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**Insight on the Diverse Cellular Pathways of Two Novel Chelators from Liposome Partition Studies**

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The understanding of the cellular pathways and targets of a given molecule which exhibits biological activity, in concert with the establishment of structure activity relationships is crucial for the design of new drugs and implementation of novel therapeutic strategies.

Our group has recently been interested in the design of novel iron chelators to target infection, by means of iron deprivation, and in this work we communicate the fluorescent and partition properties of two molecules which show different biological activity.

The two novel compounds have an identical chelating unit which has been coupled with two different fluorophores thus providing two compounds with distinct colour, hidrophylic/lipophilic balance and charge at physiological pH.

We performed comparative studies of the partition of the novel compounds and the corresponding fluorophores in large unilamellar liposomes and the results provide evidence that the biologically active compound strongly interacts with the lipid phase while the non-active does not.

The results obtained for liposomes composed by the lipids DMPC and DMPG suggest that a surface effect is quite important for the interaction with the membrane.

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**Teaching Undergraduate Biophysics using Excel**

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A new approach is presented for teaching biophysics to a broad audience, including undergraduates with no calculus background. The classic two-box system from statistical mechanics is chosen as an example, to illustrate how this approach can be implemented. In the sample exercise, students develop a simple Excel spreadsheet ''from scratch''. This spreadsheet implements a kinetic Monte Carlo (KMC) simulation algorithm. The basic transport mechanism is the transfer of a randomly selected particle from one box to the other. In a directed, activity-based exercise, students write an algorithm for the simulation, check and debug the algorithm using ''by hand'' calculations. Students then use the spreadsheet they have developed to discover for themselves the consequences of changing the number of particles ($N$) and the initial distribution of the particles. By analyzing their simulation output, students see how the system approaches equilibrium and how fluctuations in the system depend on system size. By investigating the trend in fluctuations with system size, students discover that fluctuations become negligible in macroscopic systems. Finite difference equations are derived and implemented in an Excel spreadsheet to model the kinetics of the two box system. Students then compare the theoretical predictions for the average behavior of the system with the ''random'' data from the KMC ''computer experiment'', to investigate the qualitative and quantitative differences between these microscopic and macroscopic approaches. The students also make a histogram of system states taken from an equilibrium simulation with $N$=150 particles and compare it with predictions of the binomial distribution for the same system, to demonstrate the preponderance of the most probable states in the ensemble average. This pedagogical approach is quite different from using ''canned'' computer demonstrations, as students design, implement and debug the simulations themselves - ensuring that they understand the model system intimately.

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**A Novel Approach to Measure Flip-flop of Very Long Chain Saturated Fatty Acids (VLCFA) in Model Membranes**

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VLCFA (>18 carbons) are normally present in very low proportions compared to other FA in humans, and their elevation accompanies debilitating and sometimes lethal neurological disorders. To understand whether VLCFA are causative agents or biomarkers requires detailed knowledge of their transport and metabolism, studies of which are often impeded by their extremely low aqueous solubility. Here we focus on how rapidly VLCFA can move across a protein-free phospholipid bilayer. To overcome the solubility limitation, we prepared aqueous complexes of VLCFA with methyl-β-cyclodextrin (CD), and tested whether VLCFA dissociate rapidly enough to study the kinetics of their transport in membranes. This VLCFA/CD complex was used for delivering VLCFA to small and large unilamellar vesicles (phosphatidylcholine) containing fluorescent probes, pyranine and fluorescein-phosphatidylethanolamine (FPE). Pyranine, a water-soluble pH probe, was trapped inside vesicles, and FPE, a membrane surface potential probe, was added to the outer monolayer of the vesicles. Using spectrofluorometry, FPE detected rapid binding of VLCFA (half-time <4 sec), which established that desorption from the CD carrier was at least this rapid. Pyranine detected the transbilayer movement (flip-flop) of VLCFA by a rapid decrease in pH ($t_{1/2}$ <4 sec). Together these experiments demonstrate rapid binding and flip-flop of VLCFA with 20 to 26 carbons across the bilayer. The magnitude of the intensity changes were dependent on the concentration of VLCFA added. The kinetics of flip-flop was independent of temperature, media viscosity and lipid composition of phospholipid bilayer. Our results suggest that putative proteins such as Adrenoluekodystropy protein (ALDP) are not required for the flip-flop of VLCFA between membrane leaflets and that other mechanisms for the physiological role(s) of ALDP in neuropathophysiology require investigation.

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**Cyclodextrins Deliver FA to Membranes Rapidly while Maintaining a Low Concentration of Unbound FA in Water**

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Cyclodextrins (CDs) have a polar surface and a hydrophobic cavity that can bind and solubilize long chain fatty acids (FA), while remaining soluble in water. In this study we tested the usefulness of CD for acting as a FA donor to model membranes (egg-PC vesicles). We employed i) fluorescein phosphatidylethanolamine (FPE) to detect changes in membrane surface potential when FA anions adsorb to the outer membrane leaflet ii) entrapped pyranine to measure changes in pH after FA diffusion (flip-flop) across the lipid bilayer and iii) ADIFAB to measure the concentration of unbound FA in water. Upon mixing of oleic acid (OA)/CD complexes with vesicles containing either FPE or pyranine, OA rapidly dissociated from CD, bound to the membrane and underwent flip-flop within 1s (online experiments). In the presence of vesicles, CD maintained the concentration of unbound OA in water low, at almost the same levels measured with albumin. In stopped-flow fluorometry, the mixing of OA/CD with vesicles produced a single exponential decrease in the fluorescence of FPE and pyranine ($t1/2 < 50$ ms). These kinetics reflect the FA dissociation from CD since FA binding to the membrane and flip-flop are very fast ($t1/2 < 10$ ms). The delivery of OA by CD was faster than those observed with lipid vesicles ($t1/2 = 100$ ms) and albumin ($t1/2 = 200$ ms). Thus, CD is an excellent vehicle to FA and deliver FA to membranes very rapidly while maintaining a low concentration of unbound FA.

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**Permeability of Model Stratum Corneum Lipid Membrane Measured Using Quartz Crystal Microbalance**

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The stratum corneum (SC) is the outermost layer of the epidermis. Stacked intercellular lipid membranes found in the SC play a crucial role in regulating transport of water through the skin. Despite the importance of this role of the SC lipid membranes, only a few studies have presented quantitative methods to measure the permeability of SC lipid membranes to water. In this work, we present a new method to determine the water permeability of a model SC lipid membrane using a quartz crystal microbalance (QCM). We investigate a model SC lipid membrane comprising an equimolar mixture of natural ceramide (CER), cholesterol (CHO) and palmitic acid (PA), and use QCM to determine the diffusivity ($D$), solubility ($S$) and permeability ($P$) of water vapor. Lipids are highly oriented and form lamellar stacks that are parallel to the substrate, as observed by the small angle X-ray scattering. This suggests that our model could represent some of the features that are found in native SC lipid membranes. Our QCM measurements indicate that the permeability of model membranes at different relative humidity (RH) is controlled primarily by the solubility of water within the bilayers, which increases with RH in a linear fashion. The diffusivity was found to be more or less independent on RH ($D \sim 3 * 10^{-11}$ cm$^2$/s) except for at a very high RH(>90%).