Nanometric aggregates of a covalent porphyrin dimer followed by Fluorescence Correlation Spectroscopy in aqueous buffered solution

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The design of covalently linked porphyrins envisages the goal of applying it to molecular photonic devices and artificial biomimetic light-harvesting arrays. Porphyrins are well-known for their tendency to self-aggregate in aqueous solution. The type of self-aggregation can be controlled by a series of factors that include the structure of the porphyrin (substituent groups and coordinated metal ion) and environmental conditions (pH, ionic strength, temperature, cosolvents, etc).

We have recently dealt with the aggregation properties of the anionic water-soluble porphyrin, *meso*-tetrakis(p-sulfonatophenyl)porphyrin sodium salt – TSPP induced at aqueous interfaces. It was shown that under suitable conditions of pH and ionic strength this molecule forms highly ordered molecular J and H aggregates. Interestingly, these aggregates were found to be promoted by interaction with proteins (HSA and BSA) [1], dendrimers [2] and surfactants/lipids [3, 4]. Moreover, it was possible to tune the photophysical properties of the nano-aggregates formed, within these organized systems.

The present work reports the study of the covalent dimer 5-(4-carboxyphenyl)-10,15,20-tris(3-methoxyphenyl)porphyrin with a tyrosine spacer – Dim C (Fig. 1) in aqueous solution, at controlled pH and ionic strength using fluorescence techniques. Fluorescence decays obtained in water point to the existence of small undefined porphyrin aggregates which become more organized upon salt addition (5 – 25 mM) and fluorescent particles are detected (τ_f~200 ps, Fig. 2a).

The characterization of these aggregates was further attempted making use of techniques combining both spatial (imaging) and temporal resolution. In particular, Fluorescence Correlation Spectroscopy (FCS) is a highly sensitive tool to measure concentration and diffusion coefficients from which we may determine binding/dissociation equilibria in the nanomolar range [5]. Based on the latter, a diffusion coefficient of D=6±1 $\mu m^2 s^{-1}$ (Fig. 3) could be retrieved pointing to nano-aggregates of Dim C in aqueous buffered solution. A decrease in the fluorescence lifetime of Dim C in the presence of cytochrome c occurs indicating a quenching process, possibly due to electron transfer upon binding the dimer to the protein, as the decrease in the difusional time obtained by FCS suggests (D~120 $\mu m^2 s^{-1}$).

The presence of Brij 35 nonionic micelles clearly destabilizes the porphyrin aggregates by competing hydrophobic interactions leading to incorporation of the porpyrin into the micellar moitie. This is well supported by the increase in the porphyrin lifetime (τ_f~ 12 ns; Fig. 2b) similar to that obtained in neat DMSO and the diffusion coefficient calculated agrees well with that of diffusing Brij 35 micelles (D~70 $\mu m^2 s^{-1}$).

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References:

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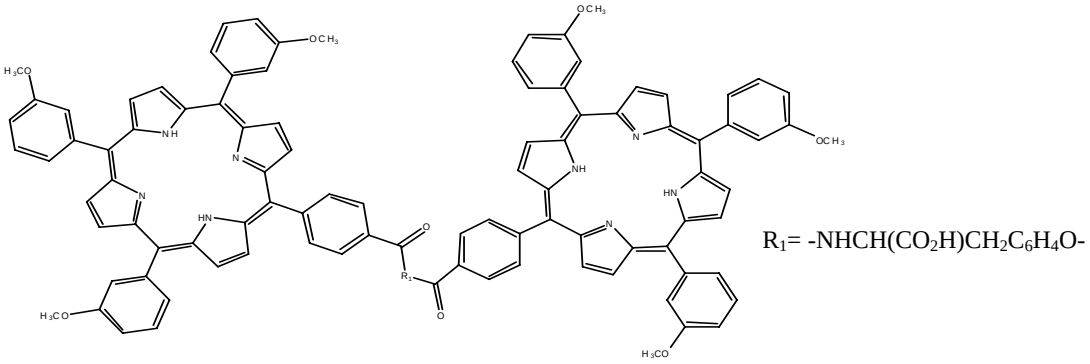

Figure 1 – Scheme of Dim C.

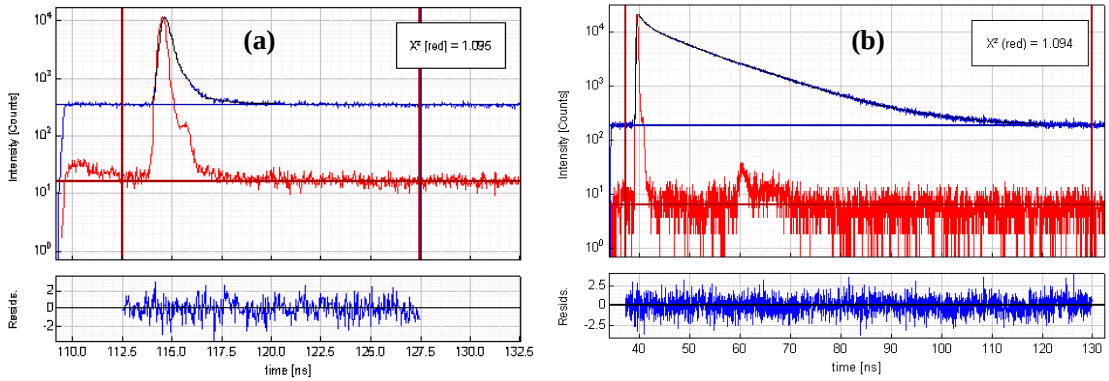

Figure 2 – Fluorescence decays of Dim C in aqueous buffer solution (a) and in the presence of Brij 35 micelles (b).

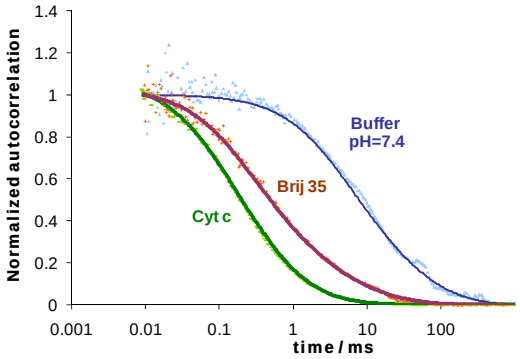

Figure 3 - Normalized autocorrelation traces (experimental – dotted lines; fitting – solid lines) of Dim C (70 nM) in aqueous solution (blue) and in the presence of Cyt c (5 μM, green) and Brij 35 micelles (1 mM; red).