EPR Course
NTU
Fall 2001
Chan

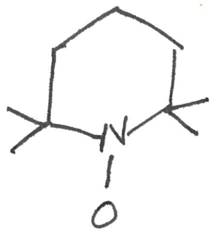
Lecture 12
January 9, 2002

ESR spin labels

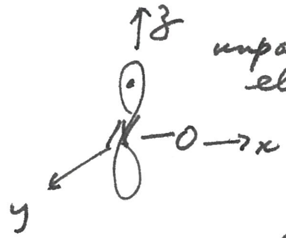
- Stable free radicals based on nitroxide
- Stable over time for weeks, even months; resistant to oxidation.
- Can therefore be followed by EPR even at room temperature; i.e., an excellent reporter group
- Have been used as a mobility indicator or motility indicator
  fluidity indicator
  solvent polarity indicator
- Have been used to follow phase separation in bilayers and biological membranes
  to follow conformational changes in proteins and nucleic acids
  to follow transmembrane phospholipid flip-flop
  to follow transmembrane potentials
  to follow lateral diffusion of phospholipids in membranes
- Applications first popularized by Harden McConnell of Stanford ~1968 – 1980
- Spin-spin interaction between spin labels can be used as a "yardstick"

Most popular spin label

TEMPO ≡ 2,2,6,6-tetramethylpiperidine 1-oxyl



g_{xx}	2.00880
g_{yy}	2.00580
g_{zz}	2.00210



unpaired electron in $2p_z$ orbital of nitrogen

$A^{^{14}N}_{xx}$ = 5.90 gauss

$A^{^{14}N}_{yy}$ = 5.40 gauss

$A^{^{14}N}_{zz}$ = 32.9 gauss

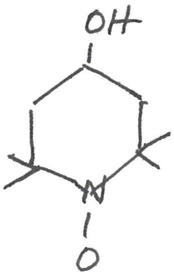
Proton hyperfine

in CCl_4

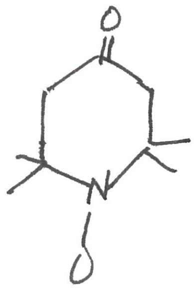
−0.23	(12 H)	CH_3's
−0.39	(4H)	3-CH_2
+0.18	(2H)	4-CH_2

small

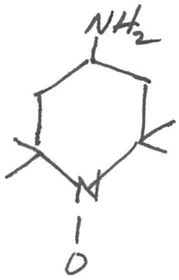
Other popular ones



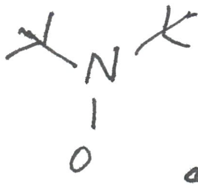
TEMPOL



TEMPONE



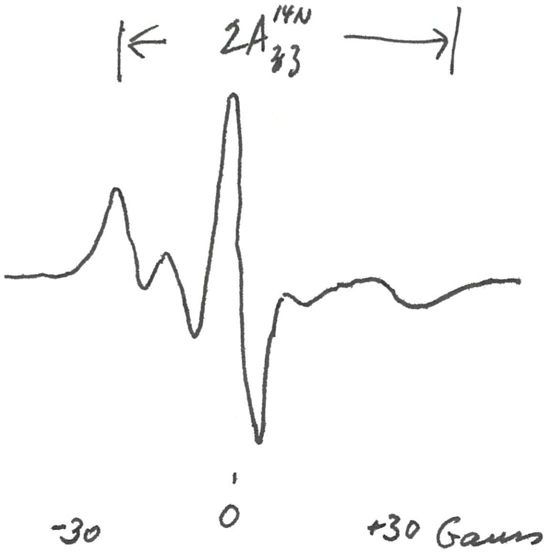
TEMPAMINE



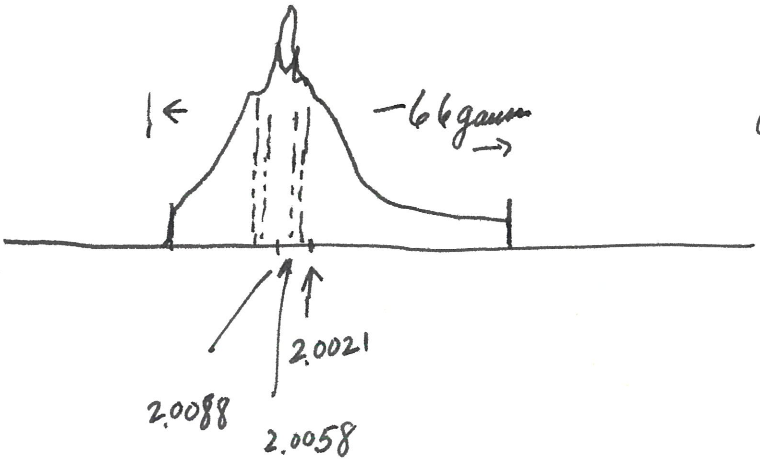
DTBN

ditert-butyl nitroxide

Frozen EPR powder



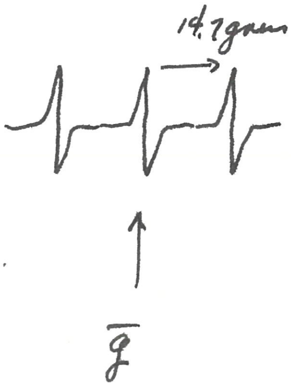
Derivative



Absorption

Solution

$m_I = 0, \pm 1$



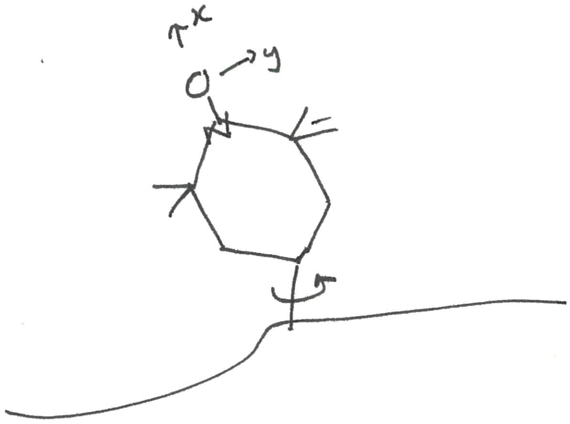
$$\bar{g} = \frac{1}{3}(g_{xx} + g_{yy} + g_{zz})$$

$$\cong 2.0056$$

$$\bar{A} = a = \frac{1}{3}(5.90 + 5.40 + 32.9)$$

$$= 14.7 \text{ Gauss}$$

Anisotropic Motion



Can be simple as well as complex

Motion restricted as well as anisotropic

Restricted : Not all solid angles covered by motion
Motion within restricted range could be fast or slow, & anisotropic

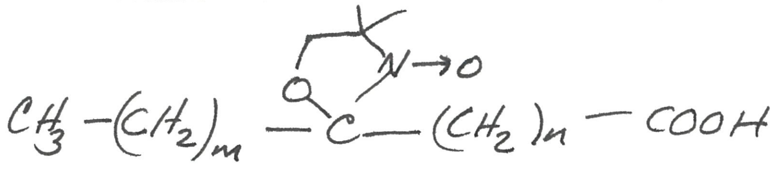
Anisotropic : Motion unrestricted, but rates of rotation about x, y, z occur at very different rates
i.e., defined by 3 different rotational diffusion coefficients

In practice, motions are complex, but different motions yield different EPR spectra. Computer programs exist to simulate EPR spectra for different kinds of motions to fit experimental spectrum

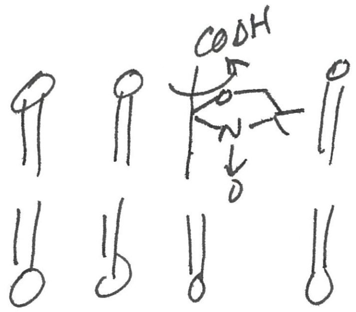
Occasionally, motions are relatively simple.

For example

a) Spin-labeled lipid chain in bilayer membrane



fatty acid spin label



spin-label fatty acid rotates rapidly about long-axis

As a result of this "axial" rotation,

x doesn't change

but g_{yy} and g_{zz} become averaged $\begin{vmatrix} 2.0058 \\ 2.0021 \end{vmatrix} \rightarrow 2.0039$

A_{yy} and A_{zz} become averaged $\begin{vmatrix} 5.40 \\ 32.9 \end{vmatrix} \rightarrow 19.1$ gauss

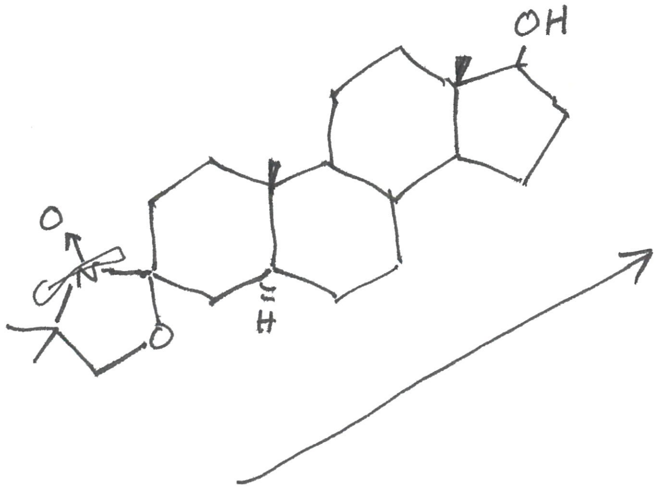
b) Spin-labeled steroid

Powder spectrum with $A_{\parallel} = 5.90$ gauss

$g_{\parallel} = 2.0088$

$A_{\perp} = 19.1$ gauss

$g_{\perp} = 2.0039$



bilayer normal

Rotation of steroid along long-axis leaves g_{zz} unchanged

& g_{xx} and g_{yy} become averaged   2.0073

A_{xx} and A_{yy} become averaged   5.65 gauss

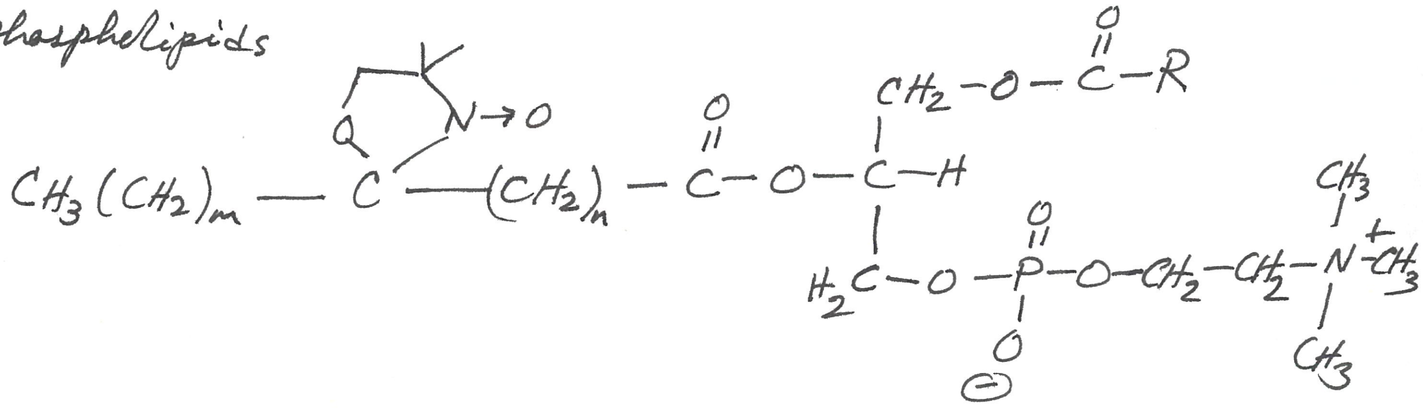
$\Rightarrow$ Powder spectrum with $A_{\parallel} = 32.9g$ $A_{\perp}$ 5.65 gauss

$g_{\parallel} = 2.0021$ $g_{\perp} = 2.0073$

Flexibility gradient in Bilayer Membrane

Wayne Hubbell & H. McConnell

Introduced nitroxide spin label into lipid chain of phospholipids



dyed in egg lecithin ; dipalmitoyllecithin

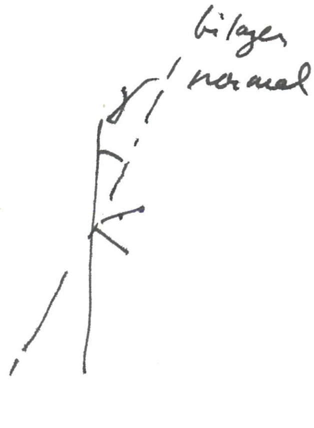
egg lecithin : Cholesterol

2 : 1

Vary m, n $\Rightarrow$ $A_{\parallel}$, $A_{\perp}$

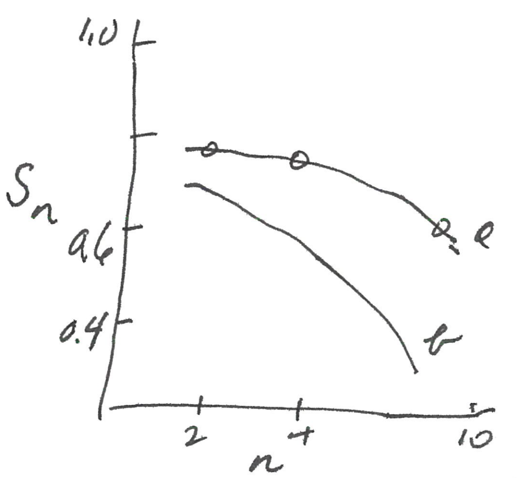
$g_{\parallel} - g_{\perp}$

Define Order parameter $\frac{1}{2}(3\cos^2\gamma - 1) = S_n$

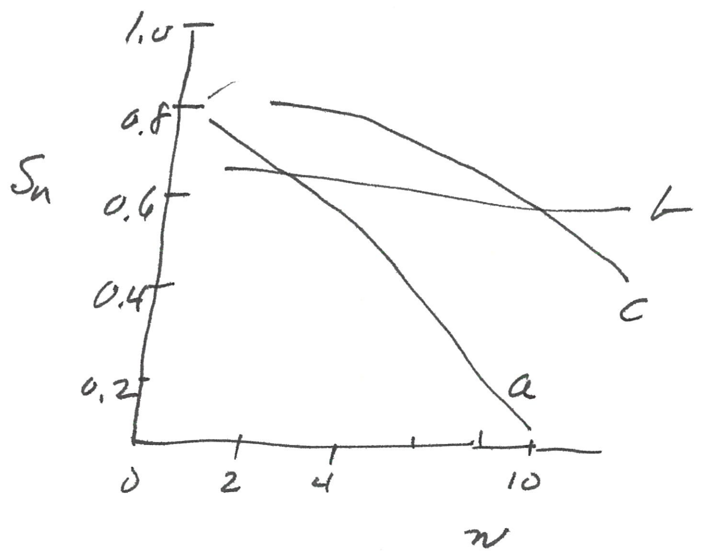


Can relate the order parameter at spin-label to probability of a gauche bond at C_n, C_{n-1}, ... + C_{n+2}, C_{n+3} etc.

Some data



Order parameter S_n as a function of n for phospholipid (m, n) in aqueous dispersions of DPL (a) 4°C below T_m; (b) 1°C above T_m ($T_m = 39°C$)



S_n for fatty acid spin label (m, n) in

(a) smectic liquid crystals of decanol-sodium decanoate

b) aqueous dispersion of egg lecithin : cholesterol (2:1 mole)

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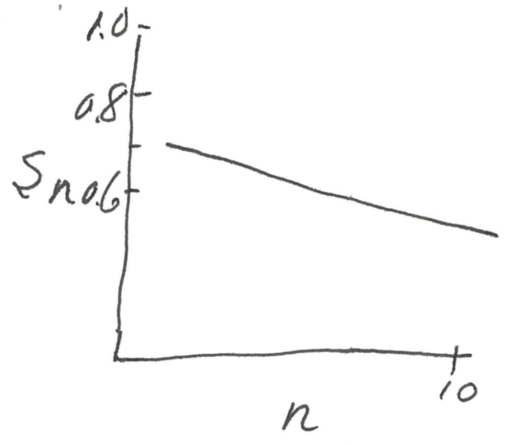
c) S_n for phospholipid spin label (m, n) in aqueous dispersion of egg lecithin : cholesterol (2:1)

Evidence for flexibility gradient of lipid chains in bilayer membrane



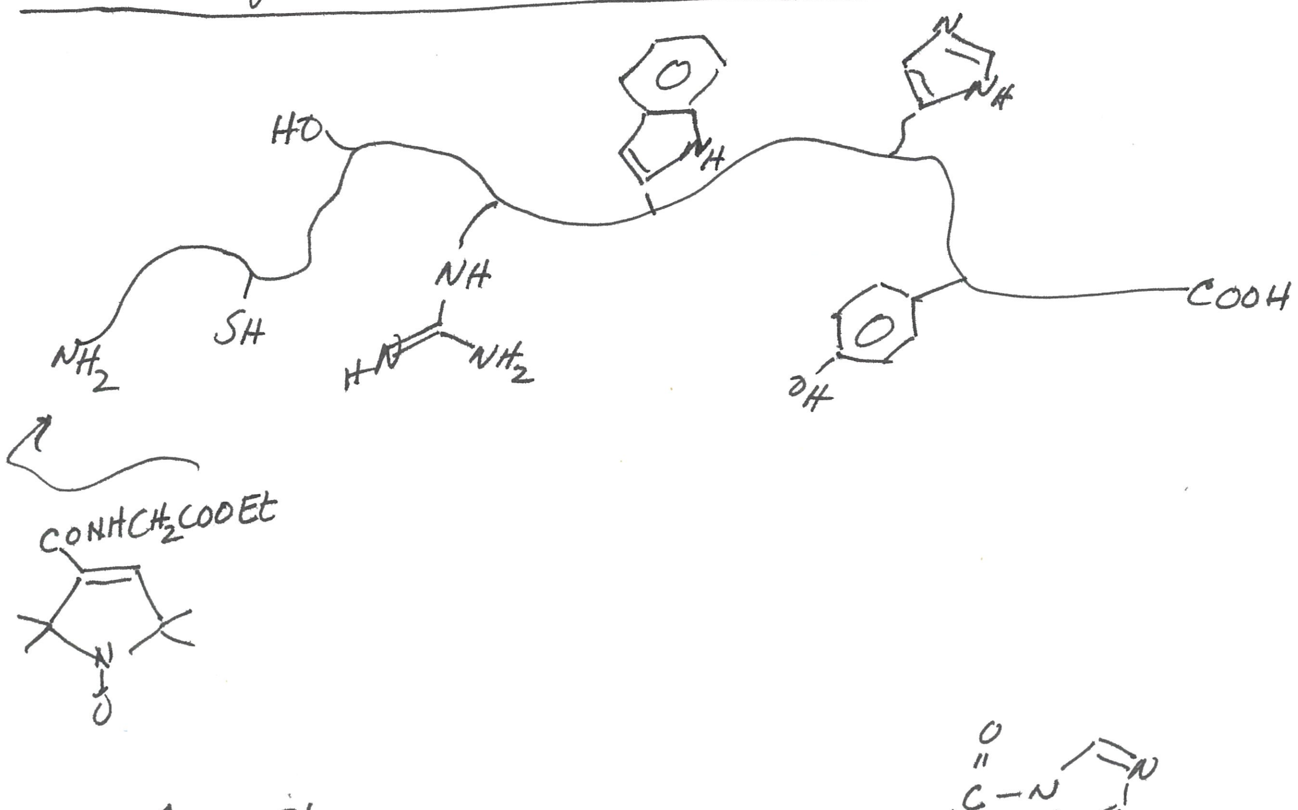
S_n for spin label fatty acid (m,n) in the walking leg nerve of Maine Lobster *Homarus americanus*

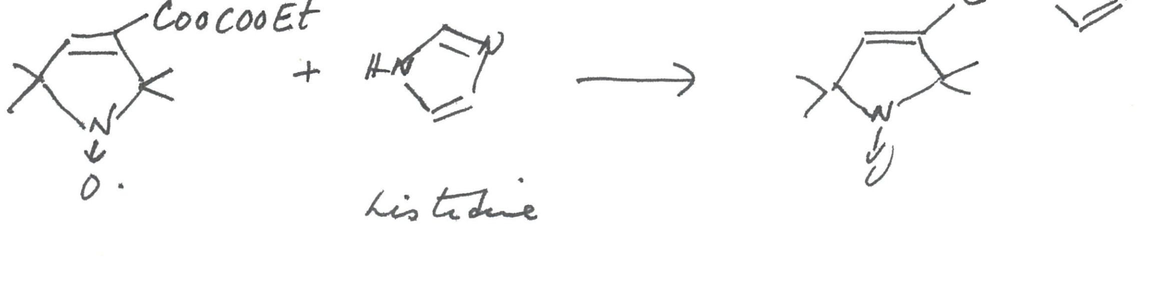
↑

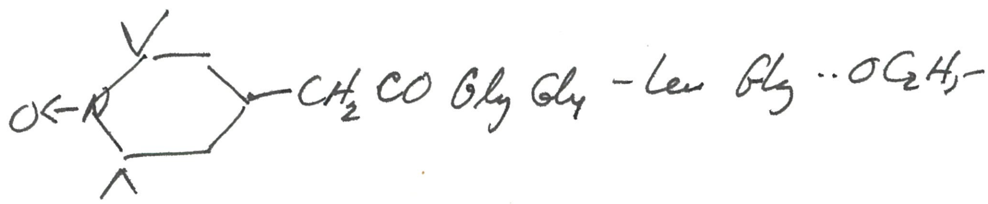
Evidence for fluid bilayer in biomembrane!

Spin label clearly an important reporter group for molecular motion!

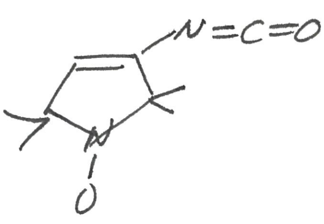
Spin labels for proteins and nucleic acids





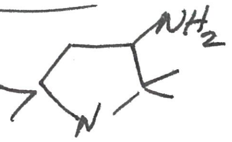


N-terminal →



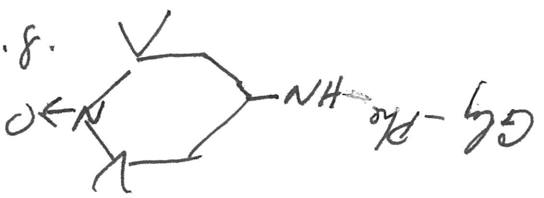
isocyanate

C-terminal

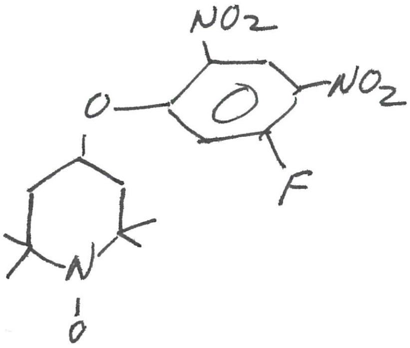


+ . . . .

e.g.

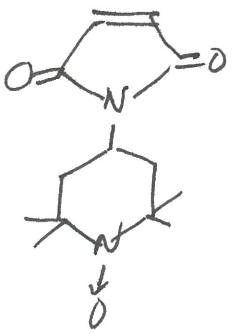


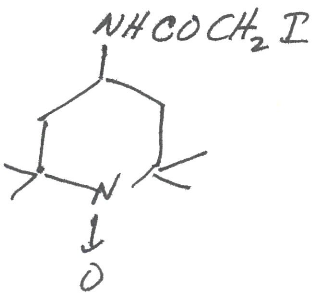
Lysine

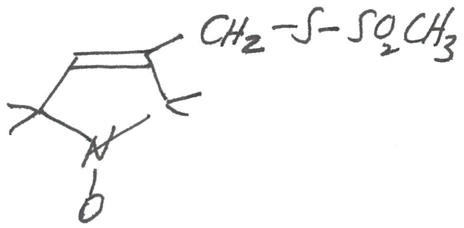


also thiol, imidazole nitrogen or phenolic OH of tyrosine

Thio (cysteine)







methane thiosulfonate

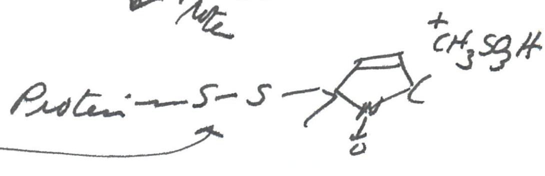
↓ P–SH

iodoacetamide

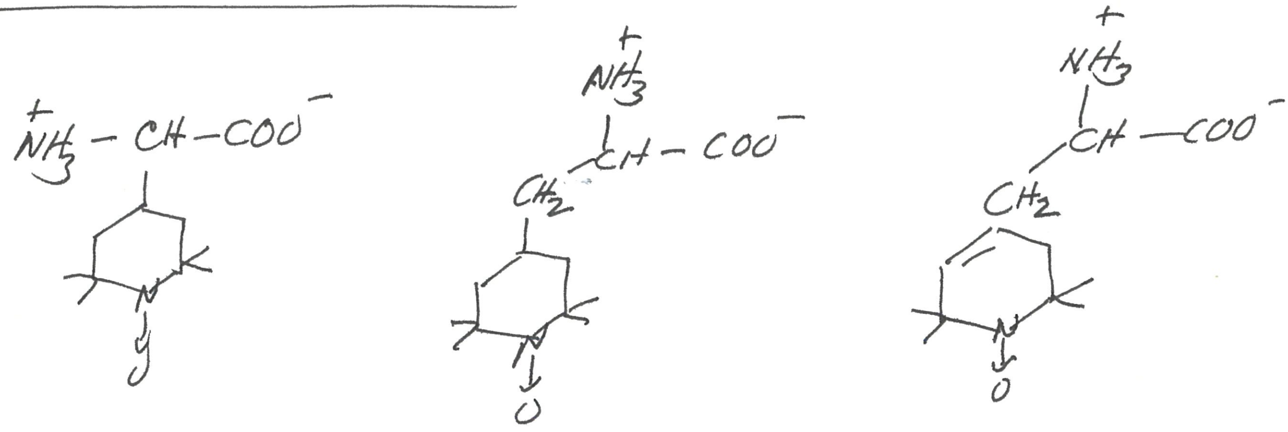
Maleimide

irreversible labels

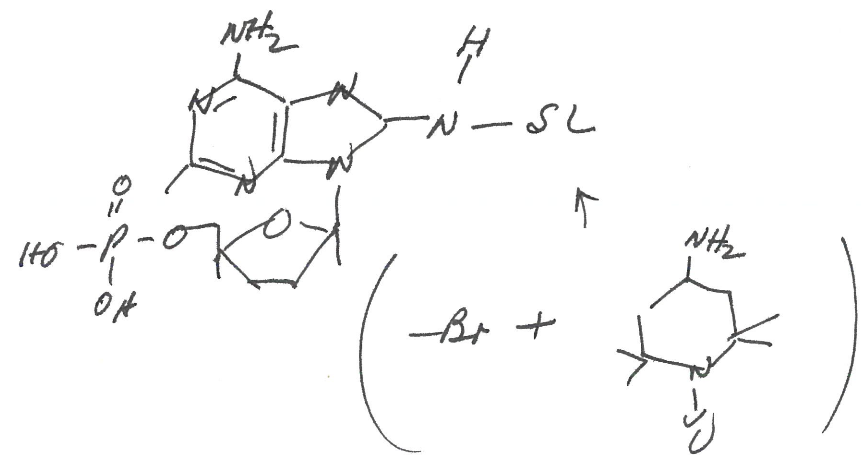
reversible



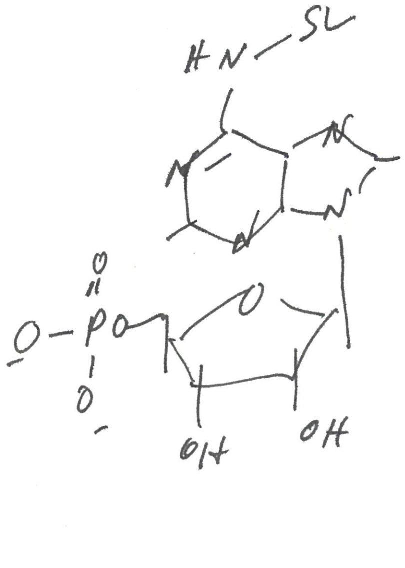
Spin-label amino acids



Nucleic Acids

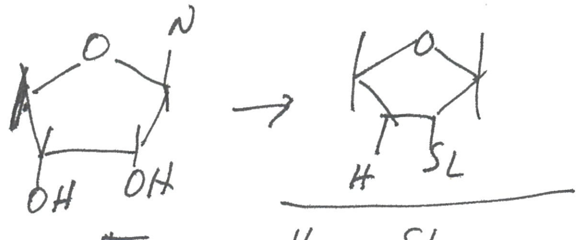


N-8 spin label



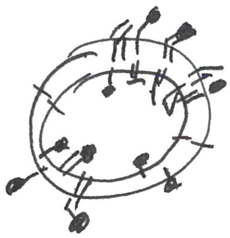
N-6 SL

also spin label



Other applications of spin labels

(1) Flip-flop rate of phospholipids in bilayers

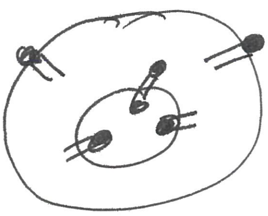


a) Incorporate spin-label PL into DPL vesicles.

b) reduce outside facing spin labels by ascorbate at 4°C

• SL

(c) Wait for transmembrane flip-flop of spin label

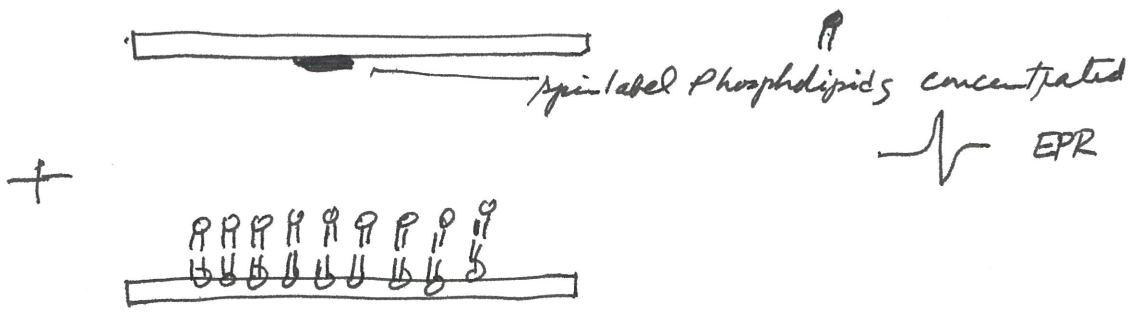


d) reduce outside-facing spin labels again

e) From decrease in spin label EPR intensity deduce flip-flop rate.

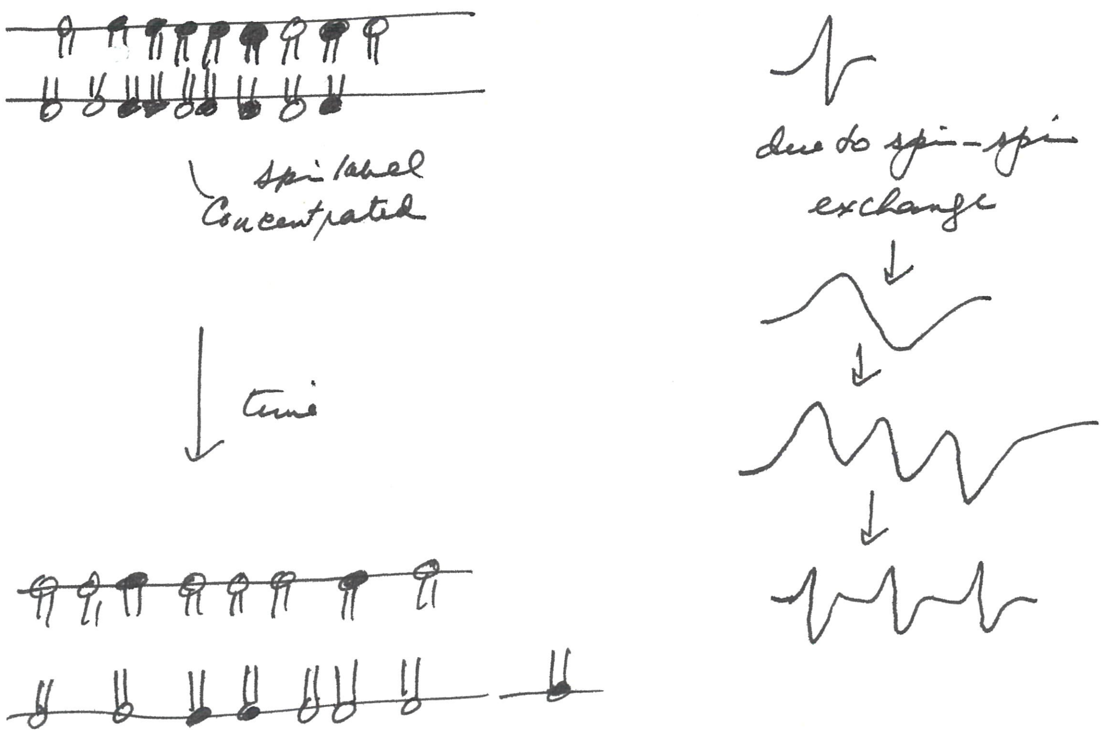
$t_{1/2} \sim 6\frac{1}{2}$ hours (McConnell)

(2) Lateral Diffusion



↓ Press

allow spinlabel phospholipids to diffuse laterally



$D \sim 10^{-8}\ cm^2/sec$

Note
$x^2 + y^2 = r^2 = 4Dt$

or 10^{-4} cm in 1 sec. **Fast** a micron in a sec!

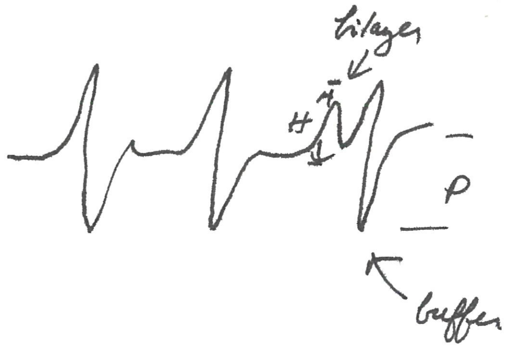
(3) Lateral phase separation in binary mixtures of lipids, e.g. DMPC + DSPC

DMPC – cholesterol

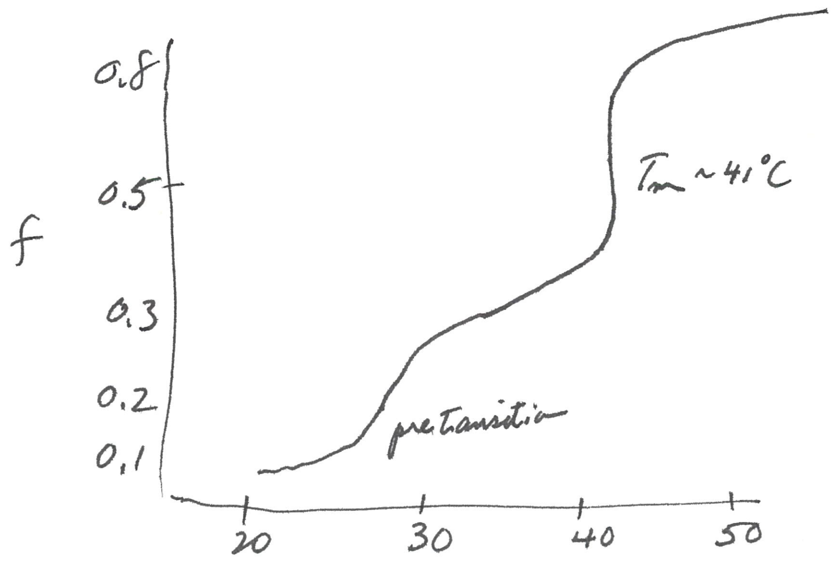
(a) Exploits different fluidity and spectrum in bilayer + H_2O (buffer)

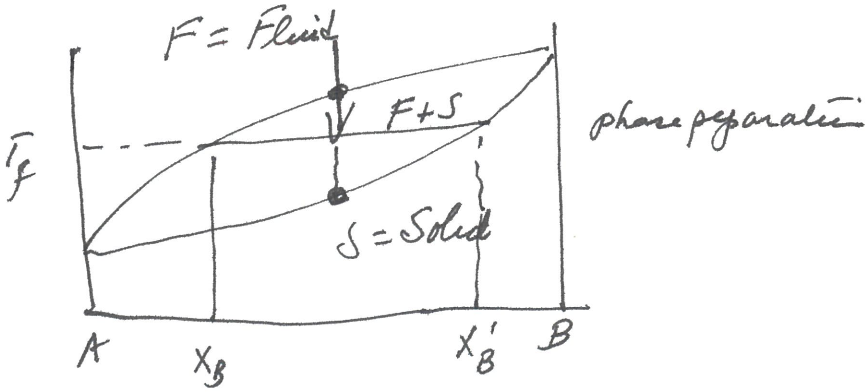
(b) different solubility of TEMPO in fluid + solid phases

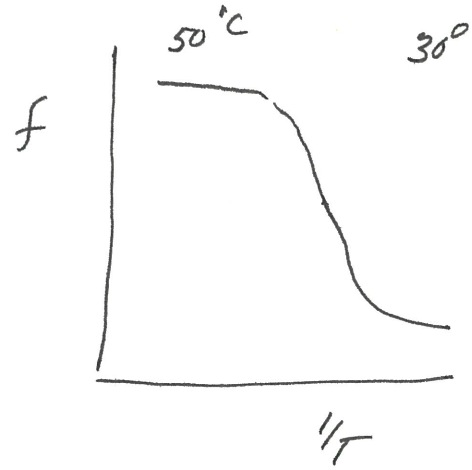
TEMPO solubility parameter in DPPL as a function of T

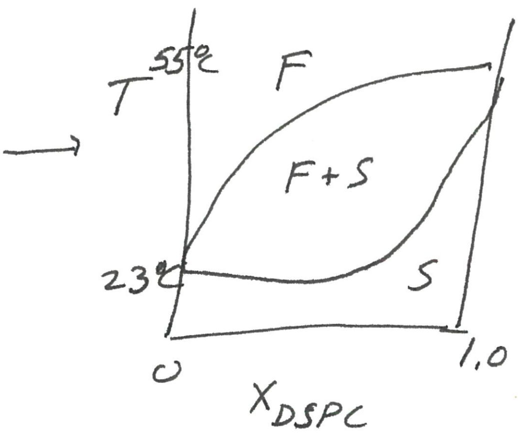


$$f = \frac{H}{H+P}$$



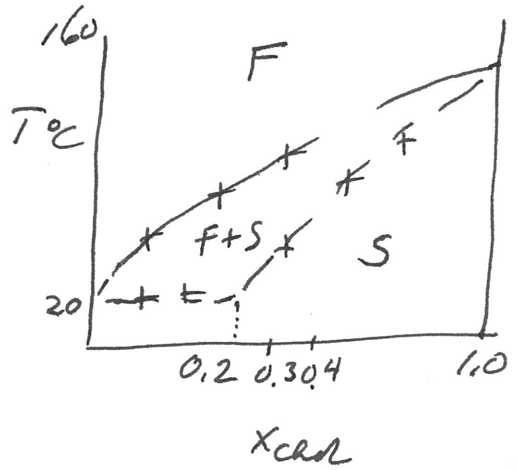






DMPC/DSPC

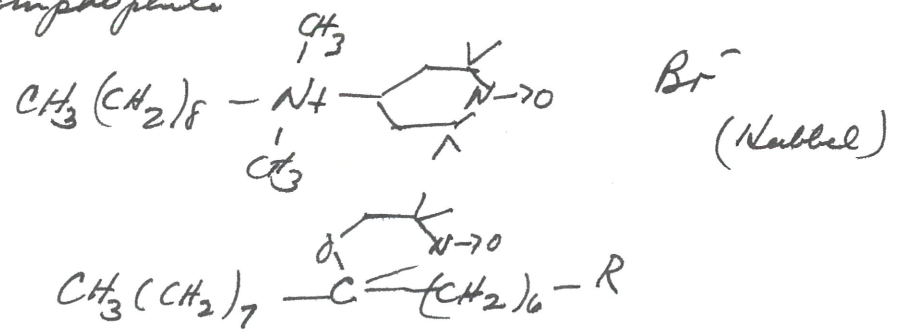
DMPC – Cholesterol



(4) Surface Potentials

PC-membrane
+ PA (phosphatidic acid)
⇒ vary σ

study distribution of spin labeled amphiphile



$R = CH_2 \overset{+}{N}(CH_3)_3$

COO^-

$$\mu_{vesicle} = \mu^{\circ}_{vesicle} + RT \ln X_{ves}\gamma_{ves} + zF\psi_{vesicle}$$

$$\mu_{aq} = \mu^{\circ}_{aq} + RT \ln X_{aq}\gamma_{aq}$$

At equilibrium, $\mu_{vesicle} = \mu_{aq}$

and $$-\frac{zF\psi_s}{RT} = \ln \frac{X_{ves}\gamma_{ves}}{X_{aq}\gamma_{aq}} + \frac{\mu^{\circ}_{ves} - \mu^{\circ}_{aq}}{RT}$$

where $\mu^{\circ}_{ves} - \mu^{\circ}_{aq}$ = free energy of transfer of a mole of spin-label from aqueous solution to vesicle phase

$$= -RT \ln K_o$$

K_o = phase partition coefficient of spin label in absence of surface potential

So, from above

$$K = K_o e^{-zF\psi_s/RT}$$

or $$\psi_s = -\frac{RT}{zF} \ln \frac{K}{K_o}$$

measure K as a function of σ

Guoy-Clapman

$$\psi_s \cong \psi_G = \frac{2kT}{e} \sinh^{-1} \frac{\sigma}{C^{1/2}} \left(\frac{500\pi}{DRT}\right)^{1/2}$$

(D = dielectric constant)