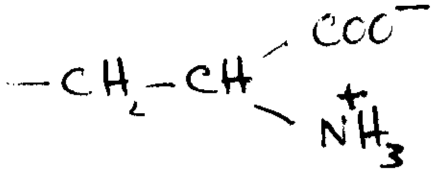
Choline



$\underline{\underline{C}}$holine ⇒ $-CH_2-CH_2-N^+(CH_3)_3$

$\underline{\underline{E}}$thanolamine $-CH_2-CH_2-\overset{+}{N}H_3$

$\underline{\underline{S}}$erine



$-CH_2-CH(COO^-)(\overset{+}{N}H_3)$

Examples: $\underline{\underline{P}}\underline{\underline{C}}$ = phosphatidylcholine (lecethins) e.g., egg yoke

SOPC 1-Stearoyl-2-Oleoyl-sn-Glycero-3-Phosphatidylcholine

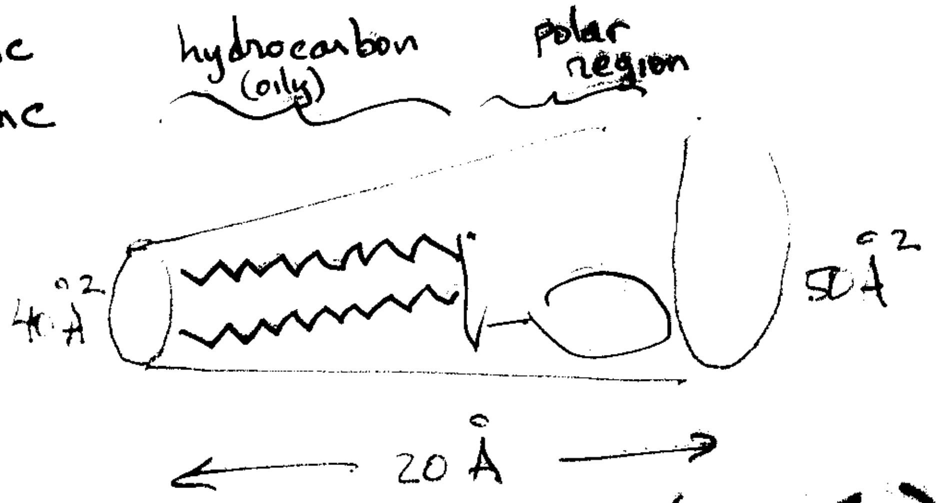
DMPC 1,2-Dimyristoyl-sn-Glycero-3-Phosphatidylcholine

PC zwitterionic

PE zwitterionic

PS cationic

Basic molecule dimensions



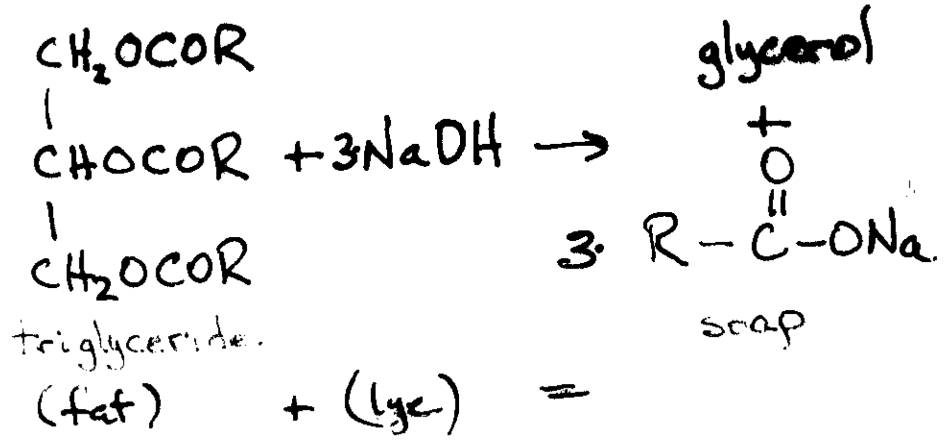
Emphasize importance for chain packing.

"AMPHIPHILIC" (amphipathic)

Each phospholipid molecule involves two fatty acids and a "head" group bonded via a glycerol.

$CH_2OH$
|
$CHOH$
|
$CH_2OH$
(glycerol)

## Aside: Relation to soaps

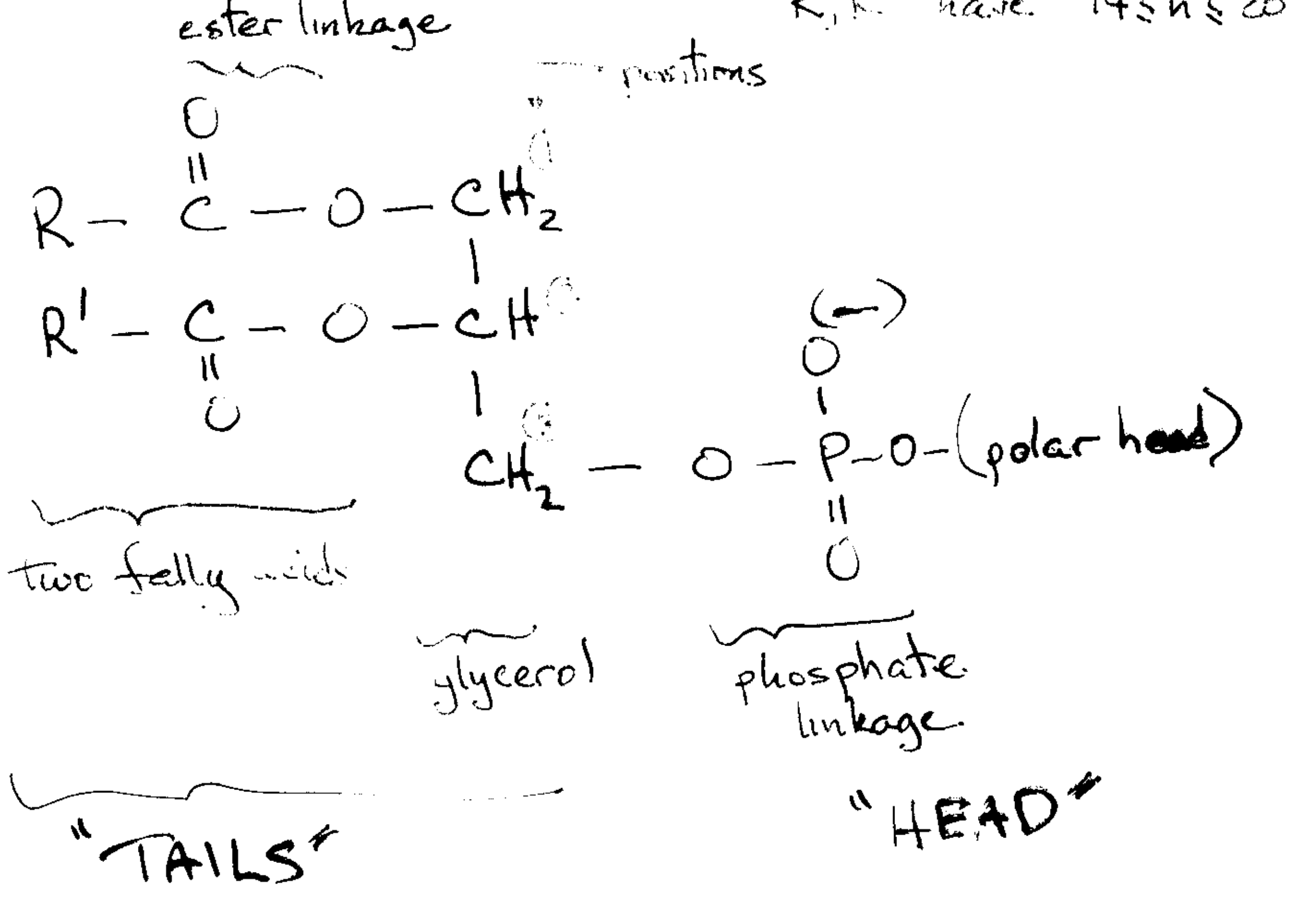


$$\begin{matrix} CH_2OCOR \\ | \\ CHOCOR \\ | \\ CH_2OCOR \end{matrix} + 3NaOH \rightarrow \text{glycerol} + 3\cdot R-\overset{\overset{\displaystyle O}{\|}}{C}-ONa$$

triglyceride (fat) + (lye) = soap

## Phospholipid Molecule

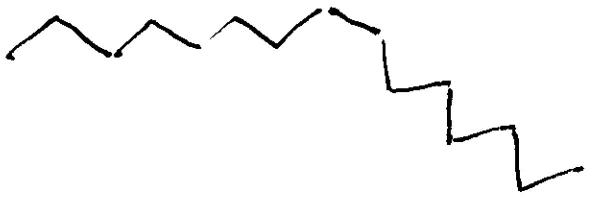
R, R' have 14 ≤ n ≤ 20



$$\begin{matrix} R-\overset{\overset{\displaystyle O}{\|}}{C}-O-CH_2 & & \\ | & & \\ R'-\underset{\underset{\displaystyle O}{\|}}{C}-O-CH & & \overset{\overset{\displaystyle (-)}{O}}{|} \\ | & & \\ CH_2 - O - & \underset{\underset{\displaystyle O}{\|}}{P} & -O-(\text{polar head}) \end{matrix}$$

Saturated chains are generally almost all-trans

A single cis double bond puts a "kink" in the direction of the chain.

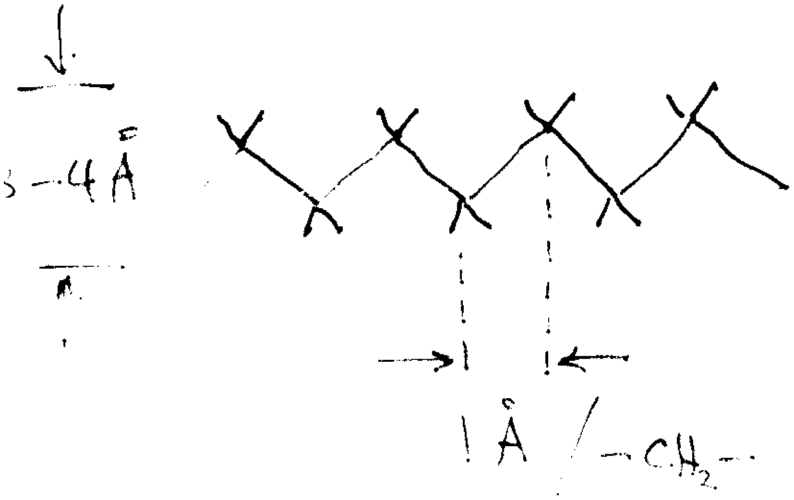


These kinks mean that chains "pack" less well.

⇒ (e.g.) decreased viscosity

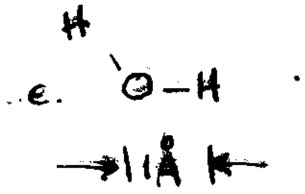
Nature uses this mechanism to adjust membrane fluidity (e.g., adjustment of SAE oil!)

Dimensions of basic saturated all-trans chain:



Covalent radii

| | |
|---|---|
| -H | 0.3 Å |
| -C | 0.77 Å |
| =C | 0.67 Å |
| -O | 0.66 Å |



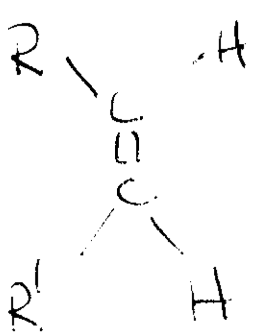
## mono-unsaturated

position of double bond counts, e.g.,

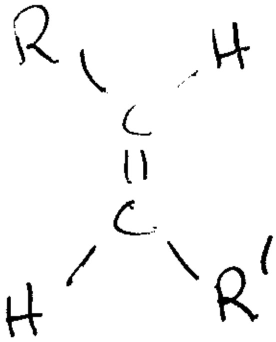
| | | |
|---|---|---|
| $n = 18$ | $CH_3(CH_2)_{10}CH = CH(CH_2)_4COOH$ | petroselenic<br>(6-cis) |
| | $CH_3(CH_2)_7CH = CH(CH_2)_7COOH$ | oleic<br>(9-cis) |
| | $CH_3(CH_2)_5CH = CH(CH_2)_9COOH$ | vaccenic<br>(11- cis or trans) |

Numbering counts carbons from acid end.

Note: The double bond does NOT rotate!

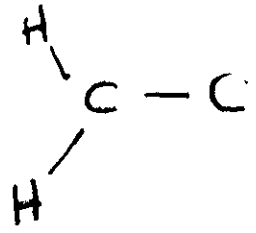


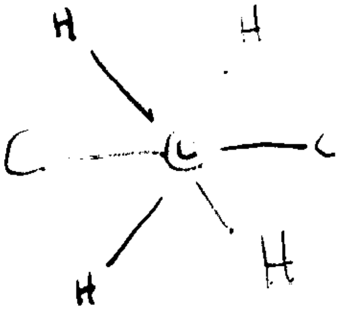
cis



trans

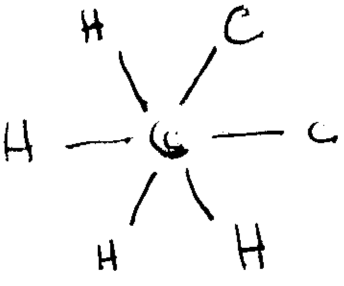
But saturated single bond can rotate:





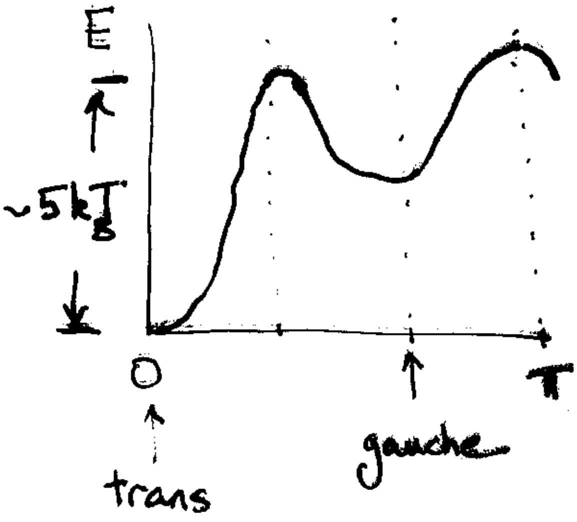
trans

$(\theta = 0)$



gauche

$(\theta = 120°)$



# Chemistry

Note: lipids are more complicated than Ar or Al, so you need to know something about them before we get started!

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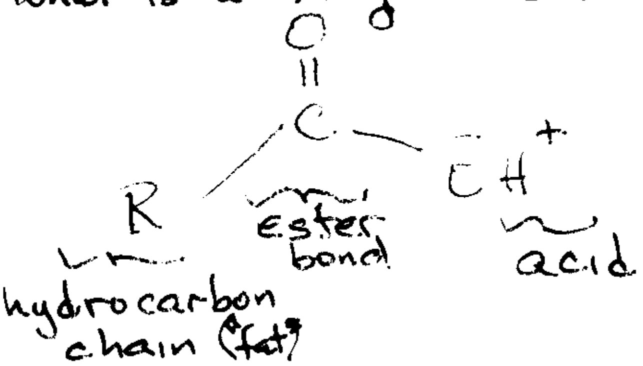
What is a phospholipid?

Chemist: lipid = ester-bonded fatty acid.

What is a fatty acid?



- R chain usually saturated or nearly saturated.
- n = total # carbon atoms

## saturated

n = 1 $H\cdot COOH$ "formic acid" (ants?)

n = 2. $CH_3COOH$ "acetic" (vinegar?)

⋮

n = 12 $CH_3(CH_2)_{10}COOH$ lauric (laurel)

n = 14 $CH_3(CH_2)_{12}COOH$ myristic (nutmeg)

n = 16 $CH_3(CH_2)_{14}COOH$ palmitic (palm oil)

n = 18 $CH_3(CH_2)_{16}COOH$ stearic (animal fat)

n = 20 $CH_3(CH_2)_{18}COOH$ arachidic

**odd-n chains exist (uncommon in natural lipids)**

# human erythrocytes

Figure 6–22 A scanning electron micrograph of human red blood cells. The cells have a biconcave shape and lack nuclei. Courtesy of Bernadette Chailley.

from B. Alberts et al.
Molecular Biology of the Cell

"biconcave disc"
(normally!)

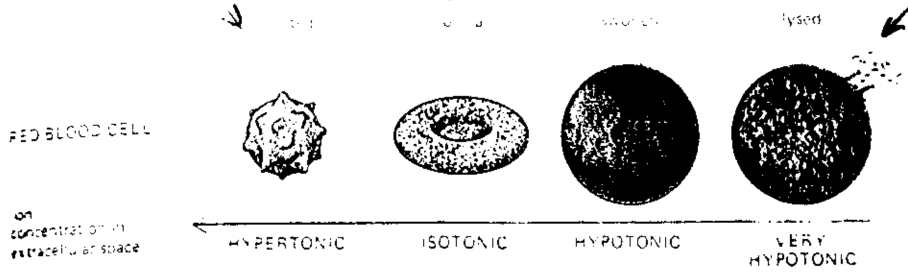
Figure 17–20 Scanning electron micrograph of mammalian blood cells in a small blood vessel. The larger, more spherical cells with a rough surface are white blood cells; the smaller, smoother, flattened cells are red blood cells. (From R.G. Kessel and R.H. Kardon, Tissues and Organs: A Text-Atlas of Scanning Electron Microscopy. San Francisco: Freeman, 1979. © 1979 W.H. Freeman and Company.)

deflated

normal

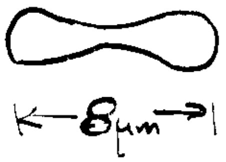
swollen

"lysis"

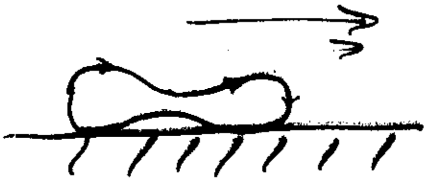


Osmotic Effects

well defined shape
very flexible



what causes this shape?
It is NOT a precast mold!



"tank motion"

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