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WATER POCKETS IN LIPID MEMBRANES 
EVALUATED BY FTIR SPECTROSCOPY

Significance of Water interphase for peptide/enzymes activities in Lipid Membranes
MEMBRANES OR WATER

MEMBRANE THEORY

Bilayer as a dielectric slab

Permeability barrier for water and ions

ASSOCIATION INDUCTION HYPOTHESIS
Water layers adjacent to lipid bilayer are 1 nm thickness with 20 water molecules per lipid.

Excluded volume for polar solutes. Exclusion zone
WATER INTERPHASE

NOT INTERFACIAL WATER
PURPOSES

To show

ELECTRICAL

THERMO DYNAMICAL

STRUCTURAL PROPERTIES

of water interphases in relation to:

Stability and reactivity of enzymes

Insertion of signal peptides

Regulation

Information
PRESENT CHALLENGES

Toxin and signal peptides with polar and positive aminoacids enter the bilayer

The dielectric slab is hypothesis not consistent

Water inside the membrane
ELECTRICAL PROPERTIES
LIPID ORGANIZED AS MONOLAYER AT AIR WATER INTERFACE

DMPC

DMPE
Expansion - Contraction

Drop volume regulation

Expansion - Contraction

C/ \mu F

Area per molecule / \AA^2

Capacitance of Lipid monolayer

Capacitance of Water on Metal

4% increase of the area
THERMODYNAMIC PROPERTIES

Surface free energy = surface tension changes
DECREASE IN SURFACE PRESSURE $\Pi_c - \Pi$

<table>
<thead>
<tr>
<th>Lipid</th>
<th>$m$</th>
<th>Cut-off</th>
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<tbody>
<tr>
<td>DMPC</td>
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<td>D(Ether)PC</td>
<td>0.352</td>
<td>31.8</td>
</tr>
<tr>
<td>D(Ether)PE</td>
<td>0.280</td>
<td>29.4</td>
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DECREASE IN SURFACE PRESSURE $P_c - P$

is related to the increase of water beyond the hydration water (confined water)

hydration water
(critical packing)

confined water
SURFACE PRESSURE PERTURBATION IS RELATED WITH WATER INTERPHASE ACTIVITY

\[ \Pi = n_w \text{ RT } \ln a_w \]

\[ K = \ln(a_w | a_{wp}) / \ln(a_c | a_{wc}) \]

Bidimensional Solution Of Hydrated Head Groups (Defay & Prigogine 1966; Evans & Skalak, 1978)
# Pairs of membrane-protein or peptides

<table>
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<tr>
<th>Membrane composition</th>
<th>K</th>
<th>Cut off</th>
<th>Protein</th>
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<tr>
<td>DMPC</td>
<td>0.264</td>
<td>41.5</td>
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<td>0.428</td>
<td>39.6</td>
<td>Aqueous protease</td>
</tr>
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<td>PC:SA (10:1)</td>
<td>0.685</td>
<td>35.18</td>
<td>Bacterial S-layer</td>
</tr>
<tr>
<td>PC:Chol:SA (10:2.5:1)</td>
<td>0.519</td>
<td>34.6</td>
<td>Bacterial S-layer</td>
</tr>
<tr>
<td>PC:Chol:SA (10:5:1)</td>
<td>0.328</td>
<td>36.64</td>
<td>Bacterial S-layer</td>
</tr>
</tbody>
</table>
Water is the common intermediary in the interaction of different proteins, peptides or aminocids with lipid membranes

\[ K = \ln \left( \frac{a_w}{a_{wp}} \right) / \ln \left( \frac{a_c}{a_{wc}} \right) \]
STRUCTURAL PROPERTIES OF WATER INTERPHASE

**OH Stretching mode**

\[ \nu = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}} \]

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[Graph showing OH stretching mode with water spectrum at 18°C]
WATER STATE AROUND THE LIPID PHASE TRANSITION
CONCLUSION

- Different water populations are found according to the lipid are in a condensed or expanded state.

- Confined water seems to appear in expanded lipid states.
Thermodynamic and structural link

\[ \Pi = n_w \, RT \, \ln \, a_w \]

Defay & Prigogine 1966; Evans & Skalak (1978)

\[ \Pi = n_w \, RT \, \ln \, g_w \, C_w \]

\[ g_w = A + BT + CT^2 + \ldots \]

A = molecules without H-bond
B = molecules with 1 H-bond
C = molecules with 2 H-bond
D = molecules with 3 H-bond
E = molecules with 4 H-bond

A variety of water populations
$K = \ln\left(\frac{\alpha_w}{\alpha_{wp}}\right)/\ln\left(\frac{\alpha_c}{\alpha_{wc}}\right)$

FRACTALS?

COHERENT DOMAINS?
Summary

• Water domains of confined water appears beyond the hard core hydration shell of lipids.

• Confined water determines the surface free energy of lipid interphases.

• The thermodynamic activity of water confined in the interphase region is correlated with different water structural arrangements according to the lipid state.

• Confined water domains appear to be modified by lateral pressure.
Water can be replaced by trehalose, glicerol, arbutin or sucrose to maintain biological structures in anhidrous state.

Water must be restored for function in order to provide confined water levels.
ENZYME ACTIVITY INCREASES 40% IN RESTRICTED WATER DOMAINS (REVERSE MICELLES OR LIPID INTERPHASES)

Enzymes activities is sensitive to the different water qualities in the different lipid membranes.
# Lipid-Water Ratio in Different Lipid Ensembles of DMPC and DMPE

<table>
<thead>
<tr>
<th></th>
<th>Water per lipid at the break of Bragg spacing (^{64})</th>
<th>Water per lipid in monolayers (^{50, 52})</th>
<th>Water per lipid in micelles (^{65})</th>
<th>Water at phosphate (FTIR) (^{66, 67})</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMPC</td>
<td>12</td>
<td>11</td>
<td>12-14</td>
<td>6</td>
</tr>
<tr>
<td>DMPE</td>
<td>ND</td>
<td>9</td>
<td>4</td>
<td>ND</td>
</tr>
</tbody>
</table>


CATALYTIC EFFICIENCY PARAMETER \( \frac{K_{\text{cat}}}{K_m} \) (mM\(^{-1}\).seg\(^{-1}\)) OF PROTEOLITIC ACTIVITY OF RENNET FROM MUCOR MIEHEI ADSORBED TO DIFFERENT LIPID INTERPHASE.
INFORMATION

Genomics $\rightarrow$ Lipidomics $\rightarrow$ aquaomics

Adapted from R. Tsenkova
SPECULATIONS

Translocons
(based on geometrical criteria)

Waterons
(based on changes in the multiple water populations in the lipid interphase with different free energy content)
EXPERIMENTS IN PROCESS

See you in 2013
MUCHAS GRACIAS-THANK YOU!