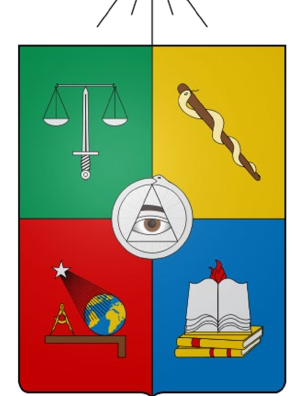

Understanding the interaction of Concanavalin A with mannoside glycoliposomes: A Surface Plasmon Resonance and Fluorescence study.

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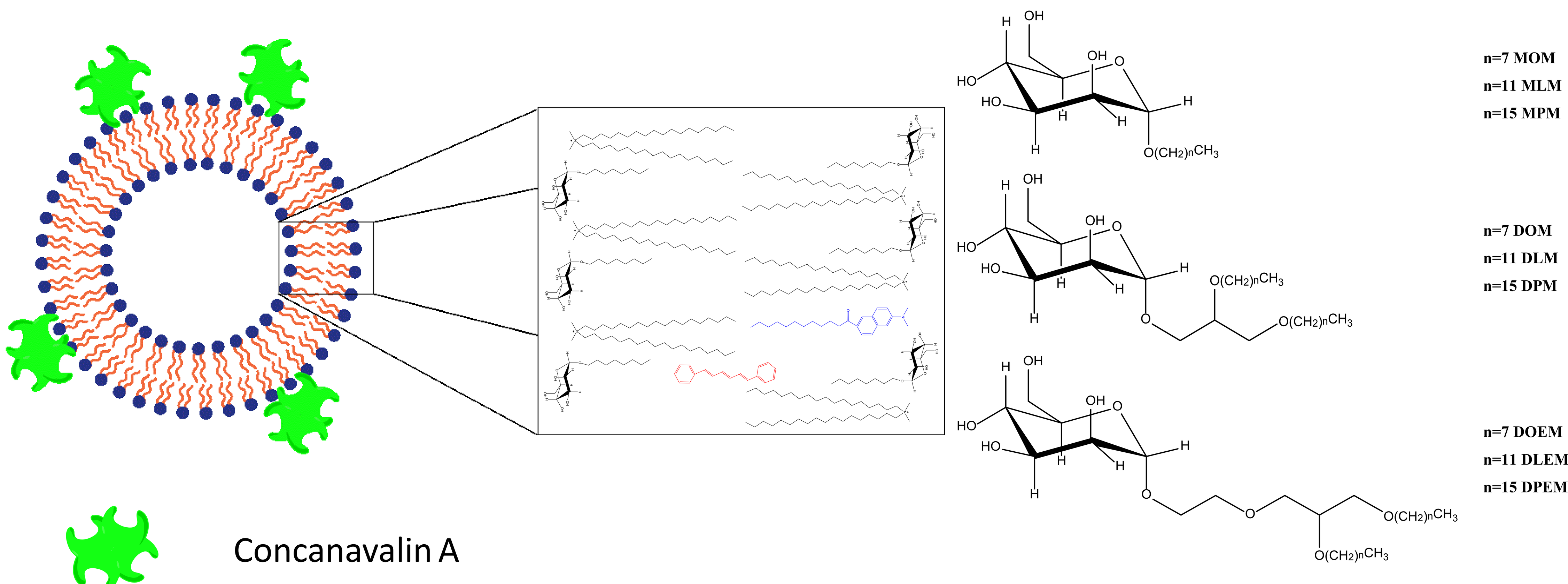
Introduction

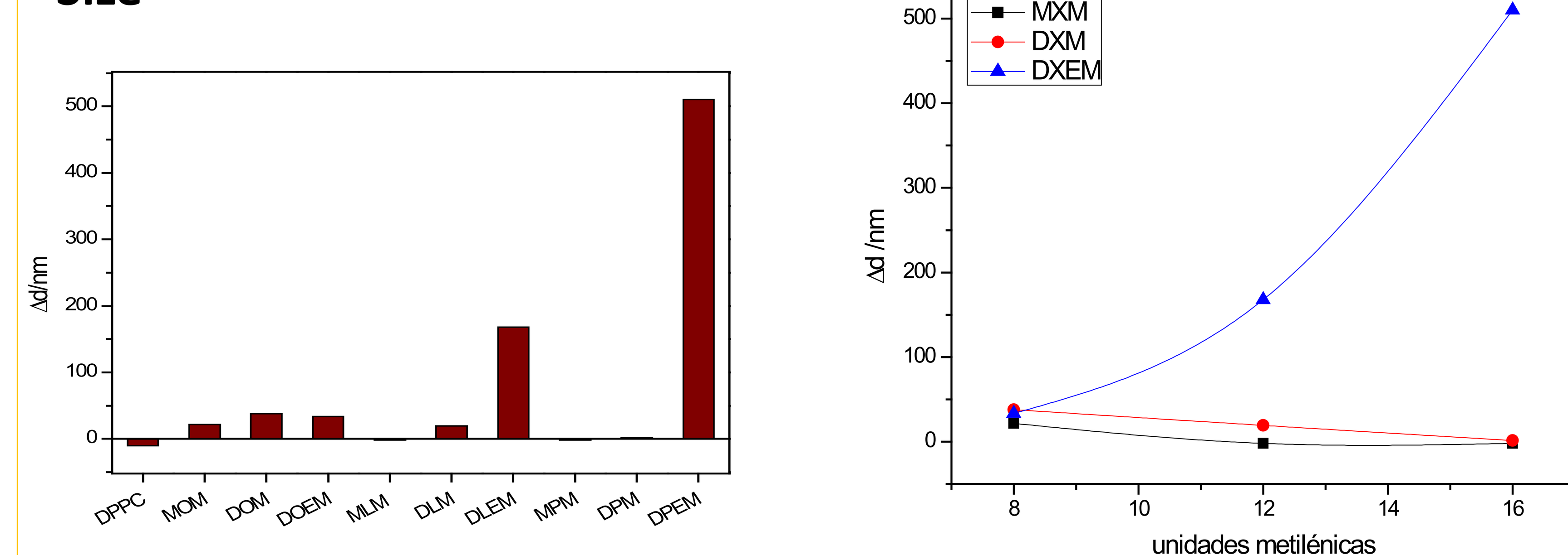
The specificity of carbohydrate-protein interaction is a key factor in a substantial number of biological processes and is the base of the technologies using glycoliposomes in drug delivery. The incorporation of glycolipids in vesicles is expected to increase their specificity toward specific targets such as lectins; however, the degree of exposure of the carbohydrate moiety at the liposome surface is a crucial parameter to be considered in the interaction.

The interaction mannosylvesicles-Con A was studied using: (i) agglutination assays; measured by dynamic laser light scattering (DLS); (ii) time resolved fluorescence methods and (iii) surface plasmon resonance (SPR) kinetic measurements. DLS data showed that an increase in hydrophobic chain length promotes a decrease of liposomes hydrodynamic radius. A longer hydrocarbon chain favors a deeper insertion into the bilayer and mannose moiety results less exposed at the surface to interact with lectin.

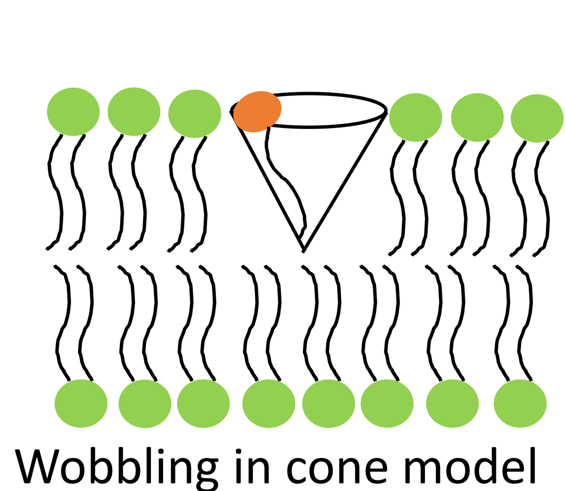
All experiments were performed using buffer Hepes 10 mM pH 7, 10mM NaCl, 0.1mM $MnCl_2$ and 0.1mM $CaCl_2$. The mole ratio lipid/protein was 100.

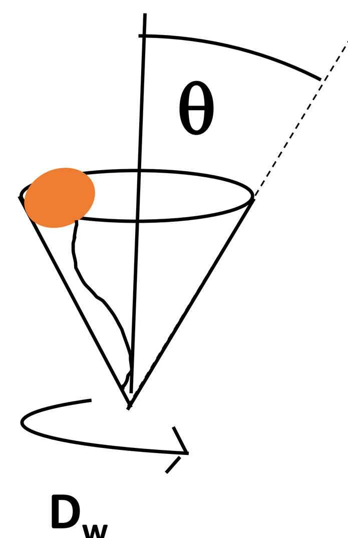


Size



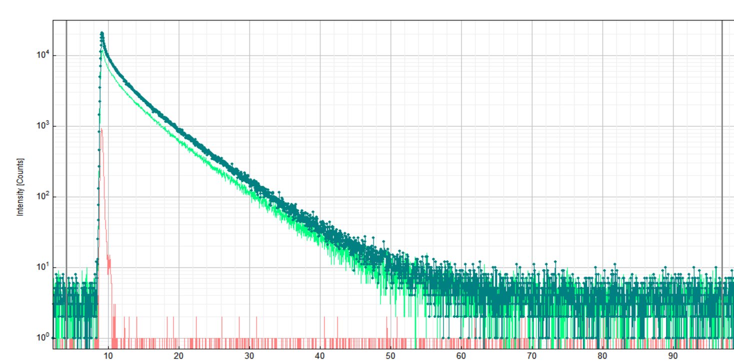
Time resolved anisotropy



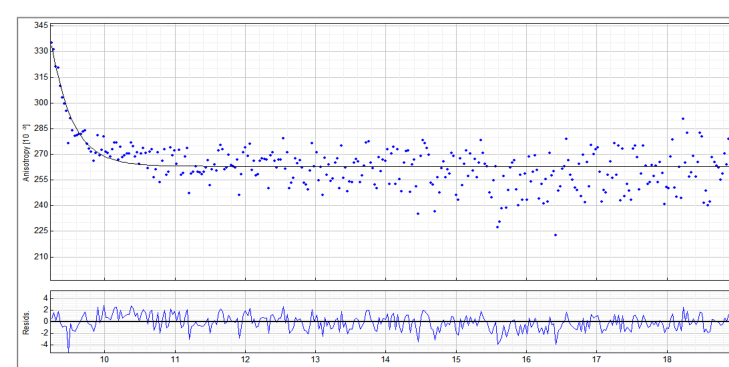


$$r(t) = r_\infty + (r_0 - r_\infty)e^{-t/\phi}$$

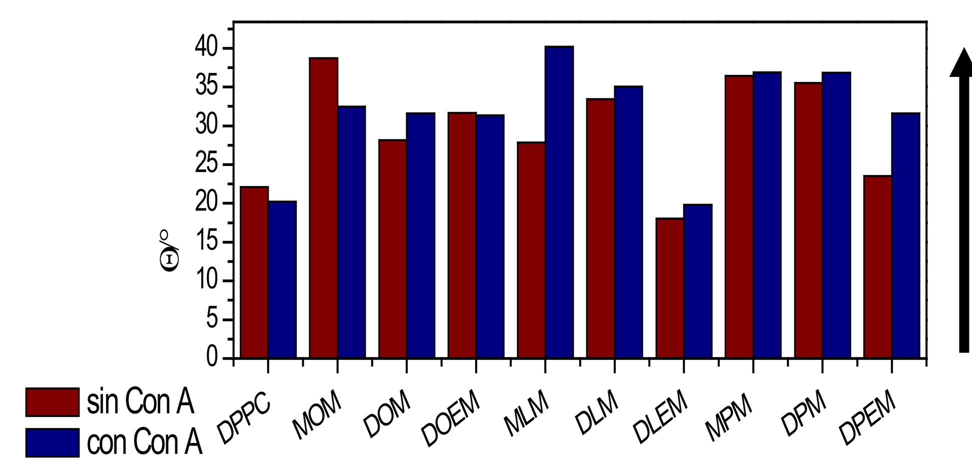
$$\frac{r_\infty}{r_0} = \frac{\cos(\theta)\,(1+\cos(\theta))^2}{4}$$



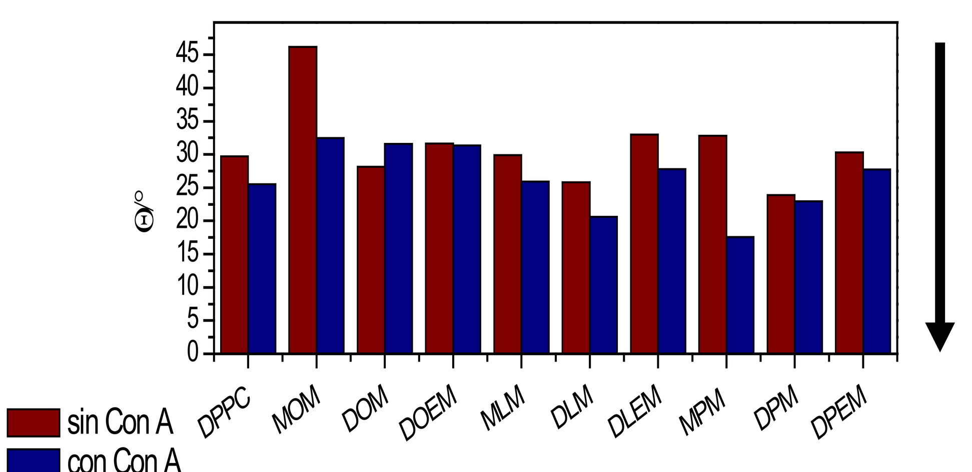
$$r = \frac{I_\parallel - GI_\perp}{I_\parallel + 2GI_\perp}$$



DPH

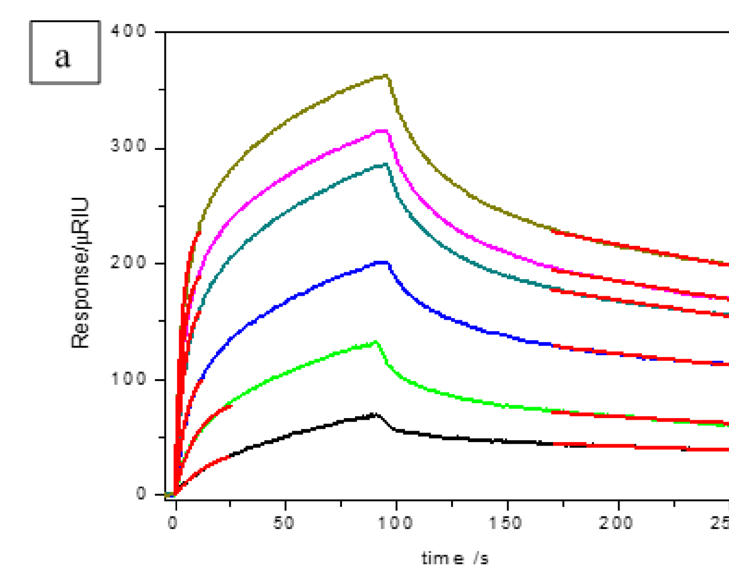


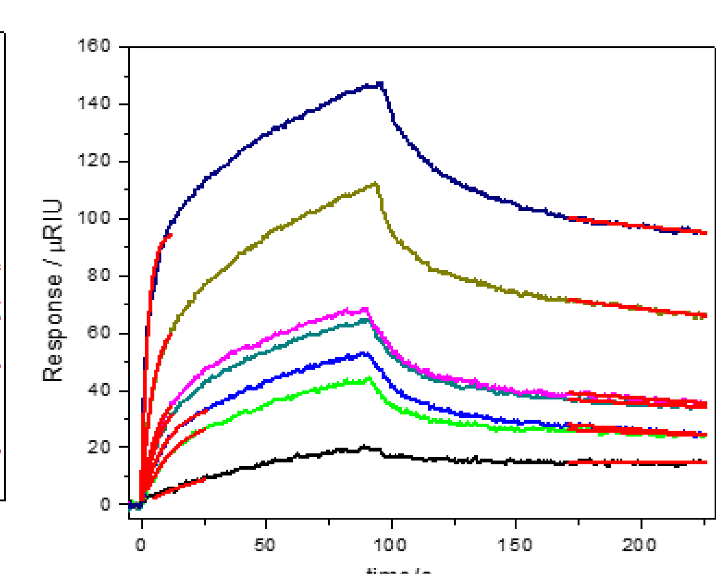
Laurdan

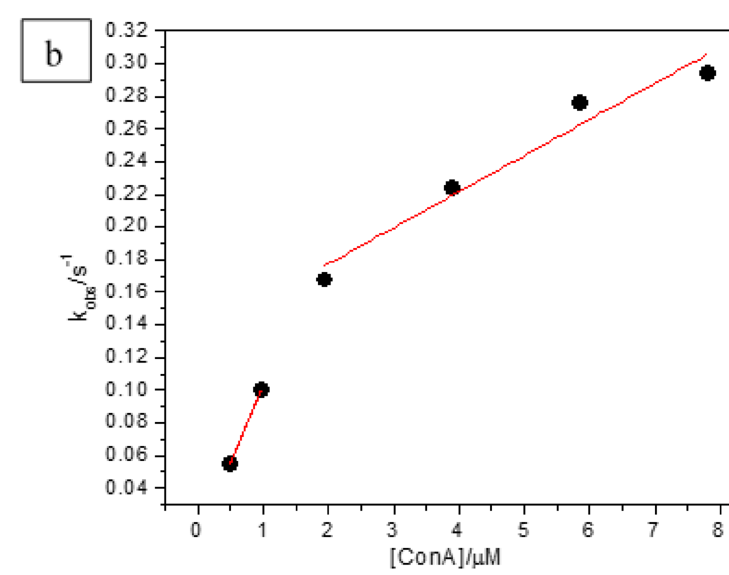


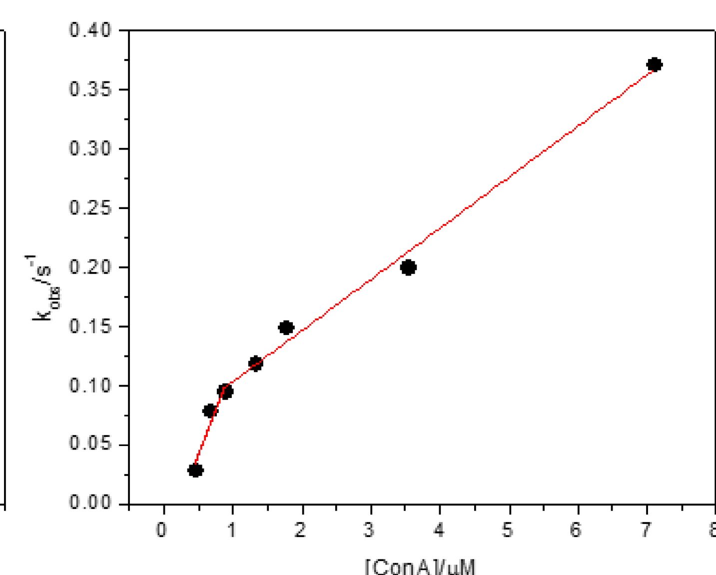
5% of DLEM

5% of DPEM









	$k_{a1}/10^4 M^{-1}s^{-1}$	$k_{a2}/10^4 M^{-1}s^{-1}$	$k_{off}/10^{-3}s^{-1}$	$K_a/10^6 M^{-1}$ Low [Con A]
DPPC	1		3	3
5% DLEM	10	2	2	50
5% DPEM	15	4	1.5	100

k_a(Me-α-Man)= $3\text{-}6\times10^4\ M^{-1}s^{-1}$

Conclusion

Fluorescence experiments demonstrated changes in the structure of glycovesicles due to the interaction with the protein. From SPR measurements the kinetic and equilibrium constants associated to the interaction of Con A with the different glycolipid synthetized were determined.

The combination of SPR and fluorescence techniques to study the interaction of a lectin with mannose glycovesicles allow to sketch and understand the interaction sensing the interface, the surface and deeper in the membrane.

Acknowledgment

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