L.4: Diffusion of Chlorin-p_6 across a lipid bilayer probed by second harmonic generation

Chlorin-p_6 (Cp_6), a porphyrin based photosensitizer has three ionizable carboxylic acid groups whose acid-base equilibrium alters the hydrophobicity of the drug and thus play an important role in the higher uptake of Cp_6 in tumors where extra-cellular pH can be slightly acidic. Factors governing the dynamics of the diffusion of Cp_6 across lipid bilayer (LB) may be useful for a better understanding of the cellular uptake of the drug.

To study the adsorption and transport of molecules across the LB a variety of methods like NMR, EPR, absorption and fluorescence spectroscopy have been used. The adsorption and transport kinetics of dye molecules and ions across a LB can also be monitored by the second harmonic generation (SHG) technique. Dye molecules in the bulk water will not generate SHG because due to their random orientation they are expected to behave as being centrosymmetric. However, when they adsorb on the outer LB, the centrosymmetricity is lost and then they can generate SH. In addition, if the size of the liposomes is of the order of the wavelength of the fundamental (~ 800 nm) radiation, the SH field generated from the dye molecules adsorbed on the outer lipid bilayer can add coherently to generate a non-zero SH field (Fig. L.4.1). When dye molecules diffuse across the LB, and get absorbed to the inner surface, the adsorbed molecules on the inner and outer surface of the LB are oppositely oriented. Since the oppositely oriented molecules are separated by the bilayer thickness (~ 5 nm) which is expected to be much less than the coherence length of the SHG process, the SHG generated from the oppositely oriented molecules will cancel each other. Thus the resulting SH field generated from the molecules adsorbed on the BL surface will be proportional to the population difference between the outer and inner surface. Therefore, the diffusion process can be monitored in real time by monitoring the SHG signal subsequent to the addition of molecules to the liposomes. Figure L.4.2 shows the time profiles of the SH signals obtained on irradiation of a buffer solution containing Cp_6 and liposomes at different pH using 800 nm femtosecond laser. On addition of liposome the intensity of the SH signal increased considerably for lower pH (3-5) values. The initial increase in the SH signal (which corresponds to the adsorption of the drug to the outer LB) is completed within the time resolution of our experiment (~ 1s). Subsequently, the intensity of the SH signal decreased with increasing time (time constants ranged from 10-100s) and for time greater than 500s it was comparable to the SH intensity obtained before addition of the liposomes. The decay constants (10-100s) are attributed to the diffusion of the neutral and charged species of the drug. For the pH 6, the increase in the intensity of the SH signal on addition of liposome was significantly lower and at physiological pH it was almost negligible.

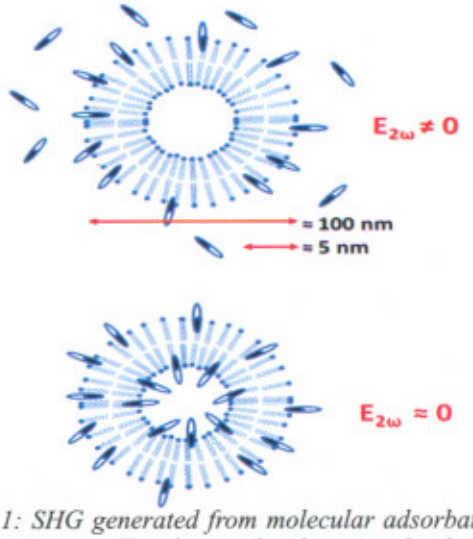

Fig.L.4.1: SHG generated from molecular adsorbates (e.g. Cp_6) on LB. Initially when molecules are adsorbed at the outer surface the SH signal increases rapidly. As the molecules diffuse through the LB, and adsorb onto the inner surface the SHG signal decreases.

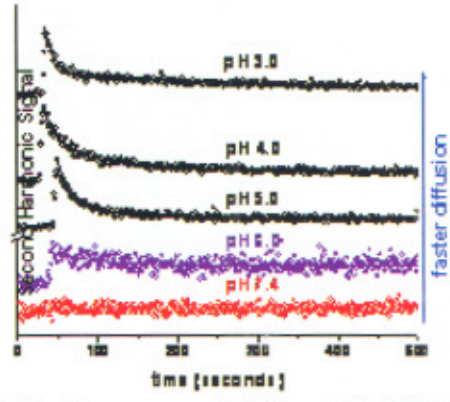

Fig. L.4.2: Decay curves at different pH of SHG signal upon addition of liposome solution into a Cp_6 solution.

These results are consistent with previous studies which show that the interaction between Cp_6 and liposomes is pH dependent. At lower pH due to the presence of the hydrophobic species of Cp_6 its interaction with liposomes is strong, and at higher pH the abundance of the negatively charged hydrophilic species decreases the interaction with the like charged liposomes. (For further details see J. Phys. Chem. B, Vol. 116, Year 2012, pages 4199-4205)

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