Electronic Supplementary Material (ESI) for RSC Advances.


# Nanoassembly of an Amphiphilic Cyclodextrin and Zn(II)-Phthalocyanine with Potential for Photodynamic Therapy of Cancer

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**S.1 Time-resolved fluorescence measurements**

The excitation source was a synchronously mode-locked rhodamine 6G dye laser (Spectra Physics 375B) which provided excitation pulses of about 2 ps full width at half-maximum at a repetition rate of 82 MHz. An excitation wavelength of 570 nm was used. The fluorescence pulses were detected with a microchannel-plate photomultiplier (Hamamatsu R1645U-01, about 200 ps rising-time) and the decay profiles at 675 nm were collected with a computer-controlled multi-channel analyzer card (EG&G Ortec Trump-8k/2k). The

collected data were then analyzed using the non linear least-squares iterative reconvolution procedures based on the Marquardt algorithm. In the case of total fluorescence decay curves, the fitting was performed on the basis of the multiexponential decay law, [1]

$$I(t) = I_0 \sum_i \alpha_i \exp(-t/\tau_i) \quad (1)$$

where I(t) is the total fluorescence decay curve, $I_0$ is the intensity at time zero, and $\alpha_i$ and $\tau_i$ are, respectively, the relative amplitudes and lifetimes of the $i^{th}$ component (the normalization condition is $\sum_i \alpha_i = 1$). In the case of time-resolved anisotropy measurements, the reconvolution fitting procedure was based on two steps. Fluorescence anisotropy r(t) is defined using the following expression:

$$r(t) = \frac{I_{VV}(t) - I_{VH}(t)}{I_{VV}(t) + 2I_{VH}(t)} = \frac{D(t)}{S(t)} \quad (2)$$

where the sum data, S(t), must be equal to the total intensity I(t). In some cases, an additive constant $r_\infty$ to r(t) was introduced to take into account a long decay contribution due to static interaction with large clusters. In the simple case of spherical molecules, each rotational correlation time, $\tau_{Rj}$, is related to the volume ($V_j$) of the rotating unit (or of the equivalent sphere) by the following equation:[2]

$$\tau_{Rj} = \frac{\eta V_j}{k_B T} \quad (3)$$

where η is the microviscosity of the medium, T is the temperature in Kelvin, and kB is the Boltzmann constant.

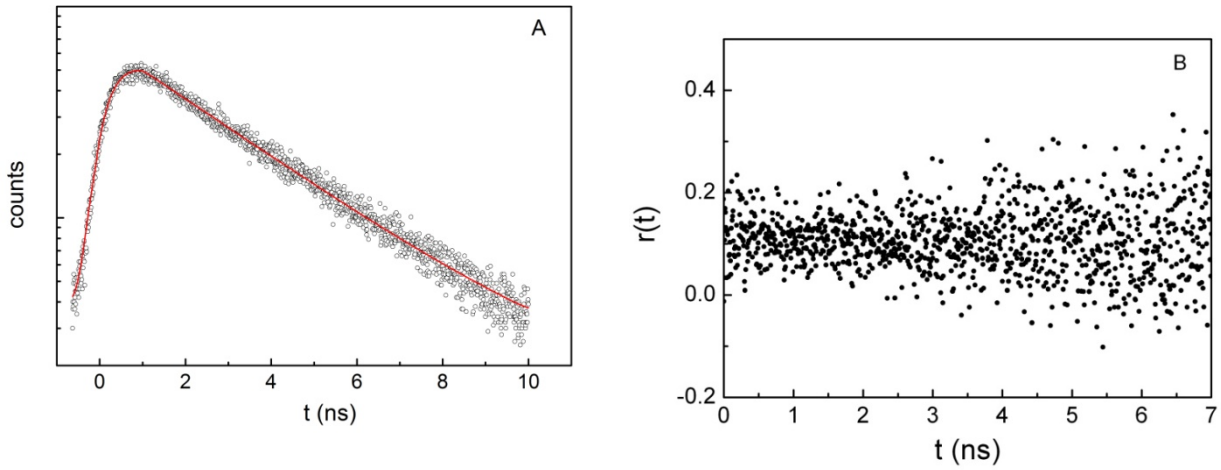


**Figure S1**. (A) Fluorescence time decay of ZnPc/SC16OH nanoassemblies dispersed in water($\lambda_{exc}$=570 nm; $\lambda_{em}$ =675 nm). The continuous red curve is the fit resulting from the convolution of the fluorescence decay and the excitation. (B) Time-resolved fluorescence anisotropy of ZnPc/SC16OH nanoassemblies dispersed in water (ZnPc concentration was fixed at 0.25 μg/mL).

**S.2 Depolarized fluorescence spectra**

Depolarized fluorescence spectra were measured by using Equation (4):[3]

$$r = \frac{I_{VV} I_{HH} - I_{VH} I_{HV}}{I_{VV} I_{HH} + 2 I_{VH} I_{HV}} \quad (4)$$

where r is the anisotropy and $I_{VV}$, $I_{HH}$, $I_{VH}$, and $I_{HV}$ are the fluorescence intensities registered with different polarizer orientations (V=vertical, H=horizontal).

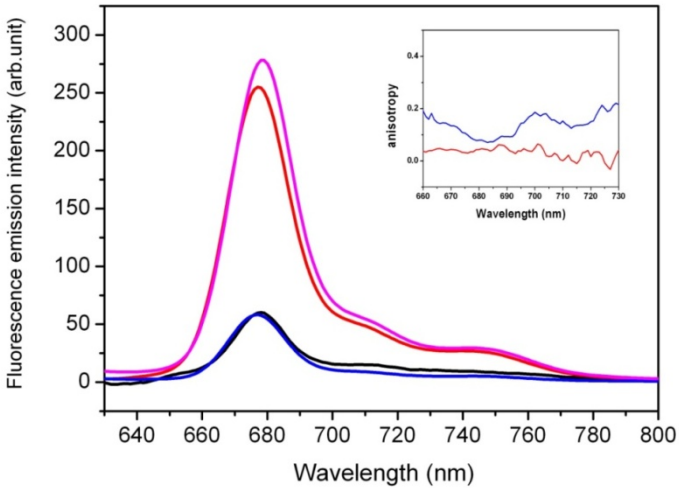


**Figure S2**: Steady-state fluorescence emission spectra of free ZnPc in DMSO (red trace), ZnPc/SC16OH nanoassemblies in DMSO (violet trace), ZnPc/SC16OH nanoassemblies in $CHCl_3$ (black trace) and ZnPc/SC16OH nanoassemblies in water (blue trace). ZnPc concentration was fixed at 0.25 μg/mL in all the dispersions, $\lambda_{exc}$= 600 nm). In the inset the anisotropy of ZnPc in DMSO (red trace) and ZnPc/SC16OH nanoassemblies in $CHCl_3$ (blue trace) is reported.

**S3.** Overall properties of Nile Red/SC16OH nanoassembly (Nr/SC16OH). SD was calculated on three different batches.

| | Nr/SC16OH |
|---|---|
| Nile Red (% w/w) | 0.2 |
| Yield (%) | 75 |
| Mean $D_H$ (nm ± SD) | 195±8 |
| P.I. | 0.198 |
| Zeta Potential (mV ± SD) | -28.3±3.5 |
| Nile Red Actual loading[a] (Ent. Efficiency [b]) | 0.199 ± 0.03 (71.2± 4.2) |

[a]Actual loading is expressed as the amount of Nile Red (mg) encapsulated per 100 mg of nanoassembly; [b] ratio between actual and theoretical loading x 100.

## References


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(3) J. R. Lackowicz, Principles of Fluorescence Spectroscopy, Kluwer Academic, Plenum Publisher, New York 1999.