“Off course Luis, I told you, it is a lipid domain...”
**Biological membranes:**

Multi-molecular bidimensional element (bilayers) composed mainly of lipids, proteins and sugars.

Membranes play a central role in both structure and function of all cells.

Membranes not only define compartments, they also determine the nature of all communications between the inside and outside.

High diversity in terms of composition, structural details and function.

- Sheetlike structures 60-100 Å thick
- Contain lipid, protein and small amount of carbohydrate
- Fluid structures
- Asymmetric
- Lipid part act as barrier to penetration
What are lipids?

Lipids are naturally occurring molecules. They have very important structural and dynamical roles in biological systems (structural components, regulatory agents, receptors).

Lipids are important components of biological membranes.

Lipids are highly diverse (in terms of molecular species):

- Fatty acid
- Ceramide
- Phospholipid
- Sphingomyelin
- Gangliosides
- Cholesterol
Lipids are amphipatic molecules, i.e. they have a hydrophilic part (soluble in water) and a hydrophobic part (insoluble in water).

When lipid molecules are dissolve in water they form aggregates, which have particular structural features. The most common structures that lipid form are called micelles and bilayers.
There are some lipids present in biological membranes that display ordered/disordered phase transitions above physiological temperatures.

DPPC, Sphyngomyelin, ceramides, cerebrosides

Lateral lipid packing
Lateral diffusion
Axial rotation
Membrane thickness

temperature, pH, ionic strength

Fluid phase
Gel phase

Fluid phase
Cholesterol
Fluid ordered phase
The model postulates that integral proteins resemble “icebergs” floating in a two dimensional lipid sea and that these proteins freely diffuse laterally in the lipid matrix unless their movements are restricted by associations with other cell components (no clear indication of the role of lipid compositional heterogeneity).

Lipids were (and still are) considered to be merely building blocks of biological membranes, although strong evidences points to the fact that the compositional diversity of these molecular species play a role in triggering particular structural phenomena in biological membranes.

Raft hypothesis: K. Simons 1997, nanoscopic domains exist in the plane of the membrane (very controversial, liquid ordered/liquid disordered phase coexistence)

Regarding lipids and membranes...

Why cells membranes contain hundreds of different molecular lipid species?

Why the molar fractions of these species vary among different membranes?

How proteins interact with these different lipid species?

Is there a coherent code for lipids (as happens with genes and proteins) still hidden and waiting to be discovered? (Hilgemann, 2003, "Getting ready for the decade of lipids" Annu. Rev. Physiol 65:697-700)

NO CLEAR ANSWER YET!!!
To better understand the basis about dynamical and structural aspects of compositionally complex membranes researchers study model lipid systems!

**Liposomes**

- **Multilamellar (MLVs)** (size vary from few tens of nm to several microns)
- **Unilamellar**
- **SUVs** (25 – 50 nm)
- **LUVs** (> 100 nm)
- **GUVs** (5 – 100 μm)

**Planar membranes**

- **5 nm**
- **glass**
- **mica (atomically flat)**

Liposome solutions are used to explore the physical aspects of membrane (lateral structure and dynamics) using an array of different experimental techniques (differential scanning calorimetry, NMR, IR spectroscopy, fluorescence spectroscopy to mention just few)
Enrico’s contribution was very important in the characterization of lipid lateral structure and dynamics. At that time researchers claimed that lipid domains were nanoscopic.
Polarity sensitive fluorescent probes*

LAURDAN Generalized Polarization (GP)

\[
GP = \frac{I_B - I_R}{I_B + I_R} \implies 1 > GP > -1
\]

LAURDAN emission spectra in a single DPPC GUV
Buffer Tris-HCl 1 mM, pH=7.4

More than 200 publications using LAURDAN in lipid experiments
Seeing is believing: fluorescence microscopy applications


Micron size domains in bilayers!
Imaging GUVs

Giant unilamelar vesicles (GUVs)

Imaging GUVs

Lipid mixtures either artificial or natural

Native membranes
DPPC/DPPE mixture

fluid phase

fluid/gel phase coexistence

gel phase

DPPE molar fraction

T (K)

DPPE molar fraction

fluid phase

30 μm

30 μm

1

2

3

4

5

1

2

3

4

5
Different domain shape depending on the nature of the binary mixture

- DPPC/DPPE 3:7 mol
- DMPC/DMPE 1:1 mol
- DMPC/DSPC 1:1 mol
- DLPC/DPPC 1:1 mol
- DLPC/DSPC 1:1 mol
- DLPC/DAPC 1:1 mol

Scale: 20 μm
Morphological and lateral characterization of lipid domains coexisting scenario using LAURDAN GP function/two photon fluorescence excitation microscopy.

DPPE/DPPC 7:3

DOPC/SUM/Chol 1:1:1

fo

fd

30 μm
Enrico's ideas and experimental results were very important to:

Visually explore the lateral structure of biological membranes

Correlate lateral structure with membrane composition and function

Visual information is key to correlate simple lipid mixtures with compositionally complex membranes.
Preparation of giant vesicles from native material: Pulmonary surfactant

DOPC/DPPC/chol

Physiological temperature

LAURDAN probe

Coexistence of fluid phases (ordered and disordered-like)

Probe free experiment:
AFM of native Pulmonary surfactant planar membranes

*J. Biol. Chem.* 279:40715-40722
Which molecular specie trigger the phase separation?

Pulmonary surfactant

- Surfactant proteins
- Cholesterol

Liquid inmiscibility in native membranes
Lipid mediated phenomenon

DLPC/DPPC
gel/fluid
The functional link: spreading experiments

Change in lateral structure influence spreading process (in presence of proteins)

pulmonary surfactant could be one of the first membranous systems reported where the coexistence of specialized membrane domains exists as a structural basis for its function.
Visualizing lipid structure and raft domains in living cells with two-photon microscopy

Katharina Gaus*,†, Enrico Gratton‡, Eleanor P. W. Kable§, Allan S. Jones§, Ingrid Gelissen*, Leonard Kritharides*¶, and Wendy Jessup*

The lateral organization of cellular membranes is formed by the clustering of specific lipids, such as cholesterol and sphingolipids, into highly condensed domains (termed lipid rafts). Hence such domains are distinct from the remaining membrane by their lipid structure (liquid-ordered vs. -disordered domains). Here, we directly visualize membrane lipid structure of living cells by using two-photon microscopy. In macrophages, liquid-ordered domains are particularly enriched on membrane protrusions (filopodia), adhesion points and cell–cell contacts and cover 10–15% of the cell surface at 37°C. By deconvoluting the images, we demonstrate the existence of phase separation in vivo. We compare the properties of microscopically visible domains (<1 μm²), with those of isolated detergent-resistant membranes and provide evidence that membrane coverage by lipid rafts and their fluidity are principally governed by cholesterol content, thereby providing strong support for the lipid raft hypothesis.

LAURDAN experiments in cells
The role of lipid composition in the skin stratum corneum (SC)
Ceramide/cholesterol/fatty acid mixtures
Human skin's stratum corneum lipid membrane composition (weight and molar percentage)

<table>
<thead>
<tr>
<th>Cholesterol</th>
<th>Ceramides</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>15</td>
<td>16</td>
</tr>
</tbody>
</table>

**FREE FATTY ACIDS**

<table>
<thead>
<tr>
<th>Chain Length</th>
<th>Ratio (mol:mol)</th>
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<tbody>
<tr>
<td>C20:0</td>
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<tr>
<td>C21:0</td>
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<td>C30:0</td>
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</tr>
</tbody>
</table>
Skin Ceramides/Chol/skin fatty acids

After electroformation

Temperature: room

No formation of spherical Vesicles but formation of Sponge-like structures.

Presence of different lipid domains may be related to skin permeability.
LAURDAN GP measurements show three different phase coexistence regimes (in agreement with DSC experiments) depending on the temperature. At skin physiological temperatures there are coexistence of two ordered phases (gel/gel).

skin Ceramides/Chol/skin fatty acids 1:0,9:0,4
Presence of ordered lipid domains at skin physiological temperatures is pH dependent. Regulation of skin permeability properties?
Side view 3D

Top view

skin Ceramides/Chol/skin fatty acids
After electroformation
Temperature: room

Stratum Corneum from skin tissue
Muchas Gracias Enrico!!
y Feliz Cumpleaños...

CONCLUSION

A novel microscopic picture of two-component lipid membranes is presented in this work. With our experimental approach, we have the advantage of correlating lipid phase behavior with the lateral topology of the GUVs. The direct observation of lipid domain characteristics such as size, shape, and dynamics in GUVs composed of phospholipid mixtures having different polar headgroups but equal hydrophobic chain length size or equal polar headgroups but different hydrophobic chain length size offers direct experimental evidence of the temperature behavior of these lipid samples. The combination of the two-photon excitation images, the polarization of the excitation light, and the fluorescent and partition properties of LAURDAN, PRODAN, and N-Rh-DPPE molecules in the different lipid phases offer a unique advantage in the study of phase coexistence in different lipid mixtures.

We thank Dr. J. D. Muller for the help with the chamber design and for the chamber construction and Dr. D. M. Jameson for critical reading of the manuscript.

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REFERENCES


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