The Skin Barrier

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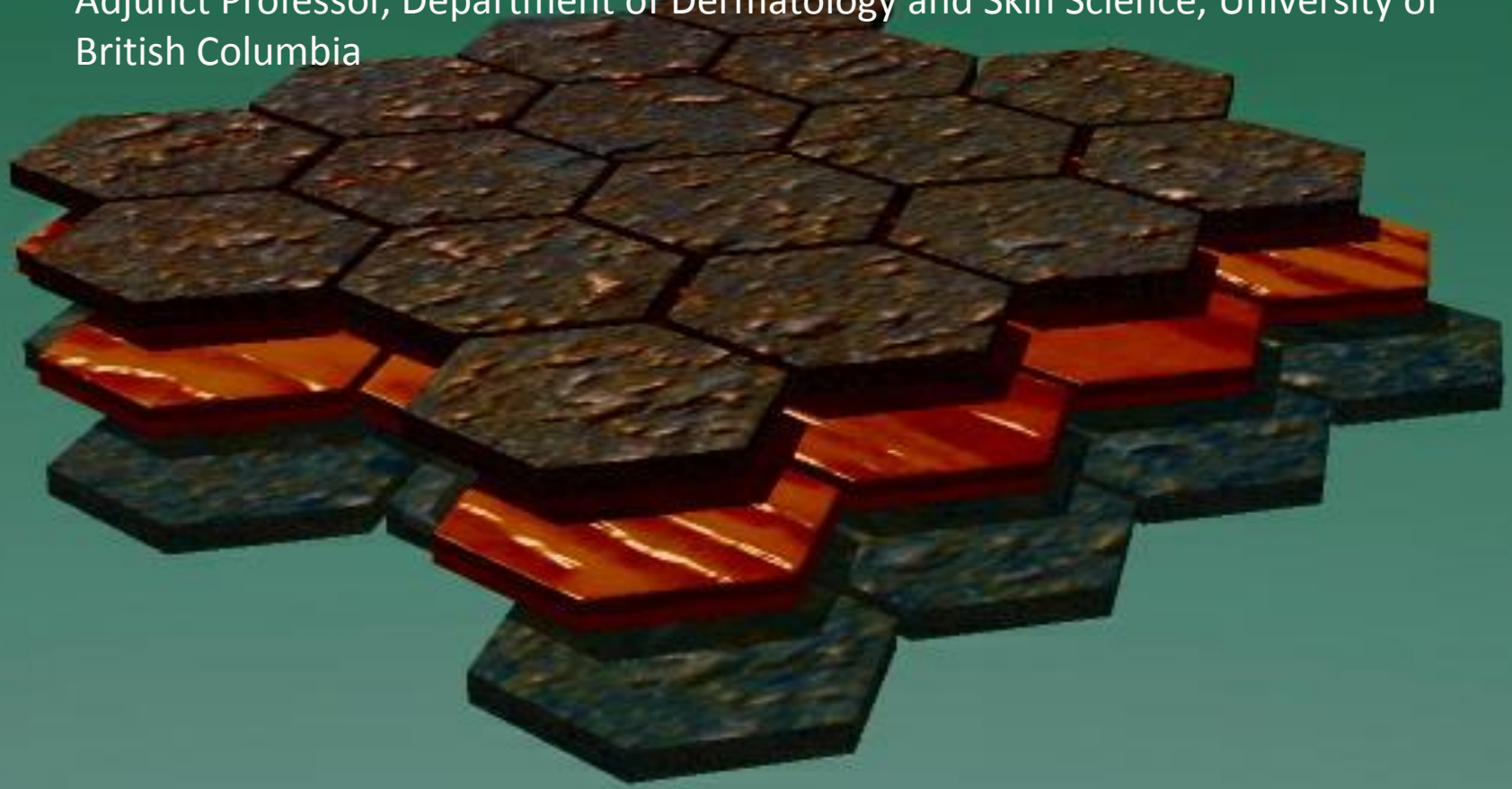
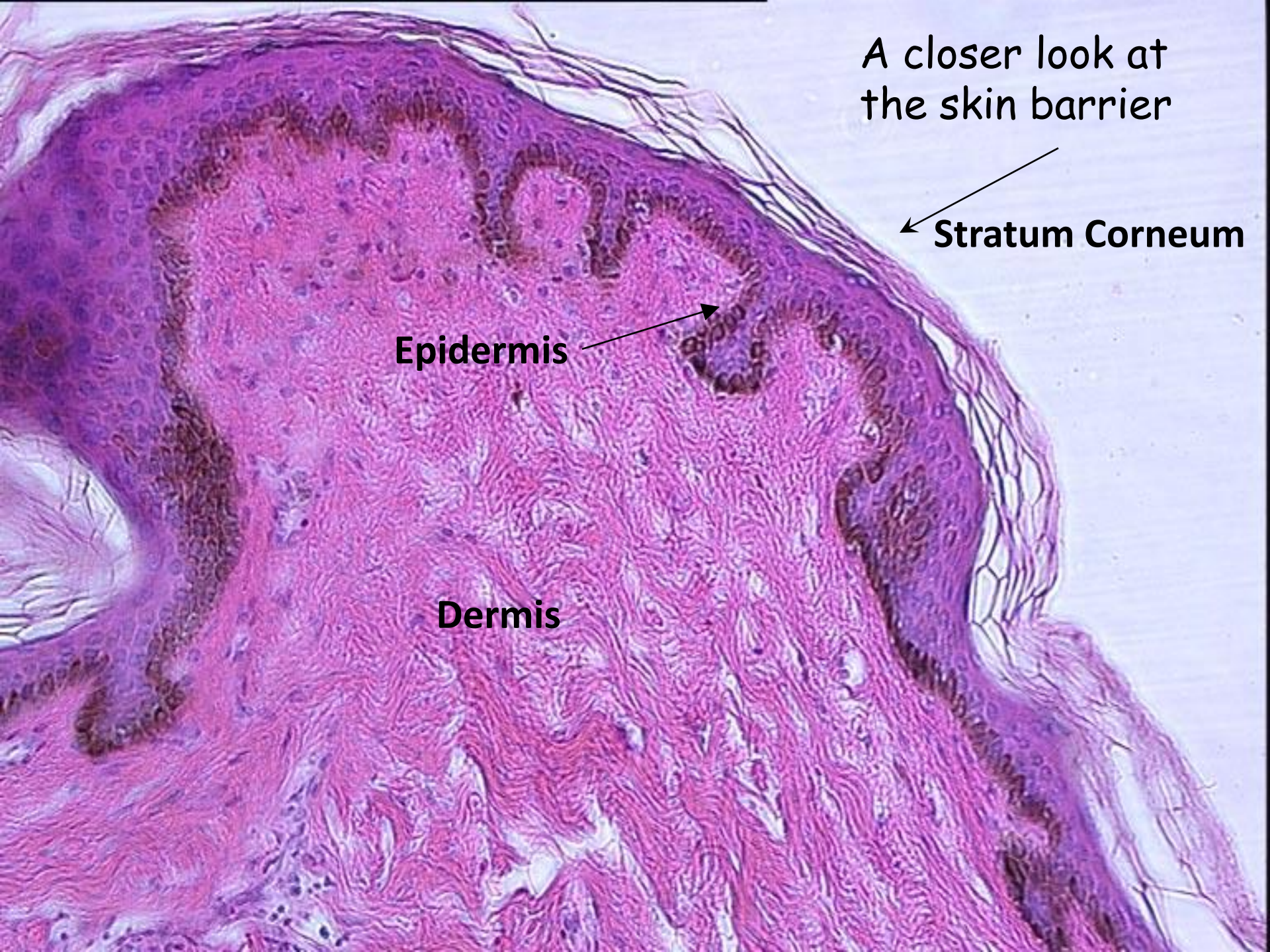
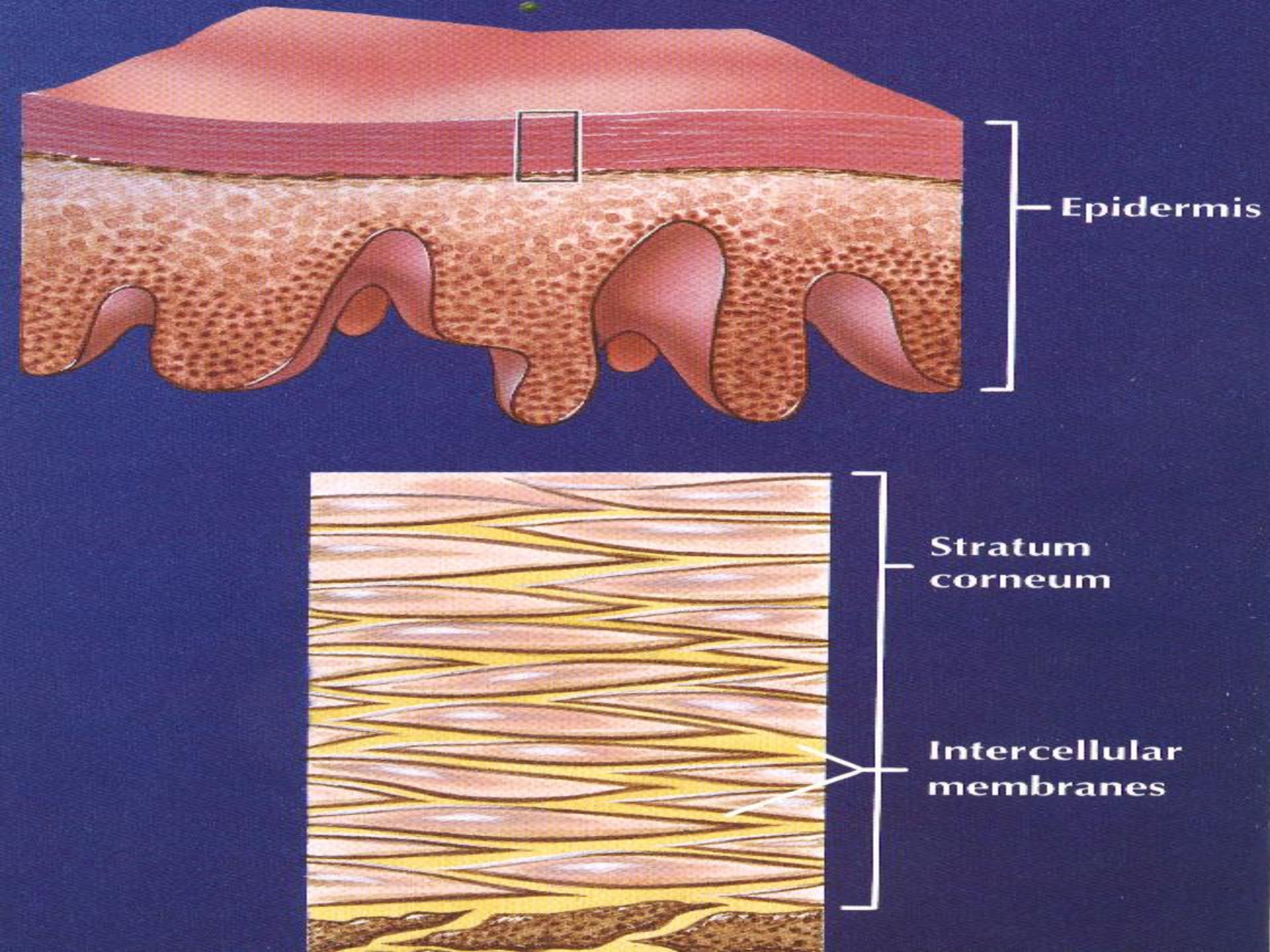


TRIUMF March 12 2011


A closer look at
the skin barrier
Stratum Corneum
Epidermis
Dermis


Epidermis
Stratum corneum
Intercellular membranes

Stratum Corneum Lipid Organization

The “stratum corneum” is the uppermost layer of the epidermis. Its lipids are responsible for skin’s barrier function.

Organization within layers

Crystalline (SOLID)

Gel (QUITE SOLID)

Liquid crystalline (PARTLY LIQUID)

Liquid ordered

Liquid disordered

Crystalline lipids are impermeable – unless packing defects are present

Gel phase lipids are less perfectly packed – low permeability

Liquid crystalline lipids are more loosely packed and mobile, but still layered
– higher permeability

Lipid mobility occurs in the plane of the membrane

DISORDERED liquid crystalline lipids – “legs” bent, layers thin

ORDERED liquid crystalline lipids – “legs” straighter, thicker layers

In the stratum corneum… LIPID ORGANIZATION MATTERS

Organization of layers

BILAYER:

layerlayerlayerlayerlayerlayerlayer

layerlayerlayerlayerlayerlayerlayer

STACKS OF BILAYERS:

bilayers bilayers bilayers

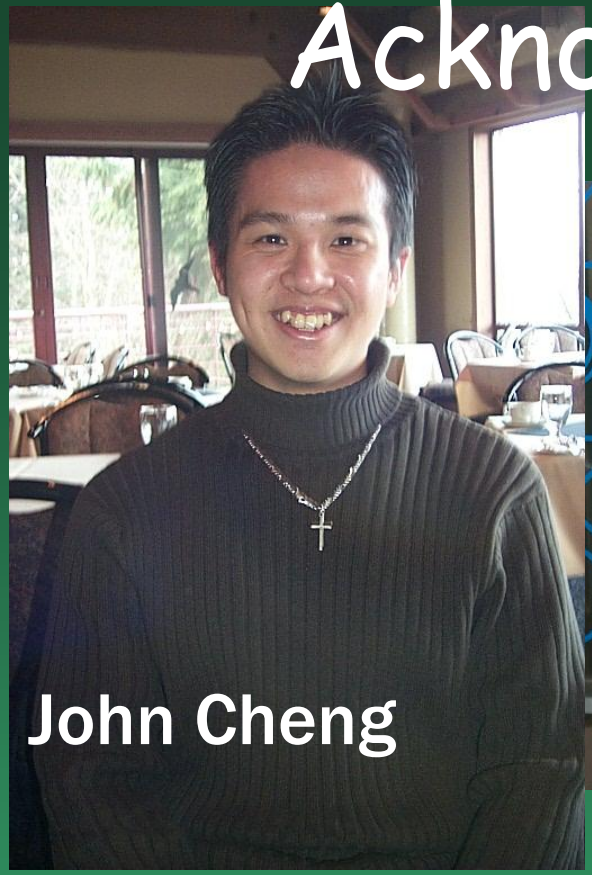
bilayers bilayers bilayers

bilayers bilayers bilayers

Acknowledgements

At SFU:

Elana Brief



John Cheng

Carolyn Young

At U de Montreal (chimie):

Sungjong Kwak & Michel Lafleur

At UBC (Dept. of Dermatology and Skin Science) :

Neil Kitson

Funding from NSERC

Characteristics of SC barrier membranes

Lipid composition: ceramides:cholesterol:fatty acids 1:1:1

pH ~5

Phase transitions observed upon heating

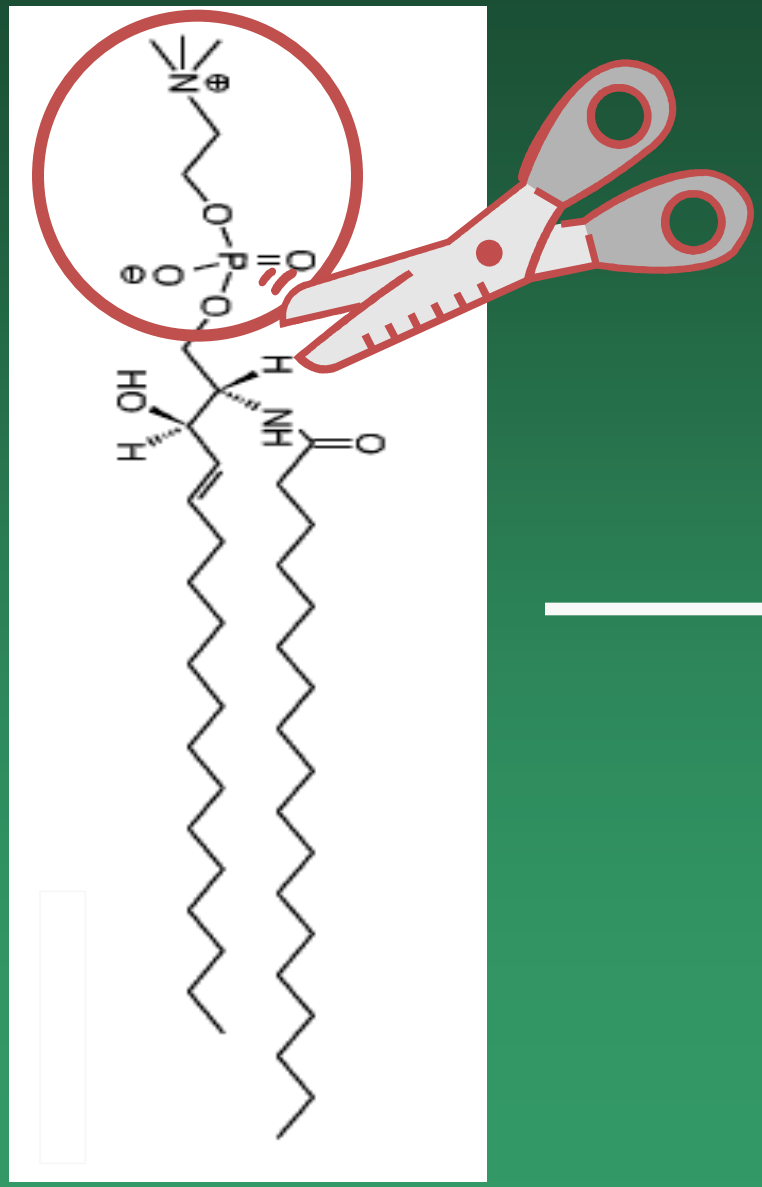
Predominantly **crystalline** packing of lipids at skin temperature. (Pilgram GS, Engelsma-van Pelt AM, Bouwstra JA, Koerten HK. *J. Invest Dermatol.* 1999, 113:403-9)

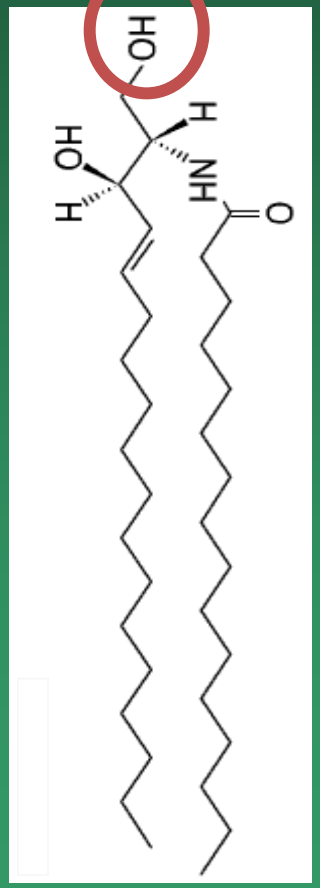
Low water content in intercellular lipid layers

Low water flux: we measure ~ 1 g/m^2/hour for pig skin at 22^{o}C (permeability coefficient 1.5 x 10^{-3} cm/hour, Wester/Maibach p.21 of 'Percutaneous Penetration Enhancers', 1995 CRC press)

Sphingomyelin is converted to Ceramide by the enzyme "acid sphingomyelinase"

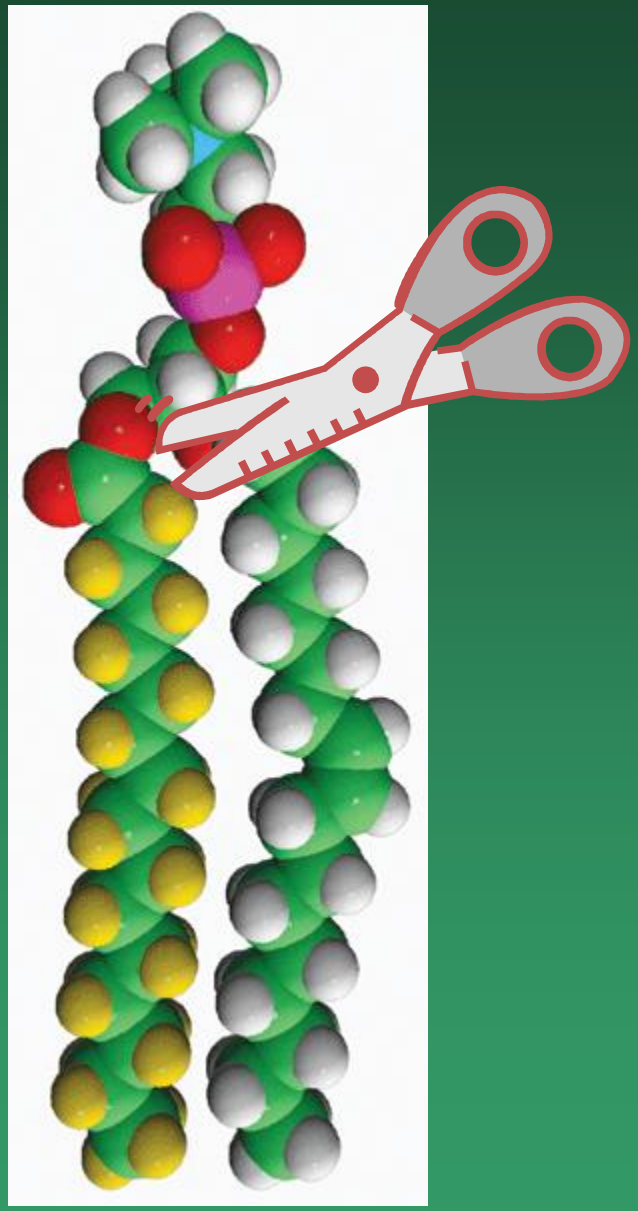
SM





Cer

Phosphatidylcholine (POPC)

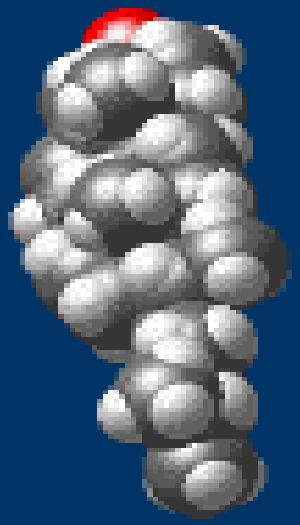


Similarly, the *saturated* fatty acyl chains of phospholipids such as POPC are cut by another enzyme (phospholipase A1) to yield free fatty acids like *palmitic acid (C16:0).*

Since the pH of the SC is low, these free fatty acids will be uncharged: *e.g.* – COOH *not*

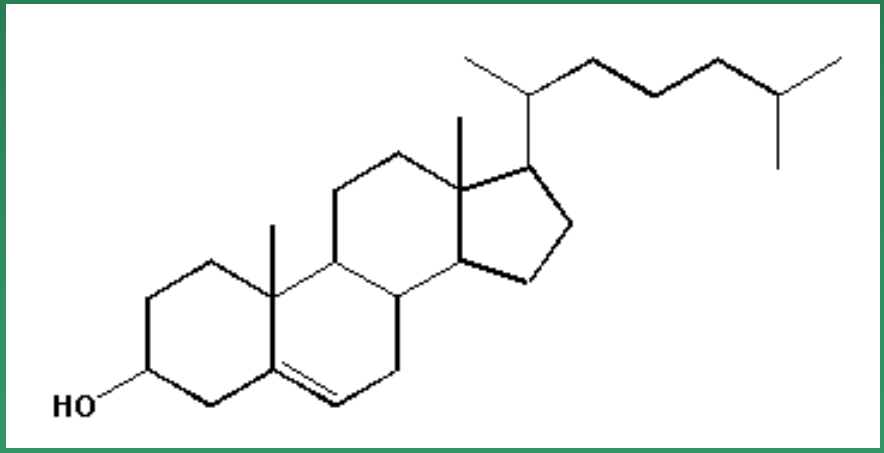
– COO^-

Such fatty acids are not forced apart by electrostatic repulsion. They can pack tightly into solid layers.



Sterols often have dramatic effects on membrane phase behaviour due to their rigid cylindrical shape: they restrict lipid acyl chain conformational freedom in liquid crystalline phases.

What role does Cholesterol play in model SC membranes?

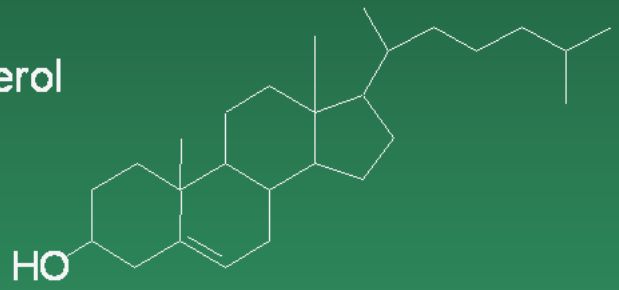


CHOLESTEROL

non-hydroxy fatty acid ceramide

SC model membranes:

cholesterol



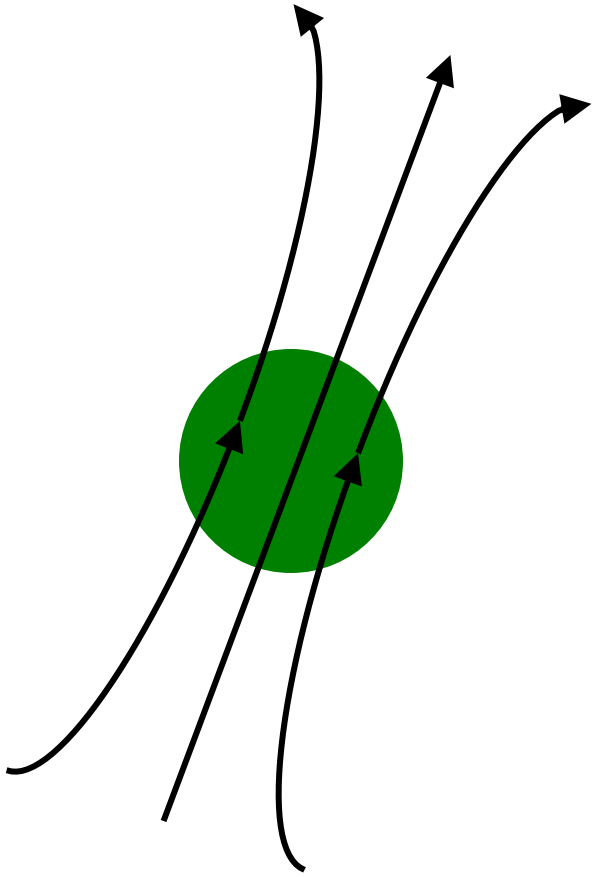
- 3 lipid components
- *hydrated* with citrate buffer, pH 5.2

palmitic acid

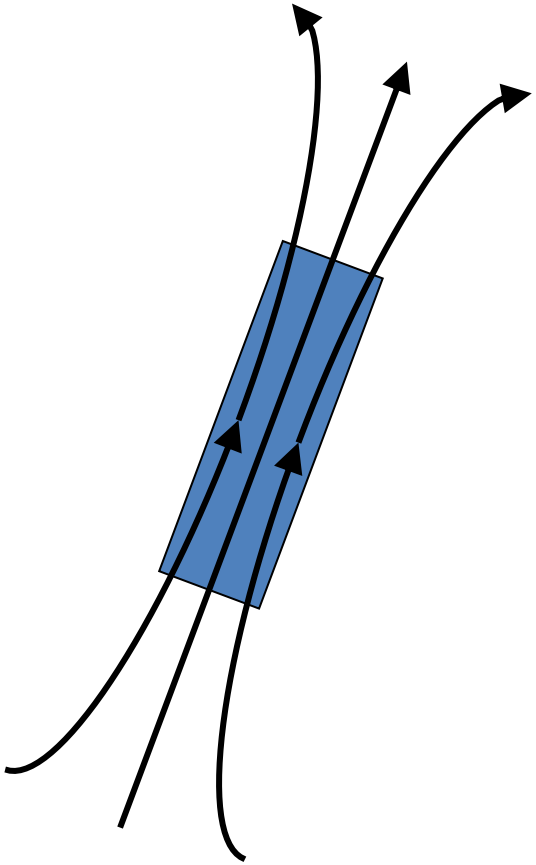
Introduction to Nuclear Magnetic Resonance:

Nuclear spin

The hydrogen nucleus (proton) has a magnetic field



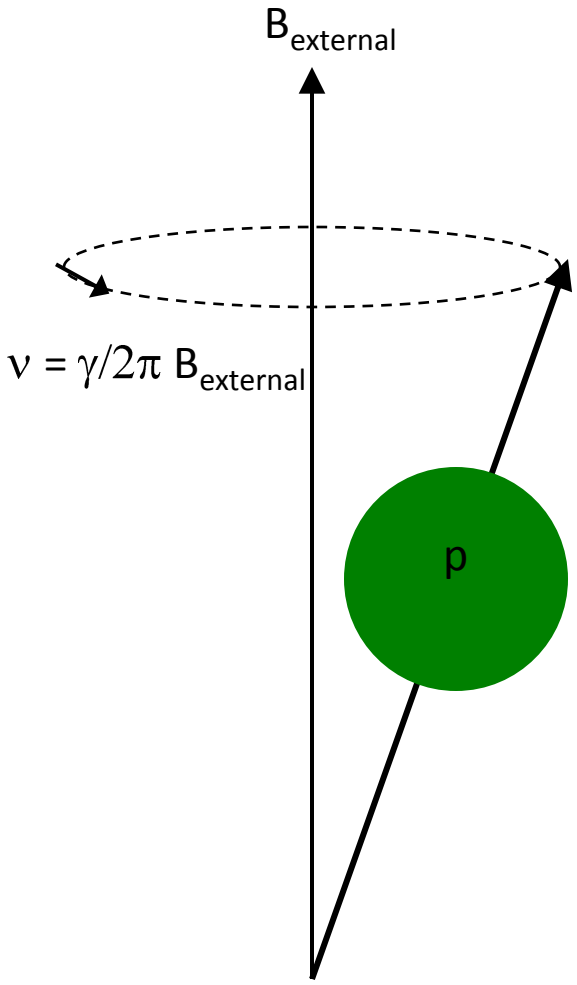
proton

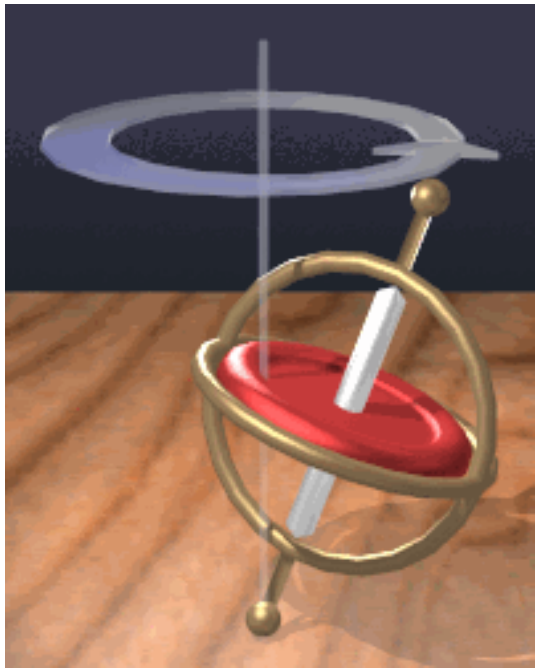


bar magnet

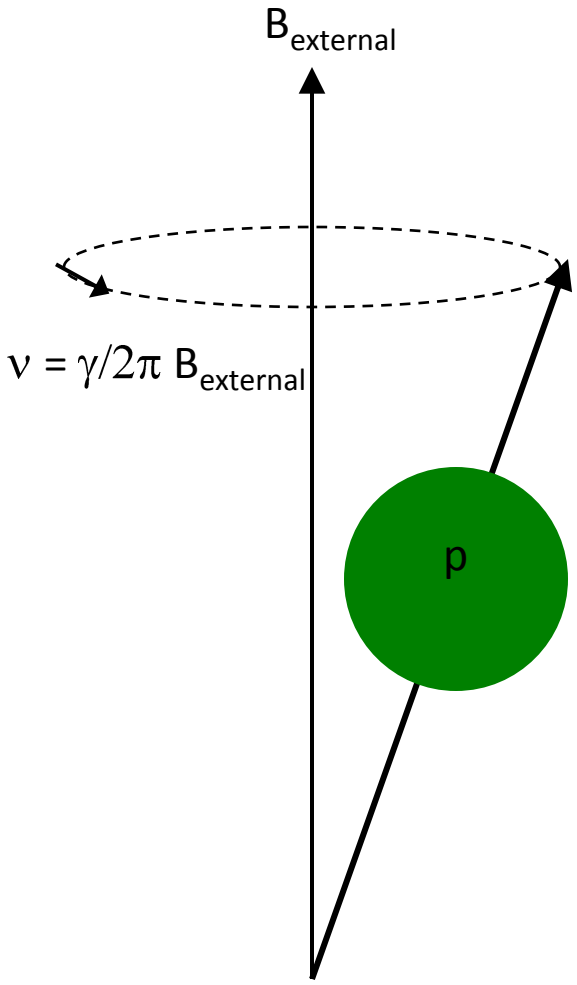
Nuclear Spin

The proton, p, precesses in an external magnetic field B like a spinning top in a gravitational field





Proton Nuclear Magnetic Resonance



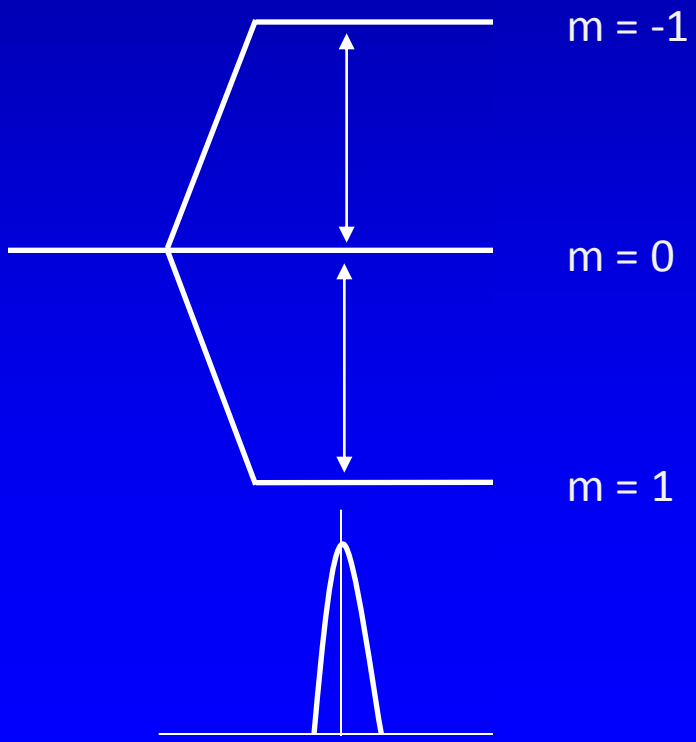
Protons in magnetic fields have two stable states, “up” and “down”. These are known as “Zeeman states”. Transitions between these states can be excited using a spectrometer that bathes the protons in energy that matches the energy difference between the up and down states of the protons: this is “NMR”.

We see a signal corresponding to the absorption of the energy: this is the “NMR spectrum”.

Introduction to Deuterium (^{2}H) NMR

Zeeman interactions: 3 possible states

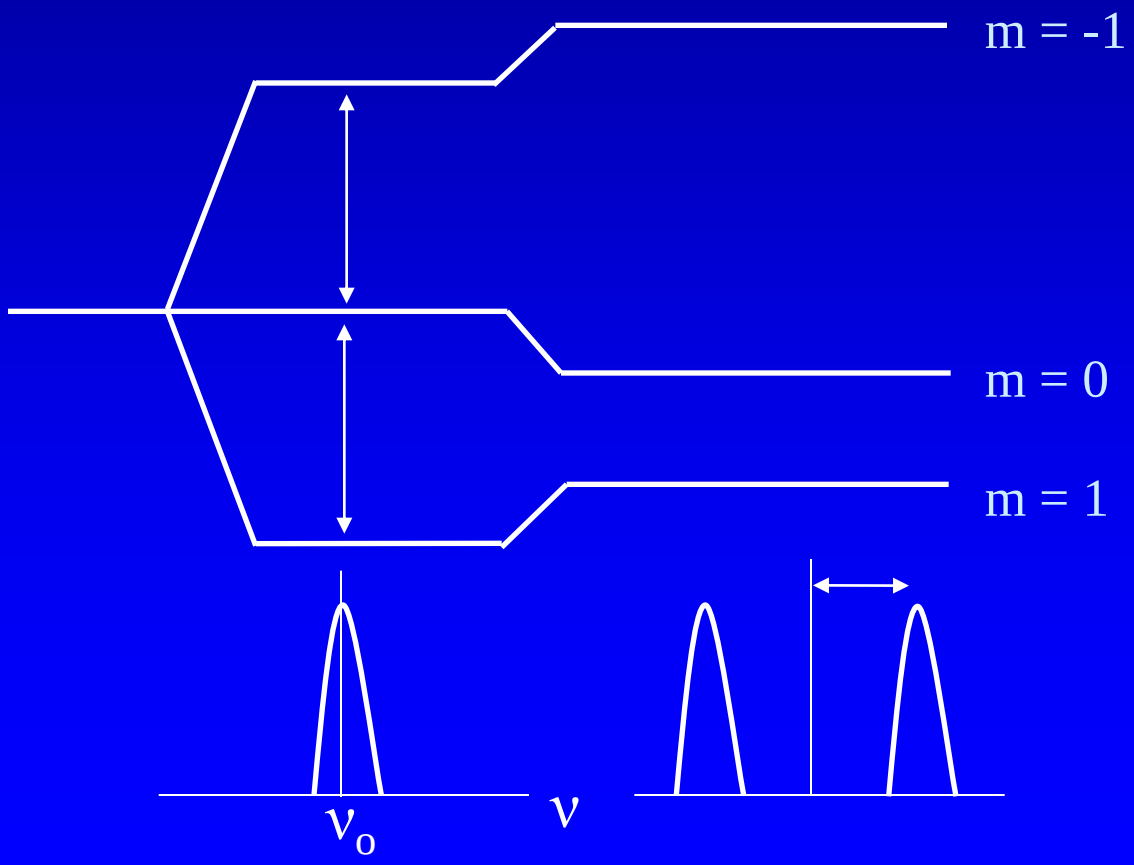
We label each energy state with an integer *m*



Deuterium (^2H) NMR

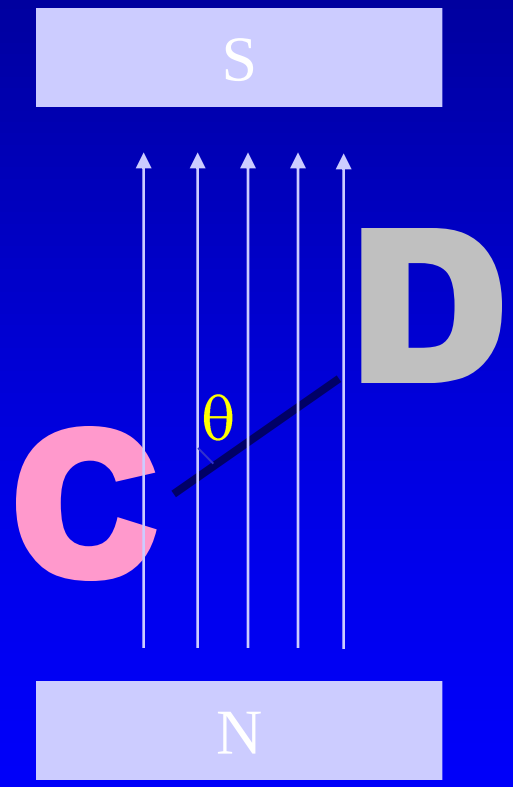
Zeeman + Quadrupolar interactions

$$E = E_Z + e^2qQ/4h\,(3m^2 - 2)$$



Orientation Dependence of Interaction: ^{2}H NMR

imagine a carbon-deuterium (CD) bond at rest in a magnetic field



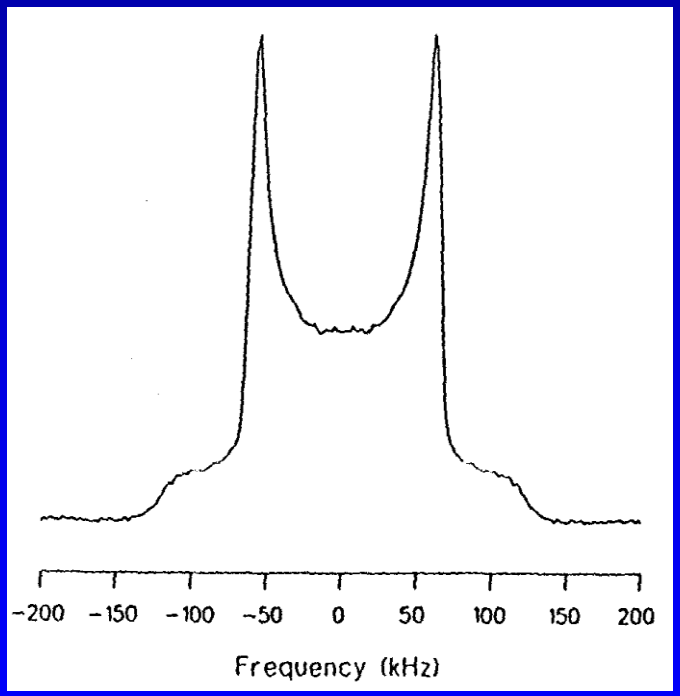
$$E_m = E_Z + (e^2qQ/4h)(3m^2-2)(3\cos^2\theta - 1)/2$$

$$\nu = \nu_o \pm (3e^2qQ/4)(3\cos^2\theta - 1)/2$$

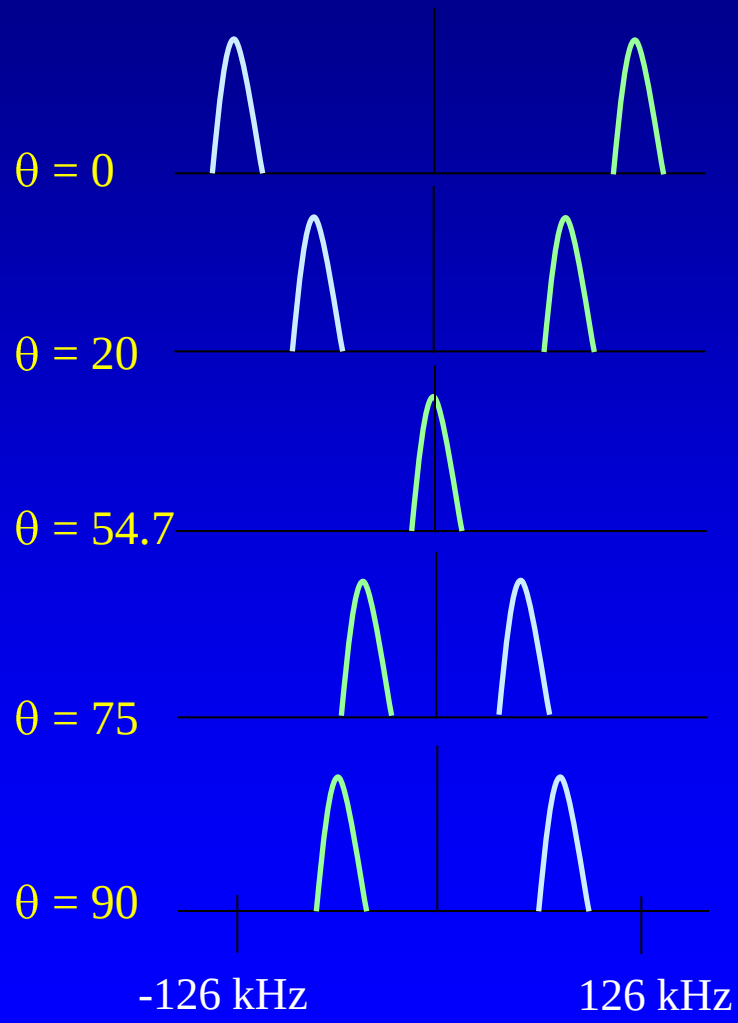
Orientation Dependence of Interaction: ^{2}H NMR

imagine changing the angle θ

$\nu = \nu_o \pm (3e^2qQ/4)(3\cos^2\theta - 1)/2$



Pake doublet



Characteristics of SC barrier membranes

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pH ~5

Phase transitions observed upon heating

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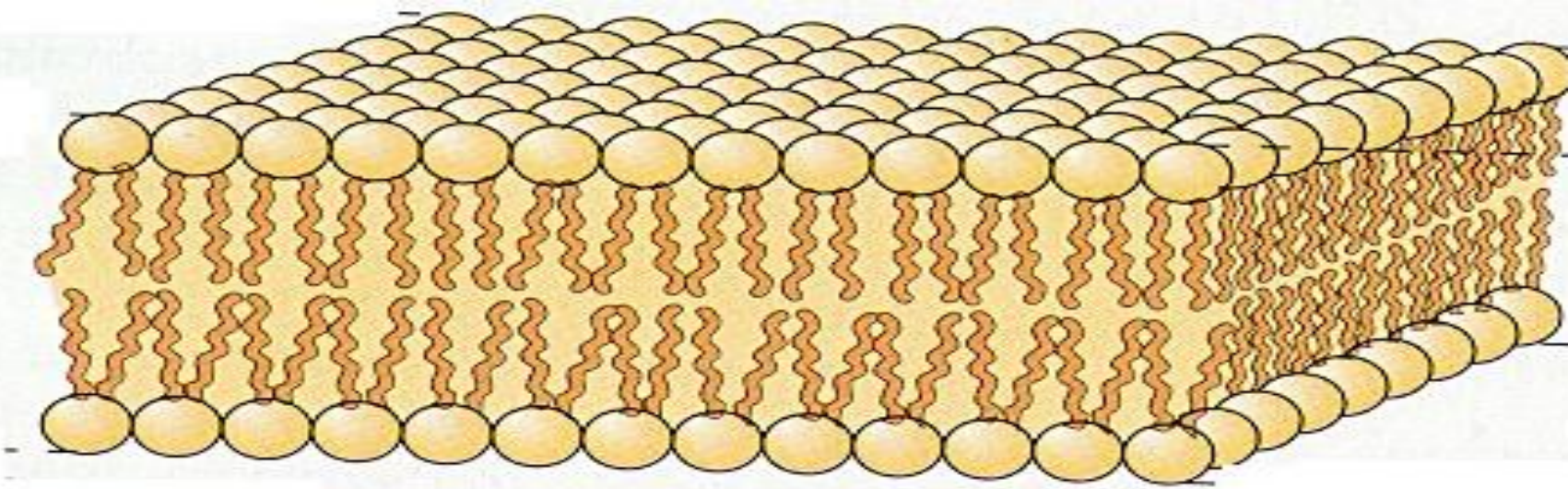
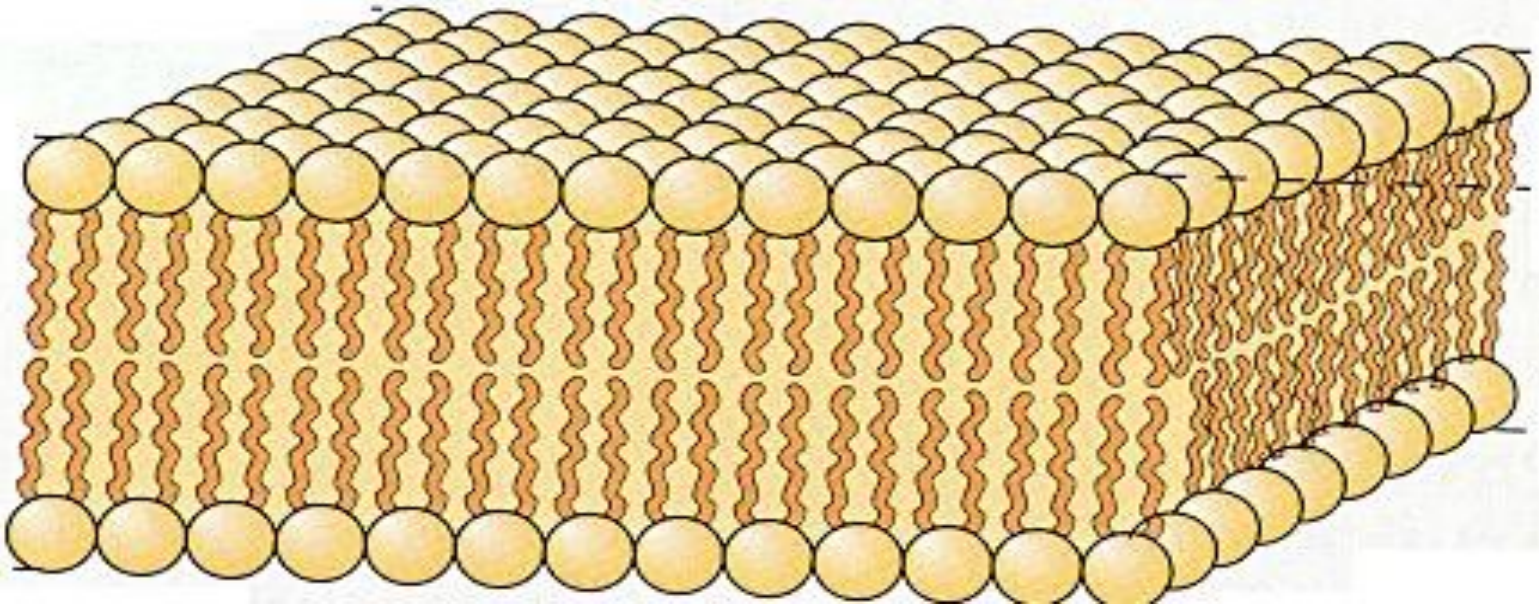
Typical bilayer: main phase transition at a temperature Tm

$T > T_m$



T_m bilayer "melts", movement is allowed

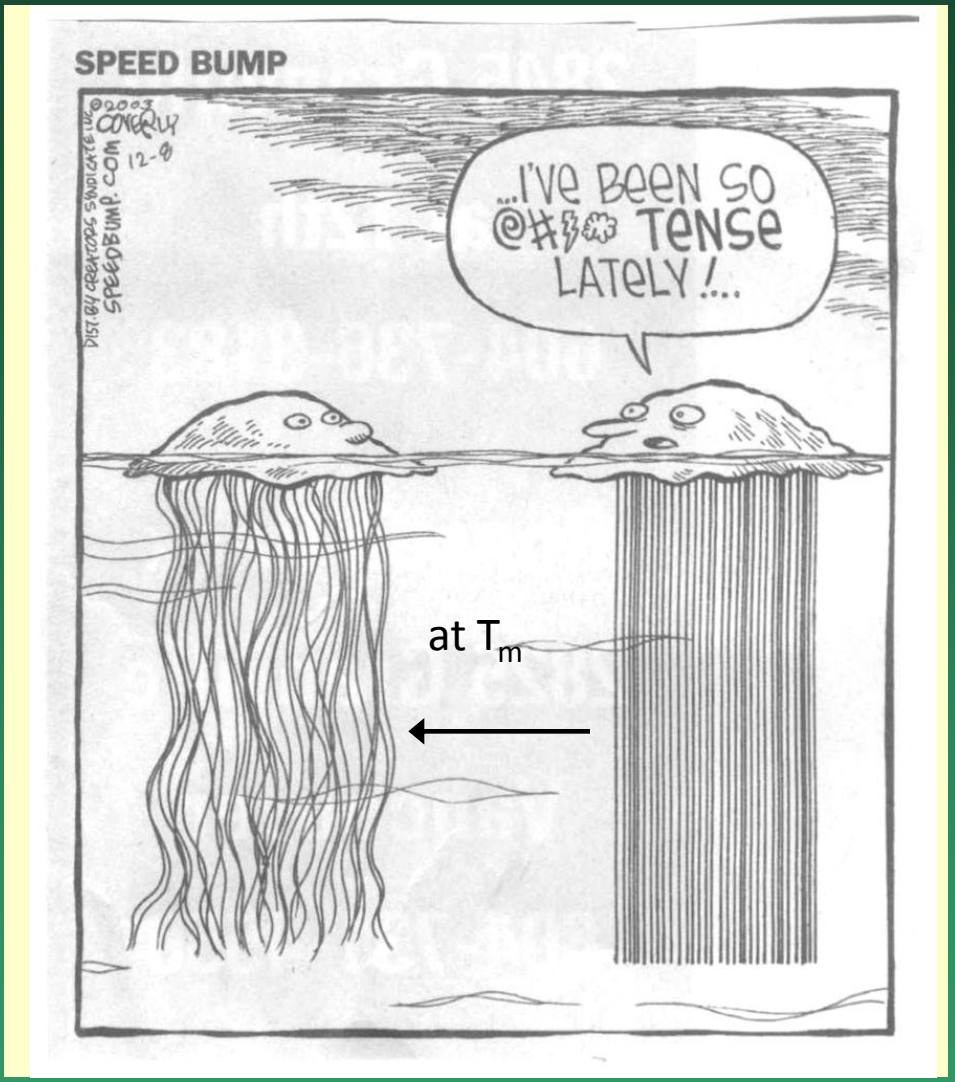
$T < T_m$



Lipid chains undergo trans-gauche isomerizations above T_m

FLOPPY CHAINS

STRAIGHT CHAINS

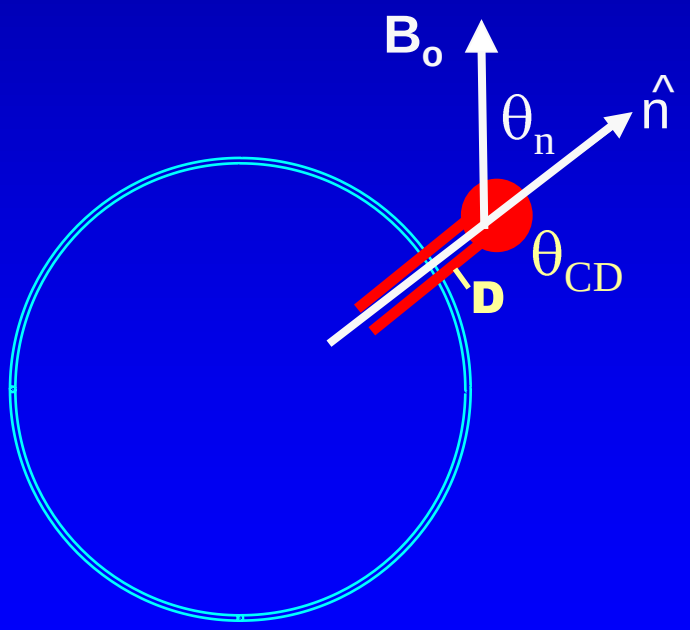


Also above T_m, lipids diffuse within the membrane plane and rotate about their long axes.

Molecular Motion Sensitivity

If there is rapid rotation about the lipid long axis in a membrane, resonances are observed at

$\nu_o \pm (3e^2qQ/4)\,[(3\cos^2\theta_n - 1)/2] < 3\cos^2\theta_{CD} - 1 >/2$



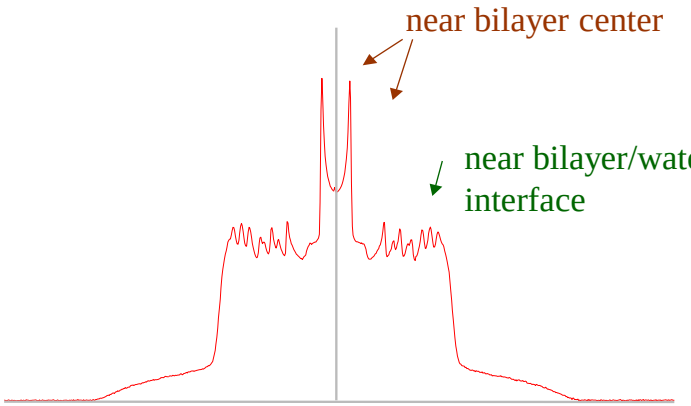
The $< 3\cos^2\theta_{CD} - 1 >/2$ term is sensitive to chain conformational freedom *i.e.* 'disorder'.

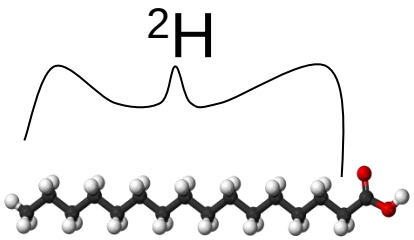
If the lipid chain is floppy the term is small and the NMR peaks are close together.

^{2}H-label the palmitic acid: PA-d31

$T > T_m$

Fully Deuterated acyl chain ⇒
Superposition of Pake doublets



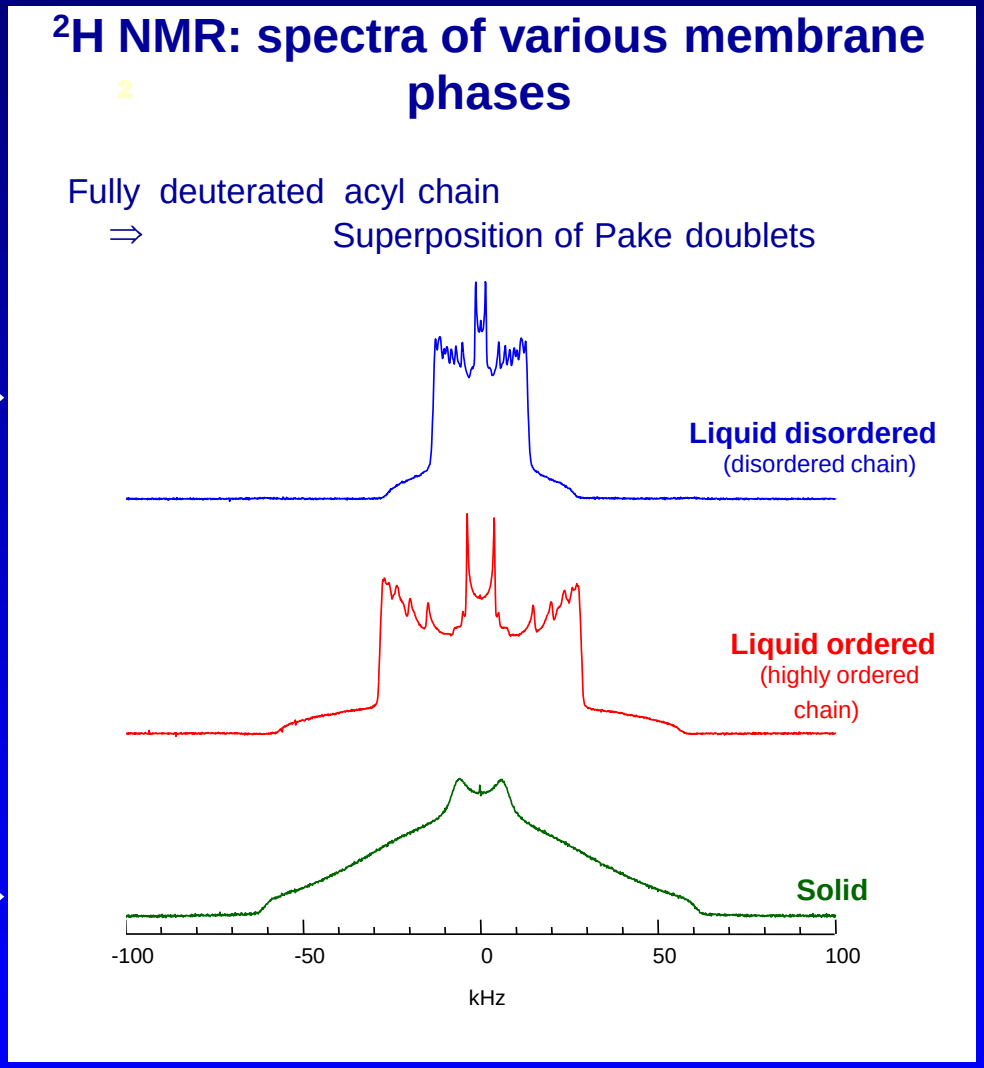


PA-d31

Phases observed in the presence of cholesterol

$T > T_m$ (little/no cholesterol) →

$T < T_m$ (little/no cholesterol) →



← Lots of cholesterol

In summary:

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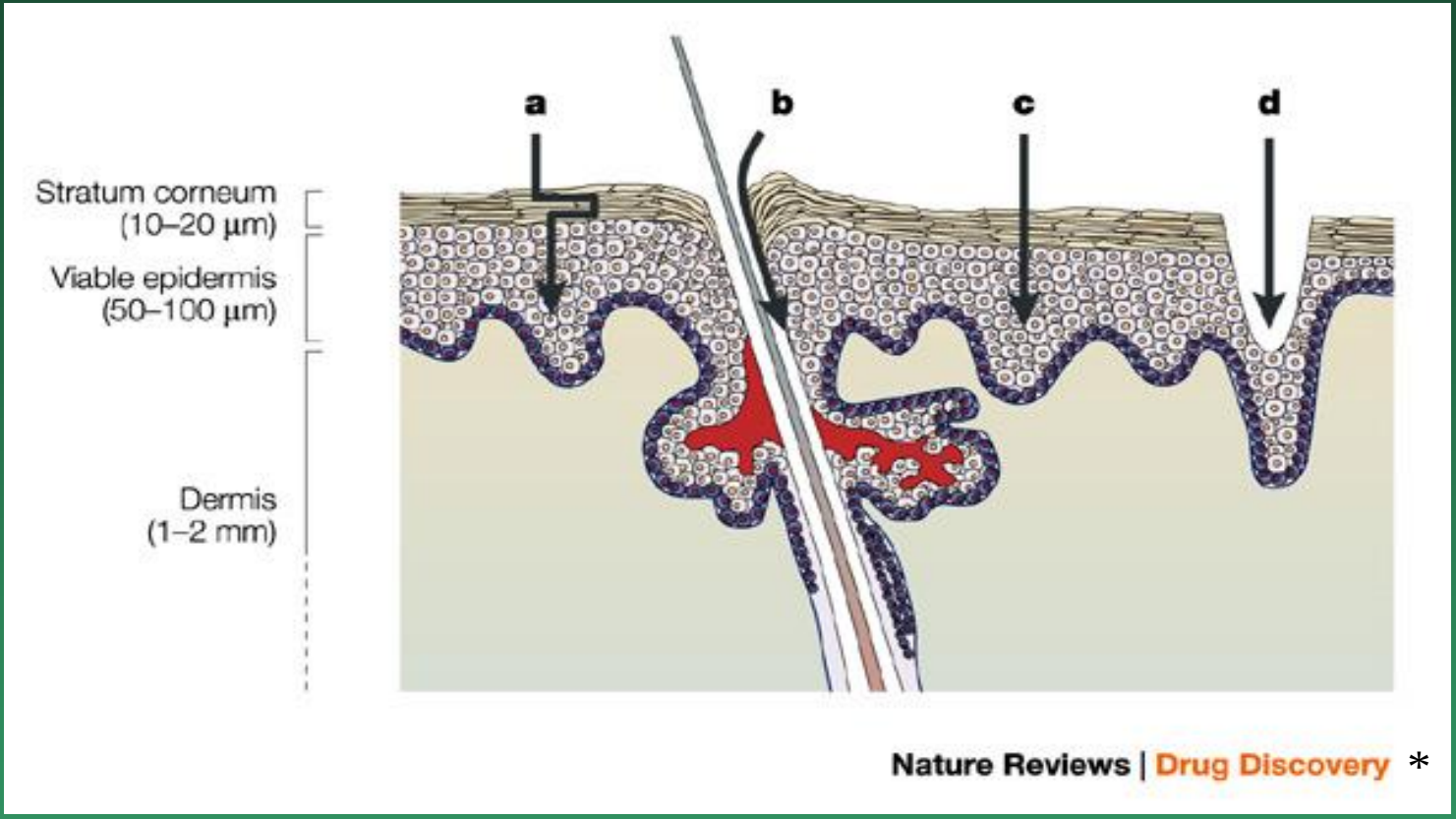
Routes through the skin barrier

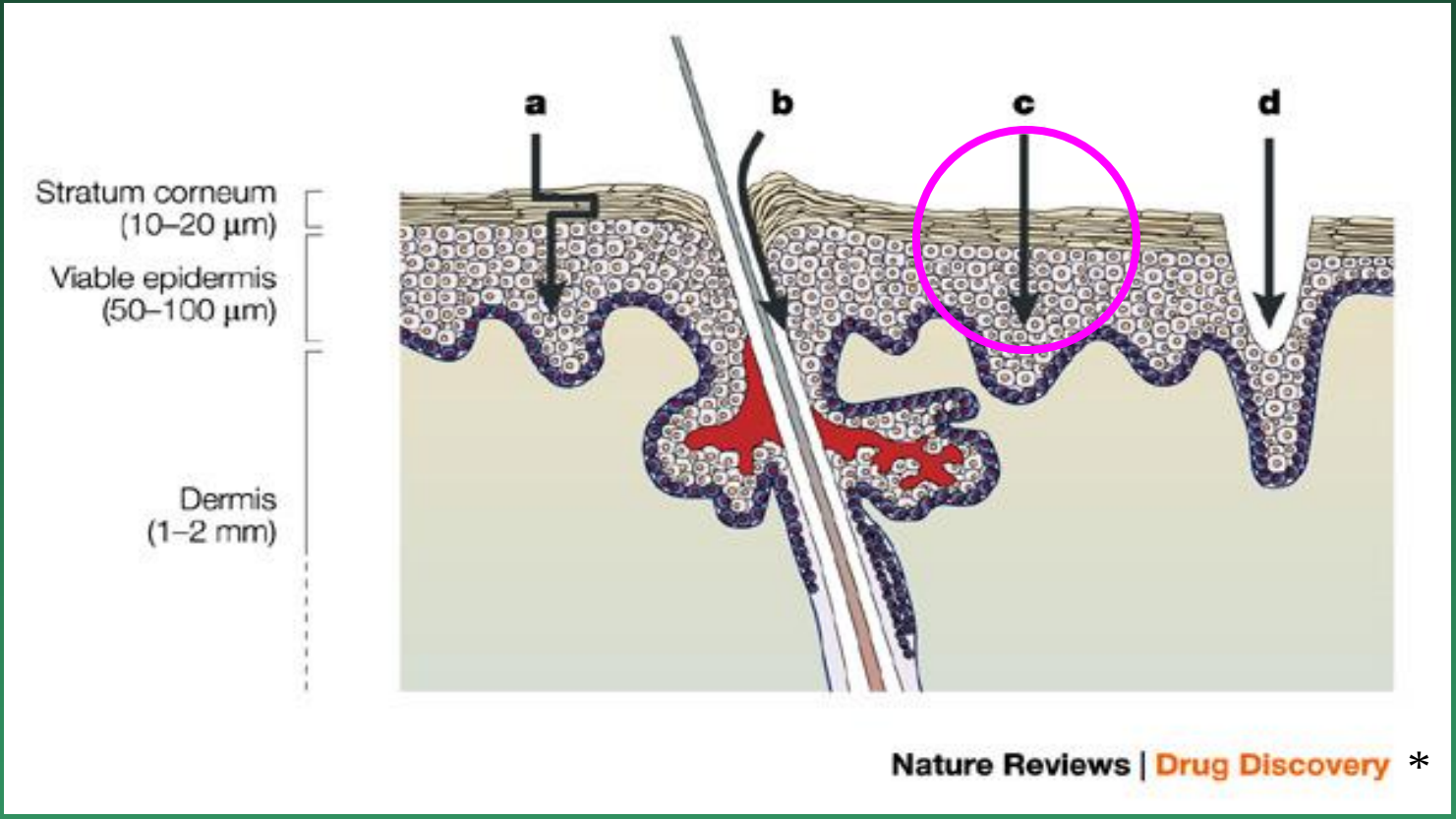


a: intact barrier (very low permeation) – SOLID LIPID MEMBRANES

b-d: possible avenues of barrier evasion/disruption

* Prausnitz et al., 2004, 3: 115-124

Routes through the skin barrier

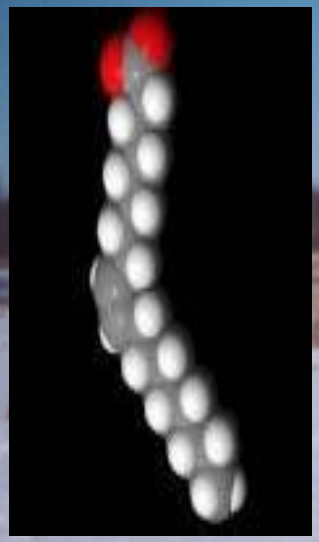


a: intact barrier (very low permeation) – SOLID LIPID MEMBRANES

c: gentle, reversible barrier disruption by changing membrane properties

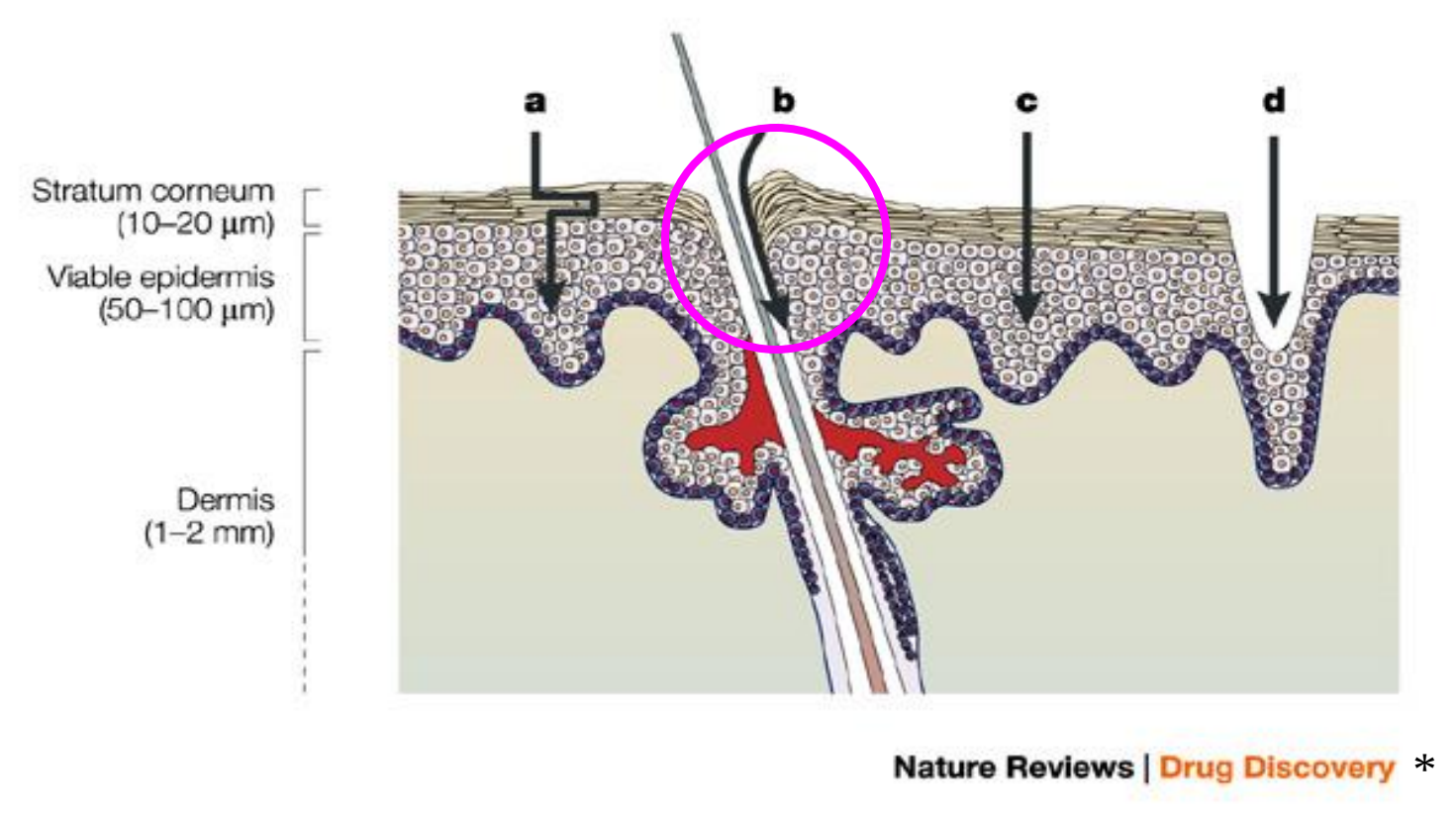
* Prausnitz et al., 2004, 3: 115-124

Oleic acid project



Amy Rowat

Routes through the skin barrier



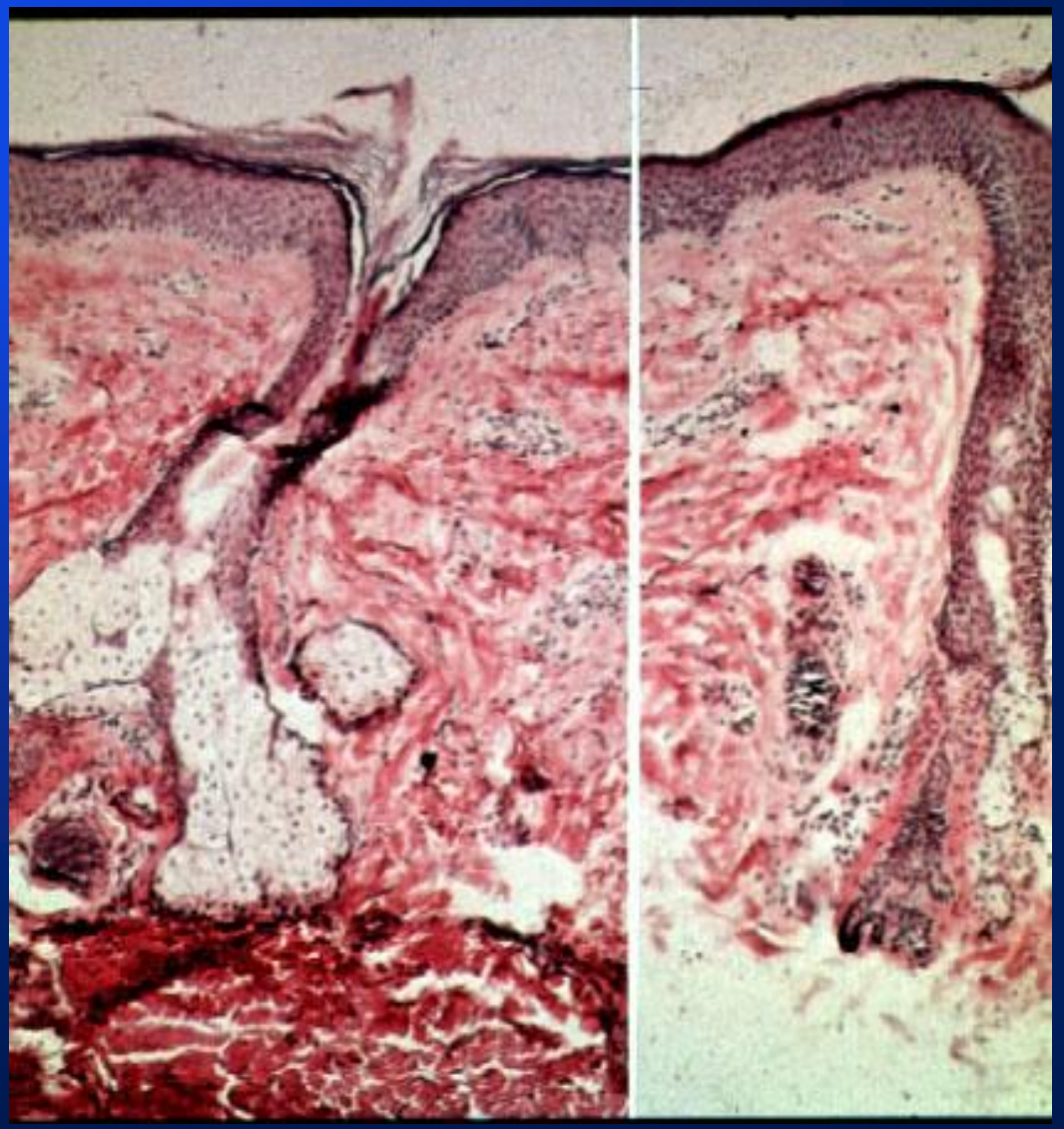
a: intact barrier (very low permeation) – SOLID LIPID MEMBRANES

b: possible avenues of barrier evasion – the hair follicle

* Prausnitz et al., 2004, 3: 115-124

Acne: The Scene of the Crime

Sebaceous Follicles



Acne slides courtesy
Dr. Neil Kitson
UBC Dermatology &
Skin Science

ACNE VULGARIS *WHAT IT ISN'T*

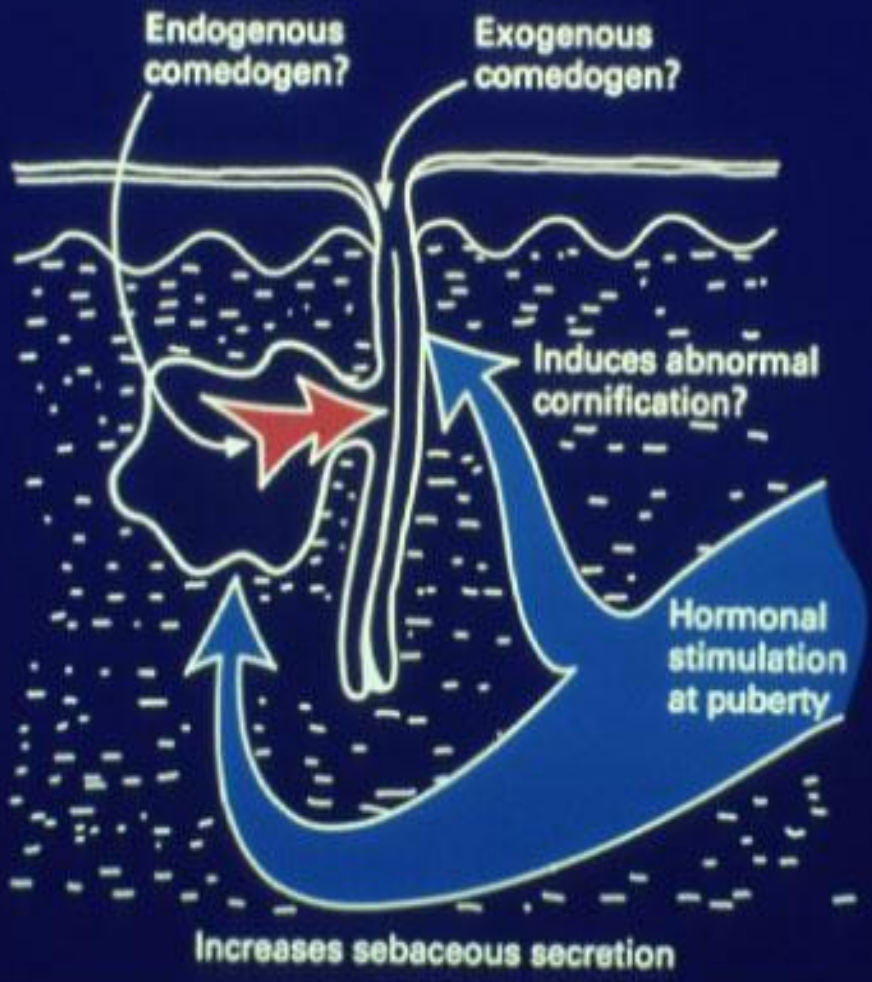
1. dirt/oiliness/washing with the wrong soap
2. dietary deficiency/excess (especially chocolate)
3. too much/not enough stress
4. not drinking enough/drinking too much water
5. not enough/too much sexual gratification

ACNE VULGARIS *WHO GETS IT?*

genetic predisposition + trigger = disease

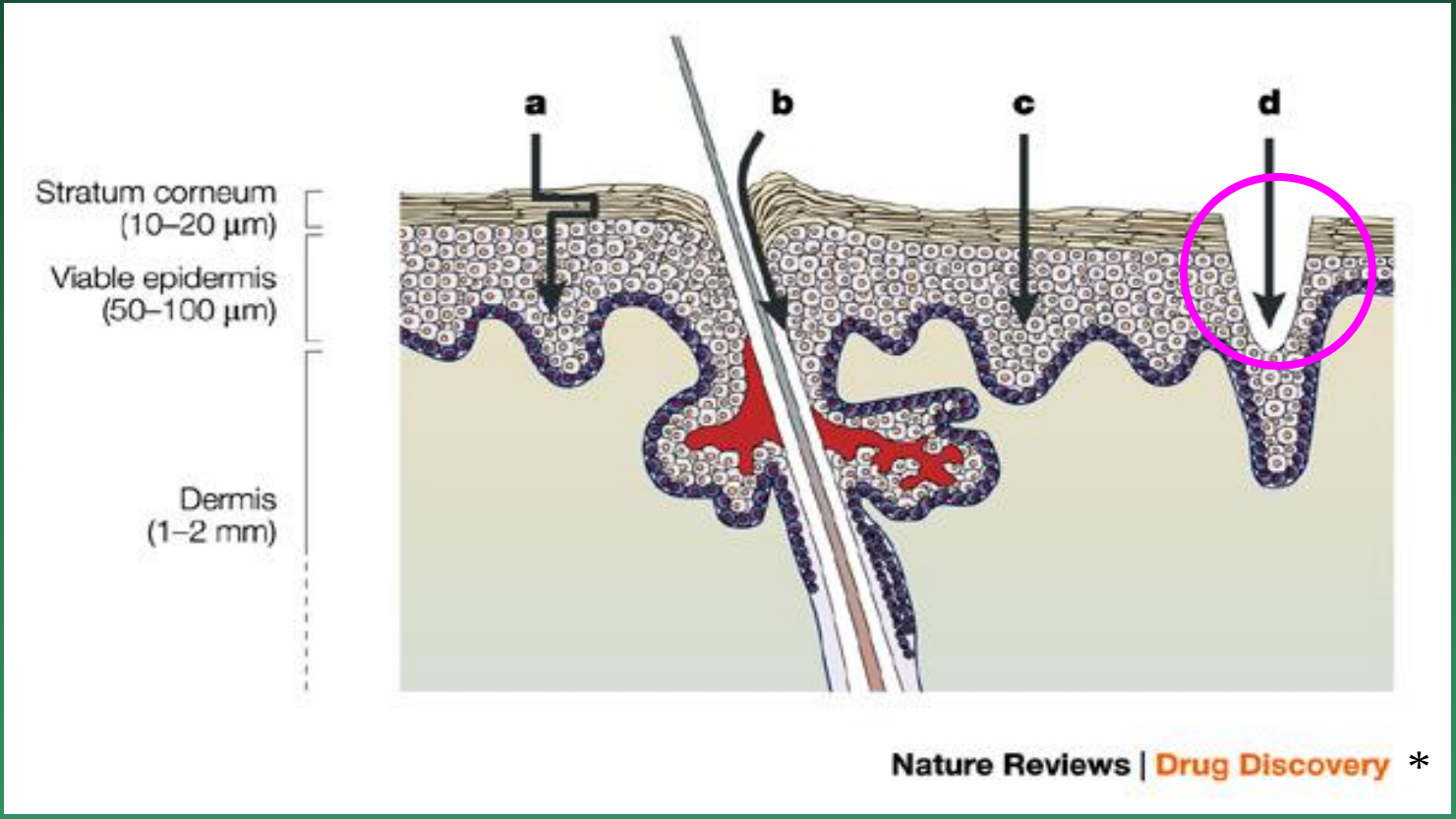
acne-prone family + sex hormones = acne vulgaris

Events in comedo formation




Eyelash Mite

Routes through the skin barrier

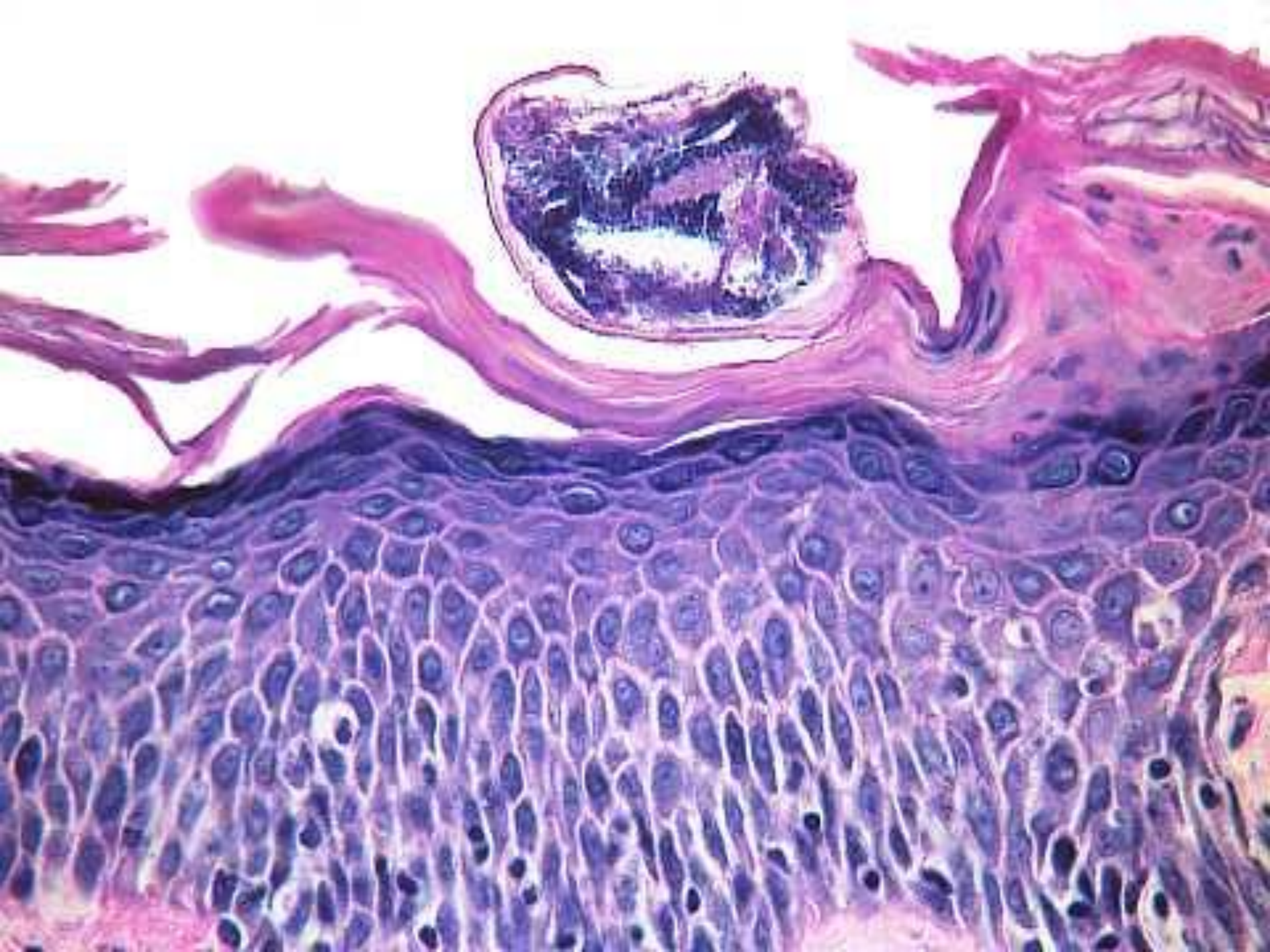


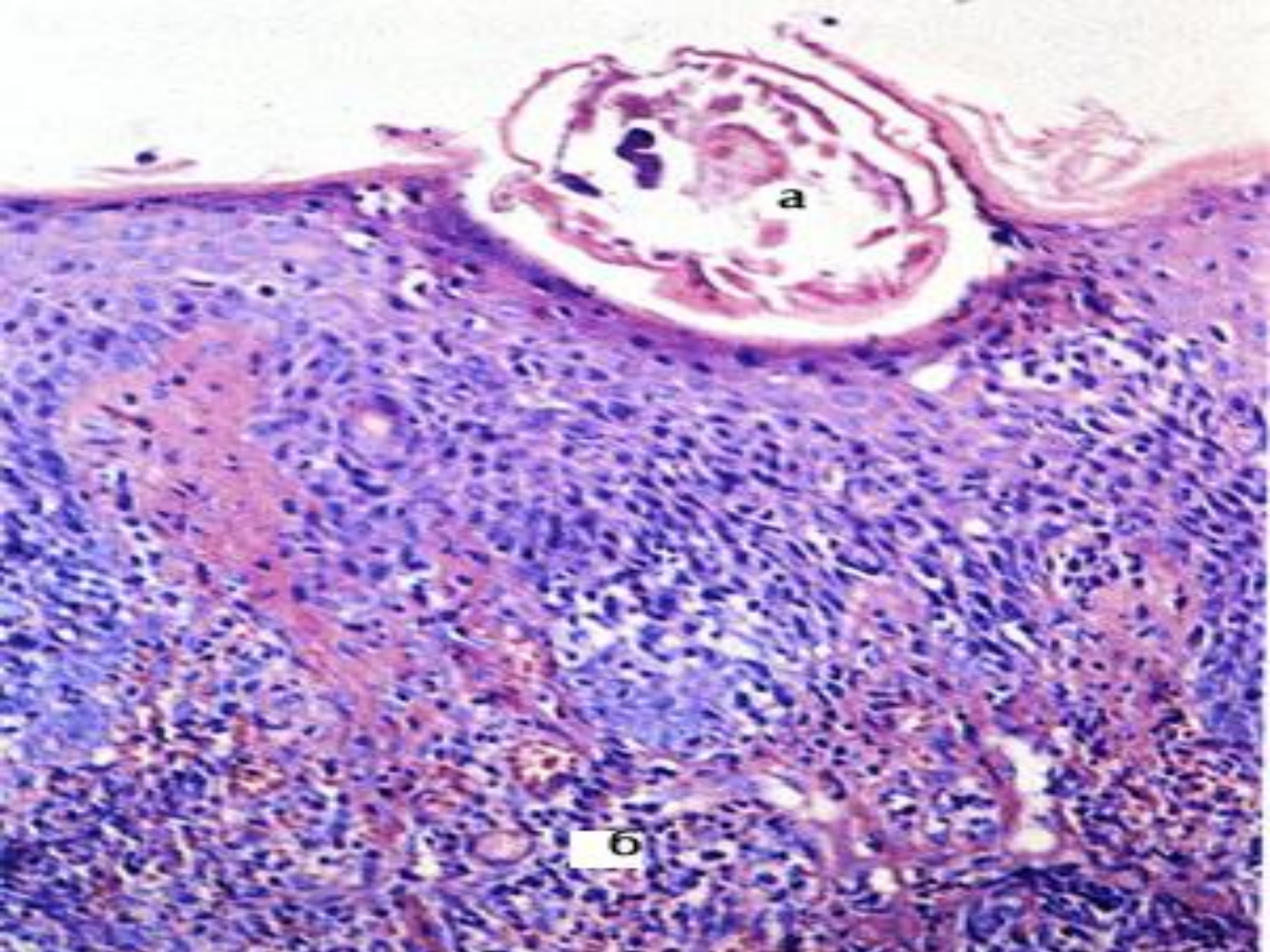
a: intact barrier (very low permeation) – SOLID LIPID MEMBRANES

d: mechanical barrier disruption, for example injection

* Prausnitz et al., 2004, 3: 115-124


A BURROW

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A.D.A.M.

Future:

SC model membranes are simplified versions of SC barrier membranes that have many of the same characteristics

Use SC model membranes to further study barrier repair, for treatments of illnesses causing impaired SC barrier function

Use SC model membranes to further study reversible barrier disruption, for transdermal drug delivery


Thank you