Glycolipids:
A Question of Balance
Glicolipidos:
una cuestión de equilibrio

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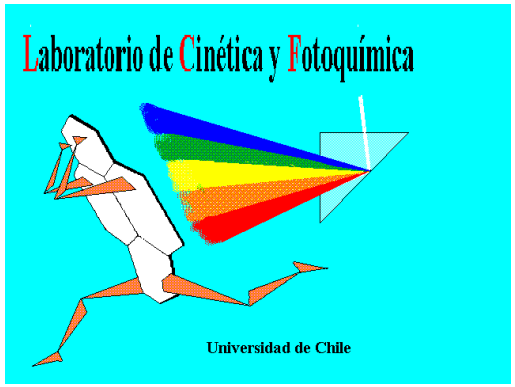


Glucolípidos:

Biomoléculas compuestas de una cadena lipídica (lipofílica) y un monosacárido u oligosacárido como grupo hidrofílico

Los glucolípidos más comunes contienen galactosa, manosa, fucosa, glucosa, glucosamina, galactosamina o ácido siálico como azúcar.

1 Esteres de Sacarosa (Monoacilados)

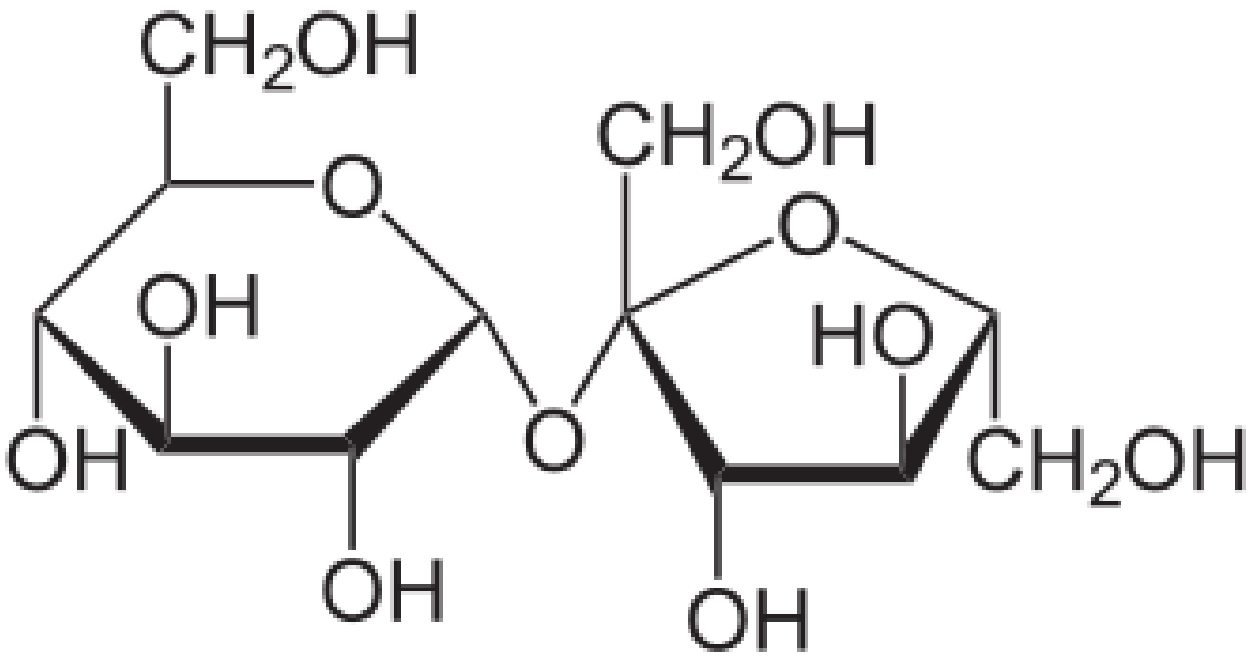
- 1.1 Solubilización de Membranas
- 1.2 Efecto de Bloqueo
- 1.3 Caracterización de micelas reversas
- 1.4 Micelas mixtas

2 Esteres de Sacarosa (Dialcoxi)

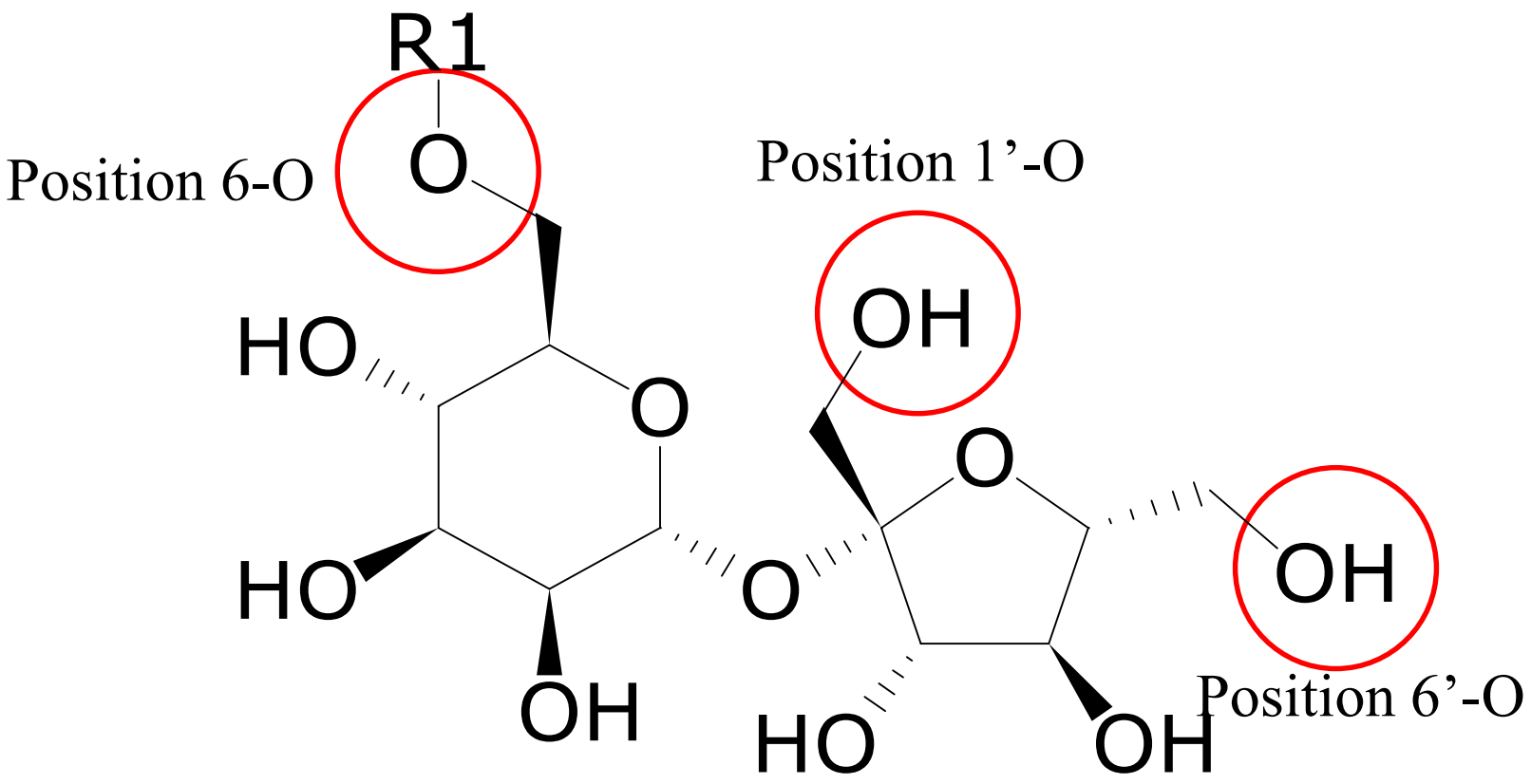
3 Derivados de Lactosa

4 Derivados de Manosa

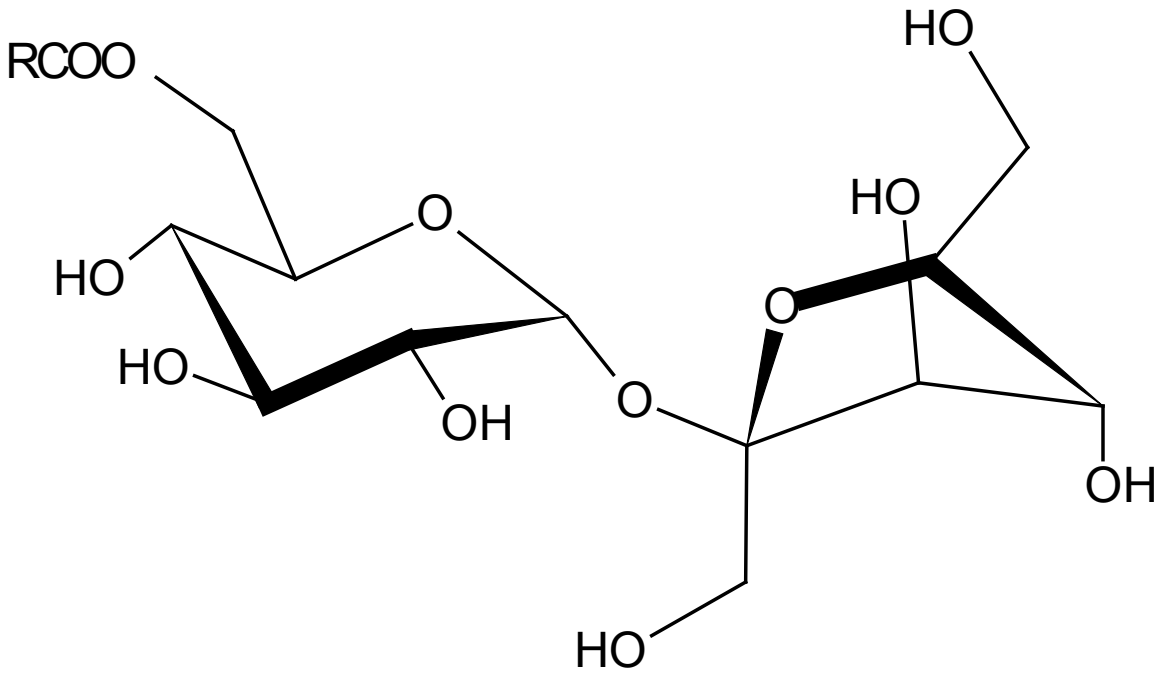
1. Sacarosa



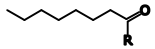
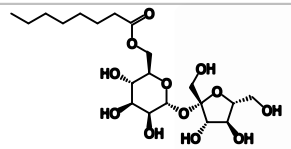
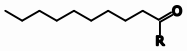
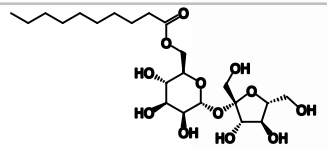
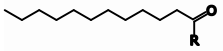
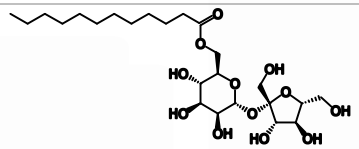
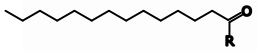
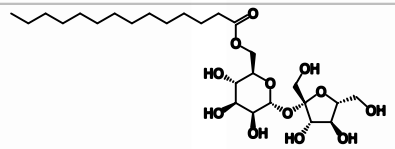
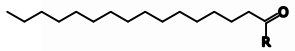
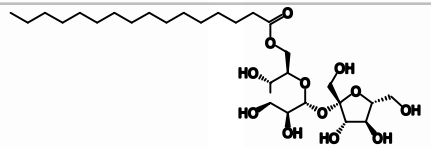
Esterificación de Sacarosa



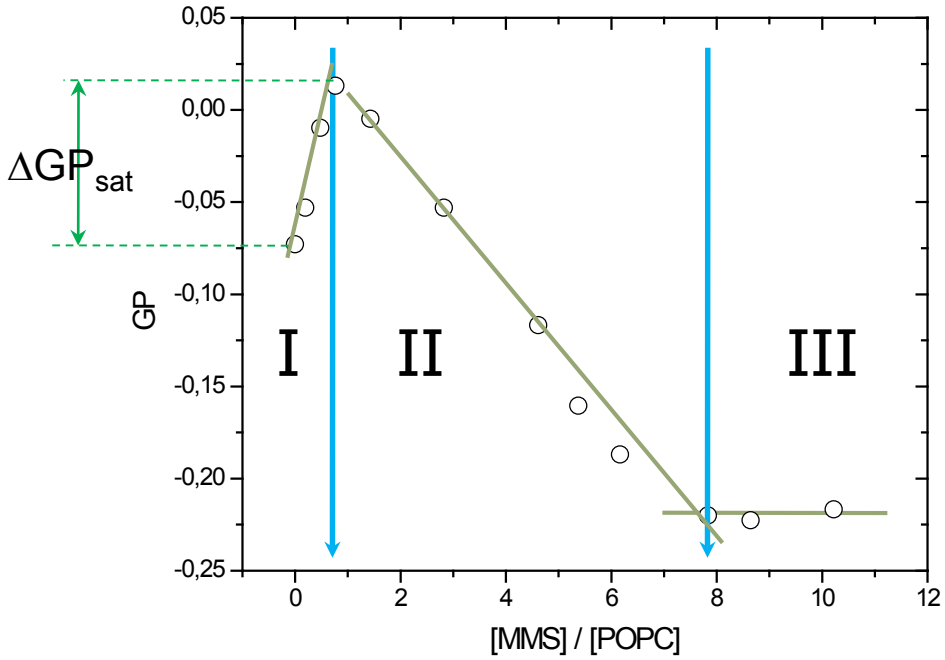
Estructura General de ésteres 6-O sacarosa



Sucrose derivatives

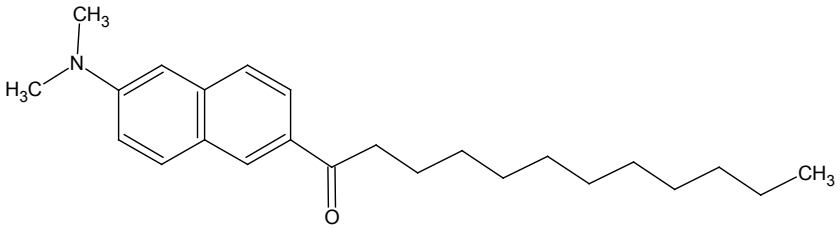
Sustituyente	nombre	Molécula
	**MOS**	
	**MDS**	
	**MLS**	
	**MMS**	
	**MPS**	

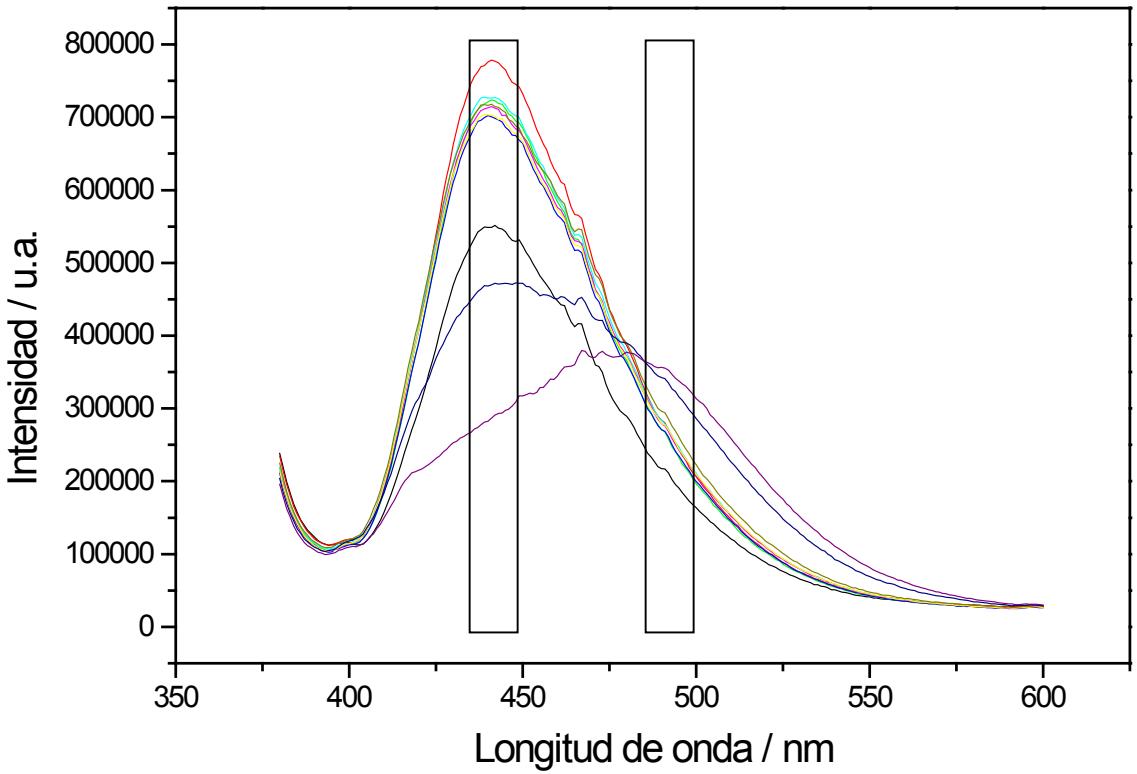
1.1 Modelo de Solubilización de tres etapas



I Saturation
II Solubilization
III Mixed Micelles

Laurdan on Cuvette





$$GP = \frac{I_{blue} - I_{red}}{I_{blue} + I_{red}}$$

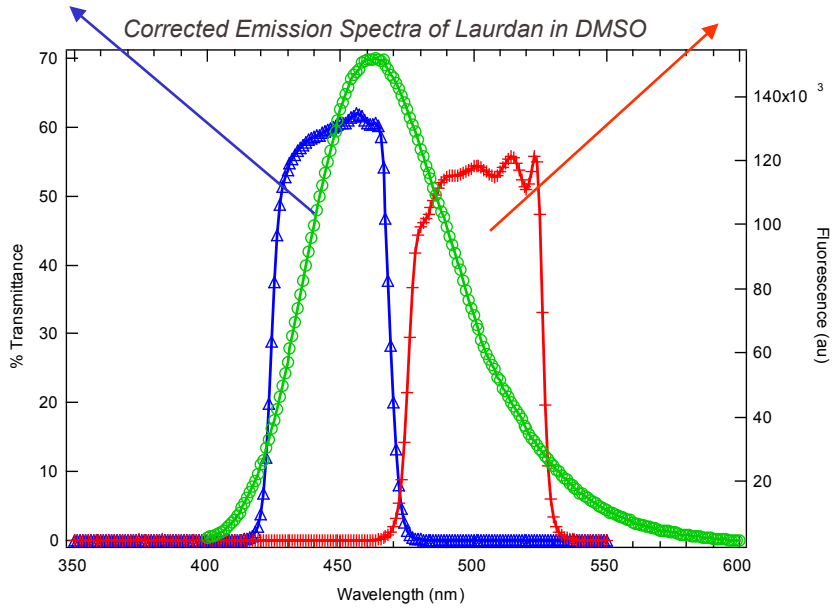
$I_{blue} = 440nm$

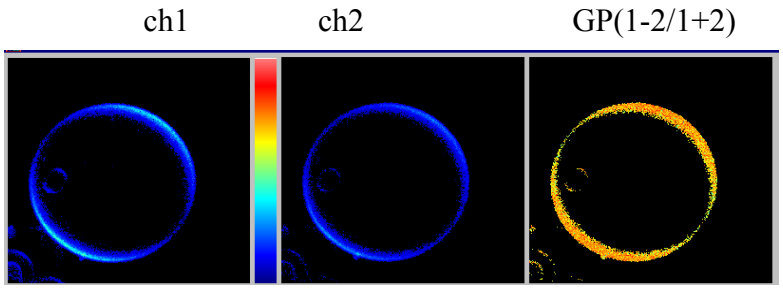
$I_{red} = 490nm$

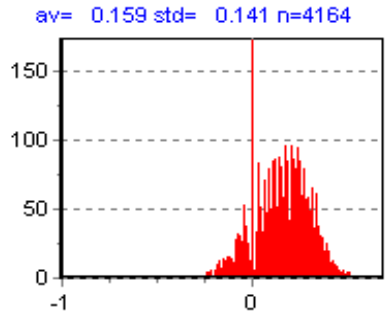
GP on the two-channel microscope

ch1
Blue filter

ch1
Red filter



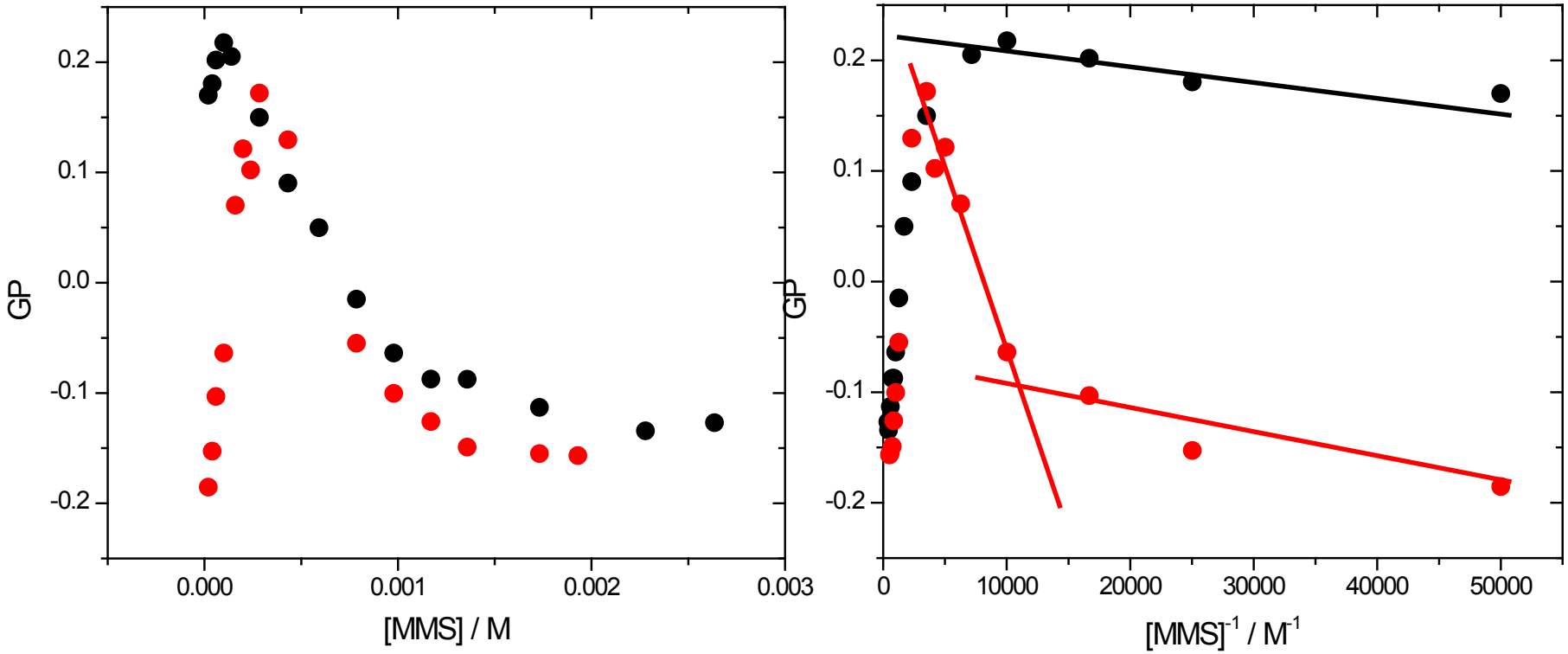




GP histogram

Measurement of Laurdan GP in the GUVs using Sim-FCS program

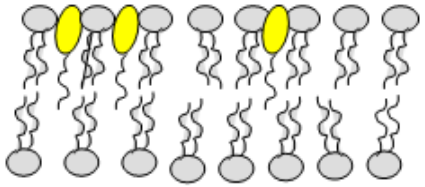
Vesicles Solubilization Profiles in the presence of Cholesterol



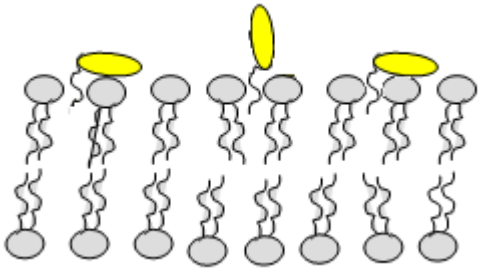
Solubilization of DODAC SUV`s (●) and POPC vesicles (●) with a 10% of cholesterol, [Lip] = 0.2mM , 25ºC.

Proposed Model

Without cholesterol

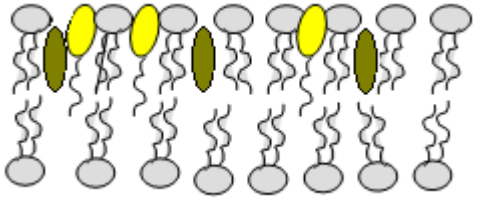


For **POPC bilayers**, the sucrose moiety of MMS could locate between the lipid heads.



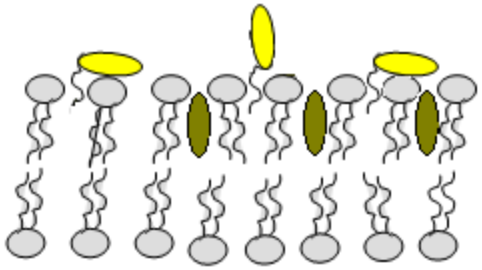
For **DODAC bilayers**, the sucrose moiety of MMS could locate over the lipid heads.

With cholesterol

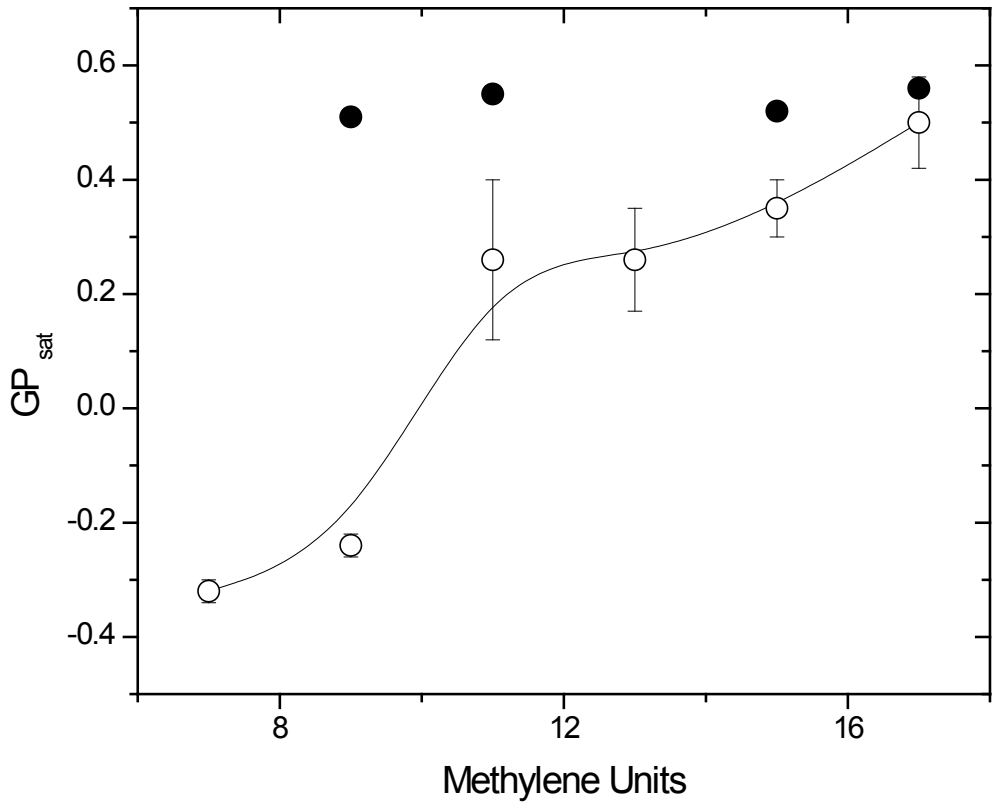


The presence of cholesterol in the bilayer of POPC liposomes would imply a major disorder when MMS is incorporated.

The presence of cholesterol for DODAC liposomes does not affect the surrounds of Laurdan.



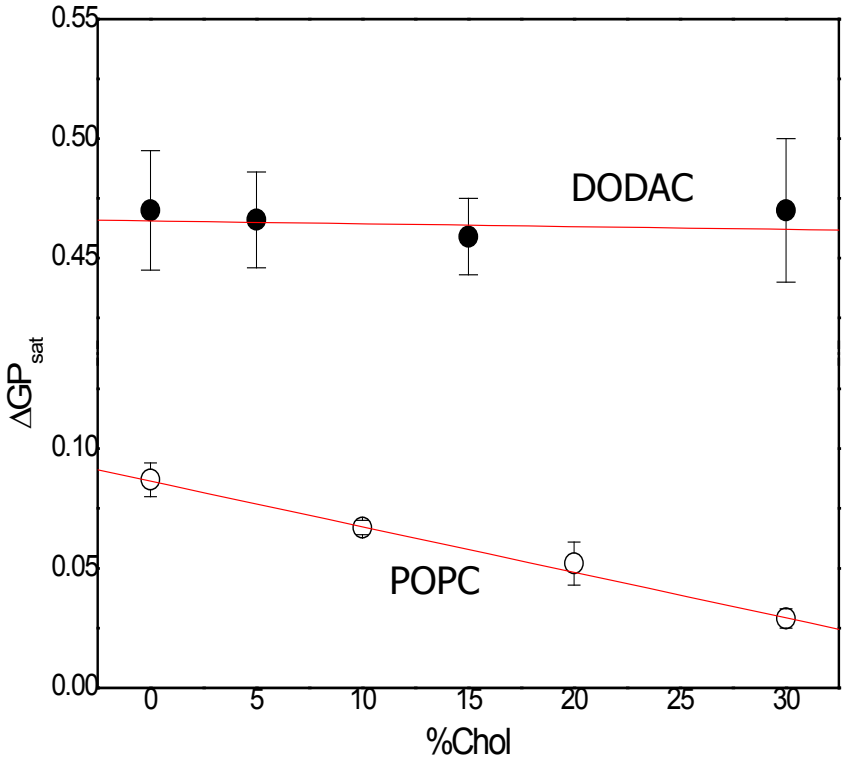
Effect of methylene units on GP_{sat}



GP_{sat} for DODAC SUV`s (○) and DPPC vesicles (●) with the series of sucrose esters at 25°C

Effect of cholesterol on ΔGP_{sat}

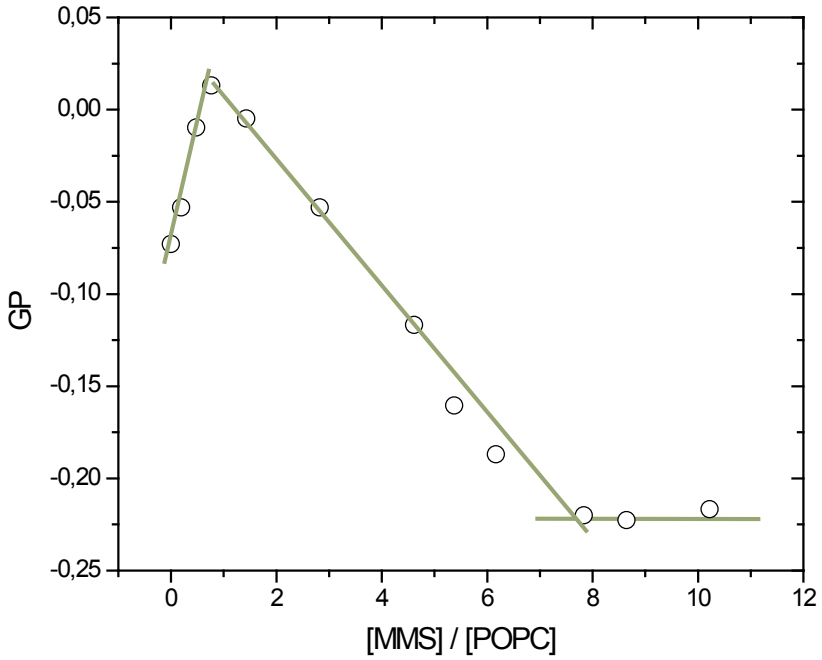
POPC-chol and DODAC-chol plus MMS



For DODAC, the ΔGP_{sat} is nearly independent of the amount of cholesterol present on the bilayer.

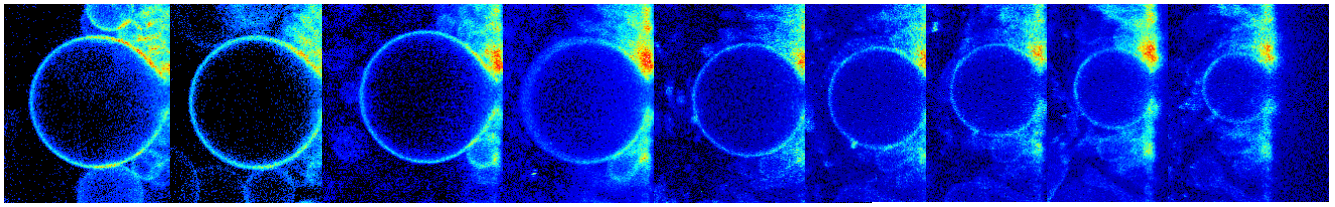
However for POPC there is a small, but clear decrease on the value of ΔGP_{sat} as cholesterol increase.

Three Stage Model of Solubilization

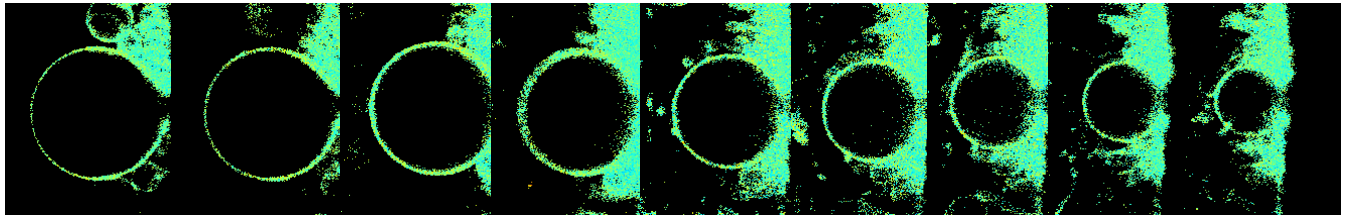


Images of Solubilization of GUVs

Two-photon intensity image: POPC plus 1μM MMS



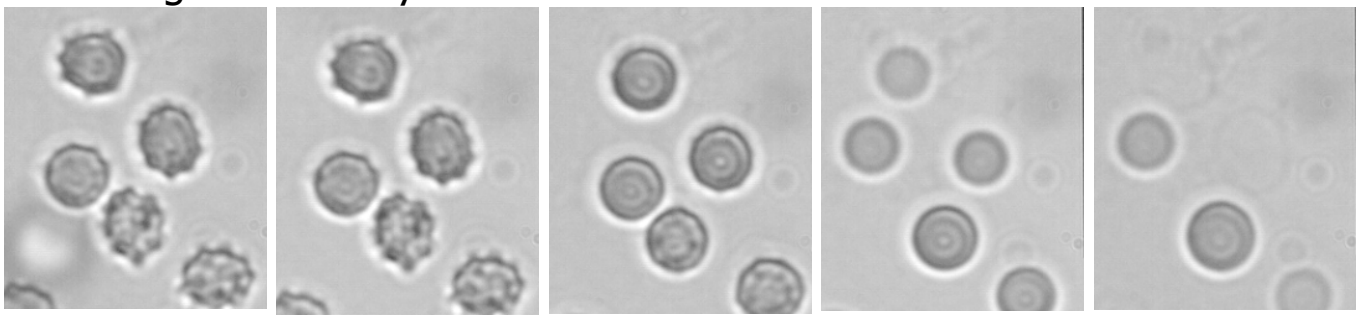
Two-photon GP image: POPC plus 1μM MMS



GP measurements indicate that there are not changes in the water content of the membrane during the solubilization process of POPC by myristyl sucrose (MMS).

Erythrocytes and MMS

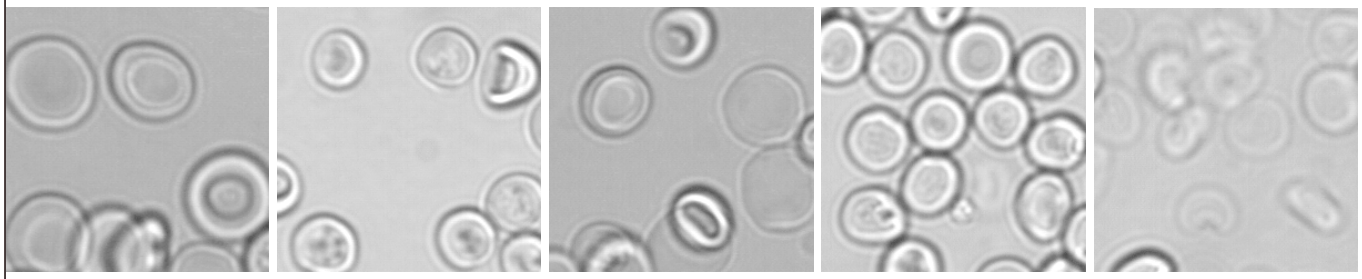
Starting as echinocytes



0 → 10 min

time

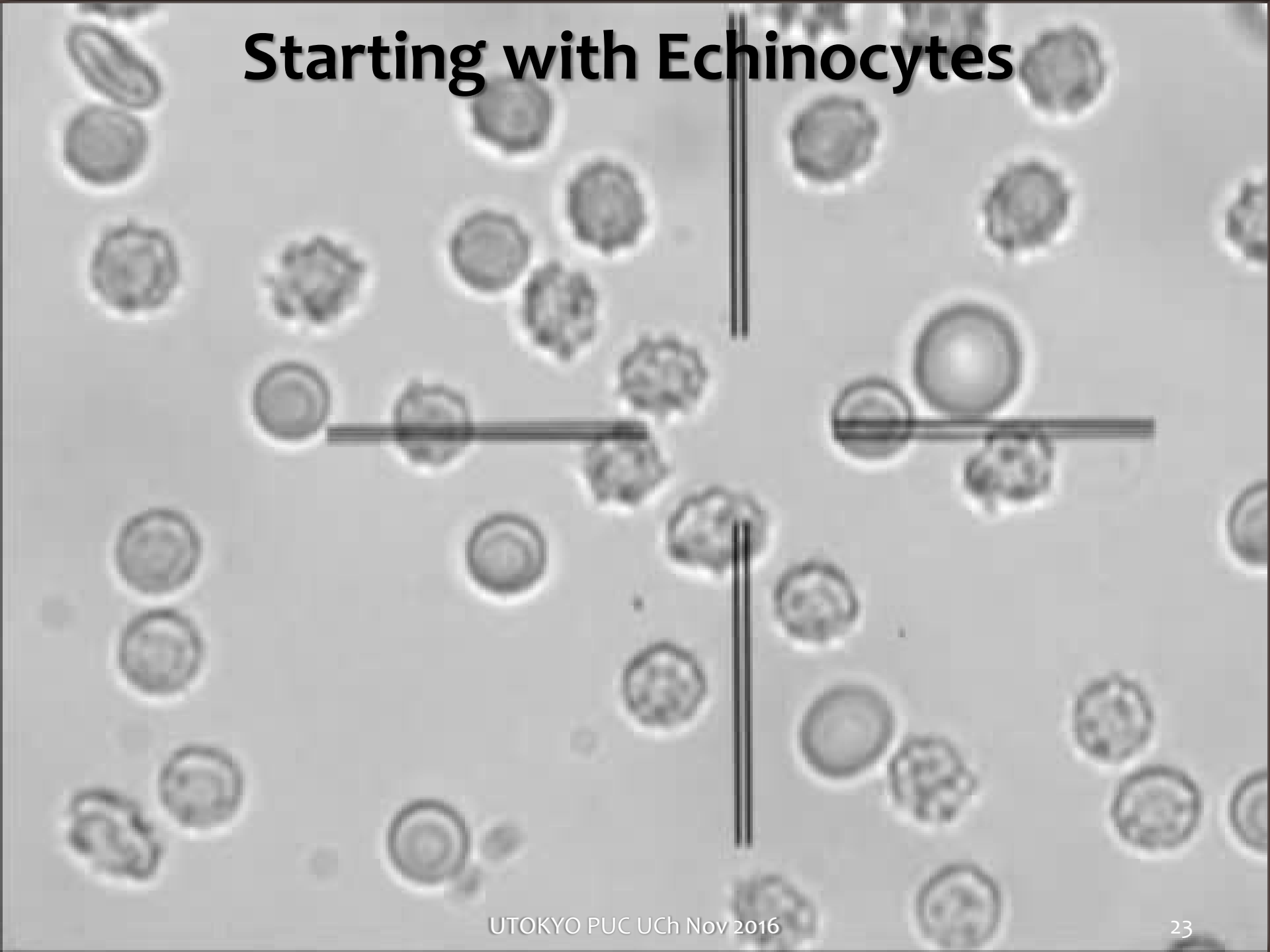
Starting as biconcave disc



0 → 8

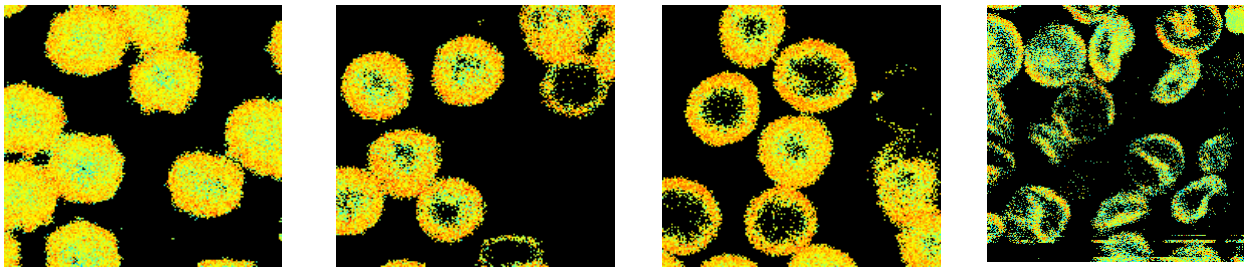
MMS concentration (uM)

Starting with Echinocytes

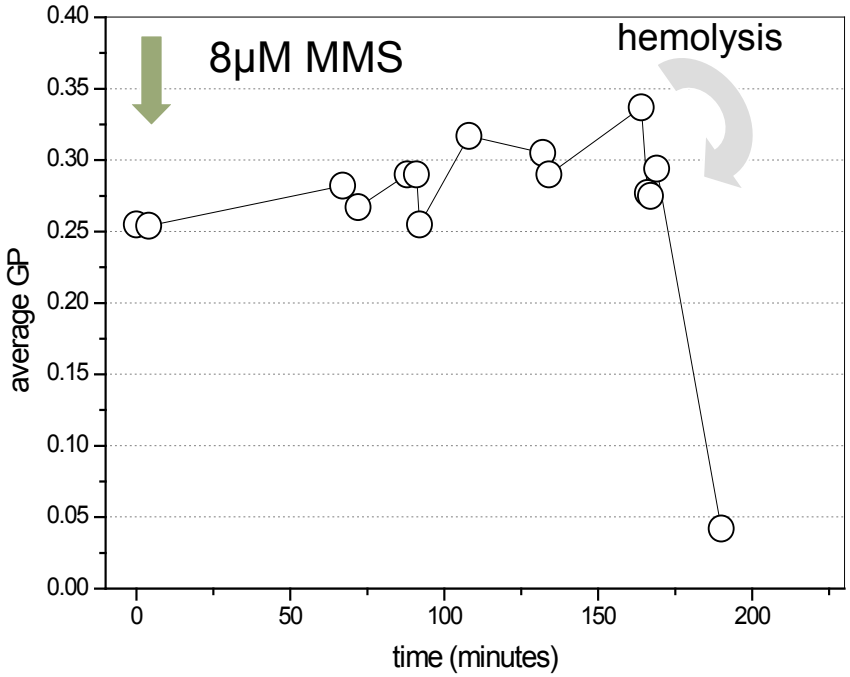


Starting with Echinocytes

Water acces in the membrane during the interaction



time

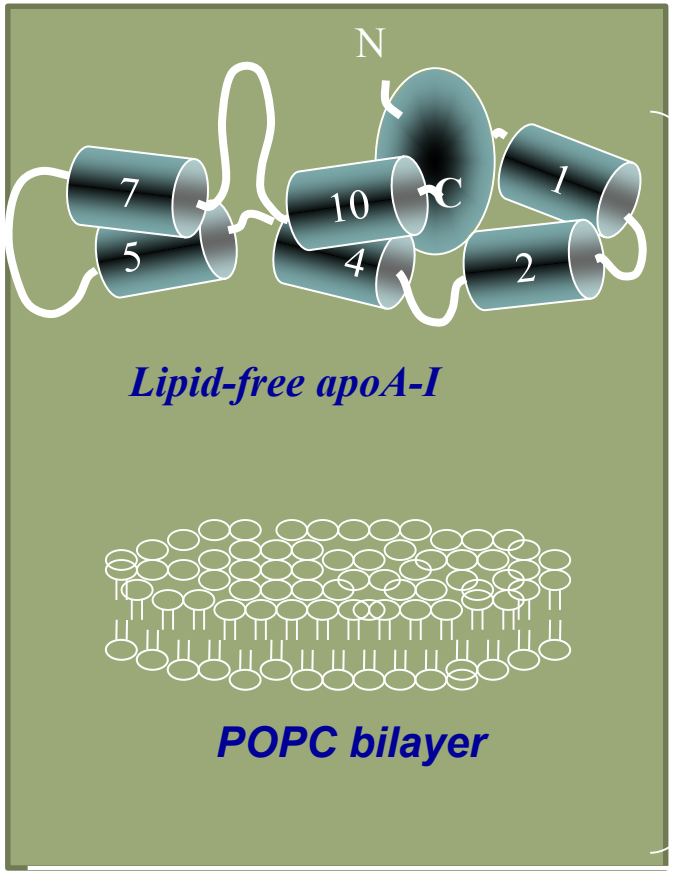


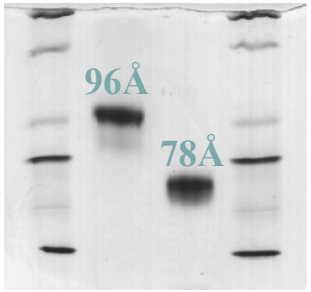
Water content increases slightly before hemolysis.

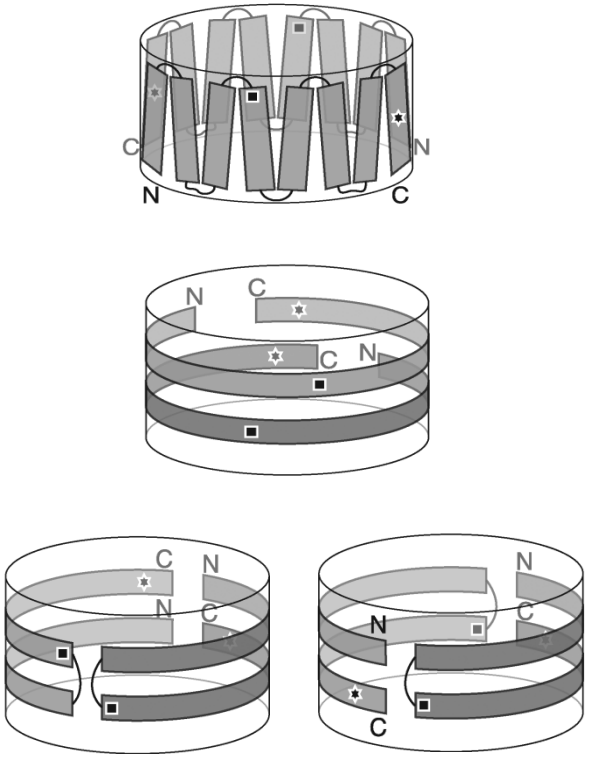
After hemolysis water content decreases.

1.2 Surface Blocking Effect

Reconstituted HDL particles: Human Apolipoprotein A-I was purified from blood plasma. Reconstituted discoidal particles containing POPC and 2 molecules of apo A-I per complex are prepared by the sodium cholate dialysis method. The homogeneity and hydrodynamic diameters of the rHDLs were estimated by native (8-25%) polyacrylamide gel electrophoresis on a Pharmacia System.



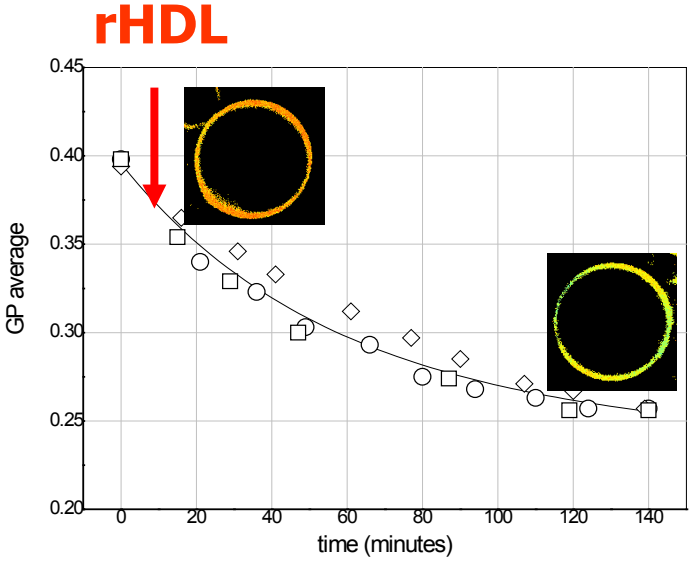


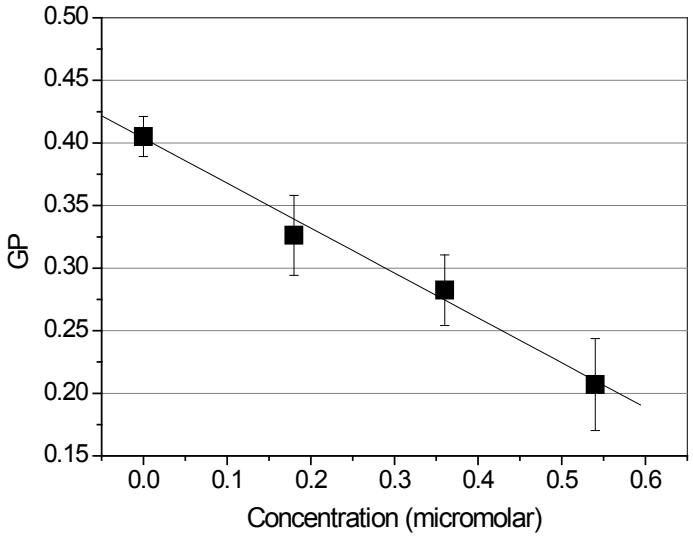


Surface Blocking Effect

GP changes after addition of rHDL to a GUV made of POPC plus 32%cholesterol

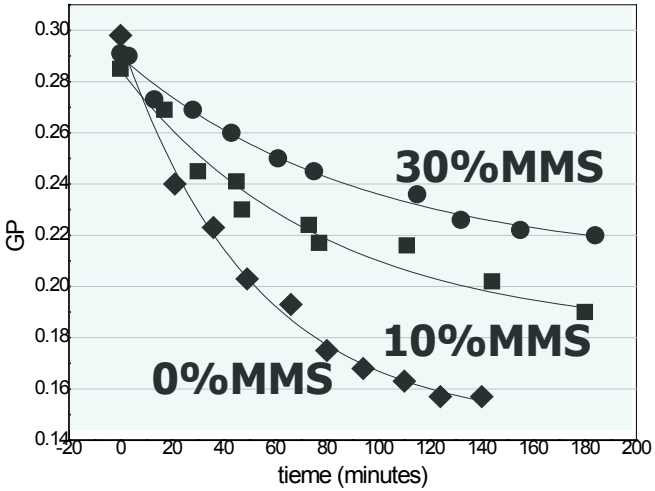
GP changes after 2 hours incubation of GUVs made of POPC plus 32% cholesterol with different concentration of rHDL





Surface Blocking Effect

Kinetics after addition of 10ug/ml rHD to GUVs.

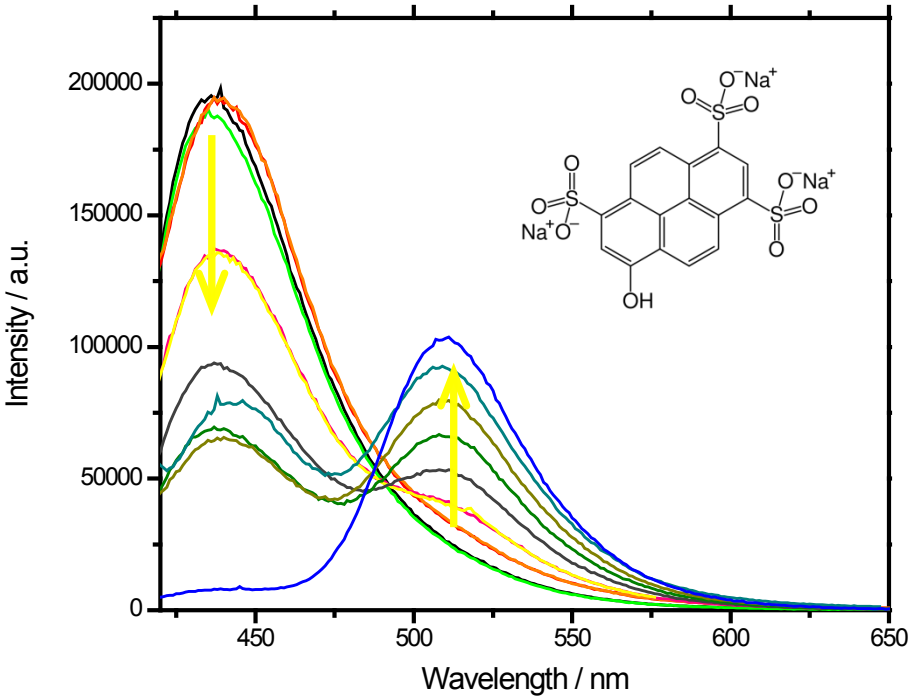


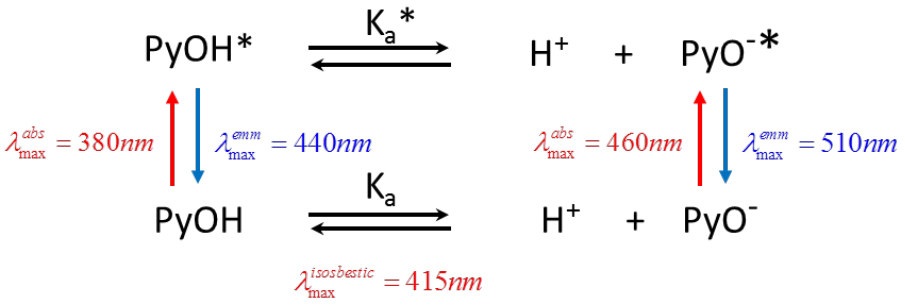
Although the GP value of the POPC-32% Chol does not change when adding MMS, the accessibility of cholesterol has been modified.

rHDL particles can detect the difference!.

1.3Reversed Micelles

Reversed Micelles

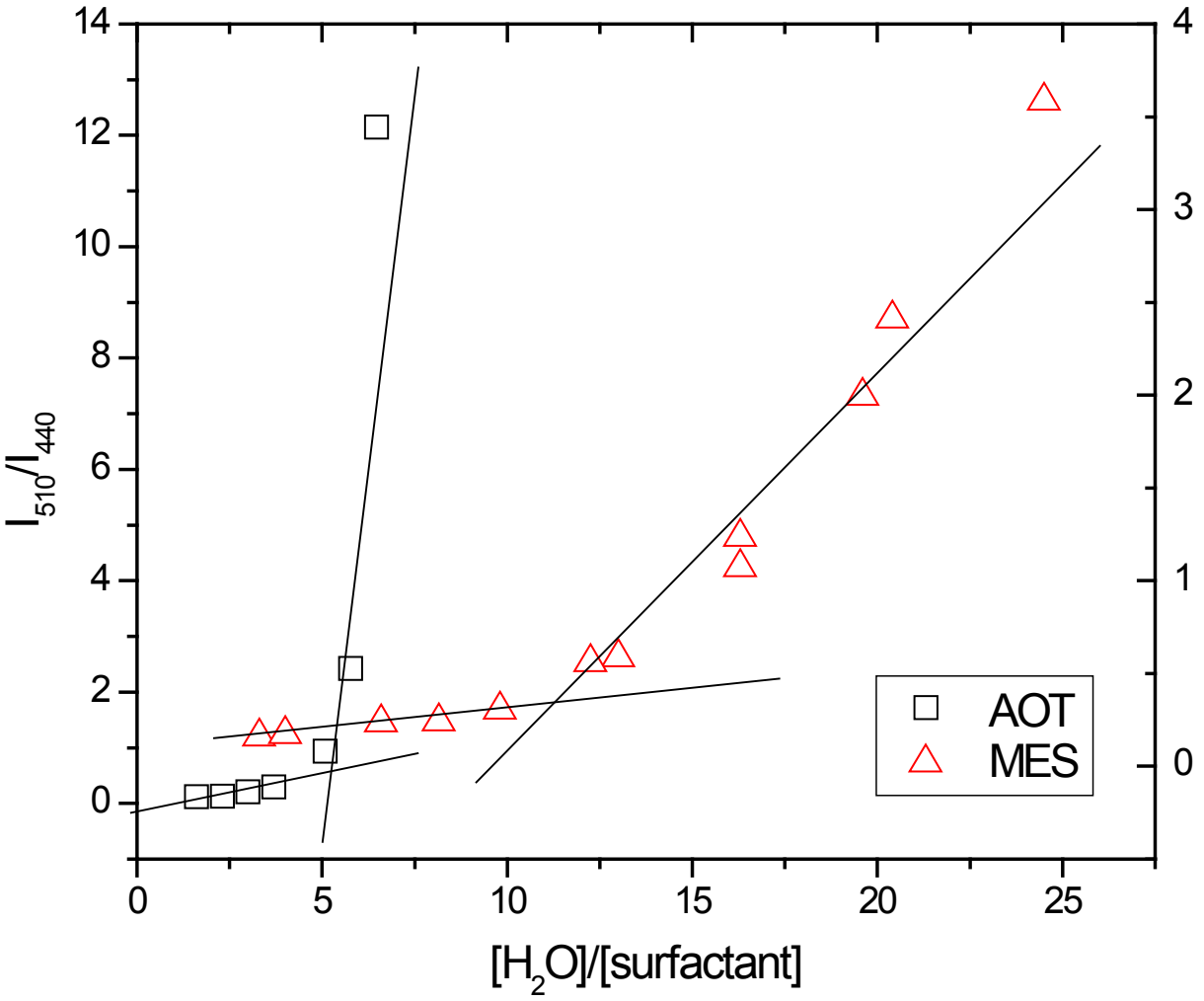




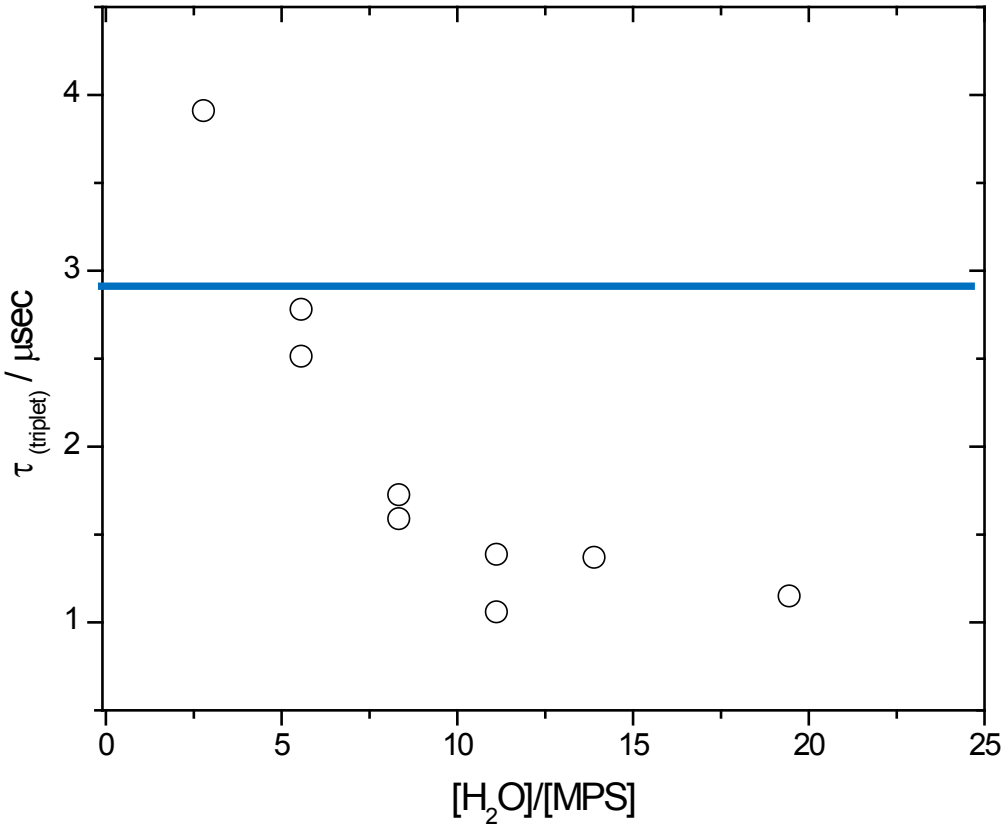
Emission spectra of pyranine located inside the aqueous pool of MPS reversed micelles in chloroform with increasing proportion of water.

Borsarelli, C. D.; Braslavsky, S. E. *Journal of Physical Chemistry B* **1997**, *101*, 6036

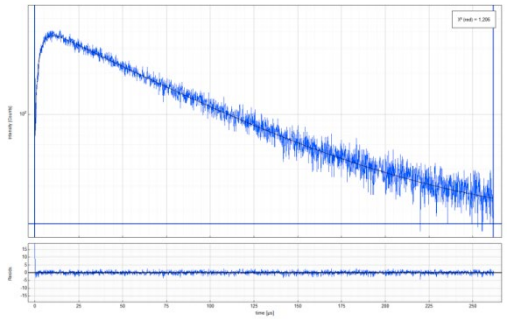
Sucrose Ester Reversed Micelles



Sucrose Ester Reversed Micelles



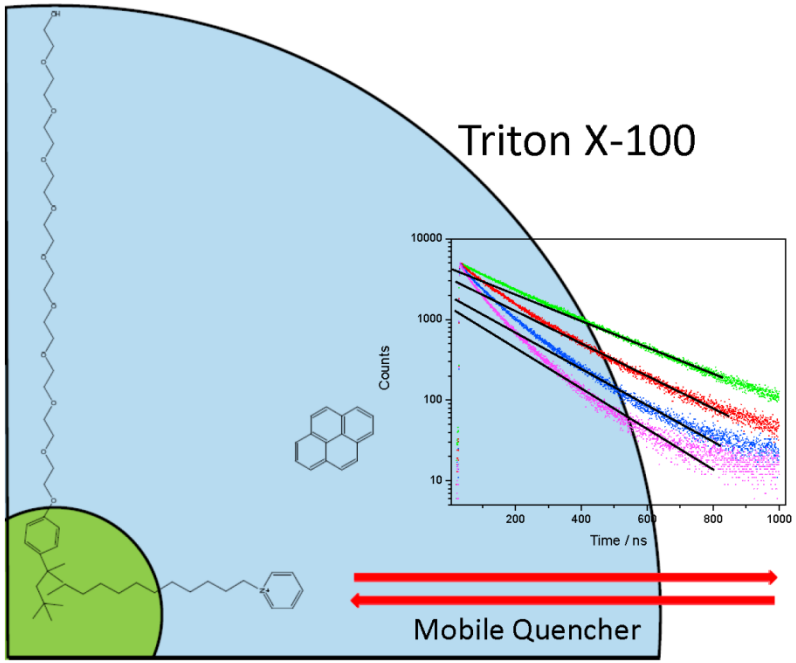
Singlet oxygen emission

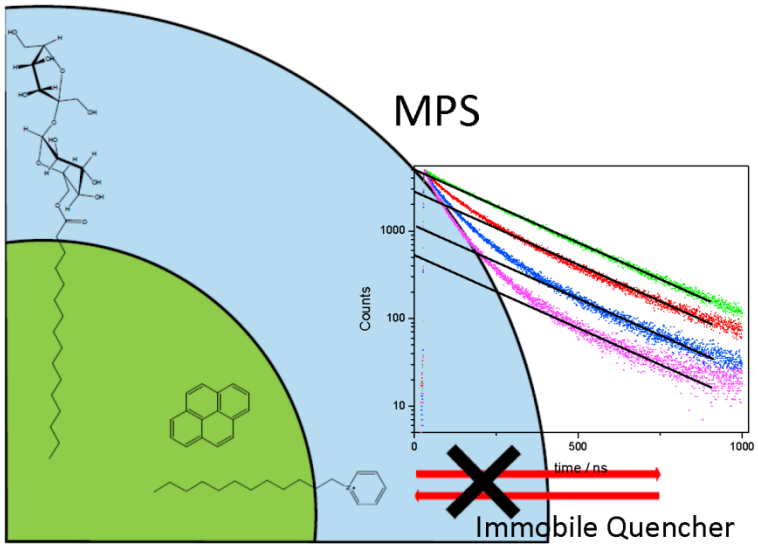


$$I(t) = A\left(e^{-t/\tau_1} - e^{-t/\tau_2}\right)$$

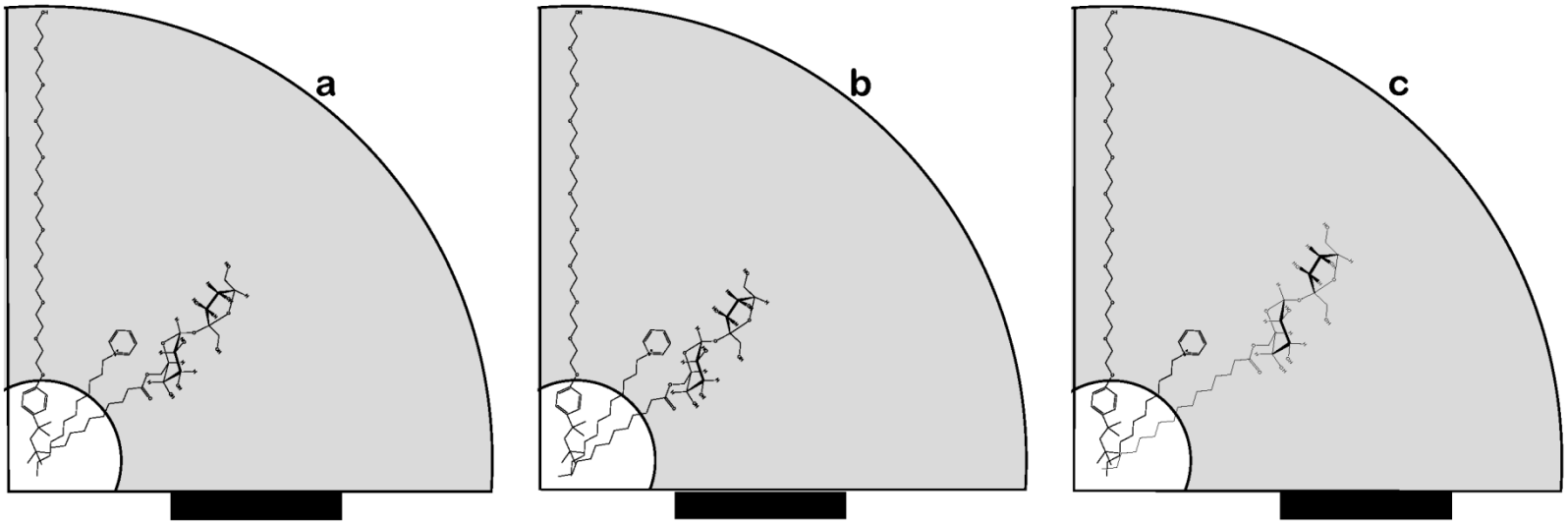
1.4Mixed Micelles

1.4 Effect on the Palisade of Direct Mixed Micelles





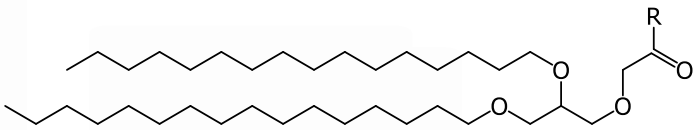
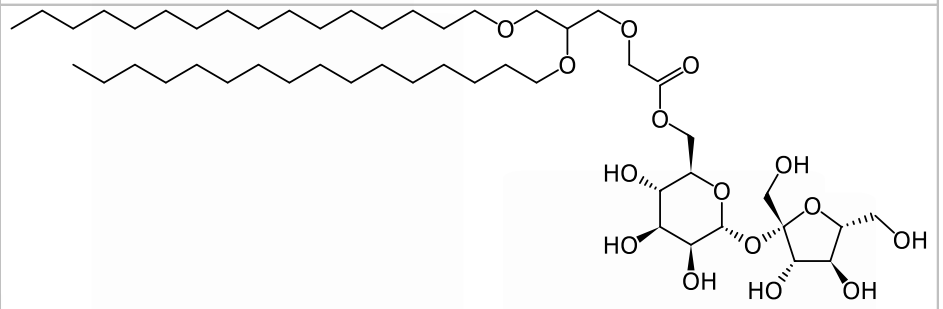
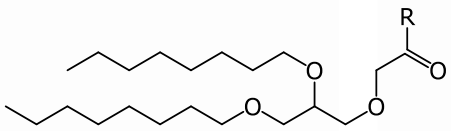
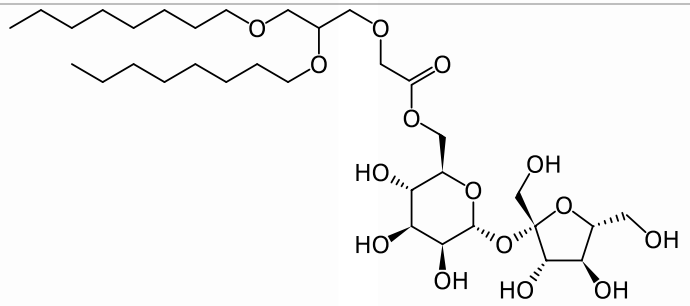
Direct Mixed Micelles



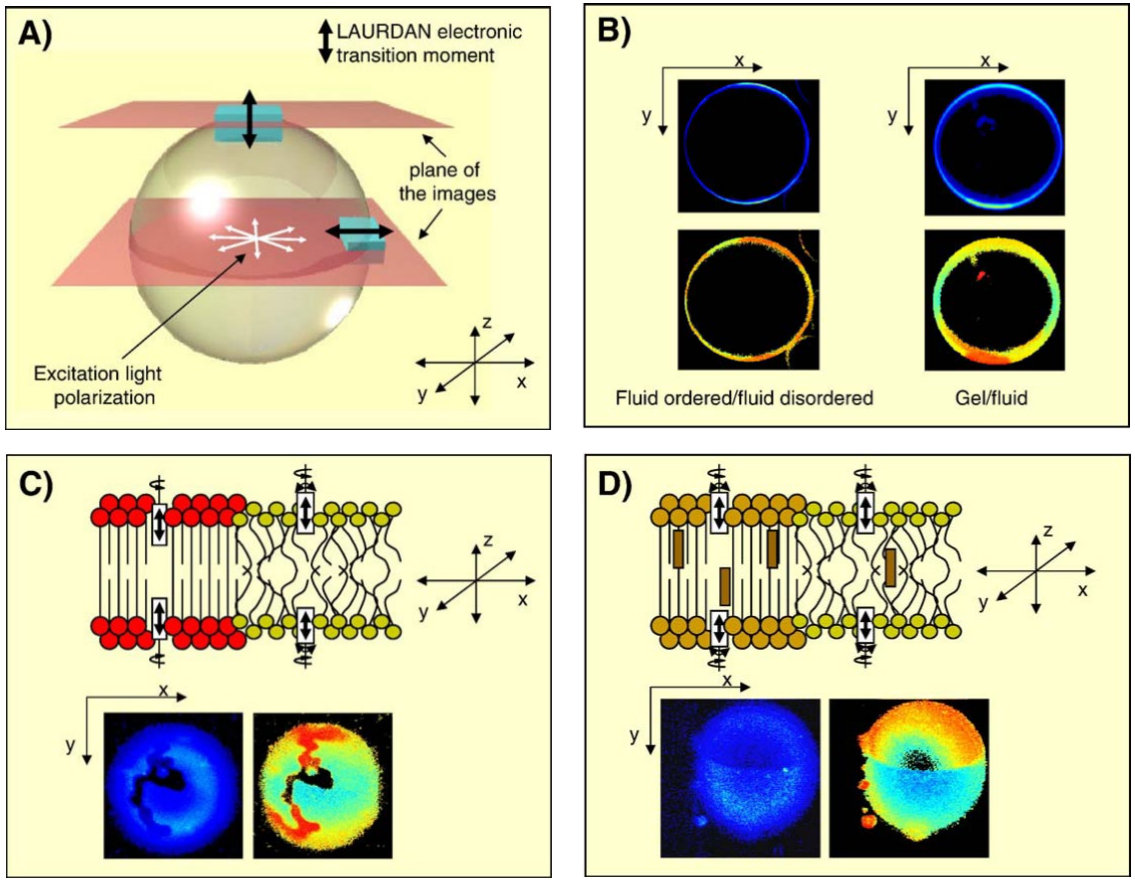
The disturbing effect on the micelle core, consequence of the difference in size of both hydrophobic tails (Triton X-100 and SE), plays an important role, and the blocking effect can be overcame when the hydrophobic tails of SEs are too short or too long compared with Triton alkyl chain.

2 Sucrose Esters (Dialcoxy)

2 Sucrose Esters (Dialcoxy)

Substituent	name	Molecule
	**DPGXS**	
	**DOGXS**	

Photoselection effect in Laurdan emission

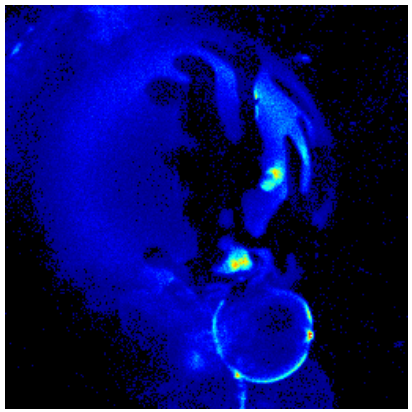


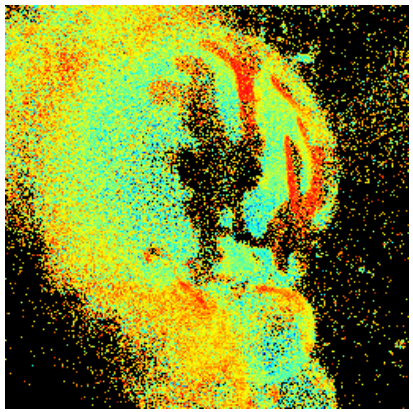
From: Bagatolli, L. A. (2006). "To see or not to see: Lateral organization of biological membranes and fluorescence microscopy." Biochimica Et Biophysica Acta-Biomembranes **1758**(10): 1541-1556.

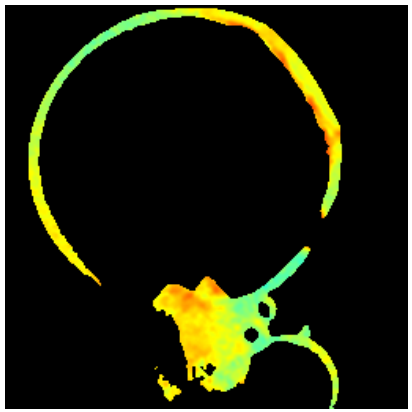
GUVs

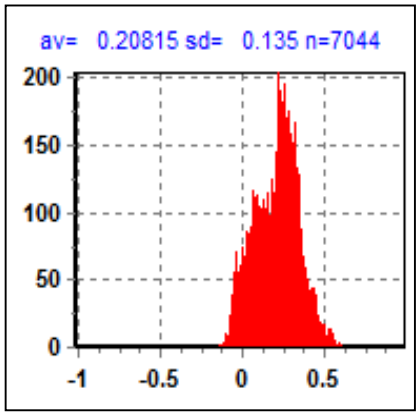
POPC:DPGXS (30% POPC) GUVs

50ºC



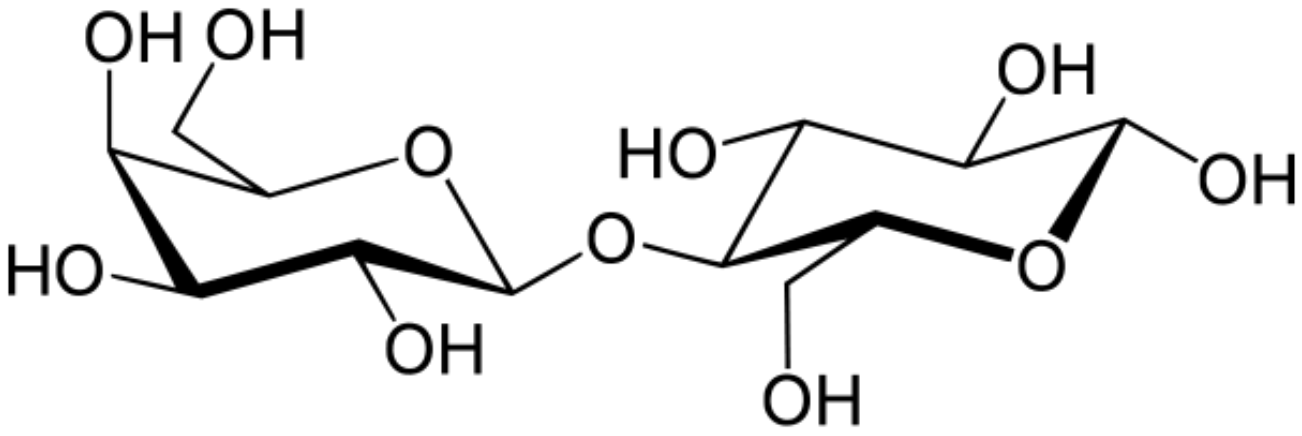




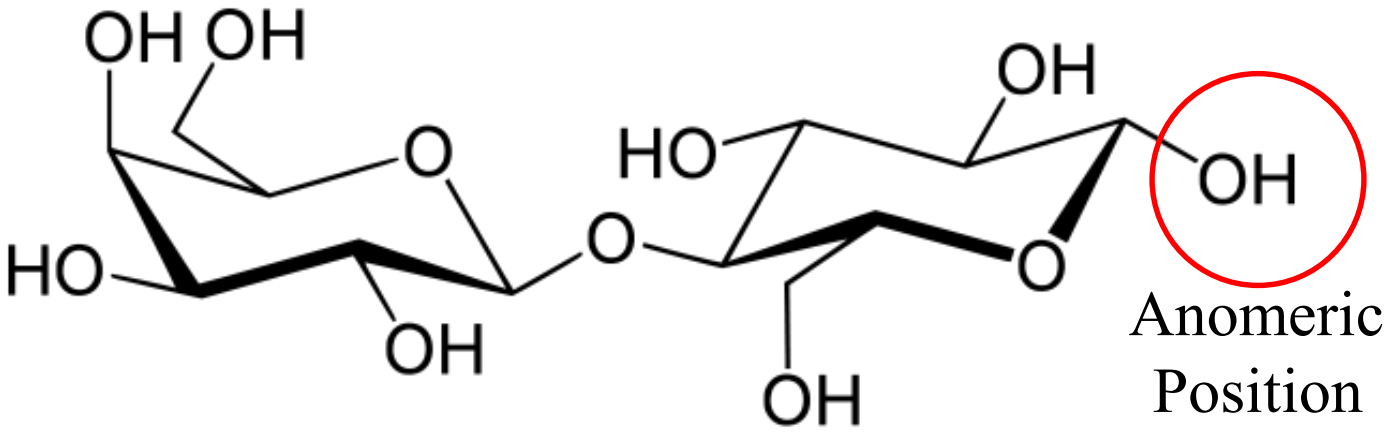


3 Lactose Derivatives

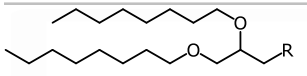
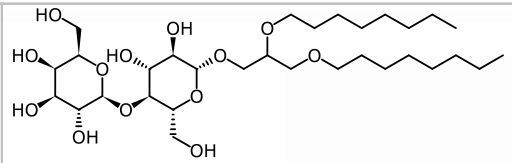
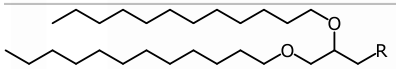
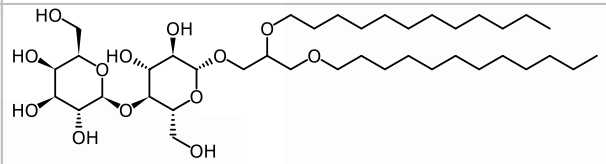
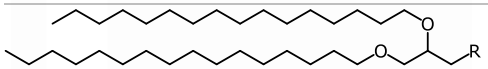
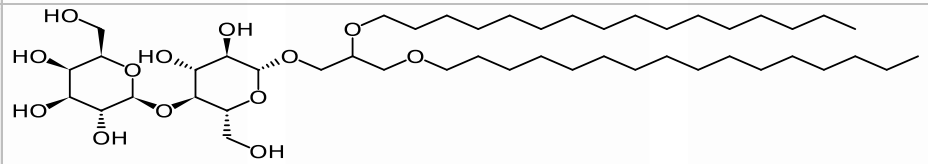
Lactose



Lactose Reaction



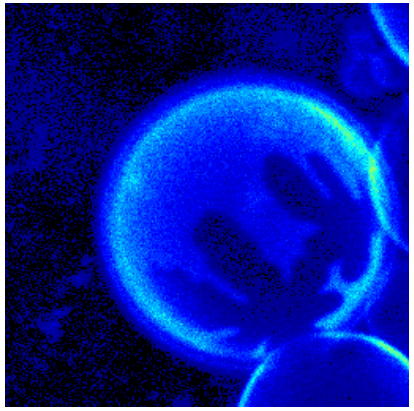
Lactose Derivatives

	DOL	
	DLL	
	DPL	

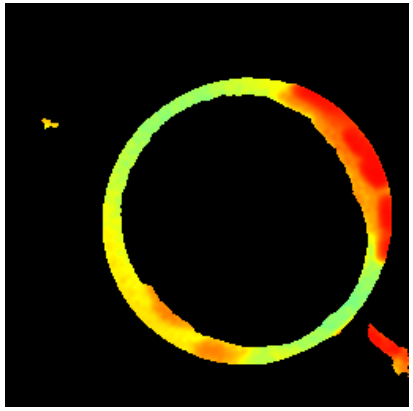
GUVs

30°C POPC:DPL (36% POPC) GUVs

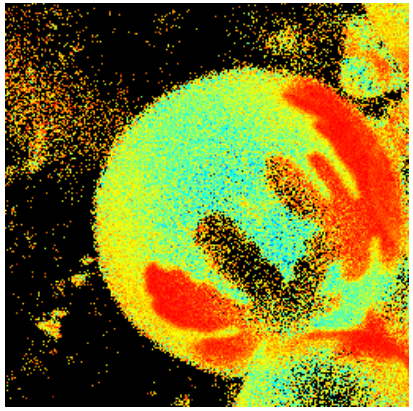
top
Intensity



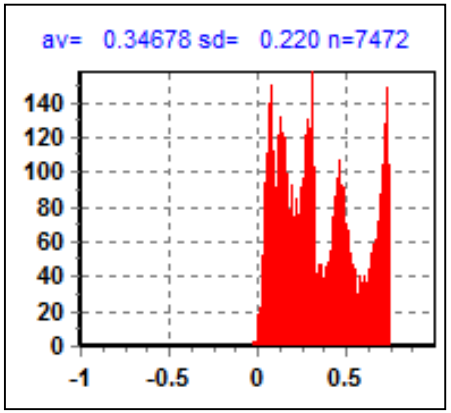
top
GP Image

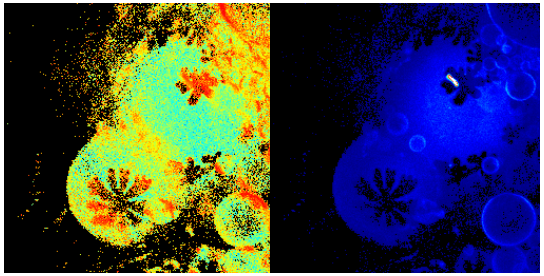


center
GP Image



GP Histogram

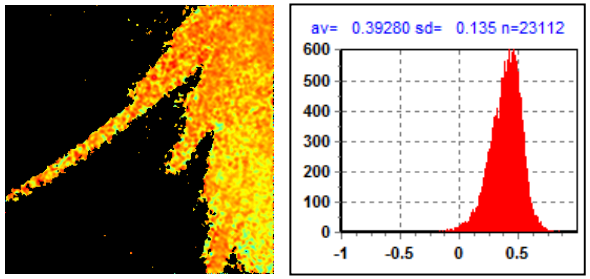


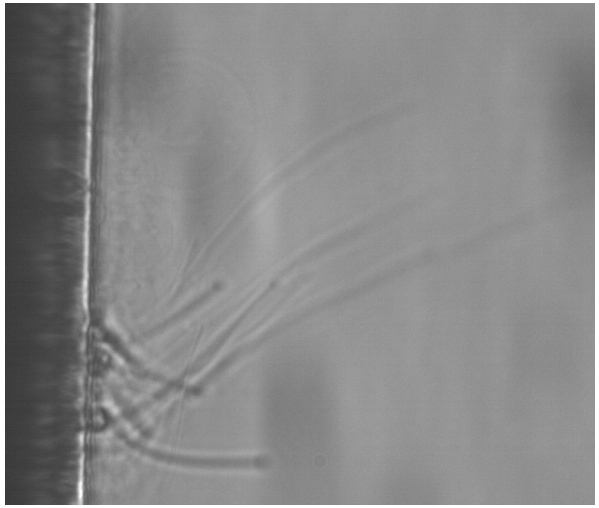


GUVs plus Tubular Structures

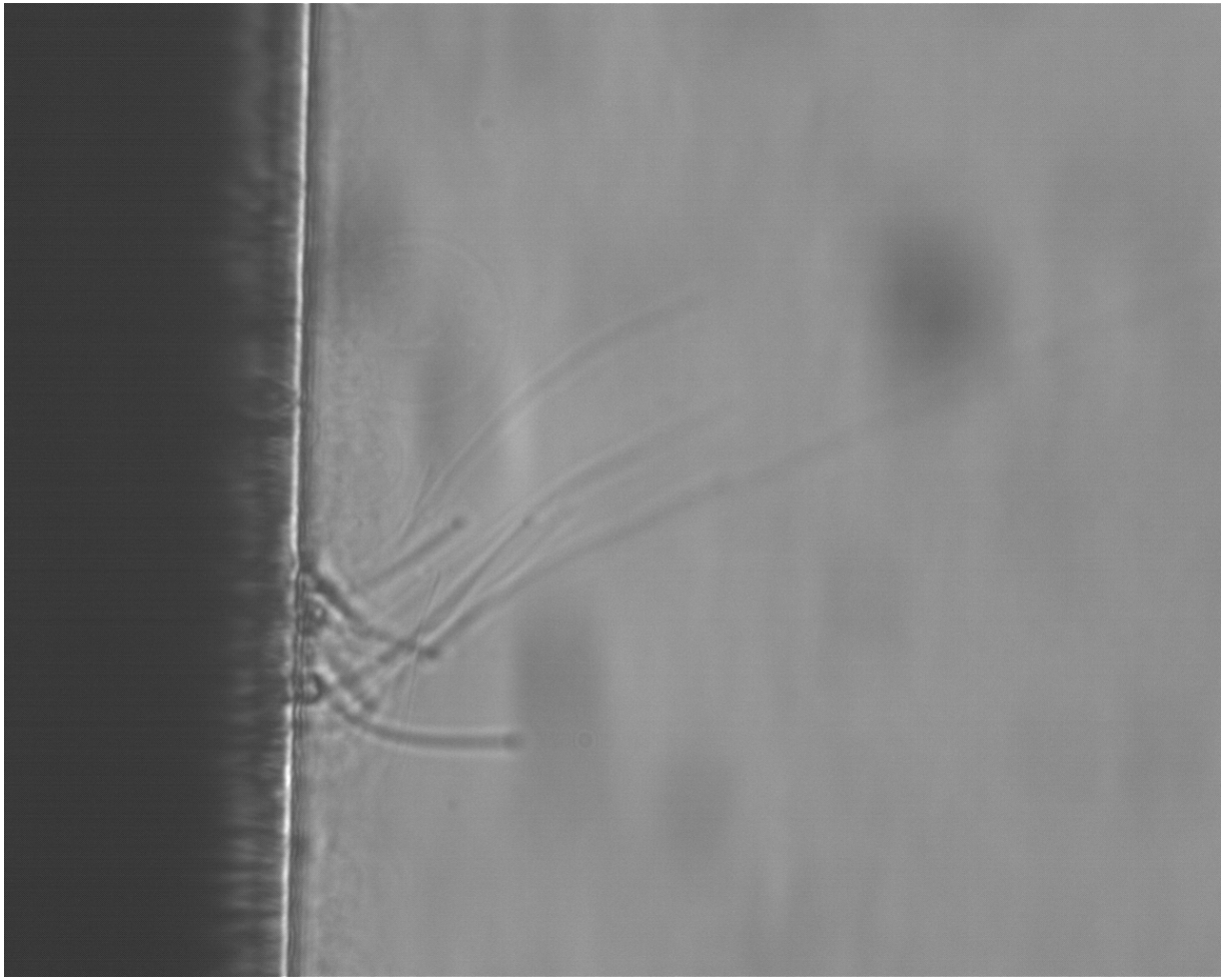
DPL:CHOL (69% Chol) GUVs

50°C



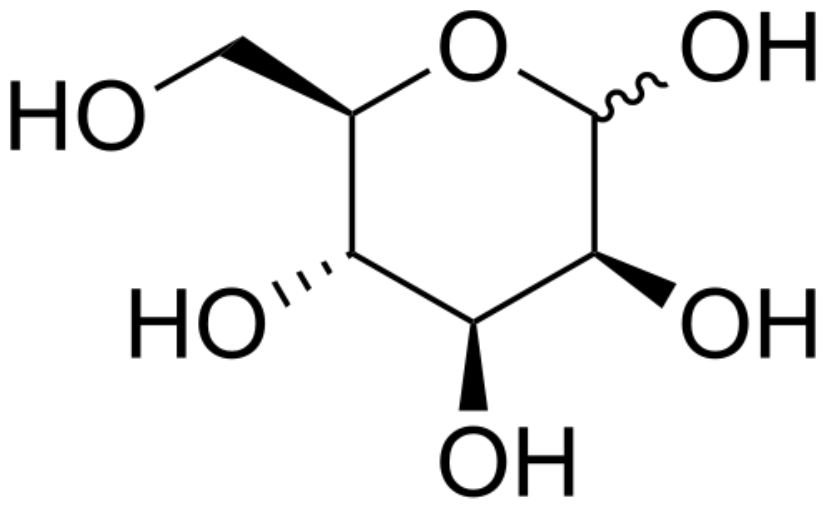


GUVs plus Tubular Structures

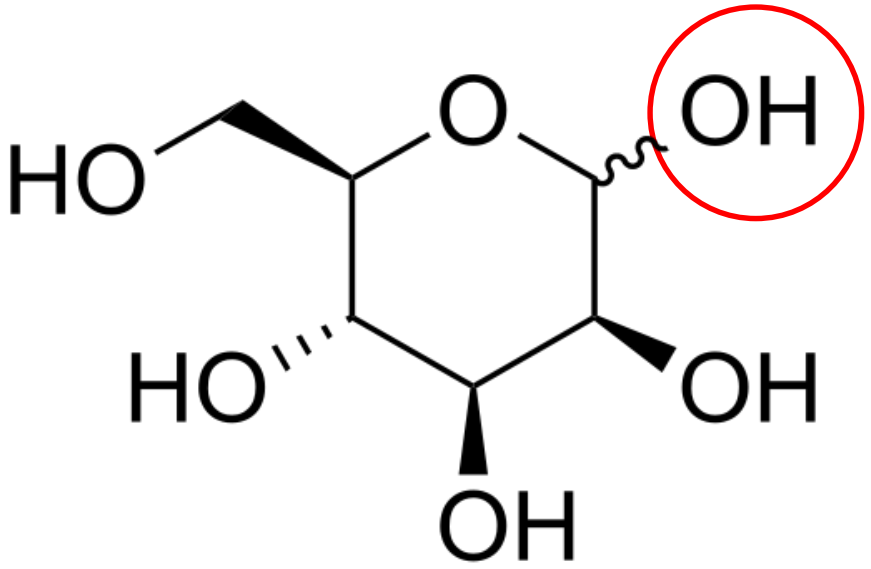


4 Mannose Derivatives

Mannose

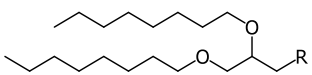
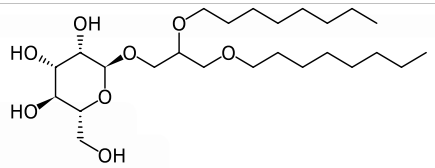
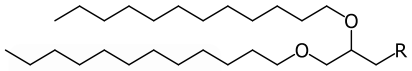
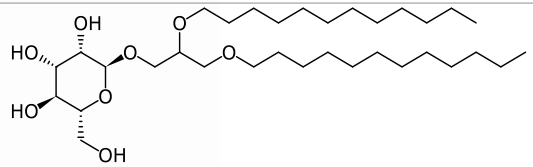
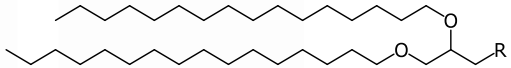
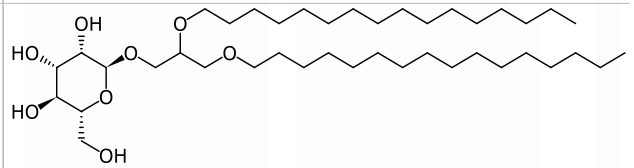
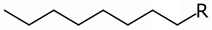
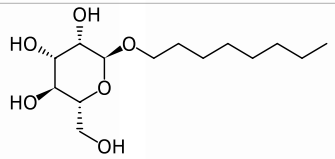
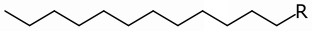
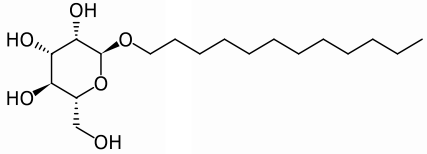
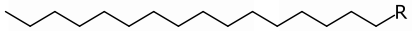
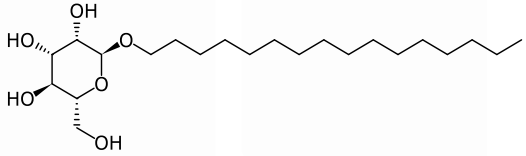


Mannose Reaction

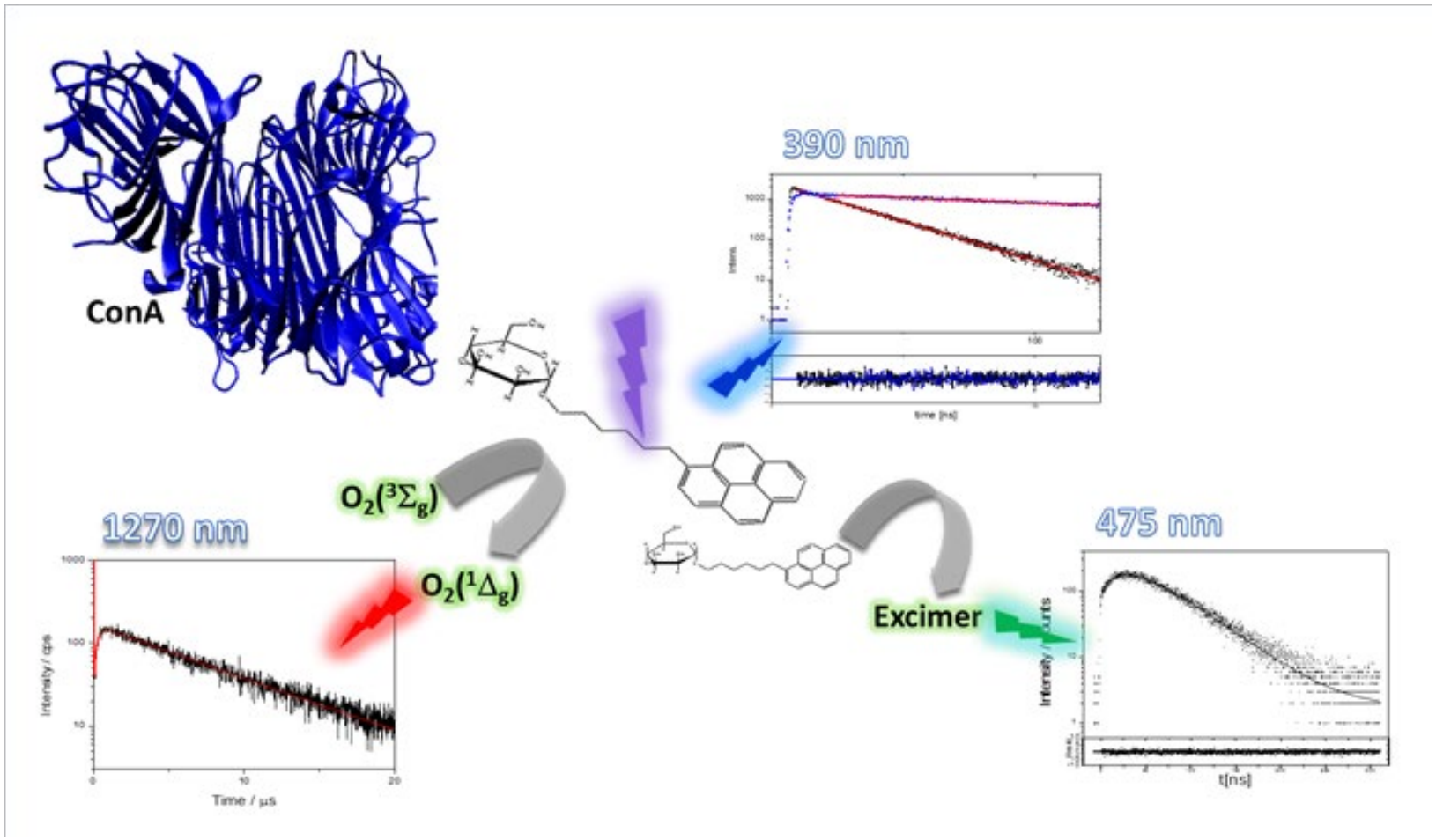


Anomeric Position

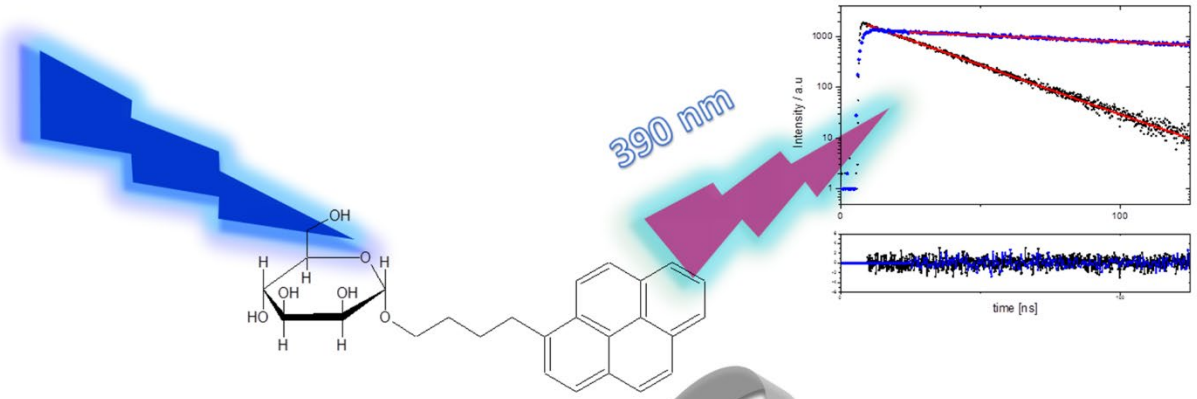
Mannose Products

	DOM	
	DLM	
	DPM	
	MOM	
	MLM	
	MPM	

Mannose plus a probe

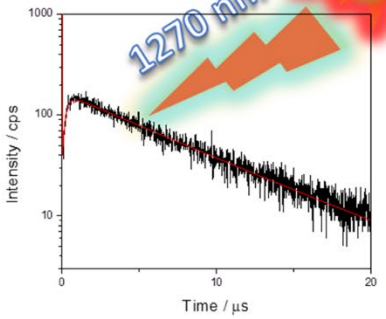


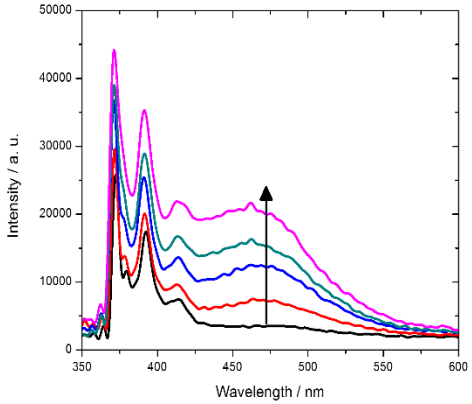
Mannose plus a probe

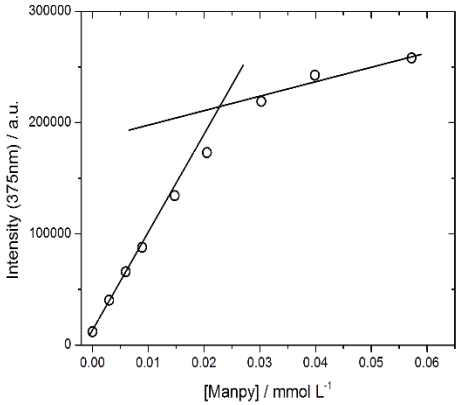


$O_2(^3\Sigma_g)$

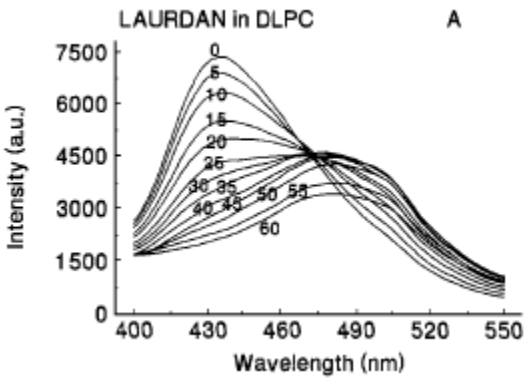
$O_2(^1\Delta_g)$

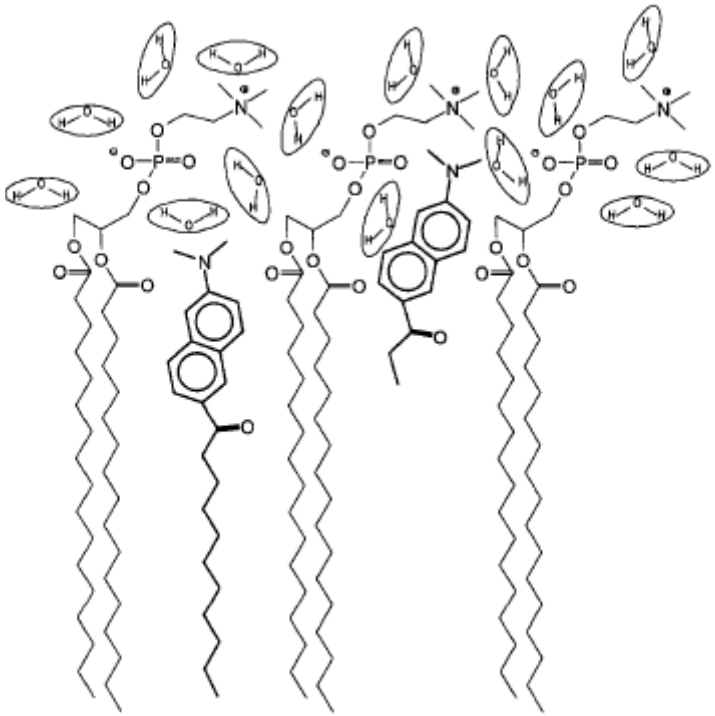


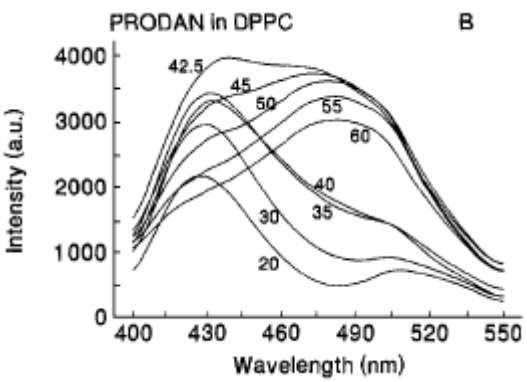




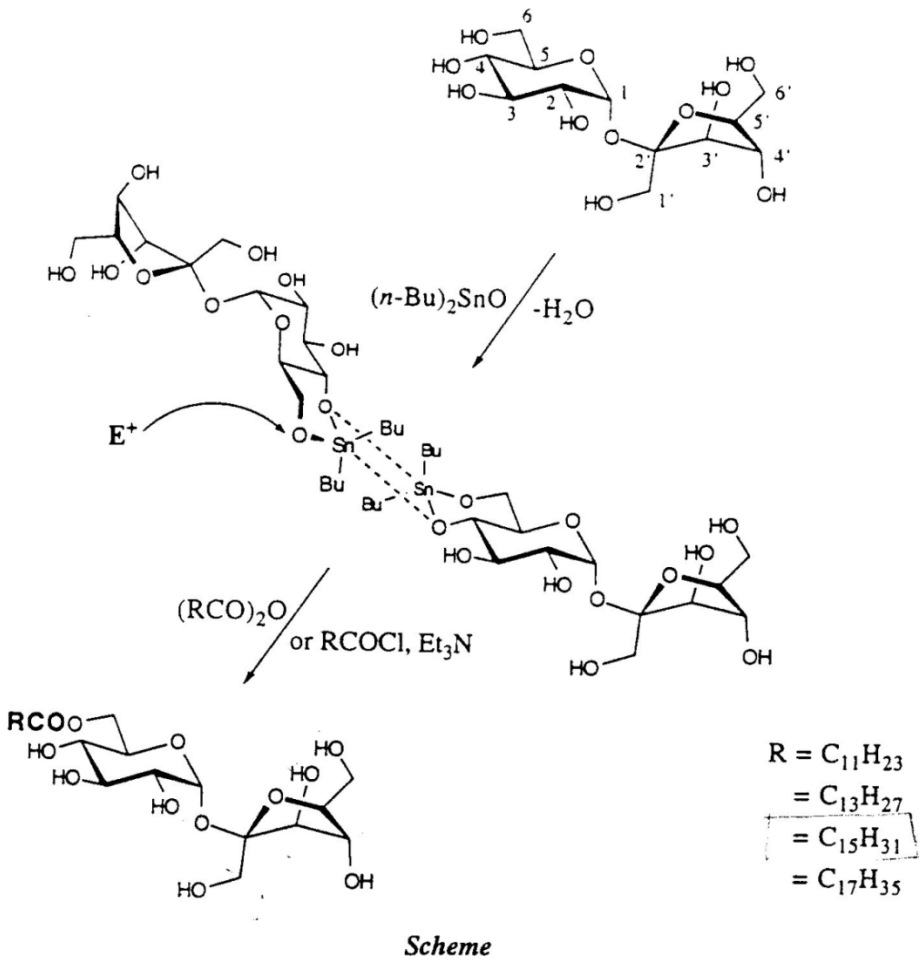
Enough


LAURDAN in DLPC
A
Intensity (a.u.)
Wavelength (nm)
0
5
10
15
20
25
30
35
40
45
50
55
60




PRODAN in DPPC
B
Intensity (a.u.)
Wavelength (nm)
42.5
45
50
55
60
40
35
30
20

Regiostereoselective synthesis of sucrose monoesters

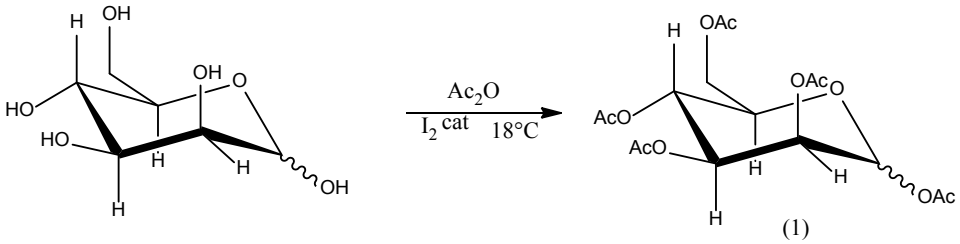


Scheme

IR. Vlahov et al., J. Carbohydrate Chemistry 16(1): 1-10 (1997).

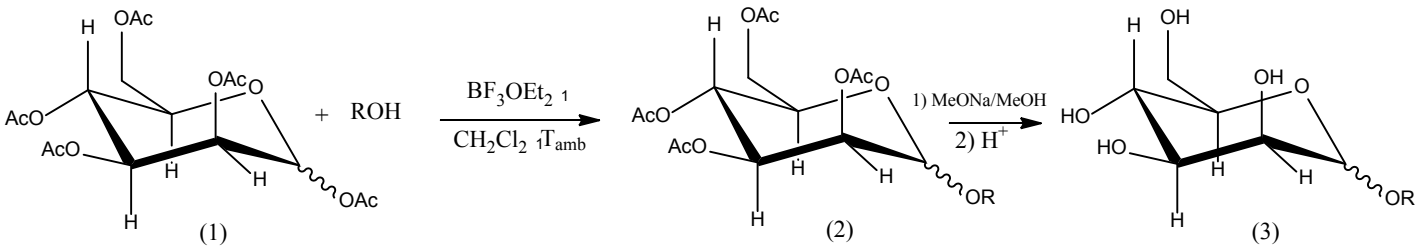
Synthesis of Lactose and mannose derivatives

1.- Protection



(1)

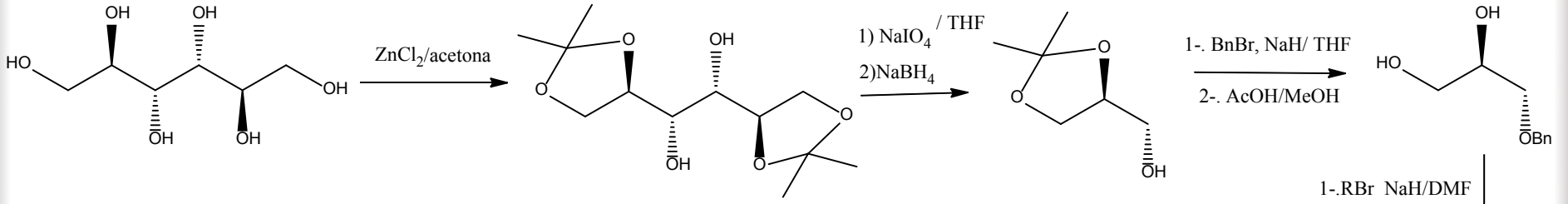
2.- Substitution



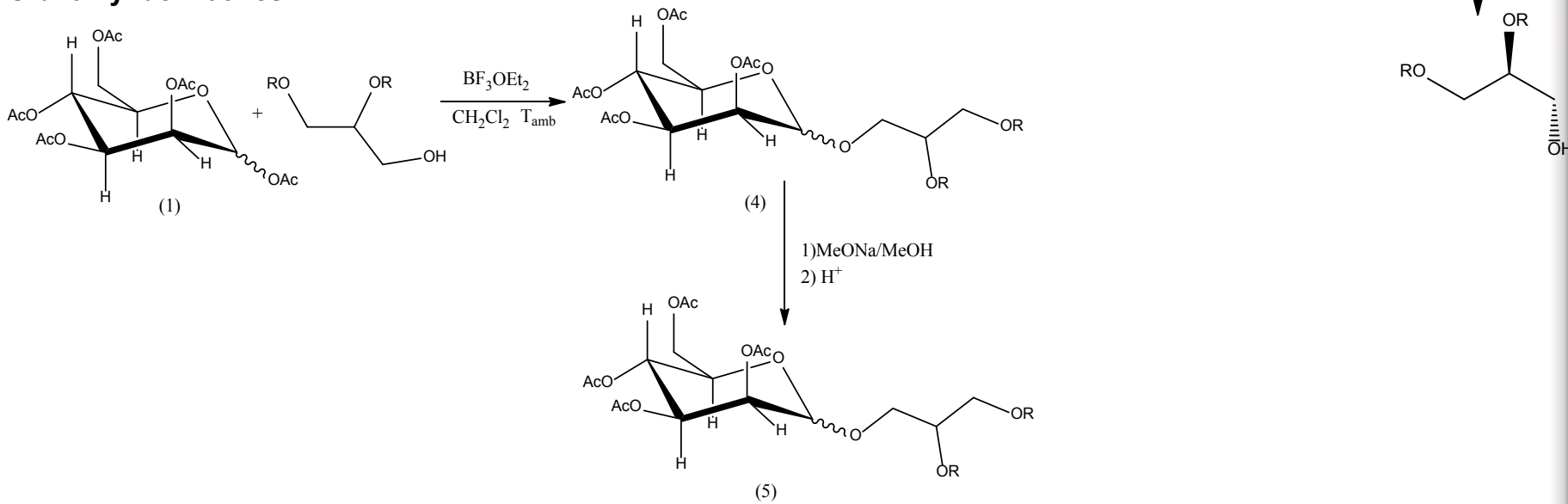
(1) (2) (3)

Synthesis of Lactose and mannose derivatives

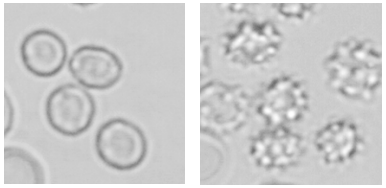
3.- Glycerol derivatives



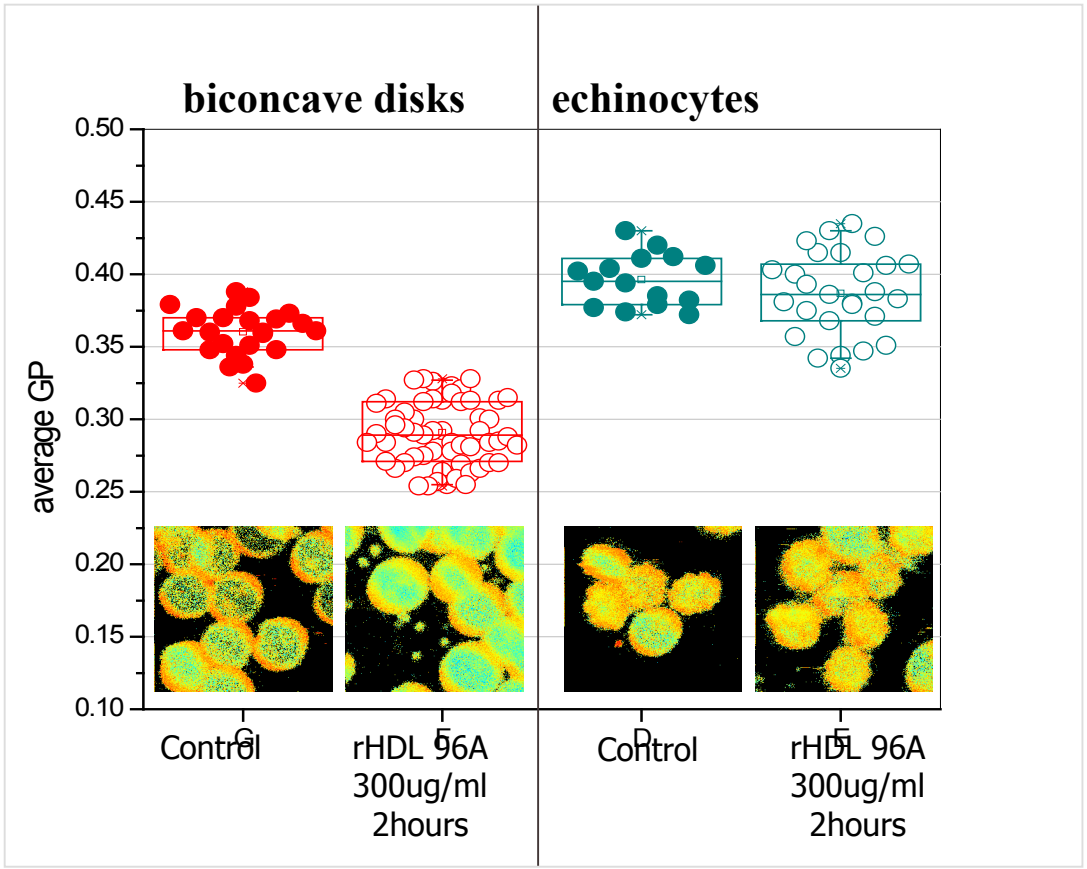
4.- 1-O-di-alkyl-derivatives



RED BLOOD CELLS



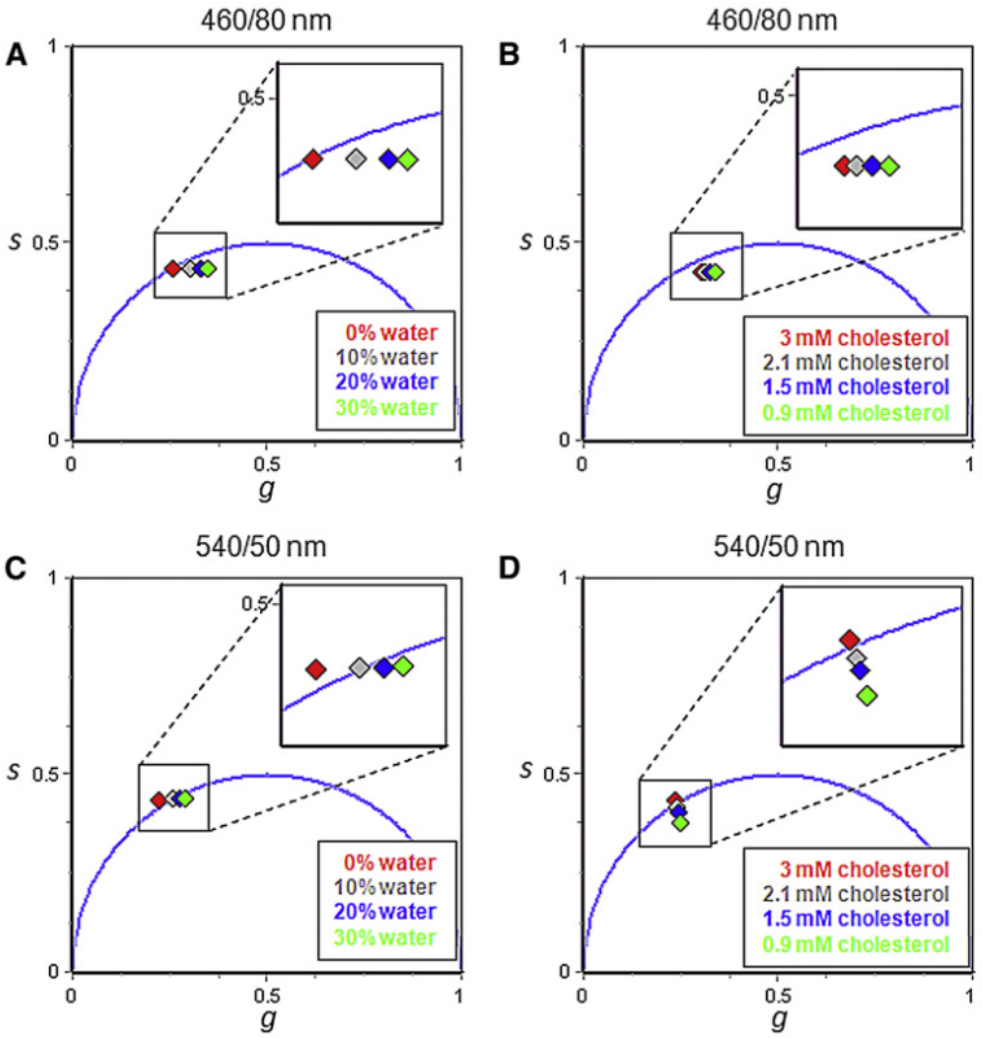
In a blood sample erythrocytes can be found as biconcave discs or as echinocytes.



The average GP value for echinocytes is slightly higher than the value of the biconcave discs.

HDL removes cholesterol more efficiently from the erythrocytes with concave-disc shape.

Water and Cholesterol effect on Phasors*



* Golfetto O., Hinde E., Gratton E. (2013) Laurdan fluorescence lifetime discriminates cholesterol content from changes in fluidity in living cell membranes. Biophys J 104: 1238-1247.