L.9: Effect of curcumin on the diffusion kinetics of an organic dye across a model membrane probed by second harmonic spectroscopy

Second harmonic generation (SHG) spectroscopy can be used to monitor the transport kinetics of certain molecules across a model membrane in real time. The principle of this technique is that the SH field generated from the dye molecules adsorbed only on the outer surface of a bilayer can add coherently to generate a measurable signal when the sizes of the liposomes are of the order of the wavelength of the fundamental (~ 800 nm) radiation. However, the SH field generated from oppositely oriented dye molecules (adsorbed on the inner and outer bilayer) will cancel out because they are separated by the bilayer thickness (~ 5 nm) which is much less than the coherence length of the process. Therefore by monitoring the time dependent SH signal, which is proportional to the population difference of the dye molecules adsorbed between the outer and inner surface the diffusion process can be monitored in real time. Using Ti-Sapphire laser as the excitation source, the SH signal from LDS-698, a positively charged dye can be used to monitor its diffusion across a negatively charged membrane in real time by the SHG technique.

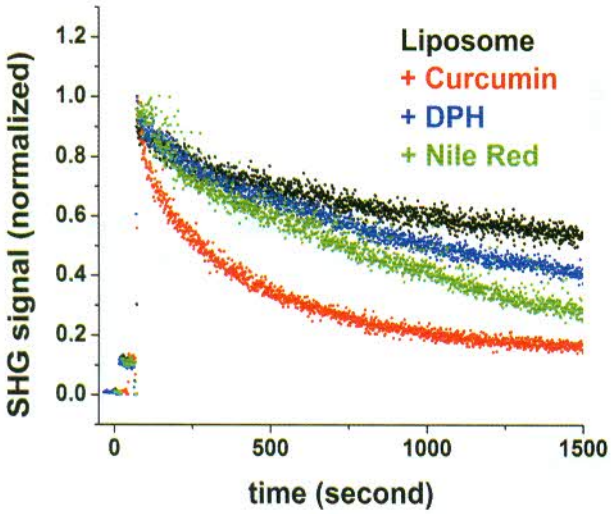

Fig.L.9.1: Normalized decays of SH signals of LDS-698 in liposomes and liposomes containing equal amounts of Curcumin, DPH and Nile Red

In Fig. L.9.1 we compare the effect of three lipophilic molecules (Curcumin, DPH and Nile Red) present in the bilayer on the transport kinetics of LDS-698. The decays of the SH signal of LDS-678 becomes faster when DPH and Nile Red (0.8 μM each) are present in the bilayer, but when compared to similar concentration of Curcumin, their effect is, clearly, less pronounced.

In Fig. L.9.2 we compare the effect of Curcumin on membrane rigidity. At room temperature (25^{0}C), the average diffusion time constant of LDS-698 changes from 780s to 14s (~56 times) in liposomes containing Curcumin. When the bilayer is made more rigid (by using Cholesterol and lowering the temperature to 2^{0}C) the effect of Curcumin were observed to be still significant.

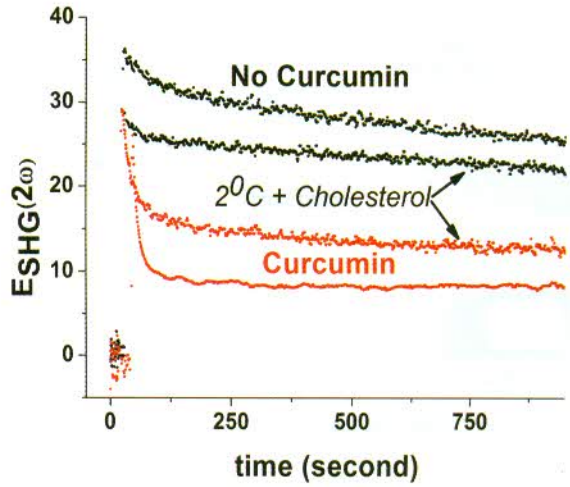

Fig.L.9..2: Decays of SH electric field of LDS-698 after addition of liposomes in absence (black) and in presence (red) of Curcumin (Curcumin:Lipid molar ratio ~0.2)

It has been proposed that lipophilic molecules may alter the physical properties of the membrane. In a recent study it has been suggested that Curcumin forms higher order oligomeric structures in the membrane that span the bilayer. Thus the bilayer environment encountered by LDS-698 in the presence of Curcumin is altered and is likely to be more polar than the one constituted by nonpolar methyelene (-CH_2-) units. Results presented here suggest that the observed diffusion kinetics of LDS-698 might depend on the altered polarity of the bilayer environment by lipophilic molecules, especially Curcumin.

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