

## Supporting Information to Accompany

### Solvatochromic Dissociation of Non-Covalent Fluorescent Organic Nanoparticles upon Cell Internalization

Contribution from

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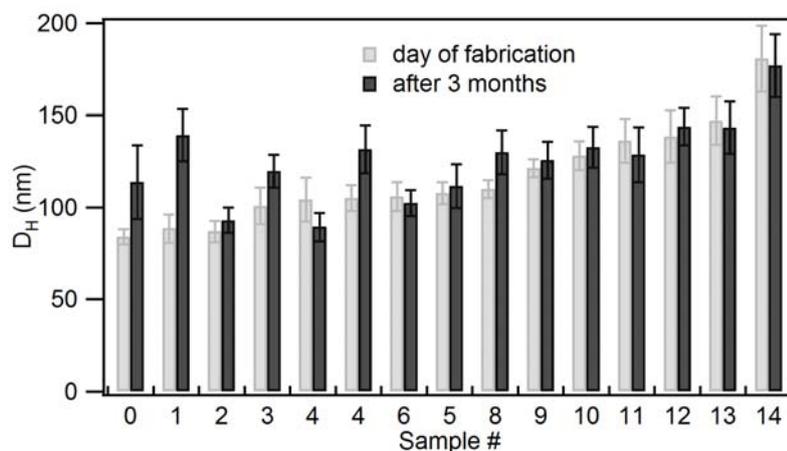
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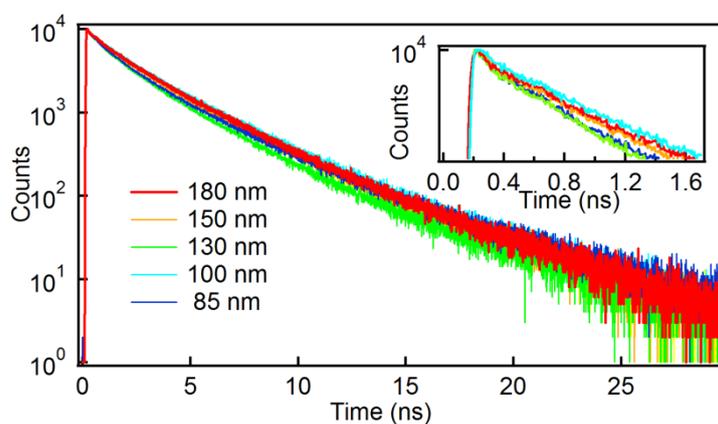
#### -List of figures-



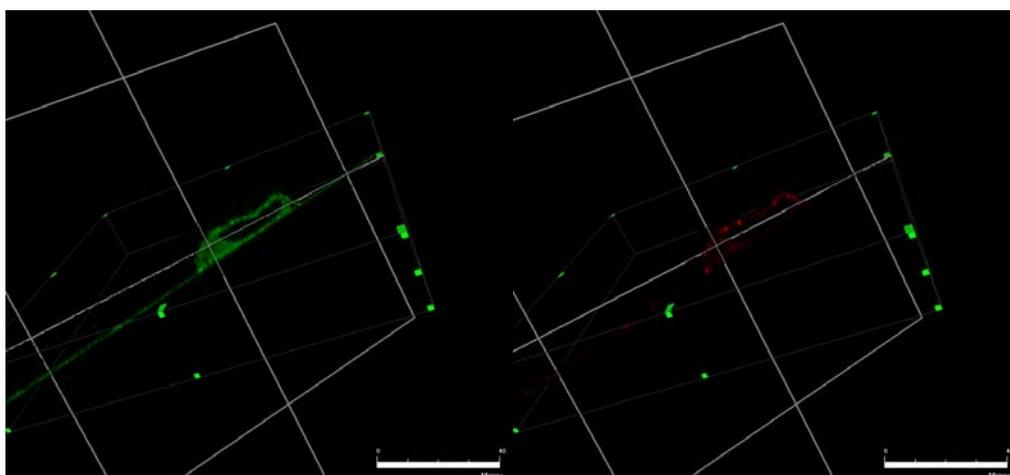
**Figure S1.** Time-stability of *fvin* nanoparticle suspensions in water fabricated by using the reprecipitation method. Suspensions were stored at 4°C.



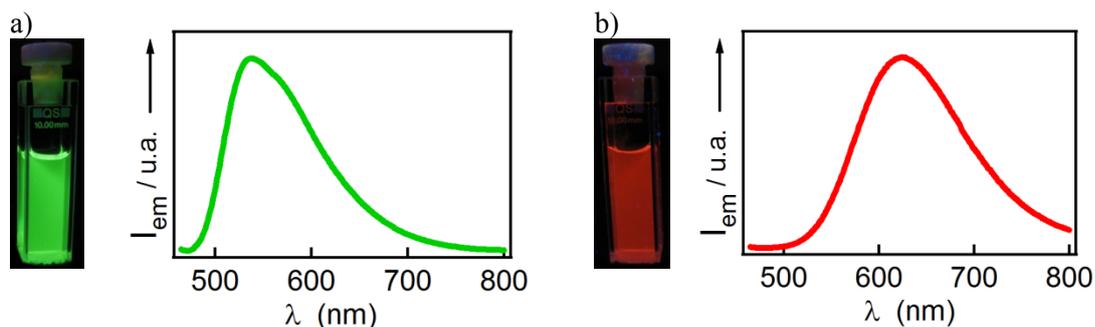
**Figure S2.** Electrophoresis on agarose gel containing 0.6 % BET after applying a 80 mV voltage for 50 min and using TBE as electrophoresis electrolyte. From left to right: DNA, fvin nanoparticle, ratio DNA to fvin 1:1, 2:1, 1:2, 4:1 and 1:4.



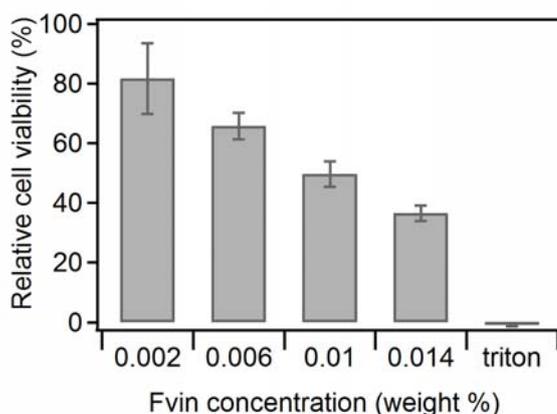
**Figure S3.** Fluorescence lifetime decays of *fvin* nanoparticles suspensions in water ( $\lambda_{\text{exc}} = 460$  nm;  $\lambda_{\text{detection}} = 630$  nm). Inset: zoom-in of the fluorescence decays.



**Figure S4.** Fluorescence confocal imaging of Z-sections of a NIH-3T3 cell incubated with *fvin* nanoparticles ( $\lambda_{\text{exc}} = 840$  nm). Left: emission recorded on [495-535] nm. Right: emission recorded on [590-640] nm for identical Z.



**Figure S5.** Fluorescence spectra ( $\lambda_{exc} = 456$  nm) of aqueous suspensions of *fvin* nanoparticles in the presence of sodium dodecylsulfate as a surfactant at a concentration a) superior ( $0.022 \text{ mol L}^{-1}$ ), and b) inferior ( $5 \times 10^{-4} \text{ mol L}^{-1}$ ) to the critical micellar concentration ( $0.008 \text{ mol L}^{-1}$  at  $25 \text{ }^\circ\text{C}$ ).



**Figure S6.** NIH-3T3 cell proliferation with various *fvin* nanoparticle concentrations assessed by MTT test after a 24 h incubation period. Control test to prove MTT activity was performed with triton-X.

### -Materials and experimental techniques-

**Reagents.** 4-di(4'-tert-butylbiphenyl-4-yl)amino-4'-dicyanovinylbenzene (*fvin*) was synthesized after a four-step procedure as reported in literature.<sup>1</sup> L- $\alpha$ -phosphatidylcholine lipids (egg- chicken 95 %) were purchased from Avanti Polar Lipids. Tris-borate-EDTA buffer (T3913), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT salt 135038), as well as all the disposable materials and products needed for cell culture were purchased from Invitrogen. Agarose (D-1 low EEO, C1349) was purchased from Eppendorf. All organic solvents were of spectroscopic grade quality and purchased from Aldrich. Ultrapure-grade water (Millipore-quality) having a conductivity of  $18.2 \mu\Omega$  was used in all experiments.

**Nanoparticles formation.** Stock solutions of *fvin* in acetone or DMSO were prepared at various concentrations depending on the targeted diameter of nanoparticles. Typically,  $100 \mu\text{L}$  of stock solution were added under vigorous stirring into  $5 \text{ mL}$  of ultra-pure deionized water (or a mixture of

water:acetone of varying composition) by means of a vortex to avoid collapse of nanoparticles onto a Teflon stirring bar if a magnetic stirrer were used.

**Dynamic Light Scattering (DLS).** DLS measurements were performed by means of a nanoparticle size analyzer DL135 (Cordouan Tcehnologies) equipped with a 15 mW diode laser, operating at 658 nm, and a photomultiplier detector collecting backscatter light at an angle of 135°. Measurements were carried out at 20 °C on aqueous solutions of NPs. For filtered solutions, filtration was performed on a 0.80 µm cellulose syringe filter. For each sample, intensity measurements were carried out in a multi-acquisition mode implying 30 correlograms, each recorded over a 25 s period. Nanoparticle mean sizes and distribution widths were obtained by fitting each correlogram with a Cumulants algorithm. The time interval and number of channels were fixed for each measurement at 12 µs and 500 respectively.

**Zetametry.** Measurements of surface potential  $\zeta$  were carried out by means of a Zetasizer Nano ZS ZEN 3600 (Malvern). The samples were placed in plastic cells. Several measurements were realized for each sample according to a predefined operating procedure.

**TEM imaging.** Nanoparticle morphology was investigated by transmission electron microscopy (TEM, JEOL 100CXII). Solutions of nanoparticles were deposited onto a carbon-coated copper grid. After solvent evaporation, the grid could be exposed to ruthenium tetroxide vapor for 10 s to stain the aromatic rings of the compounds and gain imaging contrast.

**Steady-state absorption and emission measurements.** UV-visible absorption spectra were recorded using a Varian Model Cary 5E spectrophotometer. Corrected emission spectra were obtained using Jobin-Yvon. Inc spectrofluorimeter (Fluorolog 3). Fluorescence quantum yields in solution were determined from a solution of Coumarine 540 A in EtOH ( $\Phi_f = 0.38$ ).

**Fluorescence lifetime imaging.** Fluorescence lifetime measurements were performed by means of a frequency-doubled Yb:KGW femtosecond laser (100 fs fwhm, 10 MHz, 515 nm, t-Pulse 200 Amplitude) which was directed to a Nikon 2000 TE inverted microscope in the wide-field configuration, reflected by an appropriate dichroic mirror (480-520 nm) and sent toward the sample through a high transmission microscope objective (Nikon S-Fluor, 40×, 0.90 NA). Spectral analyses of the back-collected fluorescence were performed by means of a fiber-coupled spectrometer (Ocean Optics, Inc., ZD2000). Alternatively, an ionized argon laser source at 488 nm as the excitation source was used to follow change in the emission energy and intensity in the suspended lipid bilayer.

**One- and two-photon cell imaging.** Fluorescence imaging was performed on fixed cells using a Leica SP2 inverted microscope (DMIRE2) equipped with an oil immersion objective (Zeiss, 40×, 0.75-1.25 NA) and an ultra-high pressure mercury lamp (bandpass centered at 360 nm) coupled to the

objective through a liquid light guide (Leica). Epifluorescence imaging was performed by using a digital camera (Nikon DXM 1200). Alternatively, two-photon fluorescence excitation was carried out using a Leica TCSP2 confocal system, and a Mai Tai tunable laser (Spectra Physics – 100-200 fs pulses - 80 MHz repetition range). Two-photon excitation was carried out at 840 nm and emission was collected in the 490-750 nm spectral range. Neither degradation, nor bleaching were observed during the image acquisition.

**Cell culture.** Murine fibroblasts NIH 3T3 cells were cultured as monolayers, incubated at 37°C in a humidified atmosphere with 5% CO<sub>2</sub> in Dulbecco's modified Eagle medium (Gibco Invitrogen) supplemented WITH 10% fetal bovine serum (Invitrogen), 50 units/mL penicillin, and 50 units/mL streptomycin. Cell cultures were incubated in a humidified atmosphere containing 5% CO<sub>2</sub> at 37°C.

**Incubation.** Cells were grown in 12-well sterile plates containing 30 mm round glass coverslips and incubated in the presence of nanoparticle solutions of various concentrations. After 24h, 48 h or 72 h, cells were washed three times with sterile PBS 1×, and fixed prior to imaging with 50 µL of fixing medium (4% paraformaldehyde in PBS). After 15 min. at RT and two washings with PBS, each circular coverslip was glued for 15 min onto another microscope coverslip using a drop of mowiol, after which time observation could be performed.

**In vitro cytotoxicity.** The viability of NIH 3T3 cells in the presence of *fvn* nanoparticles was tested by using a 5 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) (Invitrogen). The MTT lysis buffer consisted in a mixture of 20 % SDS solution (25 mL) and 1 M HCl solution (100 µL) in 25 mL of water. pH of the resulting solution was checked to be 4.7.  $5 \times 10^5$ /mL cells were first seeded in each well of a 96-well plate and incubated at 37 °C for 48 h in the presence of various amounts of nanoparticles prepared from 0.1 wt.% DMSO stock solutions. The plate was incubated for 48 h, after which time 100 µL of a mixture made of 10 µL fresh MTT solution (5 mg/mL) and 90 µL of culture medium were added into each well. After an additional incubation for 30 min. at 37 °C, 100 µL of the freshly prepared SDS solution were added to each well. After 2 h of incubation to complete mitochondrial reduction of MTT into a dark violet formazan precipitate, absorbance at 570 nm was measured using a Bio-Rad plate reader and subtracted from the absorbance of blank samples containing no cells. The absorbance values could be correlated to the percentage of vital cells, by comparing the data of the doped cells with those of the control cells with the individual components (i.e. either DMSO or Triton X).

**Electrophoretic migration on agarose gel.** 20 µL of nanoparticle solution mixed with 5 µL of bromophenol blue was deposited on an agarose gel (0.8 g of agarose on 100 mL TBE: Tris 1M, boric acid 0.9 M, EDTA 0.01 M). The gel was submitted to a 80 V voltage for 50 minutes. The gel is revealed with a UV lamp at 365 nm.

**Description of the microfluidic chip and fabrication of the suspended bilayer.**<sup>2</sup> The chip consisted of a PMMA block with a central circular aperture ( $\varnothing = 4$  mm,  $H = 5$  mm), and a fluidic channel ( $W = 2$  mm,  $H = 0.5$  mm,  $L = 24$  mm) terminated by two wells serving as entry and exit outlets for the injected solutions. A 2  $\mu\text{m}$ -thin poly(*p*-xylylene) (parylene) sheet obtained by plasma polymerization and ulterior piercing by standard photolithography was sandwiched between the PMMA block and a glass microscope coverslip. The parylene membrane was adjusted such that the 100  $\mu\text{m}$ -wide hole was centered regarding the PMMA central aperture. The lipid solution was obtained by dissolving 25 mg of L- $\alpha$ -phosphatidylcholine into 1 mL of *n*-decane and stored cold in the fridge when not used. 40  $\mu\text{L}$  of Millipore water were deposited on the top of the parylene aperture. 8  $\mu\text{L}$  of the lipid solution were first injected, followed by the slow injection of 80  $\mu\text{L}$  of Millipore water. A suspended lipid bilayer spontaneously formed across the aperture when the lipid solution flowed along. The lipid bilayer could be observed by means of an inverted optical microscope (IX-71, Olympus, Japan) in a DIC mode.

- (1) Ishow, E.; Brosseau, A.; Clavier, G.; Nakatani, K.; Tauc, P.; Fiorini-Debuisschert, C.; Neveu, S.; Sandre, O.; Leaustic, A. *Chem. Mater.* **2008**, *20*, 6597.
- (2) Le Pioufle, B.; Suzuki, H.; Tabata, K. V.; Noji, H.; Takeuchi, S. *Anal. Chem.* **2008**, *80*, 328.