Electronic Supplementary Information (ESI)

Multiple dye-doped NIR-emitting silica nanoparticles for both flow cytometry and *in vivo* imaging

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Contents

Materials

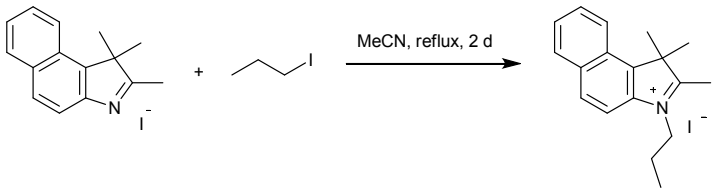
1-Iodopropane (99%), malonaldehyde bis(phenylimine) monohydrochloride (97%), acetic anhydride (≥ 99%), 6-Bromohexanoic acid (97%), DMF (≥ 99.8%, over molecular sieve, H_2O ≤ 0.01%), Pyridine (≥ 99.8%, over molecular sieve (H_2O ≤ 0.005%)), 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU, 97%), IR-813 perchlorate (80%), (3-Mercaptopropyl)triethoxysilane (≥ 80%), MeCN (99.8%), diethyl ether (≥ 99.8%), MeOH (≥ 99.8%), dichloromethane (≥ 99.8%), Ethyl acetate (≥ 99.5%), Pluronic F127, tetraethyl orthosilicate (TEOS, 99.99 %), chlorotrimethylsilane (TMSCl, ≥ 98 %), acetic acid (HOAc, ≥ 99.7 %), HCl (≥ 37 %), reagent grade dimethylformamide (DMF), diethylether (Et_2O) and acetone were purchased from Sigma-Aldrich. 1,1,2-Trimethylbenz[e]indole (≥ 98%), triethylamine (TEA, ≥ 99.5%), (3-Aminopropyl)triethoxysilane (APTES, ≥ 98%), *N*,*N*-Diisopropylethylamine (DIPEA, ≥ 98%), NaCl and Silica on TLC Alu foils (4 × 8 cm, with fluorescent indicator 254 nm) were purchased from Fluka. Reference Cy5.5 commercial dye (Cy5.5 GE) was purchased from GE Healthcare (Amersham FluoroLink™ Cy5.5 Monofunctional dye, Code: PA25501).

UF tubes Amicon Ultra-0.5mL, cut-off 100 KDa, were purchased from Millipore. Dialysis was performed vs. water at room temperature under gentle stirring with regenerated cellulose dialysis tubing (Sigma, mol wt. cut-off > 12 KDa, avg. diameter 33 mm).

Synthesis

Synthesis of Cy5.5

3-propyl-1,1,2-trimethyl-1H-benz[e]indolium iodide salt (2). Compound **2** was synthesized adapting reported procedures.[1] In a 100 mL flamed round bottom flask dried under vacuum, to a solution of 1,1,2-Trimethylbenz[e]indole (**1**) (1.0 g, 4.8 mmol) in MeCN (36 mL) was added 1-Iodopropane (702 µL, 7.2 mmol) and the reaction mixture was heated under reflux for two days.

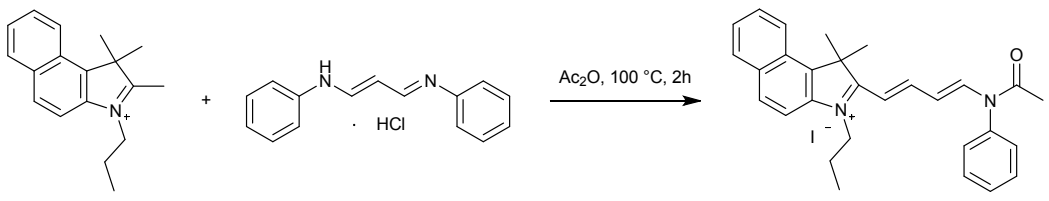


1 **2**

After cooling down to room temperature, the reaction mixture was concentrated in vacuo and the resulting oil was washed several times with diethyl ether to obtain compound **2** as dark brown solid (1.34 g, yield 97 %).

^{1}**H-NMR** (400 MHz, $CDCl_3$, 25°C) δ = 8.08 (2H, t, *J* = 8 Hz, ar. C*H*) and 8.04 (1H, d, *J* = 8 Hz, ar. C*H*) part. overlapped, 7.92 (1H, d, *J* = 8 Hz, ar. C*H*), 7.83 (1H, d, *J* = 8 Hz, ar. C*H*), 7.66 (1H, t, *J* = 8 Hz, ar. C*H*), 4.80 (2H, t, *J* = 8 Hz, -NCH_2-), 3.20 (3H, s, -CH_3), 2.11-2.02 (2H, m, -$NCH_2CH_2CH_2$), 1.87 (6H, s, -CH_3), 1.11 (3H, t, *J* = 8 Hz, -$NCH_2CH_2CH_3$); **^{13}C-NMR** (100 MHz, $CDCl_3$, 25°C) δ = 195.3, 189.7, 150.1, 138.5, 138.0, 136.9, 133.5, 132.1, 131.2, 130.0, 127.3, 55.8, 55.2, 22.6, 21.6, 16.8, 11.2; **ESI-MS** m/z calculated for $C_{18}H_{22}N^+$ 252.17; obs.: 252.2.

2-[4-(N-Phenyl-N-acetylamino)1,3-pentadienyl]-1,1-dimethyl-3-propyl-1H-benz[e]indolium iodide salt (3). Compound **3** was synthesized adapting reported procedures.[1, 2] In a flamed 50 mL round bottom flask dried under vacuum, a suspension of compound **2** (1.0 g, 3.5 mmol) and malonaldehyde bis(phenylimine) monohydrochloride (906 mg, 3.5 mmol) in acetic anhydride (17 mL) was heated at 100 °C for 2h.

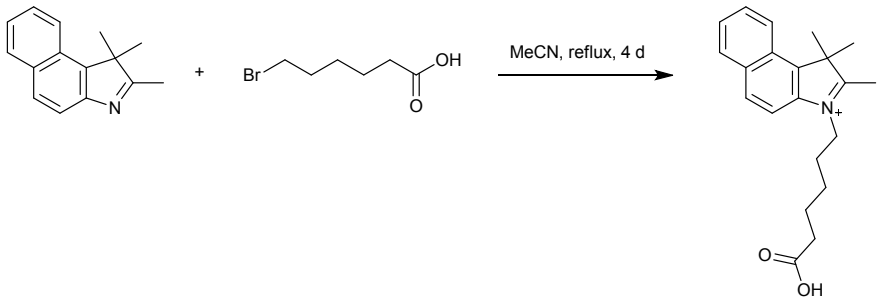


2 **3**

After cooling down to room temperature, the reaction mixture was poured into 190 mL of diethyl ether. The dark brown precipitate (1.51 g, yield 79%) was then filtered and used in the next step without further purification.

ESI-MS m/z calculated for $C_{29}H_{31}N_2O^+$ 423.24; obs.: 423.2.

1,1,2-Trimethyl-3-(6-carboxylatohexyl)benz[e]indolium bromide salt (4). Compound **4** was synthesized adapting reported procedures.[1, 2] In a flamed 100 mL round bottom flask dried under vacuum, to a solution of 1,1,2-Trimethylbenz[e]indole (**1**) (1.0 g, 4.8 mmol) in MeCN (40 mL) was added 6-Bromohexanoic acid (1.21 g, 6.2 mmol) and the reaction mixture was heated under reflux for four days. After cooling down to room temperature, the reaction mixture was poured into 150 mL of diethyl ether and kept in fridge overnight.



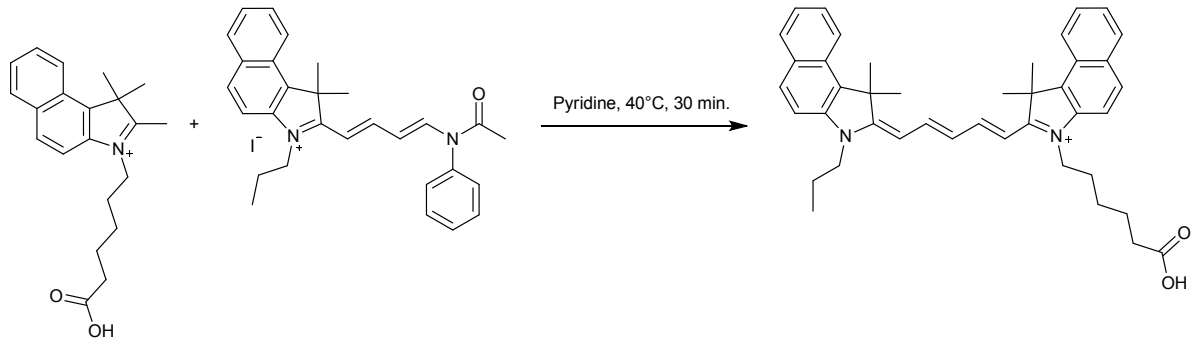
1 **4**

The obtained solid was filtered and washed three times with diethyl ether to obtain the title compound as blue-green solid (0.522 g, yield 34%).

^{1}H NMR (400 MHz, MeOH-d_4, 25°C) δ = 8.33 (1H, d, *J* = 8 Hz, ar. C*H*), 8.25 (1H, d, *J* = 8 Hz, ar. C*H*), 8.17 (1H, d, *J* = 8 Hz, ar. C*H*), 8.01 (1H, d, *J* = 8 Hz, ar. C*H*), 7.83-7.79 (1H, m, ar. C*H*), 7.75-7.71 (1H, m, ar. C*H*), 4.64 (2H, t, *J* = 8 Hz, –NCH_2-), 2.36 (2H, t, *J* = 6 Hz, -CH_2COOH), 2.10-2.01 (2H, m, -NCH$_2$CH_2), 1.85 (6H, s, -CH_3), 1.79-1.76 (2H, m, -CH_2CH$_2$COOH), 1.62-1.54 (2H, m, -CH$_2$CH_2CH$_2$-); **^{13}C-NMR** (100 MHz, $CDCl_3$, 25°C) δ = 197.7, 175.8, 139.9, 138.9, 135.4, 132.6, 131.2, 129.9, 129.29, 128.93, 124.57, 113.92, 57.5, 34.52, 34.41, 28.9, 27.21, 27.16, 25.58, 25.55, 22.5; **ESI-MS** m/z calculated for $C_{21}H_{26}NO_2^+$ 324.2; obs.: 324.2.

2-[7-(1,3-Dihydro-1,1-dimethyl-3-propylbenz[e]indolin-2-ylidene)-1,3,5-pentatrienyl]-1,1-dimethyl-3-(6-carboxylatohexyl)-1H-benz[e]indolium inner salt (5). Compound **5** was

synthesized adapting reported procedures.[1, 2] In a flamed 10 mL round bottom flask dried under vacuum, to a solution of 1,1,2-Trimethyl-3-(6-carboxylatohexyl)benz[e]indolium bromide salt (**4**) (0.150 g, 0.46 mmol) in Pyridine (3 mL) was added 2-[4-(N-Phenyl-N-acetylamino)1,3-pentadienyl]-1,1-dimethyl-3-propyl-1H-benz[e]indolium iodide salt (**3**) (0.255 g, 0.46 mmol) and the reaction mixture was heated at 40°C for 30 min.



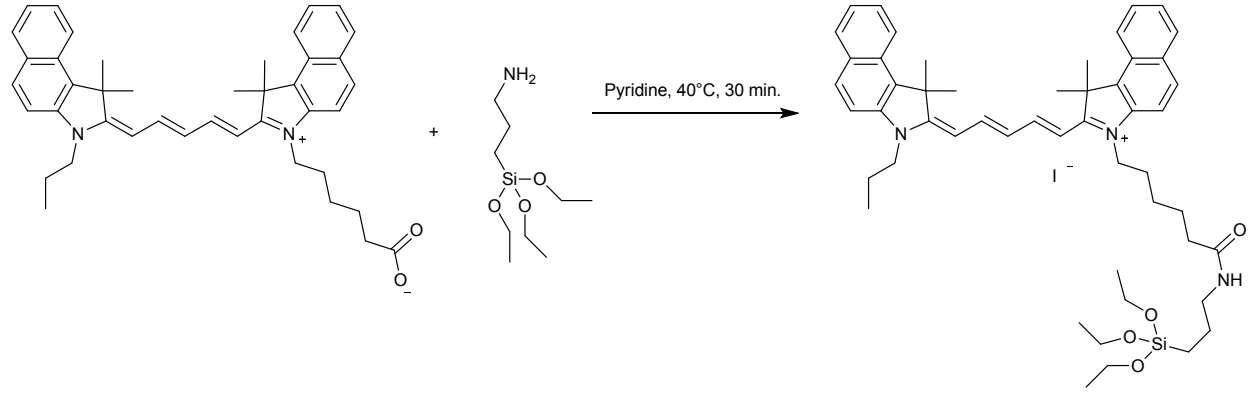
4 **3** **5**

After cooling down to room temperature, the reaction mixture was concentrated in vacuo and the residue was purified by silica gel chromatography eluting with CH_2Cl_2/MeOH (99:1 - 90:10) to give the compound **5** as blue solid (0.147 g, yield 49%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C) δ = 8.33-8.11 (4H, m, ar. C*H*), 7.90 (4H, d, *J* = 8 Hz, ar. C*H*), 7.59 (2H, q, *J* = 6.7 Hz, ar. C*H*), 7.45 (2H, q, *J* = 6.7 Hz, ar. C*H*), 7.32 (2H, t, *J* = 8 Hz, C*H*), 6.99 (1H, t, *J* = 12 Hz, C*H*), 6.54 (1H, d, *J* = 12 Hz, C*H*), 6.37 (1H, d, *J* = 16 Hz, C*H*), 4.19 (2H, t, *J* = 8 Hz, -NC*H*$_2$-) and 4.15 (2H, t, *J* = 8 Hz, -NC*H*$_2$-) par. overlapped, 2.15 (2H, t, *J* = 8 Hz, -C*H*$_2$COOH), 2.05-2.03 (12H, d, -C*H*$_3$), 1.93-1.86 (4H, m, -NCH$_2$C*H*$_2$CH$_2$ and -C*H*$_2$CH$_2$COOH), 1.80-1.76 (2H, m, -NCH$_2$C*H*$_2$), 1.61-1.57 (2H, m, -CH$_2$C*H*$_2$CH$_2$-), 1.08 (3H, t, *J* = 8 Hz, -NCH$_2$CH$_2$C*H*$_3$); **^{13}C-NMR** (100 MHz, $CDCl_3$, 25°C) δ = 11.7, 15.1, 21.5, 24.3, 26.4, 28.1, 34.1, 44.6, 46.3, 51.2, 51.4, 58.6, 103.7, 104.4, 110.6, 110.8, 110.9, 120.1, 122.4, 122.5, 123.6, 124.5, 125.2, 125.3, 125.4, 127.0, 128.1, 129.2, 130.3, 130.8, 130.9, 135.9, 152.2, 173.9, 202.0; **ESI-MS** m/z calculated for $C_{42}H_{47}N_2O_2^+$ 611.36; obs.: 611.2.

2-[7-(1,3-Dihydro-1,1-dimethyl-3-propylbenz[e]indolin-2-ylidene)-1,3,5-pentatrienyl]-1,1-dimethyl-3-[6-[*N*-(3-(triethoxysilyl)propyl]hexanamide]-1H-benz[e]indolium iodide salt (6). Compound **6** was synthesized adapting reported procedures.[1] In a flamed 25 mL round bottom flask

dried under vacuum, to a solution of 2-[7-(1,3-Dihydro-1,1-dimethyl-3-propylbenz[e]indolin-2-ylidene)-1,3,5-pentatrienyl]-1,1-dimethyl-3-(6-carboxylatohexyl)-1H-benz[e]indolium inner salt (**5**) (0.118 g, 0.19 mmol) in dichloromethane (12 mL) was added 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU) (0.087 g, 0.24 mmol), (3-Aminopropyl)triethoxysilane (0.049 mL, 0.21 mmol) and triethylamine (TEA) (0.053 mL, 0.38 mmol) and the reaction mixture was stirred at room temperature for 2h.



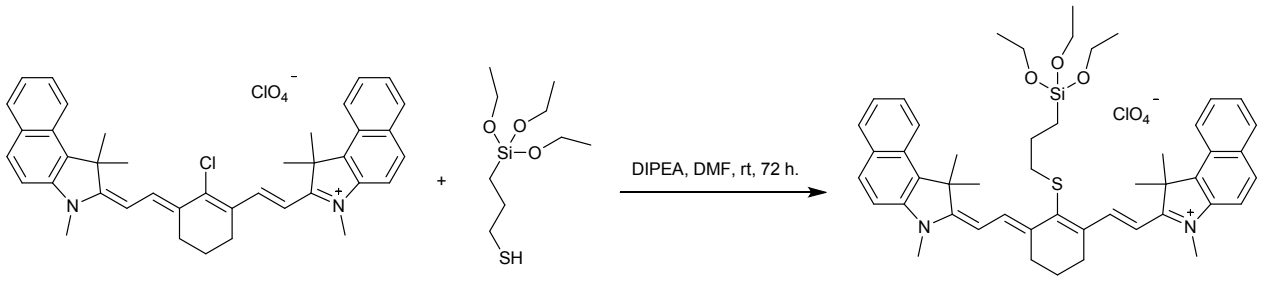
5 **6**

The reaction mixture was concentrated in vacuo and the residue was purified by silica gel chromatography eluting with ethyl acetate/methanol (95:5-80:20) to give the title compound **6** as blue solid (0.102 g, yield 66%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C) δ = 8.33-8.11 (4H, m, ar. C*H*), 7.90 (4H, d, *J* = 8 Hz, ar. C*H*), 7.59 (2H, q, *J* = 6.7 Hz, ar. C*H*), 7.45 (2H, q, *J* = 6.7 Hz, ar. C*H*), 7.32 (2H, t, *J* = 8 Hz, C*H*), 6.99 (1H, t, *J* = 12 Hz, C*H*), 6.54 (1H, d, *J* = 12 Hz, C*H*), 6.37 (1H, d, *J* = 16 Hz, C*H*), 4.19 (2H, t, *J* = 8 Hz, -NCH_2-) and 4.15 (2H, t, *J* = 8 Hz, -NCH_2-) par. overlapped, 3.77 (6H, q, *J* = 6.7 Hz, –OCH_2CH$_3$) - (6H, s, N-CH_3) overlapped, 2.15 (2H, t, *J* = 8 Hz, -CH_2COOH), 2.05-2.03 (12H, d, -CH_3), 1.96-1.92 (2H, m, -NHCH$_2$CH_2CH$_2$-) and 1.93-1.86 (4H, m, -NCH$_2$CH_2CH$_2$ and -CH_2CH$_2$COOH) overlapped, 1.80-1.76 (2H, m, -NCH$_2$CH_2), 1.81-1.74 (2H, q, -CH$_2$CH_2CH$_2$-), 1.61-1.57 (2H, m, -CH$_2$CH_2CH$_2$-), 1.17 (9H, t, *J* = 6 Hz, –OCH$_2$CH_3), 1.08 (3H, t, *J* = 8 Hz, -NCH$_2$CH$_2$CH_3), 0.75-0.70 (2H, m, -CH_2CH$_2$CH_2Si); **^{13}C-NMR** (100 MHz, $CDCl_3$, 25°C) δ = 12.1, 13.9, 18.5, 21.6, 23.5, 24.4, 25.0, 25.3, 27.5, 27.7, 34.3, 43.1, 49.4, 49.7, 52.8, 53.3, 58.4, 104.5, 104.8, 113.7, 117.6, 121.0, 122.0, 124.9, 125.5, 125.6, 125.8, 126.1, 126.3, 126.5, 126.9, 127.0, 128.6, 130.4, 132.5, 132.6, 133.5, 137.4, 143.1, 148.4, 170.7, 172.8; **ESI-MS** m/z calculated for $C_{51}H_{68}N_3O_4Si^+$ 814.5; obs.: 816.4. **ESI-MS** m/z calculated for $C_{51}H_{68}N_3O_4Si^+$ 814.5; obs.: 816.4.

Synthesis of Cy7

2-[2-[2-Thio-[3-(triethoxysilyl)propyl]-3-[2-(1,3-dihydro-1,1,3-trimethyl-2*H*-benzo[e]-indol-2-ylidene)-ethylidene]-1-cyclohexen-1-yl]-ethenyl]-1,1,3-trimethyl-1*H*-benzo[e]indolium perchlorate (7). Compound **7** was synthesized adapting reported procedures.[3]



IR-813 **7**

In a flamed 25 mL round bottom flask dried under vacuum, to a solution of 2-[2-[2-Chloro-3-[2-(1,3-dihydro-1,1,3-trimethyl-2*H*-benzo[e]-indol-2-ylidene)-ethylidene]-1-cyclohexen-1-yl]-ethenyl]-1,1,3-trimethyl-1*H*-benzo[e]indolium perchlorate (IR-813 perchlorate, 0.064 g, 94 μmol) in DMF (6 mL) was added *N*,*N*-Diisopropylethylamine (DIPEA, 20 μL, 113 μmol) and (3-Mercaptopropyl)triethoxysilane (68 μL, 282 μmol) and the reaction mixture was stirred at room temperature for 72h. The reaction mixture was then diluted with brine and extracted three times with dichloromethane. The combined organic phases are dried over sodium sulfate, concentrated with the rotary evaporator and desiccated in vacuum, affording a green solid substance. Purification was carried out by silica gel chromatography eluting with Ethyl acetate/methanol (9:1-1:9) to give the title compound **7** as a dark green solid (0.053 g, yield 64%).

^{1}**H NMR** (400 MHz, $CDCl_3$) δ = 8.91 (2H, d, *J* = 16 Hz, C*H*), 8.11 (2H, d, *J* = 8 Hz, ar. C*H*), 7.93 (4H, d, *J* = 8 Hz, ar. C*H*), 7.47-7.43 (2H, m, ar. C*H*), 7.41 (2H, d, *J* = 8 Hz, ar. C*H*), 6.23 (2H, d, *J* = 12 Hz, C*H*), 3.77 (6H, q, *J* = 6.7 Hz, –OCH_2CH$_3$) - 3.77 (6H, s, N-CH_3) overlapped, 2.83 (2H, t, *J* = 8 Hz, -SCH_2-), 2.68 (4H, t, *J* = 8 Hz, -CH_2CH$_2$CH_2-), 2.03 (12H, s, -CH_3), 1.96-1.92 (2H, m, -SCH$_2$CH_2CH$_2$-), 1.81-1.74 (2H, q, -CH$_2$CH_2CH$_2$-), 1.17 (9H, t, *J* = 6 Hz, –OCH$_2$CH_3), 0.75-0.70 (2H, m, -CH_2CH$_2$CH_2Si); **^{13}C-NMR** (100 MHz, $CDCl_3$, 25°C) δ = 139.8, 139.2, 135.7, 132.5, 131.5, 128.1,

125.6, 124.9, 124.5, 123.1, 105.0, 70.0, 64.5, 57.4, 30.7, 29.3, 27.14, 27.13, 27.0, 17.6, 7.9; **ESI-MS** m/z calculated for $C_{51}H_{68}N_3O_4Si^+$ 814.5; obs.: 816.4.

Photophysical measurements

UV-VIS absorption spectra were recorded at 25°C by means of Perkin-Elmer Lambda 45 spectrophotometer. The fluorescence spectra were recorded with a Perkin-Elmer Lambda LS 55 fluorimeter and with a modular UV-vis-NIR spectrofluorimeter Edinburgh Instruments FLS920 equipped with a photomultiplier Hamamatsu R928P. The latter instrument connected to a PCS900 PC card was used for the Time Correlated Single Photon Counting (TCSPC) experiments (excitation laser λ = 640 nm). Corrected fluorescence emission and excitation spectra (450 W Xe lamp) were obtained with the same instrument equipped with both a Hamamatsu R928P P photomultiplier tube (for the 500-850 nm spectral range) and an Edinburgh Instruments Ge detector (for the 800-1600 nm spectral range). Quartz cuvettes with optical path length of 1 cm were used for both absorbance and emission measurements.

Nanoparticles solutions were diluted with Milli-Q® water. Luminescence quantum yields (uncertainty ± 15%) were recorded on air-equilibrated solutions and cyanine IR-125 (from Acros Organics) in ethanol (Φ = 0.05).[4] Corrections for instrumental response, inner filter effects and phototube sensitivity were performed.[5] All fluorescence anisotropy measurements were performed on an Edinburgh FLS920 equipped with Glan-Thompson polarizers. Anisotropy measurements were collected using an L-format configuration, and all data were corrected for polarization bias using the G-factor. Four different spectra were acquired for each sample combining different orientation of the excitation and emission polarizers: I_{VV}, I_{VH}, I_{HH}, I_{HV} (where V stands for vertical and H for horizontal; the first subscript refers to the excitation polarizer and the second subscript refers to the emission one). The spectra were used to calculate the G-factor and the anisotropy *r*: $G = I_{HV}/I_{HH}$, $r = (I_{VV} - GI_{VH})/(I_{VV} + 2GI_{VH})$.

Photophysical properties. Nanoparticles samples, having different doping levels, present the spectral properties of the doping dyes Cy5.5 (**6**) and Cy7 (**7**): the absorption and emission spectra of samples **NP-1**, **NP-2**, **NP-3** and **NP-4** are reported in Fig. S1-S4.

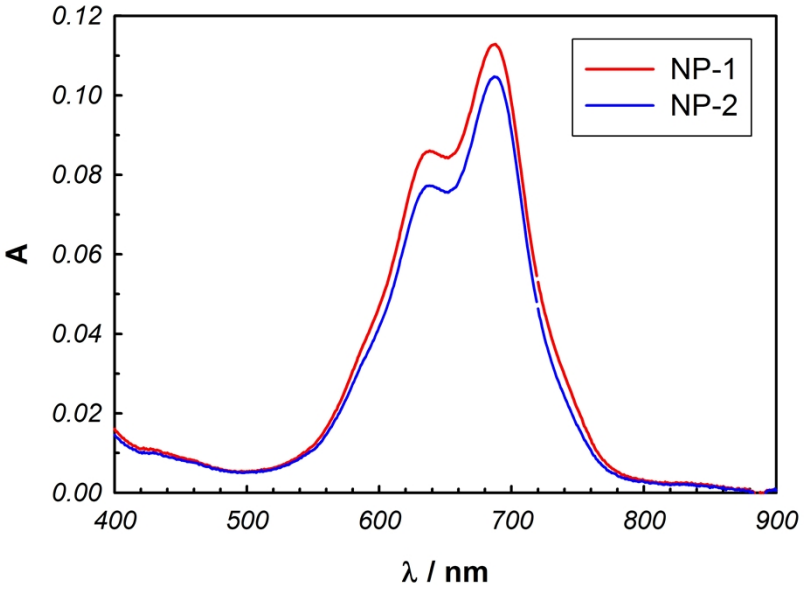


Figure S1: Absorption spectra of aqueous suspensions of nanoparticles samples **NP-1** and **NP-2** ([**NP-1**] = 0.76 μM; [**NP-2**] = 0.58 μM).

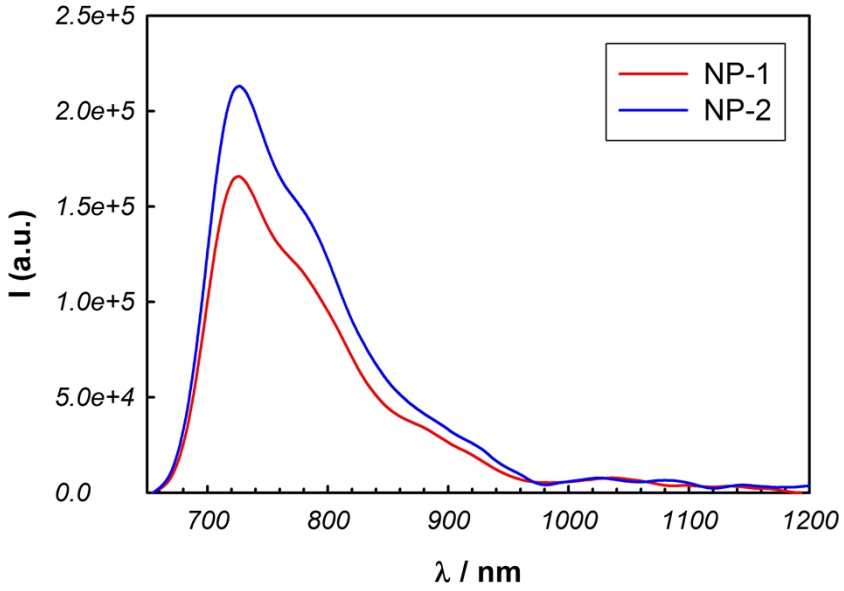


Figure S2: Emission spectra of aqueous suspension of nanoparticles samples **NP-1** and **NP-2** (λ_{ex} = 640 nm).

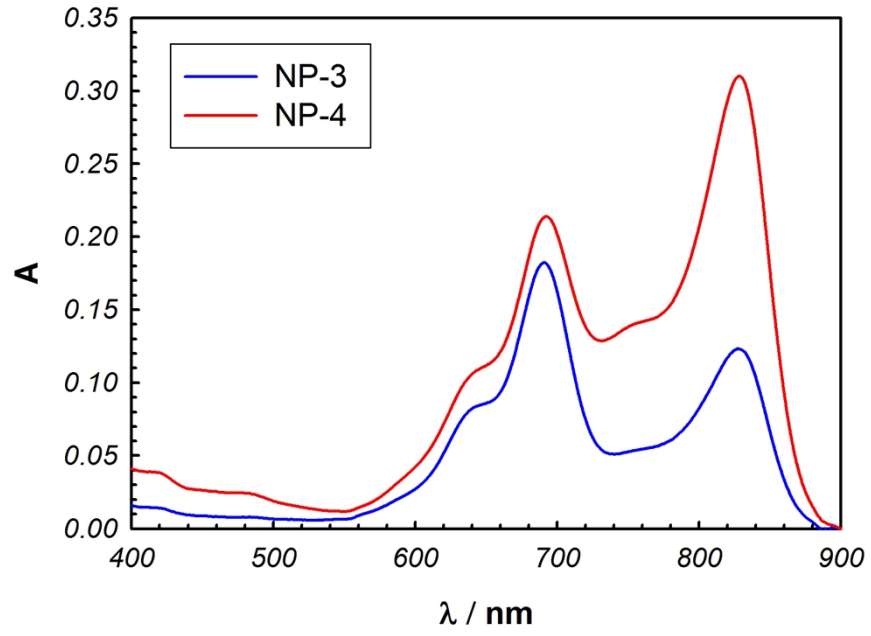


Figure S3: Absorption spectra of aqueous suspensions of nanoparticles samples **NP-3** and **NP-4** ([**NP-3**] = 0.4 μM; [**NP-4**] = 0.4 μM).

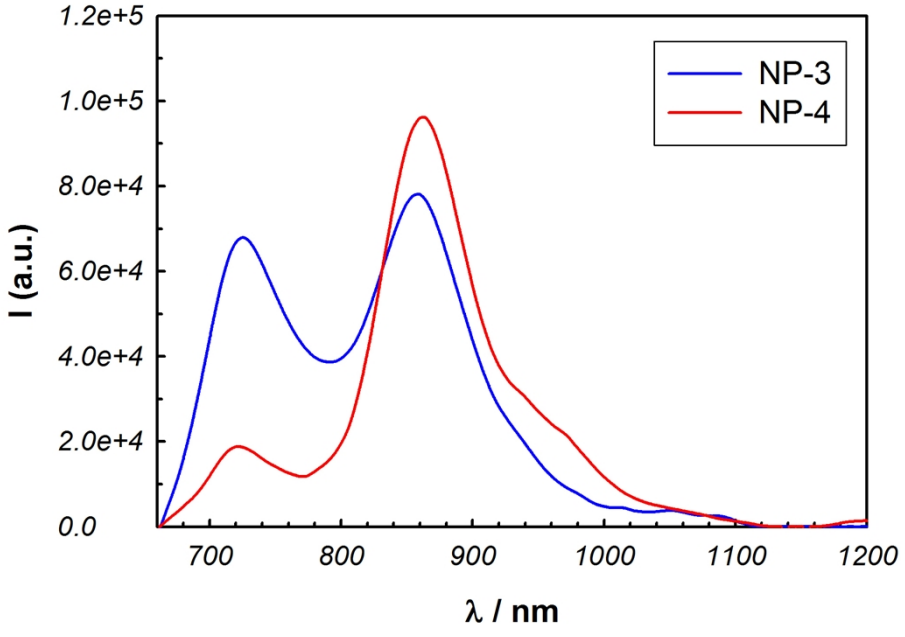


Figure S4: Emission spectra of aqueous suspension of nanoparticles samples **NP-3** and **NP-4** (λ_{ex} = 640 nm).

The main spectral and emission properties are summerized in **Table S1**.

Table S1: spectral and emission properties of nanoparticles samples

Sample	*Absorption maximum /nm*	*Emission maximum [a] / nm*	Φ_{em}	τ [b,d]	τ [c]	*r* ($\Delta\lambda$ / *nm*)	*r* [e] ($\Delta\lambda$ / *nm*)	η_{ET} (%)
Cy5.5 (5)	685	717	0.07	1.6	-	-	-	-
Cy5.5 GE	683	707	0.2	1.4	-	-	-	-
Cy7 (7)	819	860	0.03	-	-	-	-	-
NP-1	645, 688	722	0.02	1.5	-	0.3 (720-730) [d]	-	-
NP-2	645, 688	722	0.03	1.4	-	0.008 (720-730) [d]	-	-
NP-3	645, 693, 828	724, 860	0.08	1.0	1.1	0.4 (720-730) [e]	0.4 (850-870)	38
NP-4	645, 693, 828	724, 860	0.06	0.5	0.7	0.4 (720-730) [e]	0.0064 (850-870)	70

[a] λ_{ex} = 650 nm; [b] λ_{em} = 724 nm; [c] λ_{em} = 860 nm; [d] λ_{ex} = 640 nm; [e] λ_{ex} = 650 nm.

The efficiency of the energy transfer from Cy5.5 (**6**) to Cy7 (**7**) was evaluated using the following equation:[6]

$$\eta_{ET} = 1 - \frac{\tau_{DA}}{\tau_D}$$

where τ_{DA} is the lifetime of the donor in the presence of the acceptor, and τ_D is the lifetime of the donor itself. The energy transfer efficiency varies from 38% for 0.1% nanoparticles to 70% for nanoparticles **NP-3** and **NP-4** respectively.

Trasmission Electron Microscopy (TEM) images

A Philips CM 100 transmission electron microscope operating at 80 kV was used. For TEM investigations a 3.05 mm copper grid (400 mesh) covered by a Formvar support film was dried up under vacuum after deposition of a drop of nanoparticles solution diluted with water (1:50). The DDSN TEM images show that only the silica cores present sufficient contrast to appear in the images. The size distribution was obtained analyzing images with a block of several hundred

nanoparticles. The obtained histogram was fitted according to a Gaussian distribution obtaining the average diameter for the silica nanoparticles core.

Table S2: mean silica core DDSNs - diameter ± SD - determined by TEM analysis.

Sample	$(d_{core} \pm SD)$ / nm
NP-1	10 ± 2
NP-2	9 ± 2
NP-3	10.5 ± 1.5
NP-4	11.0 ± 1.5

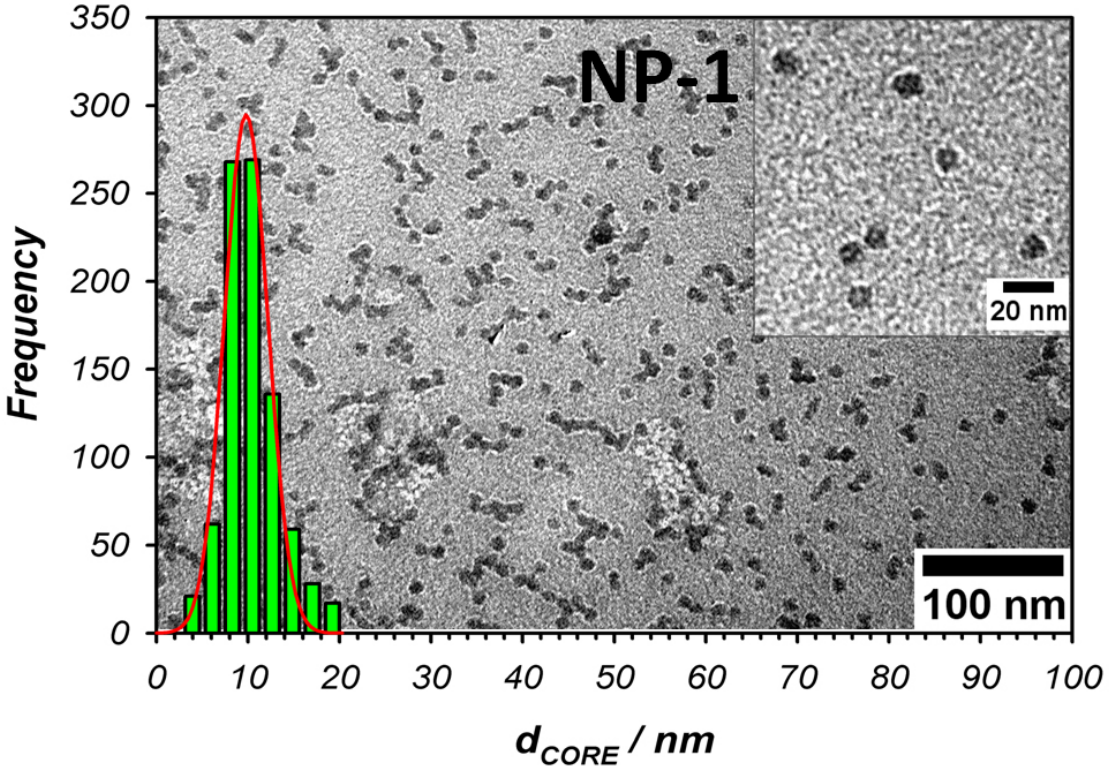


Fig. S5: **NP-1**, TEM images and silica core size distribution: d = (10 ± 2) nm.

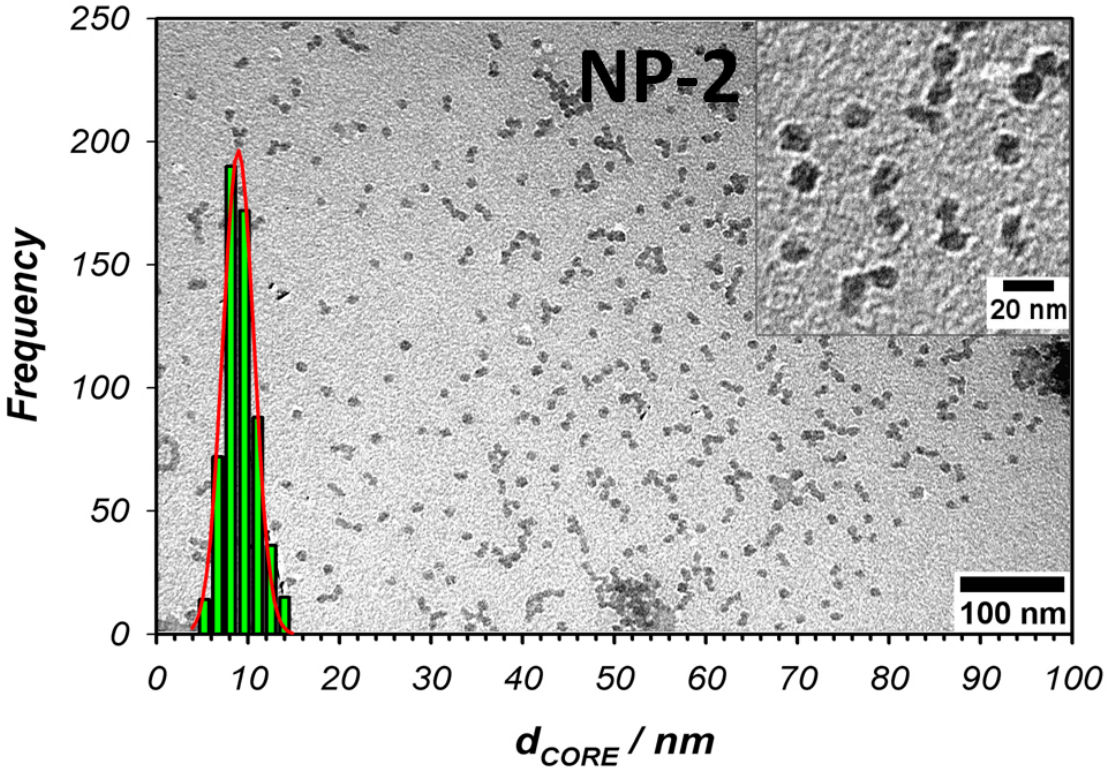


Fig. S6: **NP-2**, TEM images and silica core size distribution: d = (9 ± 2) nm.

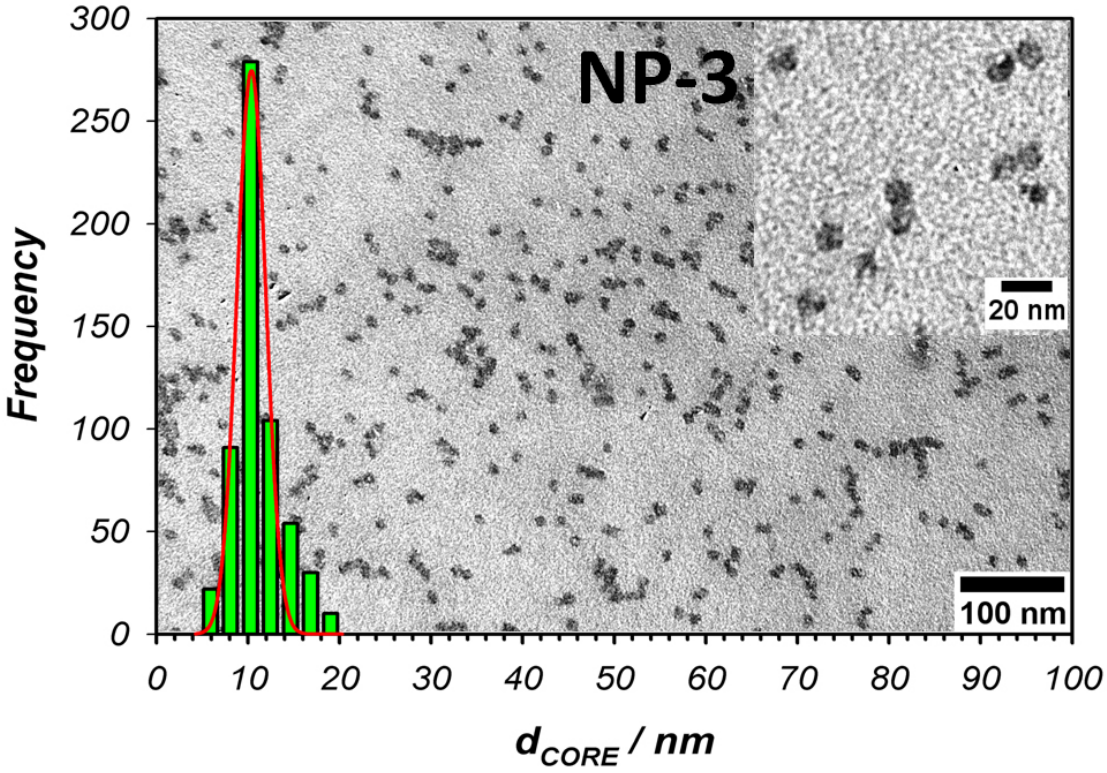


Fig. S7: **NP-3**, TEM images and silica core size distribution: d = (10.5 ± 1.5) nm.

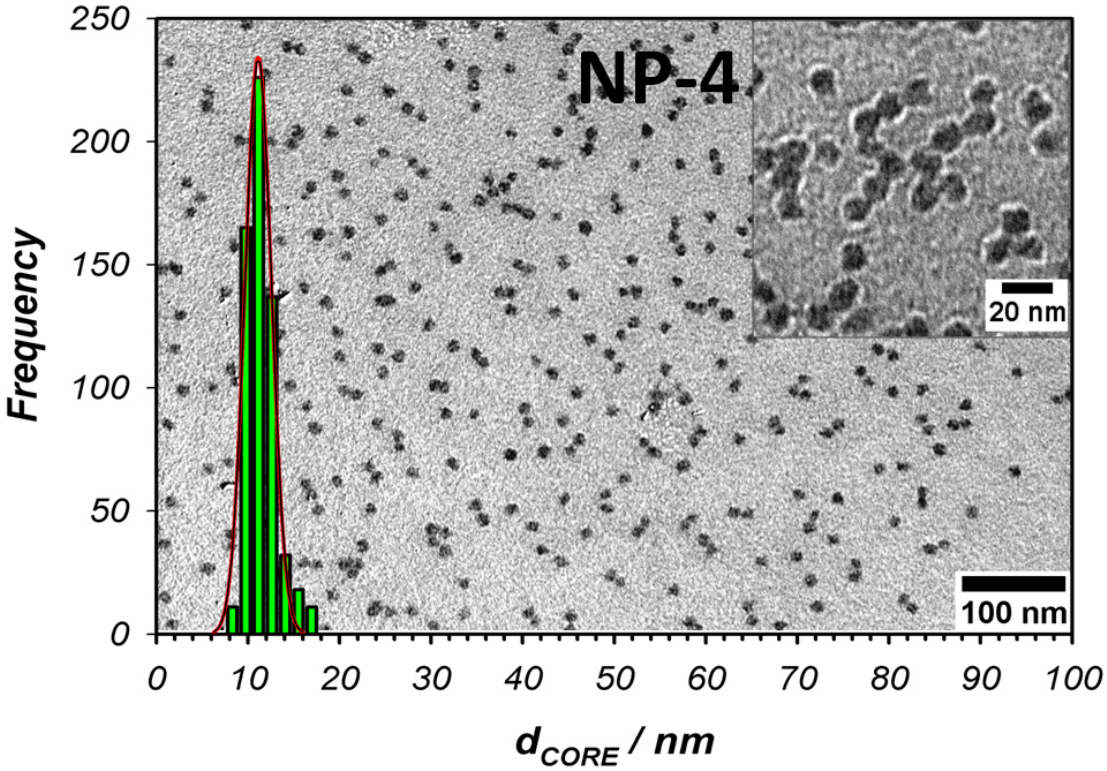


Fig. S8: **NP-4**, TEM images and silica core size distribution: d = (11.0 ± 1.5) nm.

Dynamic Light Scattering

DDSNs hydrodynamic diameter (d_H) distributions determination was carried out at 25°C through Dynamic Light Scattering measurements using a NICOMP Model 370 Submicron Particle Sizer (Nicomp International, Orlando, FL) equipped with a 488 nm Argon laser. This laser source and equipment was used to minimize interferences due to absorption-emission processes during the DLS measurements. Samples were housed in small disposable glass test tubes, using water as solvent. The width of DLS hydrodynamic diameter distribution is indicated by the standard deviation of five different measurement. The Polydispersion Index values (in case of monomodal distribution $PdI=(\sigma/Z_{avg})^2$, where σ is the width of the distribution and Z_{avg} is average diameter of the particles population respectively) are also reported. Representative hydrodynamic distributions by intensity in water are showed in Fig. S7-S10. DLS measurements showed no aggregation of the DDSNs even after several months.

Table S3: DLS Hydrodynamic diameter values for the nanoparticles samples described in this work. Standard deviation was calculated on five different measurements.

Sample	$d_H \pm SD$ *(nm)*	*PdI*
NP-1	25 ± 13	0.27
NP-2	27 ± 14	0.27
NP-3	24 ± 11	0.21
NP-4	27 ± 15	0.31

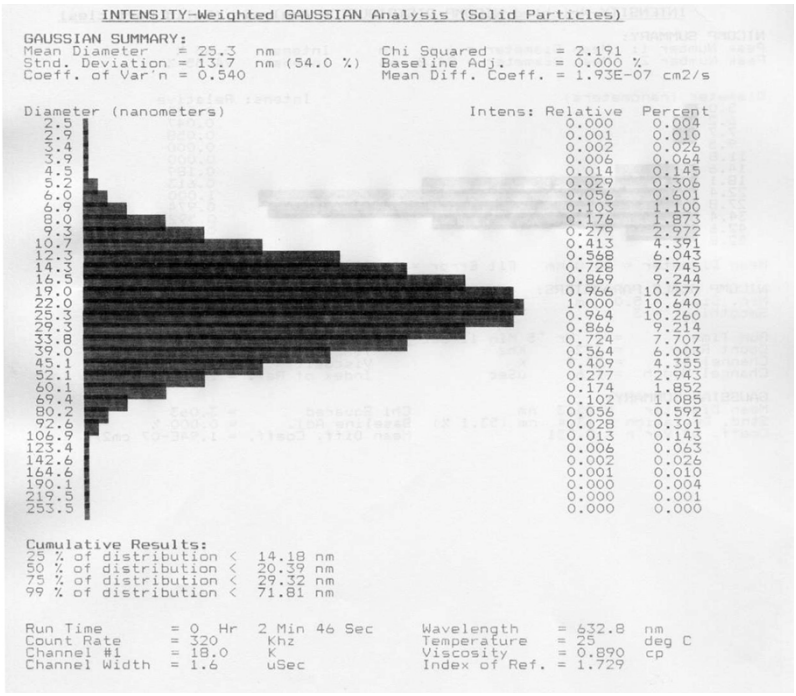


Fig. S9: Representative dynamic light scattering diameter distribution by intensity of **NP-1**.

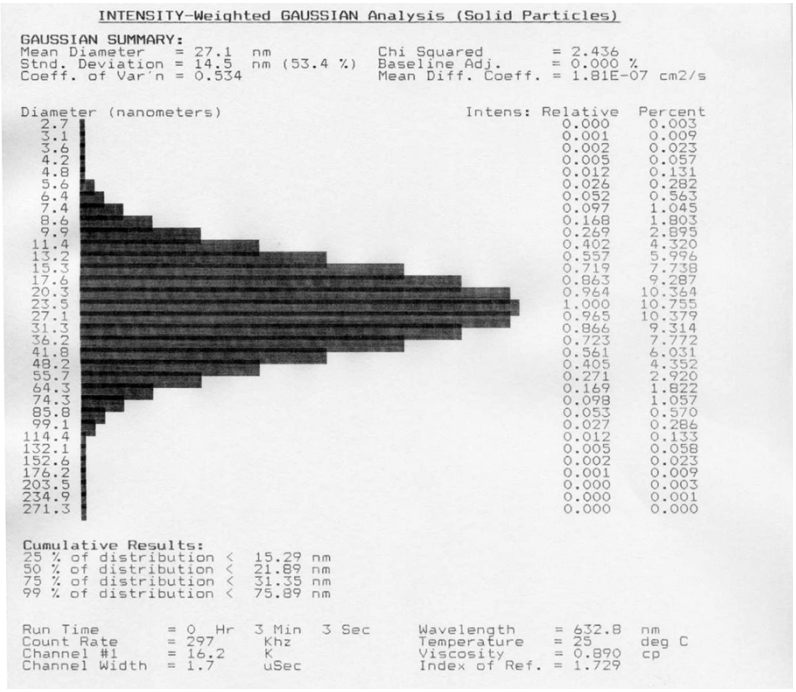


Fig. S10: Representative dynamic light scattering diameter distribution by intensity of **NP-2** (d_H = 27 ± 14 nm; water, 25°C).

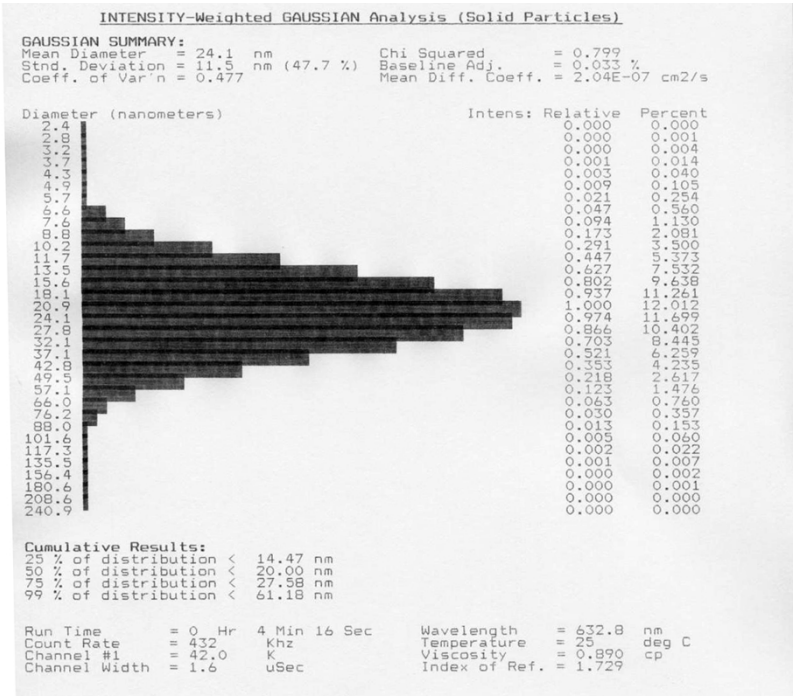


Fig. S11: Representative dynamic light scattering diameter distribution by intensity of **NP-3** (d_H = 24 ± 11 nm; water, 25°C).

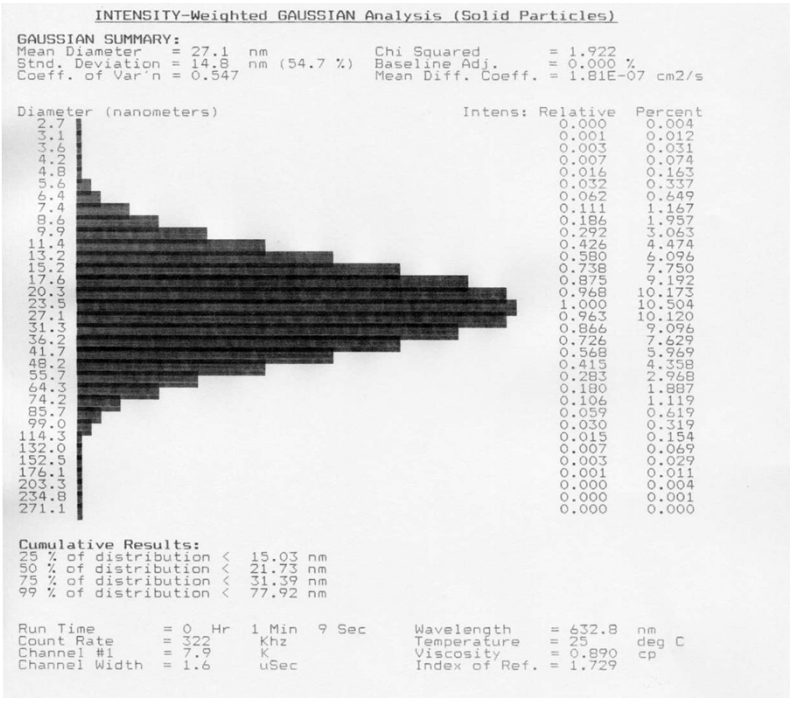


Fig. S12: Representative dynamic light scattering diameter distribution by intensity of **NP-1** (d_H = 27 ± 15 nm; water, 25°C).

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