Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Information

Evaluation of Fluorescent Polysaccharide Nanoparticles for pH-Sensing

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Supplementary Methods

Apparatus

The fluorescence quantum yield of FITC was calculated according to Demas and Crosby[1] relative to rhodamine 6G in ethanol (Uvasol, Merck) used as a standard ($\varphi_f = 0.95$). The absorbance at the excitation wavelength was kept below 0.05 for the samples and the reference. The absolute photoluminescence quantum yield of FITC incorporated in nanoparticles using the home made set-up, was determined according to methods of de Mello et al.[2] and Palsson et al.[3] with an integrating sphere inserted in the fluorescence spectrometer CD900FS (Edinburgh Analytical Instruments). A custom designed integrating sphere (Mod. 05-105, AMKO) with an inner diameter of 105 mm, coated inside with $BaSO_4$ was used. The excitation light source was a tungsten lamp of variable intensity in order to achieve suitable conditions for measuring the fluorescence of the sample and the scattered excitation light with the same slit widths of the spectrometer.

The kinetics of fluorescence was investigated with a CD900 time correlating single photon counting spectrometer (Edinburgh Analytical Instruments). The excitation source was a hydrogen filled nanosecond flash-lamp, which yielded an instrument response pulse of 1.3 ns FWHM. The kinetics of fluorescence were recorded at the emission maxima with excitation at the absorption maxima of the longest wavelength. The spectral slit width was 18 nm. Decay curves were accumulated until 10^3 counts in the maximum with at least 10^3 occupied channels were achieved. The channel width corresponded to 50 ps. In order to calculate the fluorescence lifetime, the LEVEL 1 (up to 4 exponentials) package implemented in the Edinburgh Instruments software was used. Plots of weighted residuals and of the autocorrelation function and values of reduced residuals χ^2 were used to judge the quality of the fit. χ^2 values larger than 1.3 were not accepted.

Calculations

Förster radius

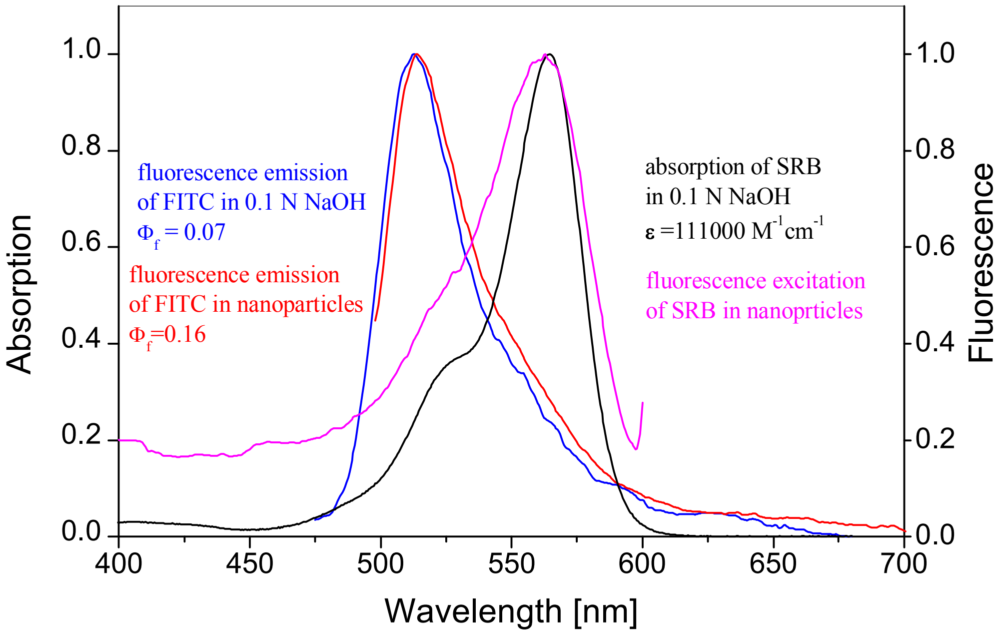

Figure S-1. Normalized fluorescence emission spectra of the donor FITC in 0.1 N NaOH and in nanoparticles suspended in aqueous solution of pH 11 (adjusted with NaOH). Normalized absorption spectrum and fluorescence excitation spectrum of the acceptor SRB in 0.1 N NaOH solution and immobilized in nanoparticles suspended in aqueous solution of pH 11 (adjusted with NaOH).

The Förster radius for the FITC-SRB donor-acceptor pair was calculated[4] using the fluorescence emission spectra of the donor(FITC) and the absorption spectra of the acceptor (SRB) in solution.

$$R_0 = 0.2108\left(\kappa^2 \cdot n^{-4} \cdot \phi_D \cdot J(\lambda)\right)^{1/6} \qquad \text{(S1)}$$

$$\text{and } J(\lambda) = \frac{\int F_{corr}(\lambda) \cdot \varepsilon_A(\lambda) \cdot \lambda^4 d\lambda}{\int F_{corr}(\lambda) d\lambda} \qquad \text{(S2)}$$

According to equation (S1) and (S2), where κ^2 = 2/3 (relative orientation of the donor/acceptor dipoles), n = 1.33 (refractive index of water), Φ_D = 0.16 (quantum yield of the donor immobilized inside the nanoparticles), ε_A =111000 $M^{-1}cm^{-1}$ (absorption coefficient of the acceptor) and $J(\lambda)$ = $3.3 \cdot 10^{15}$

M^{-1} cm^{-1} nm^{4} (integral of overlap from donor emission and acceptor absorption in solution), the critical transfer distance R_0 is determined to be 46 Å.

Theoretical fluorophore loading of the particles

The particle diameter of around 680 nm was converted to the volume of a single nanobead

$$V_{NP} = 4/3 \cdot \Pi \cdot r_{NP}^{3} = 1.65 \cdot 10^{8}\, nm^{3}. \qquad \text{(S3)}$$

With a particle density of 1.1 g/cm³ (obtained from helium-pycnometry) the mass of a single nanoparticle was obtained: $m_{NP} = 1.81 \cdot 10^{-13}$ g. The amount of FITC-dextran propionate and SRB-dextran propionate in a nanoparticle was calculated from the weight ratio (FITC-dextran propionate : SRB-dextran propionate = 1:7) used during preparation of the nanoparticles. consequently, the mass of the substituted dextran units were:

$m_{\text{FITC-dextran propionate}} = 2.26 \cdot 10^{-14}$ g and

$m_{\text{SRB-dextran propionate}} = 1.58 \cdot 10^{-13}$ g.

The molar masses of both dye-dextran propionate units were calculated as the following:

$M_{\text{FITC-dextran propionate}} = M_{\text{dextran}} + DS_{\text{propionate}} \cdot M_{\text{propionate}} - DS_{\text{propionate}} \cdot M_{H} + DS_{\text{FITC}} \cdot M_{\text{FITC}}$

$M_{\text{FITC-dextran propionate}} = (162 + 1.81 \cdot 57 - 1.81 \cdot 1 + 0.0265 \cdot 389.4)\ g \cdot mol^{-1} = 273.68\ g \cdot mol^{-1}$ and

$M_{\text{SRB-dextran propionate}} = M_{\text{dextran}} + DS_{\text{propionate}} \cdot M_{\text{propionate}} - DS_{\text{propionate}} \cdot M_{H} + DS_{\text{SRB}} \cdot M_{\text{SRB-Cl}}$

$M_{\text{SRB-dextran propionate}} = (162 + 3 \cdot 57 - 3 \cdot 1 + 0.00145 \cdot 545.2)\ g \cdot mol^{-1} = 330.79\ g \cdot mol^{-1}$.

The mass and the molar mass of the dextran propionates gave the amount of substance for each dye-dextran propionate and thus the number of substituted dextran units in a single nanoparticle.

$N_A = 6.022 \cdot 10^{23}\ mol^{-1}$ (Avogadro number)

$n_{\text{FITC-dextran propionate}} = m_{\text{FITC-dextran propionate}} / M_{\text{FITC-dextran propionate}} = 8.27 \cdot 10^{-17}$ mol

$N_{\text{FITC-dextran propionate}} = n_{\text{FITC-dextran propionate}} \cdot N_A = 4.98 \cdot 10^{7}$

$n_{\text{SRB-dextran propionate}} = m_{\text{SRB-dextran propionate}} / M_{\text{SRB-dextran propionate}} = 4.78 \cdot 10^{-16}$ mol

$N_{\text{SRB-dextran propionate}} = n_{\text{SRB-dextran propionate}} \cdot N_A = 2.88 \cdot 10^{8}$

With the degree of substitution of dyes (DS_{FITC} and DS_{SRB}) at each dextran propionate unit the number of FITC and SRB molecules per particle was obtained:

$N_{FITC} = N_{\text{FITC-dextran propionate}} \cdot DS_{FITC} = 4.98 \cdot 10^{7} \cdot 0.0265 \approx 1{,}320{,}000$

$N_{SRB} = N_{\text{SRB-dextran propionate}} \cdot DS_{SRB} = 2.88 \cdot 10^{8} \cdot 0.00145 \approx 420{,}000$

Theoretical FRET efficiency

Supposed that all fluorophore molecules were statistically distributed in the nanoparticle the volume around each dye molecule was described with:

$$V = \frac{V_{NP}}{N_{FITC} + N_{SRB}} = \frac{1.65 \cdot 10^{8}\, nm^{3}}{1{,}320{,}000 + 420{,}000} \approx 95\, nm^{3}. \qquad \text{(S4)}$$

The mean distance *R* between the dye molecules in a particle followed by using the radius of the sphere around the fluorophore

$$R = 2\sqrt[3]{\frac{3\,V}{4\,\Pi}} \approx 5.6\, nm. \qquad \text{(S5)}$$

With the critical Förster distance $R_0 = 4.6$ nm (equation S1) and the mean distance *R* the theoretical FRET efficiency for FITC-SRB donor-acceptor pair was estimated according to:

$$E = \frac{1}{\left(R/R_0\right)^{6} + 1} = \frac{1}{\left(5.6\, nm / 4.6\, nm\right)^{6} + 1} \approx 23\,\%. \qquad \text{(S6)}$$

References

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[4] Lakowicz, J.R. *Principles of Fluorescence Spectroscopy* (2nd edition)., Kluwer Academic/ Plenum Publishers: New York, 1999