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ELECTRONIC SUPPLEMENTARY INFORMATION

High performance NIR fluorescent silica nanoparticles for bioimaging

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CONTENTS

(A) Materials and Methods

Materials:

Absolute ethanol (Panreac, 99.5%), ammonium hydroxide solution 25% (Fluka), tetraethyl orthosilicate (TEOS, Aldrich, 98%), (3-aminopropyl)triethoxysilane (APTES, Aldrich, 98%), chloroform (Aldrich, 99.8%), toluene spectroscopic (Aldrich, 99.9%) and 1,4-dioxane spectroscopic (Aldrich, 99%) were used as received. Commercial hexane and acetone were distilled prior to use. Deionized water from a Millipore system Milli-Q g18 MΩ cm was used for fluorescence measurements and endocytosis experiments. Perylenediimide derivatives **1** (chart)[1] and **3** (Scheme 1)[2] were synthesized according to the literature. Silica nanoparticles doped with **2** were synthesized by the Stöber method.[3] Wheat germ agglutinin (WGA) coupled to Alexa Fluor (AF)-594, and DiIC18(5) and DiIC18(7) were purchased from Invitrogen (Carlsbad, CA).

Methods:

- Absorption spectra were recorded on a Shimadzu UV-3101PC UV-vis-NIR spectrophotometer and fluorescence measurements were obtained on a Horiba Jobin Yvon Fluorolog 3-22 spectrofluorometer. For the quantum a yield, DiIC18(5) was used as standard.[4] The fluorescence emission spectra were corrected to the photomultiplier response. The spectra of the nanoparticles in water and ethanol dispersion were obtained in plastic cells with a I-shaped cross section in order to minimize the distortion of the spectra by light scattering.

- Time-resolved picosecond fluorescence intensity decays were obtained by the single-photon timing method. The setup consists of a diode-pumped solid state Nd:YVO4 laser (Milennia Xs, Spectra Physics) that can synchronously pump a mode-locked Ti:sapphire laser (Tsunami, Spectra Physics, with tuning range 700-1000 nm, output pulses of 100 fs, and 80 MHz repetition rate that can be reduced to 4 MHz by a pulse picker) or a cavity dumped dye laser (701-2, Coherent, delivering 3-4 ps pulses of ca. 40 nJ pulse-1 at 3.4 MHz) working with rhodamine 6G. Intensity decay measurements were made by alternating collection of impulse and decays with the emission polarizer set at the magic angle position. Impulses were recorded slightly away from the excitation wavelength with a scattering suspension. For the decays, a cutoff filter was used to effectively remove excitation light. Emission light was passed through a depolarizer before reaching the monochromator (Jobin-Yvon HR320 with a 100 lines/mm grating) and detected using a Hamamatsu 2809U-01 microchannel plate photomultiplier. No less than 10 000 counts were accumulated at the maximum channel. The decay curves were analyzed using a nonlinear least squares reconvolution method.[5]

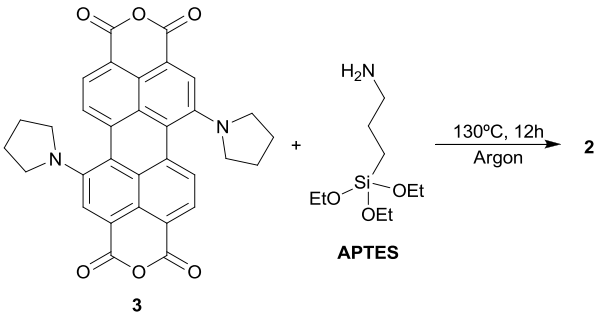
- TEM images were obtained on a Hitachi transmission electron microscope (Model H-8100 with a LaB6 filament) with an accelerator voltage of 200 kV. The freeze-dried particles were dispersed in water or ethanol. One drop of dispersion was placed on a carbon grid and dried in air before observation.

- Hydrodynamic particle radii were obtained by dynamic light scattering (Brookhaven Instruments: BI-200SM goniometer, BI-APD avalanche photodiode detector and BI-9000AT autocorrelator; Spectra Physics He-Ne laser with 35 mW at 632.8 nm) of diluted particle dispersions in water (0.005 wt%). The autocorrelation functions were analyzed by Laplace inversion (CONTIN) and the cumulant method (BI-ZP software package from Brookhaven).

- HEK 293 cells were cultured in an atmosphere of 5% CO_2/95% air at 37°C. Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (GIBCO, Grand Island, NY) was used. Transfection was performed in serum-free medium with pEGFP-N1 (Clontech, Palo Alto, CA), and 24 hours after cells were seeded in 8 well Ibidi μ-slides (Martinsried, Germany). Gencarrier-2 from Epoch Biolabs (Sugar Land, TX, USA) was used as the transfection reagent. Transfection medium was removed after 6 hours and cells were incubated with nanoparticles (250 μg/ml) in complete medium for another 12 hours. Immediately before imaging, cells were stained with Alexa Fluor 594-conjugated wheat germ agglutinin (AF594-WGA) (5 μg/ml in PBS or DiIC18(7) (5 μM in PBS) at 37ºC for 10-20 minutes. Excess dye was removed by washing 3 times with culture medium.

- Confocal laser scanning microscopy (CLSM) images were obtained with a Leica TCS SP5 laser scanning microscope using an HCX PL APO CS 1.20 W 63× water immersion objective and at a resolution of 512×512 pixels. Optical slice thickness for all confocal images displayed was 1 airy unit. Images were processed with ImageJ (http://rsbweb.nih.gov/ij/). The laser power at the sample was measured with a PM100D optical power meter with an S120C sensor (Thorlabs, NJ).

(B) Synthesis of N,N'-Bis(3-(triethoxysilyl)propyl)-1,7-Dipyrrolidinylperylene-3,4:9,10-tetracarboxylic acid bisimide (2)



40 mg (0.07 mmol) of 1,7-Dipyrrolidinylperylene-3,4:9,10-tetracarboxylic acid bisanhydride (**3**)[2] and 0.33 g (1.50 mmol) of APTES were added into a Schlenk flask. The flask was repeatedly evacuated and flushed with argon. The reaction mixture was stirred for 5 min, then heated to 130ºC, and kept it for 12 h. After cooling to room temperature, the mixture was purified by column chromatography (chloroform:hexane:acetone / 10:1:9), yielding 43 mg of **2** (67%) as a green solid.

- ^{1}H RMN ($CDCl_3$) δ 0.81 (t, 4H), 1.25 (t, 18H), 1.87-1.95 (m, 4H), 2.03-2.10 (m, 8H), 2.88 (m, 4H), 3.79 (m, 4H), 3.85 (q, 12H), 4.25 (t, 4H), 7.76 (d, J = 8.0 Hz 2H), 8.45 (d, J = 8.0 Hz, 2H) and 8.52 ppm (s, 2H).

- ^{13}C NMR ($CDCl_3$): δ 8.0, 18.3, 21.6, 25.8, 42.9, 52.2, 58.4, 118.2, 119.2, 120.8, 121.9, 122.4, 123.9, 126.7, 130.1, 134.5, 146.6, 164.1 and 164.2 ppm.

- HRMS (ESI): *m/z* for $C_{50}H_{64}N_4O_{10}Si_2$ calcd. 936.4155; found 936.4178 (M+)

(C) ^{1}H-, ^{13}C-NMR and MS spectra of PDI 2

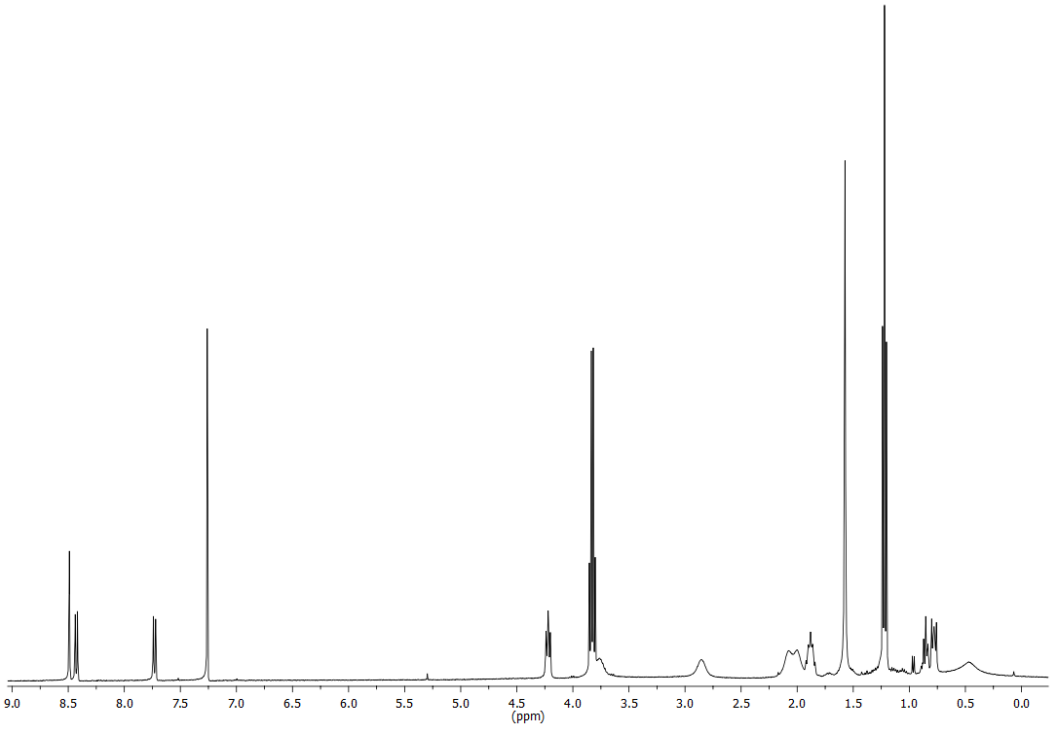


Figure S1 – ^{1}H NMR spectra of PDI **2**.

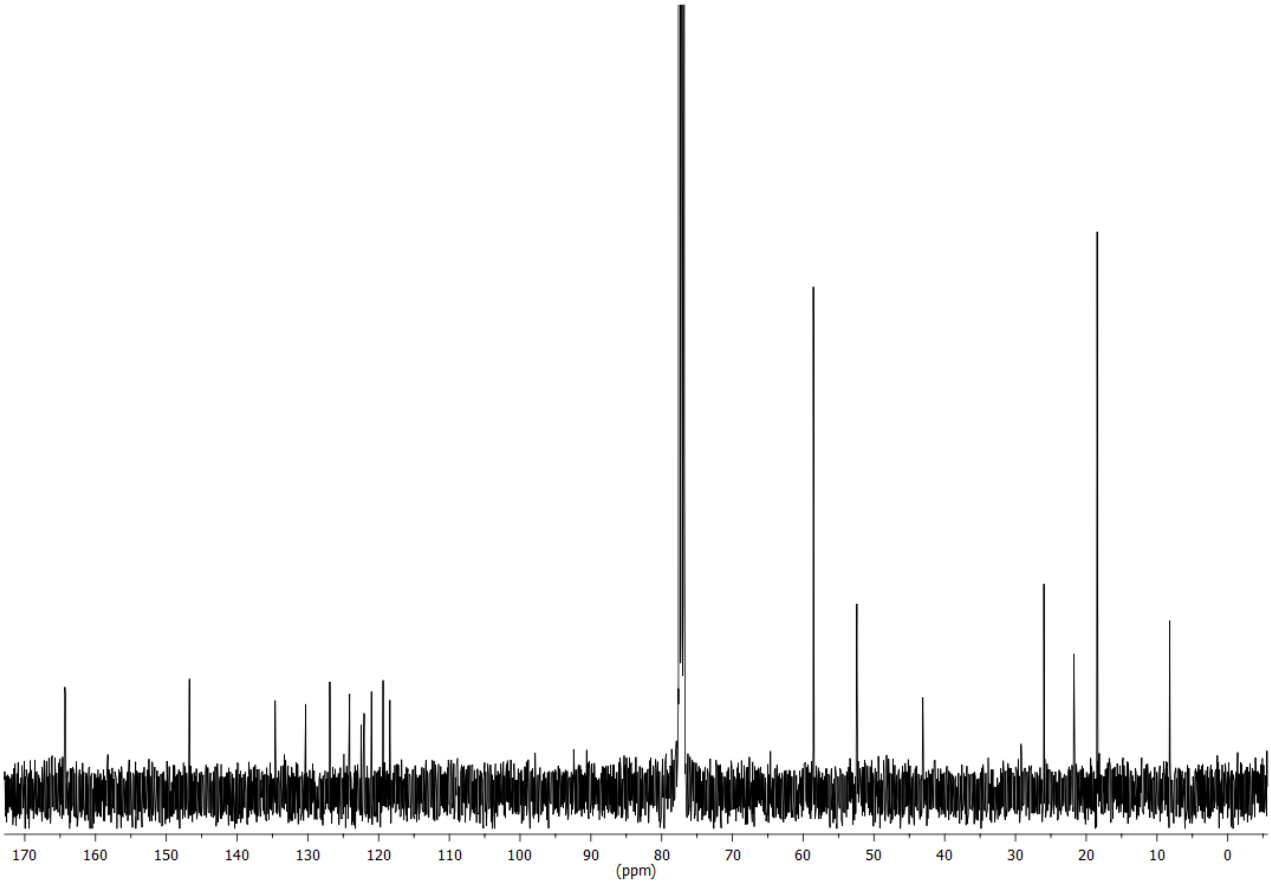


Figure S2 – ^{13}C NMR spectra of PDI **2**.

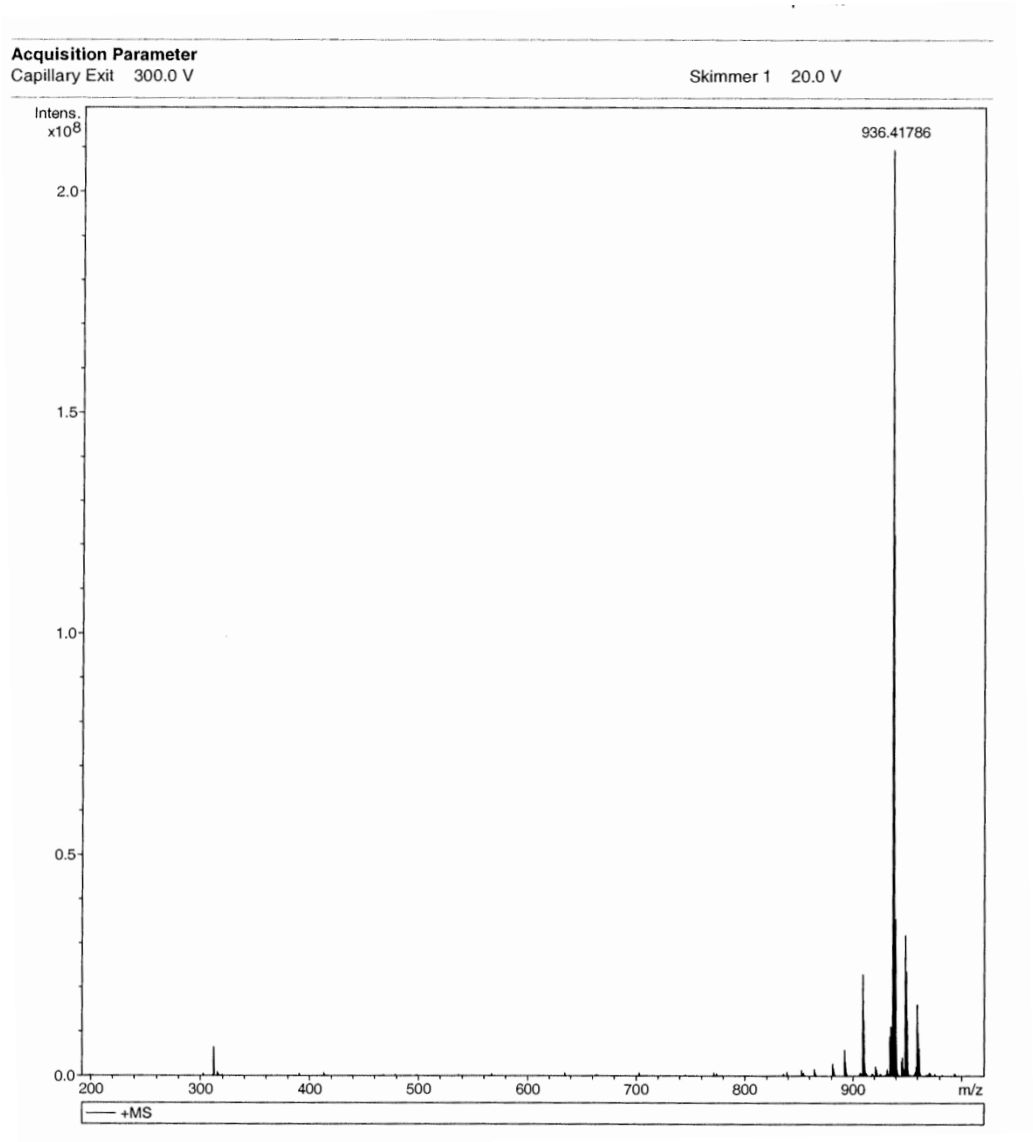


Figure S3 – HRMS-ESI spectra of PDI **2**.

(D) Synthesis of 2-SiNP

Water (6.0 g) absolute ethanol (23.33 g) and ammonia solution (2.52 mL) were mixed in a plastic flask, and stirred at 30ºC. TEOS (1.04 mL) and **2** (2.2 mg) were dissolved in absolute etanol (3 g) and added at the same time to this mixture. The reaction was kept for 24 hours at 30ºC with magnetic stirring. After that time, the particles were centrifuged during 30 minutes at 10000 rpm for 3 cycles and dispersed in ethanol or dried in vacuum.

(E) TEM and DLS of 2-SiNP

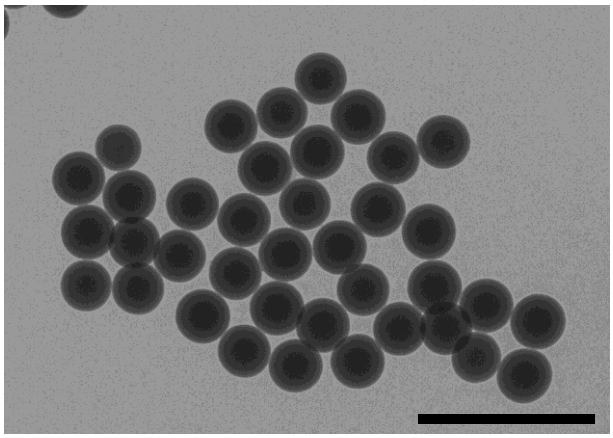


Figure S4 - TEM images of **2-SiNP** with an average diameter of 299±10 nm (obtained from more than 200 nanoparticles; scale bar: 1000 nm)

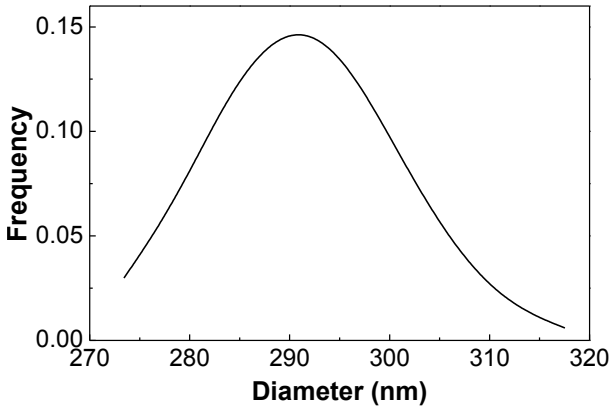


Figure S5 - Diameter distribution of **2-SiNP** with 288±2 nm (histogram obtained from several measurements by dynamic light scattering).

(F) Fluorescence data of PDI 2 and 2-SiNP

Table S1 - Photophysical parameters of PDI **2** in several solvents.

	λ_{max}^{abs} (nm)	λ_{max}^{exc} (nm)	λ_{max}^{emi} (nm)	ε at absorption maximum ($M^{-1}cm^{-1}$)	Φ_F	Brightness [a] ($\times 10^4$ M^{-1} cm^{-1})	τ (ns)
1,4-dioxane	685	684	728	41,700	0.24	1.00	4.0
Toluene	688	686	724	37,200	0.31	1.11	4.6
Ethanol	700	700	770	37,800	0.04[b]	0.14	1.0

[a] The product between the Absorptivity and the quantum yield.

[b] Photoinduced electron transfer from amines to fluorescent dyes, and consequent fluorescence quenching, is more favorable in polar solvents like ethanol.

Table S2 - Photophysical parameters of **2-SiNP** in several solvents.

	λ_{max}^{exc} (nm)	λ_{max}^{emi} (nm)	τ (ns)
1,4-dioxane	683	734	3.8
toluene	683	732	--[a]
ethanol	698	764	1.0
water	--[a]	765	1.4

[a] not determined due to low intensity/scattering ratio.

(G) Photostability of PDI 2 and 2-SiNP

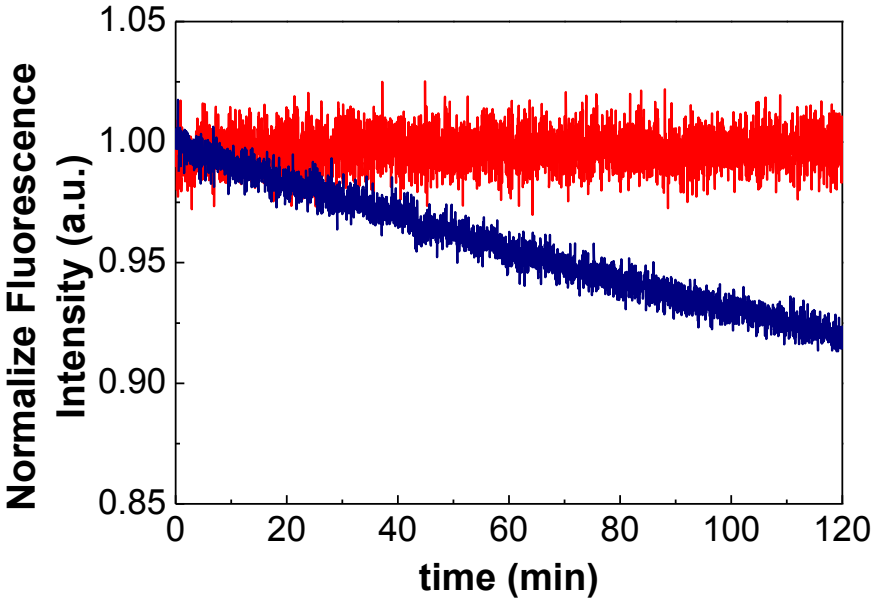


Figure S6 - Fluorescence intensity of a carbocyanine 5 derivative (DiIC18(5), *blue*) and PDI **2** (*red*) in 1,4-dioxane (with similar dye concentration) upon continuous irradiation at λ_{exc}= 615 nm using a 450 W Xe lamp. The fluorescence intensity of the DiIC18(5) drops almost 9% after 2 h, while no change is detected for PDI **2** A dispersion of **2-SiNP** in 1,4-dioxane with similar PDI **2** dye concentration was irradiated under the same conditions and exhibit the same profile than free PDI **2** (result not shown to avoid overlapping).

(H) Confocal Fluorescence Microscopy Imaging of 2-SiNP

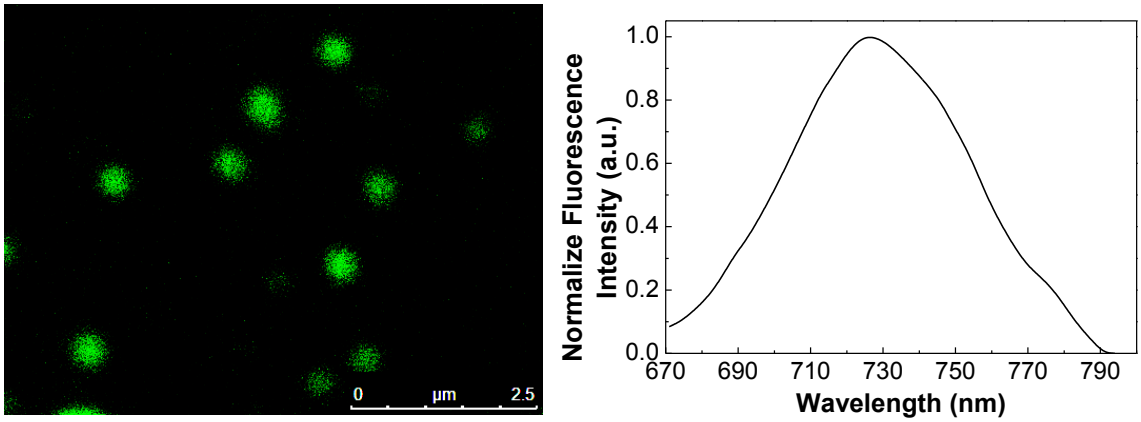


Figure S7 - Confocal fluorescence microscopy image (*left*) of **2-SiNP** casted from dioxane dispersions onto glass slides and using the HeNe (633 nm) laser as excitation source. The diameter is similar to the values recovered from TEM and DLS. The fluorescence emission spectrum obtained for the individual particles by laser scanning confocal microscopy (*right*), exibits a maximum at 730 nm, similar to the ensemble spectrum obtained for **2-SiNP** dispersed in dioxane. (reported by other authors to exhibit physical characteristics close to those of biological micro-environments).[6]

(I) References

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