Electronic Supplementary Information (ESI)

A Versatile Strategy for Tuning the Color of Electrochemiluminescence Using Silica Nanoparticles

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Experimental Part

Chemicals: All reagents and solvents were used as received without further purification: non ionic surfactant Pluronic® F127, tetraethyl ortosilicate (TEOS, 99.99%), chlorotrimethylsilane (TMSCl, ≥98%), Acetic acid (≥99.7%), DBAE (2-(Dibutylamino)ethanol, 99%) and DPA (9,10-Diphenylanthracene, 97%) were purchased from Aldrich. Reagent grade dichloromethane, and NaCl were purchased from Fluka. Bromurated cyanine 5 **C** (2-((1E,3Z,5E)-3-bromo-5-(1-ethyl-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-1-ethyl-3,3-dimethyl-3H-indol-1-ium iodide) was supplied by Cyanagen S.r.l. (Bologna, Italy).

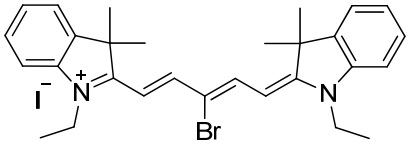


Figure S1 Cyanine 5 **C** (2-((1E,3Z,5E)-3-bromo-5-(1-ethyl-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-1-ethyl-3,3-dimethyl-3H-indol-1-ium iodide).

A Milli-Q Millipore system was used for the purification of water (resistivity ≥18 MΩ).

Synthesis of the triethoxysilane derivative R: Triethoxysilane derivative of rhodamine B **R** was synthesized as previously reported.[1]

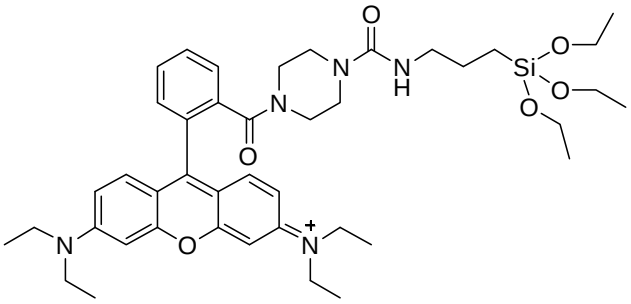
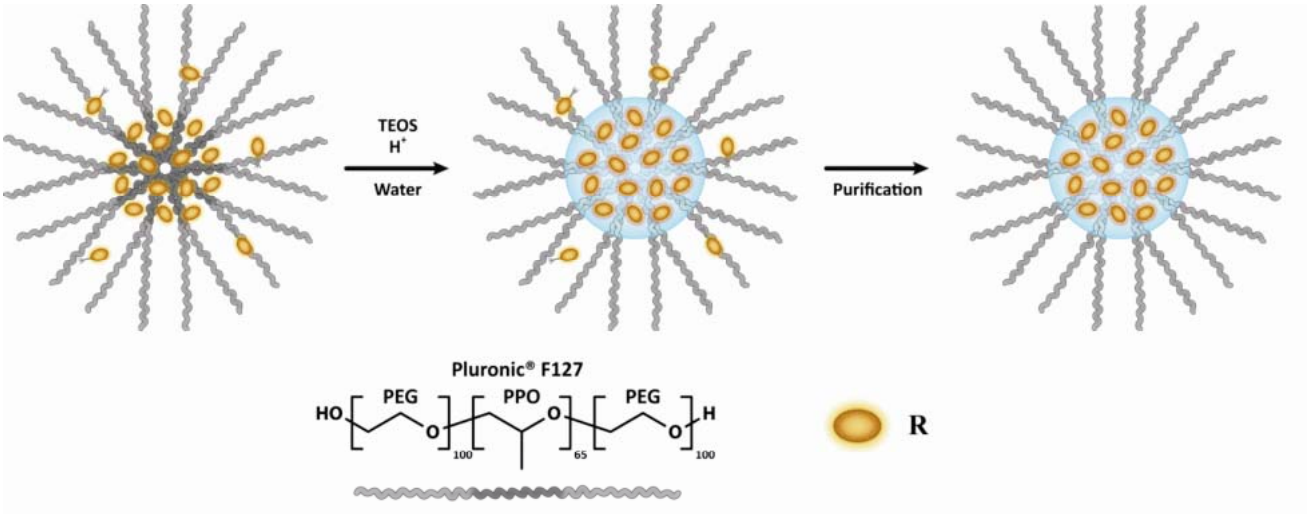


Figure S2 Triethoxysilane derivative of rhodamine B **R**.

Synthesis of Covalently Doped Core-Shell Nanoparticles: Core-shell silica-PEG (polyethyleneglycole) nanoparticles were synthesized adapting previously reported procedures (see scheme S1).[1] In a typical preparation 1.000 g of Pluronic F127 and 13.6 mg of the silanized dye **R** (0.0017 mmol) were carefully solubilized with few mL of dichloromethane in a 20 mL glass scintillation vial. The solvent was evaporated from the homogeneous solution by means of a gently nitrogen flow and subsequently under vacuum at room temperature. NaCl (0.685 g) was added to the solid residue and the mixture was solubilized at 25°C under magnetic stirring with 15.65 mL of acetic acid 1 M for 1h. TEOS (1780 µL, 7.97 mmol) was then added to the resulting aqueous homogeneous solution followed by TMSCl (100 µL, 0.8 mmol) after 180 min. The mixture was kept under stirring for 48 h at 25°C before dialysis treatments. The dialysis purification steps were carried out versus water on a precise amount of nanoparticles solution (15.00 mL) finally diluted to a total volume of 50 mL with water (concentration 20 µM).



Scheme S1: Synthesis of Core-shell silica-PEG nanoparticles doped with **R**.

Synthesis of Undoped Core-Shell Nanoparticles: In a 20 mL glass scintillation vial, 1.000 g of Pluronic F127 and 0.685 g of NaCl were carefully solubilized at 25°C under magnetic stirring with 15.65 mL of acetic acid 1 M for 1h. TEOS (1780 µL, 7.97 mmol) was then added to the resulting aqueous homogeneous solution, followed by TMSCl (100 µL, 0.8 mmol) after 180 min. The mixture was kept under stirring for 48 h at 25°C before dialysis treatments. The dialysis purification steps were carried out versus water on a precise amount of nanoparticles solution (15.00 mL) finally diluted to a total volume of 50 mL with water (concentration 20 µM).

Ultrafiltration (UF) and dialysis experiments Nanoparticles ultrafiltration was carried out in a 75 mL stainless steel-glass, solvent-resistant stirred cell purchased from Millipore (47 mm filters). The ultrafiltration experimental setup included Amicon regenerated cellulose membranes (100 kDa cut-off) and an auxiliary reservoir (800 mL) equipped with a concentration selector valve. Utrafiltrations were carried under nitrogen at a pressure of 0.5 atm. Concentration of the dialyzed nanoparticles suspensions (20 µM) to the final concentration of 100 µM was obtained measuring the volume of the filtrate obtained during the ultrafiltration experiments.

Nanoparticle UF by centrifugation was carried out by means of Amicon Ultra 0.5 mL centrifugal filters (RC 100 KDa).

Dialysis was performed vs. water at room temperature under gentle stirring with regenerated cellulose dialysis tubing (Sigma, mol wt. cut-off >12000 Da, avg. diameter 33 mm).

Inclusion of dyes in the nanoparticles shell.

DPA: 4.0 mg of **DPA** were added to 4 mL of a 100 µM nanoparticles suspension. The mixture was equilibrated under magnetic stirring overnight (25°C), and finally filtered three times using a 0.20 µm PTFE syringe filter unit.

C: the desidered amount of dye **C** (0.85 mg) was added to 4 mL of a 100 µM NP sample. The mixture was then equilibrated under magnetic stirring overnight (25°C) and finally filtered three times using a 0.20 µm PTFE syringe filter unit.

Dynamic Light Scattering: the determination of the nanoparticles hydrodynamic diameter distributions was carried out through Dynamic Light Scattering measurements employing a Malvern Nano ZS instrument equipped with a 633 nm laser diode. Samples were housed in disposable polystyrene cuvettes of 1 cm optical path length, using water as solvent.

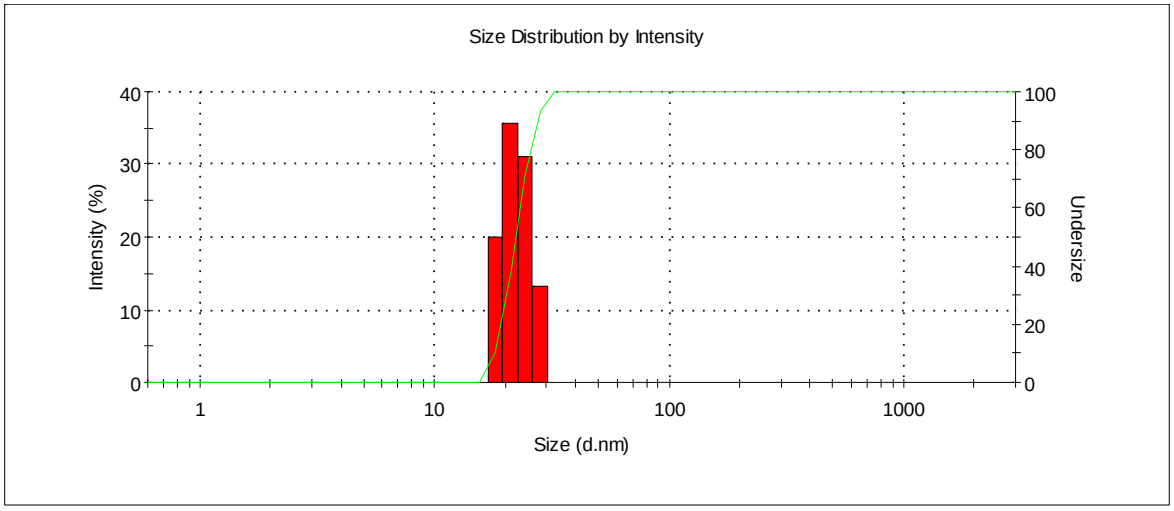


Figure S3 Typical Dynamic light scattering diameter distribution of Core-shell silica-PEG nanoparticles (d_H= 23 nm, PdI = 0.09).

The width of DLS hydrodynamic diameter distribution is indicated by PdI (Polydispersion Index). In case of a mono-modal distribution (gaussian) calculated by means of cumulant analysis, $PdI=(\sigma/Z_{avg})^2$, where σ is the width of the distribution and Z_{avg} is average diameter of the particles population respectively.

DLS measurements showed no aggregation of the NPs even after several months.

TEM Experiments: 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 NPs 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, fig. S4 (left). The obtained histogram was fitted according to a Gaussian distribution obtaining an average diameter of (11 ± 3) nm for the silica nanoparticles core.

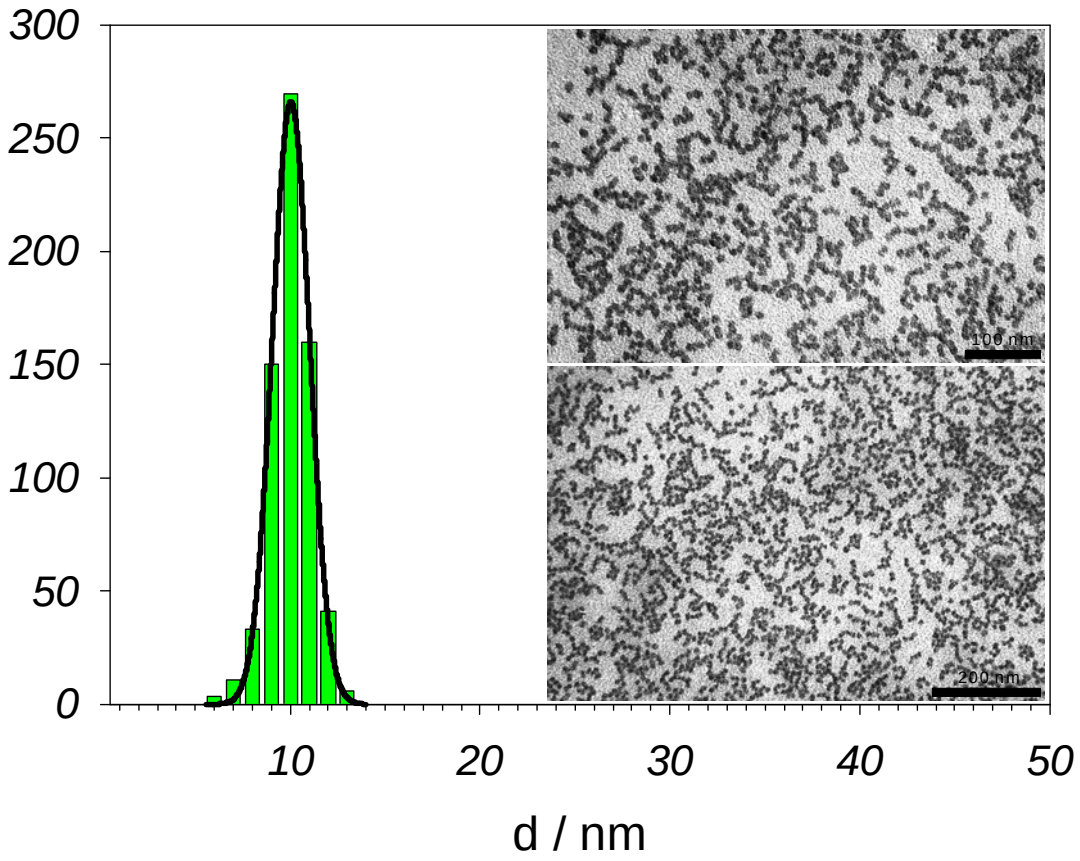


Figure S4 TEM images of Core-shell silica-PEG nanoparticles, and silica core size distribution, d = (11 ± 3) nm, (scale bars 100 nm and 200 nm)

^{1}H-NMR: Measurements of Pluronic F127 and of undoped core-shell nanoparticles were carried out with a Varian 400 MHz spectrometer, using D_2O as solvent/suspendant. The D_2O suspension of undoped core-shell nanoparticles was prepared through a threefold filtration starting from 500 uL of dialyzed nanoparticles suspension with an Amicon Ultra 0.5 mL centrifugal filter (RC 100 KDa; 10000 rpm/10 min). Similar results were obtained using standard solvent suppression/saturation techniques.

The entire ^{1}H-NMR spectra of Pluronic F127 is reported for reference purposes:

^{1}H NMR (400 MHz, D_2O, 25 °C, δ *ppm*): 1.17 (s, -OCH_2CHCH_3O-) 189H; 3.56 (s, -OCH_2CHCH_3O-) 65H; 3.63-3.78 (m, -OCH_2CH_2O-) 926H, 3.93-3.95 (m, -OCH_2CH_2OH) 4H.

^{1}H-NMR analysis is a useful and simple way to verify the nanoparticles core-shell structure, in which the more lipophilic central PPO (polypropyleneoxide) part of Pluronic F127 is strictly bounded to the central silica core of the particle, while the more hydrophilic PEG parts are outstretched toward the external water solution, in which they can preserve most of their mobility. ^{1}H-NMR spectra clearly show how the signal intensity decreasing and broadening typical of nanoparticle immobilized subunits mainly concern the proton signals attributable to the PPO moiety (-OCH_2CHCH_3O-; -OCH_2CHCH_3O-).

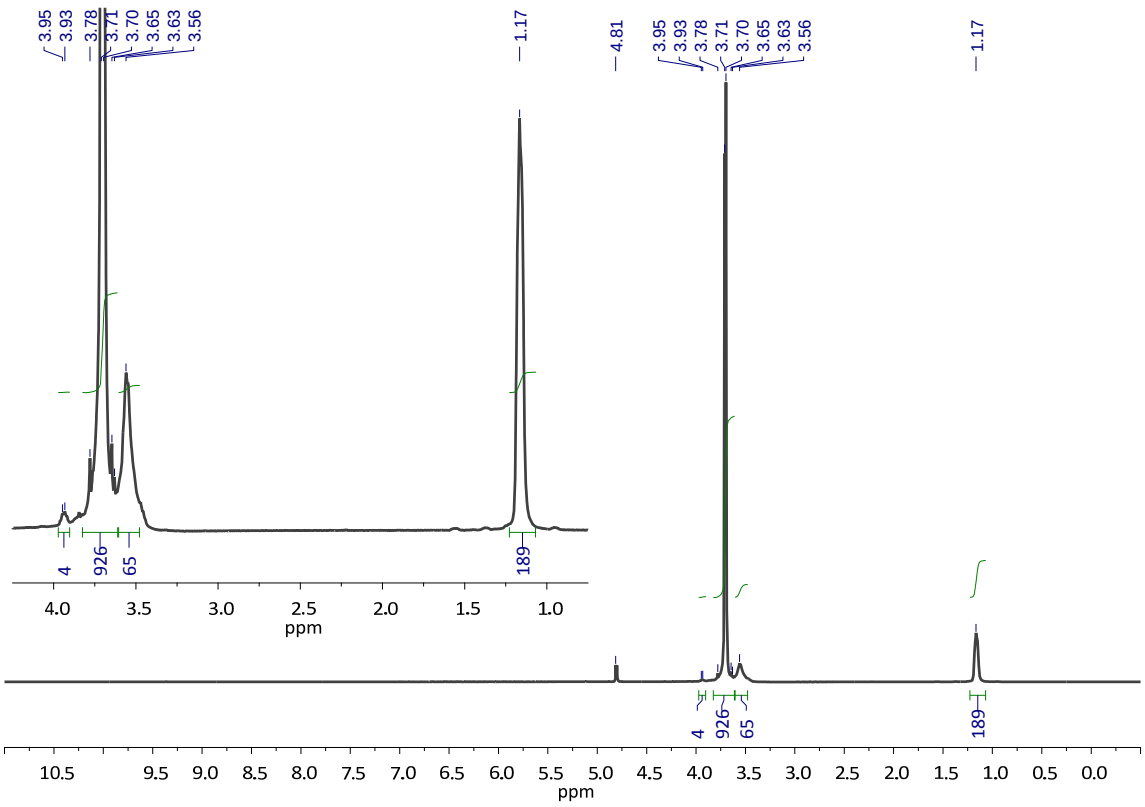


Figure S5 ^{1}H-NMR spectra of Pluronic F127 (400 MHz, D_2O, 25 °C).

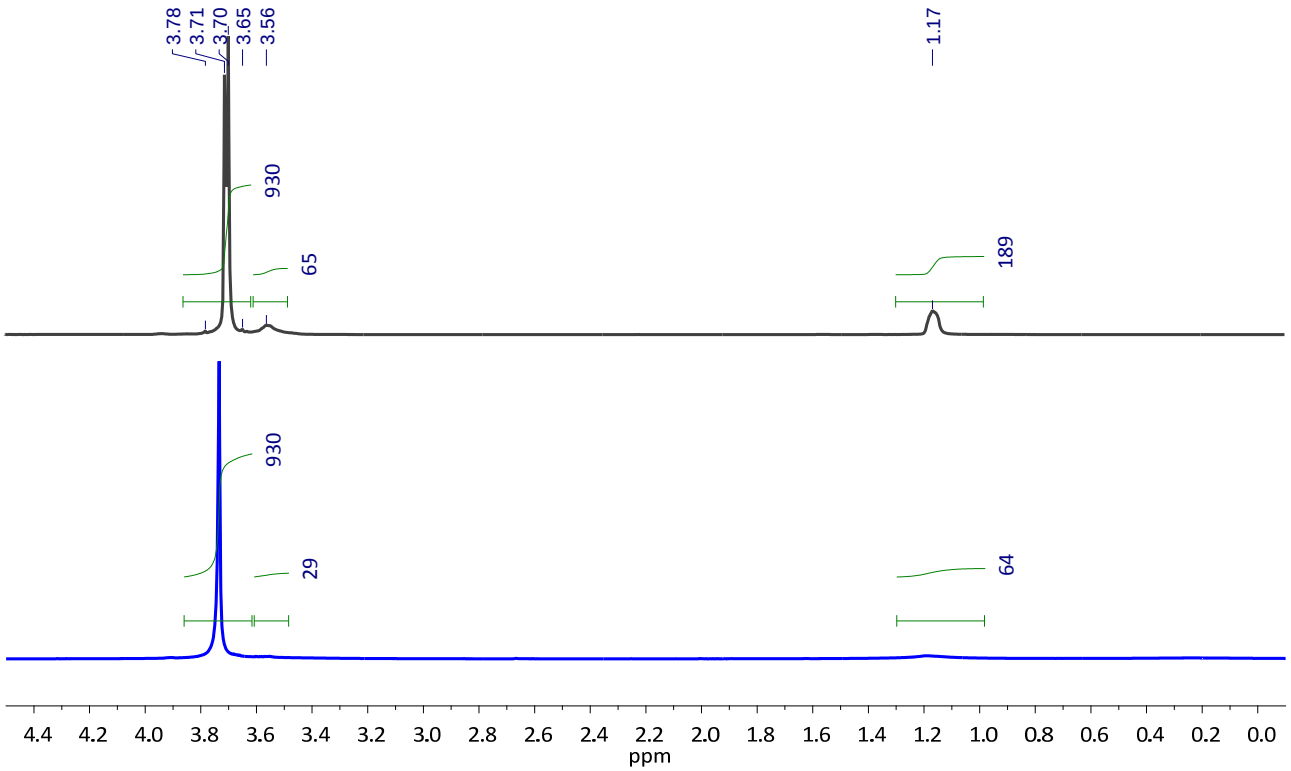


Figure S6 ^{1}H-NMR spectra of Pluronic F127 (black) and of core-shell silica-PEG nanoparticles (blue). (400 MHz, D_2O, 25 °C).

Thermogravimetric Analysis: A Q5000 IR thermogravimetric analyzer from TA Instruments, equipped with 100 µL high temperature Platinum-HT pans was used. All the experiments were carried out on 2-5 mg of solid samples with the following temperature program: 100.00 °C (10 min, N_2); ramp 10.00 °C/min to 900.00 °C (N_2); ramp 10.00 °C/min to 1000.00 °C (air); 1000 °C (1.00 min, air).

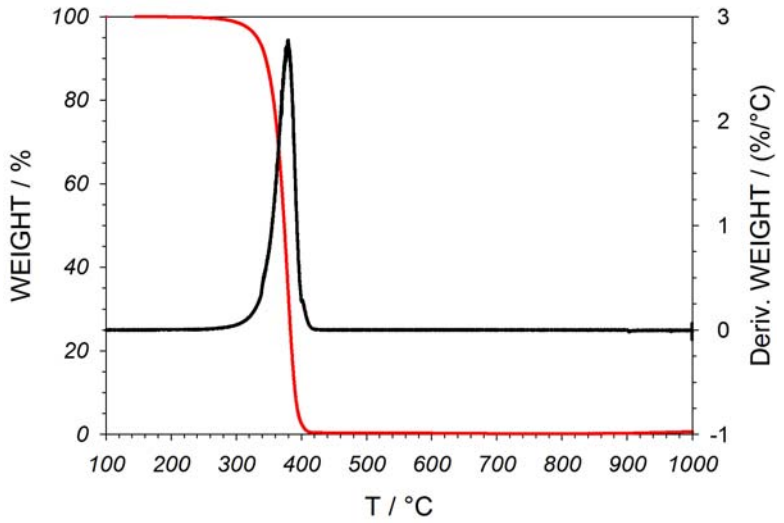


Figure S7 Thermo gravimetric analysis of Pluronic F127 surfactant (inflexion point 380.20 °C, 33.5%).

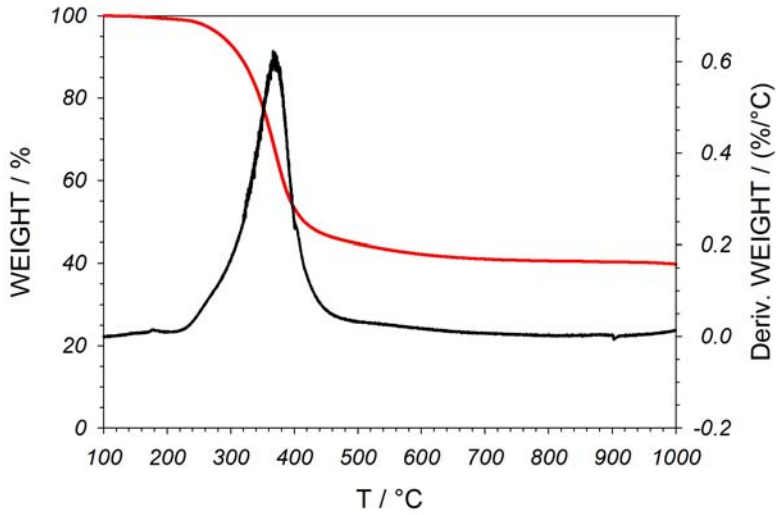


Figure S8 Thermogravimetric analysis of a Core-shell silica-PEG nanoparticles obtained from a sample of 100 µM nanoparticles suspension (inflexion point 371.75 °C, 65.24%).

Thermogravimetric analysis shows that the percentage amount of silica in nanoparticles samples is about 40%. This result does not change for samples subjected to ultrafiltration treatments with a 100 KDa membrane (Pluronic F127 MW = 12600), and confirm that the silica core of the particles and the surfactants units are tightly bounded each other.

Photophysical measurements: Except for the absence of coreactant, photophysical measurements were carried out on identical solution of the ECL ones. This choice was motivated by the need to reproduce the same experimental conditions of the ECL measurements.

UV-VIS absorption spectra were recorded at 25°C by means of Perkin-Elmer Lambda 45 spectrophotometer, using detachable windows Hellma 136-QS quartz cuvettes (optical path lenght 0.01 mm). The fluorescence spectra were recorded with an Edinburgh FLS920 fluorimeter equipped with a photomultiplier Hamamatsu R928P. The same instrument connected to a PCS900 PC card was used for the Time Correlated Single Photon Counting (TCSPC) experiments. Quartz cuvettes with optical path length of 0.01 mm were used for both absorbance and emission measurements. In the latter case the experiments were made with a front face configuration in order to avoid auto-absorption phenomena.

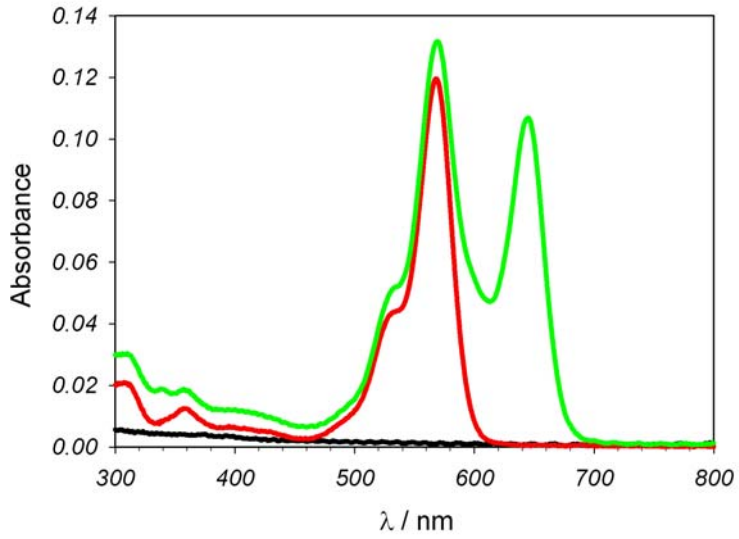


Figure S9 UV-VIS absorption spectra of nanoparticles sample **DPA@NP** (black line), **DPA+R@NP** (red line) and **DPA+R+C@NP** (green line); for all the spectra the optical pathlength (b) is 0.01 mm.

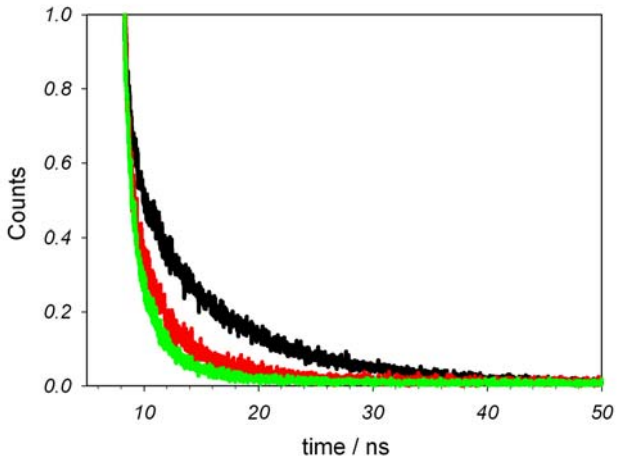


Figure S10. Fluorescence decays of **DPA@NP** (black) in comparison to **DPA+R@NP** (red) and **DPA+C+R@NP** (green) ones (λ_{exc} 340 nm (led); λ_{em} 420 nm). The decays clearly show the quenched state of **DPA** (the donor) in the presence of **R** and **C** (the acceptors) resulting from ET*.

Table S1: lifetime fitting for **DPA@NP, DPA+R@NP** and **DPA+C+R@NP** samples.[a]

	τ (ns) - λ_{em} 420 nm*	τ (ns) – λ_{em} 610 nm*
DPA@NP	8.5	--
DPA+R@NP	4.8	4.9
DPA+R+C@NP	3.5	0.9

[a] Owing to the presence of an inter-dyes distances distributions in the NPs, the fluorophores exhibit multiexponential fluorescence decays.[1] To prove the occurrence of any ET processes from the dyes **R**, **DPA** and **C** located in the different discricts of the **NPs**, we can however consider the average lifetimes decays of the donors dyes **DPA** and **R**. On account of the time resolution limitation in our Time Correlated Single Photon Counting instrumental setup, it was not possible to measure with sufficient accuracy the decay of species quenched by more than 90 % (t < 600 ps). This instrumental limitation leads to a general underestimation of the ET efficiency values in the nanoparticles samples.

Electrochemiluminescence measurements: ECL and electrochemical measurements were carried out with an AUTOLAB electrochemical station (Ecochemie, Mod. PGSTAT 30). The working electrode consisted of a platinum side-oriented 2 mm diameter disk sealed in glass or ITO electrode while the counter electrode was a platinum spiral and the reference electrode was a SCE electrode. Nanoparticles dialyzed solution was diluted with a posphate buffer solution (PB) to adjust the pH in the 7.5-8.5 range and to add the appropriate concentration of supporting electrolyte. For ECL generation, 30 mM DBAE (2-(dibutilamino) ethanol was added as oxidative coreactant. ECL was obtained in single oxidative steps (pulse steps or sweep steps) by generating, at the same time, the amine and the luminophore in their oxidized forms according to known mechanisms.
The ECL signal generated by performing the potential step program was measured with a photomultiplier tube (PMT, Hamamatsu R4220p) placed a few millimeters from the cell, and in front of the working electrode, inside a dark box.

Possible self-absorption contributions to the observed phenomenon were discarded on the basis of both the photoluminescence experiments and of a complementary electrochemical experiment carried out with an optically-transparent (ITO) working electrode where the ECL signal were measured directly from the back of the ITO electrode in order to minimize, or totally avoid, any possible self-absorption of the luminescence signal from the solution. While the ECL signals obtained with this configuration displayed, not unexpectedly, lower intensities, the recorded spectrum was in close agreement with that obtained with a front configuration, shown in Figure 1, thus confirming the occurrence of the above intra-NP ET process.

A voltage of 750 V was supplied to the PMT. The light/current/voltages curves were recorded by collecting the preamplified PMT output signal (by a ultra-low noise Acton research mod. 181 by a Keithley Mod. 6485 picoamperometer) using the second input channel of the ADC module of the AUTOLAB instrument. The ECL spectra were recorded by inserting a Liquid Crystal Tunable Filter (LCTF VIS-10-20-STD from VariSpec) between the PMT and the optical window of the electrochemical cell.

For spectra acquisition, the ECL signal was generated by single pulse step and integrating the ECL intensity for 1 second. In order to stabilize and maximise the ECL emission the electrode surface was electrochemically polished applying a negative potential (-1 V vs. SCE) and generating hydrogen bubbles immediately before to each recording.[2] The ECL signals were acquired every 5 nm selecting the wavelength by the tunable filter. The intensities related to different wavelengths are then corrected by the transmittance of the filter.

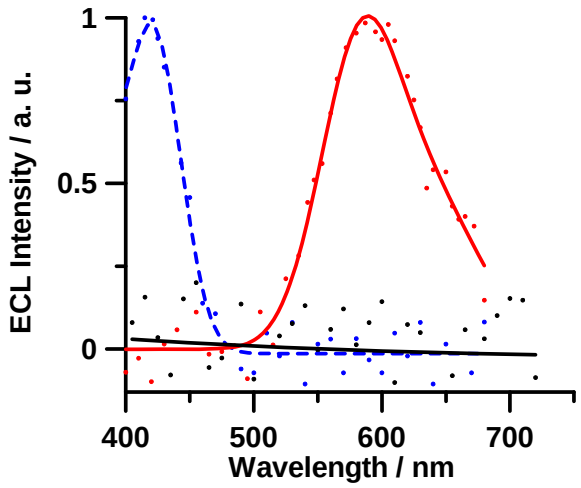


Figure S11. Normalized electrochemiluminescence spectra (dots) and fits (lines) of **DPA@NP** (blue trace), **R@NP** (red trace) and **C@NP** (black trace). DBAE 30 mM as coreactant; 100 µM PB; single potential step program: E = 1.60 V (E vs SCE, first oxidation), pulse width 1 s, PMT bias 750 V.

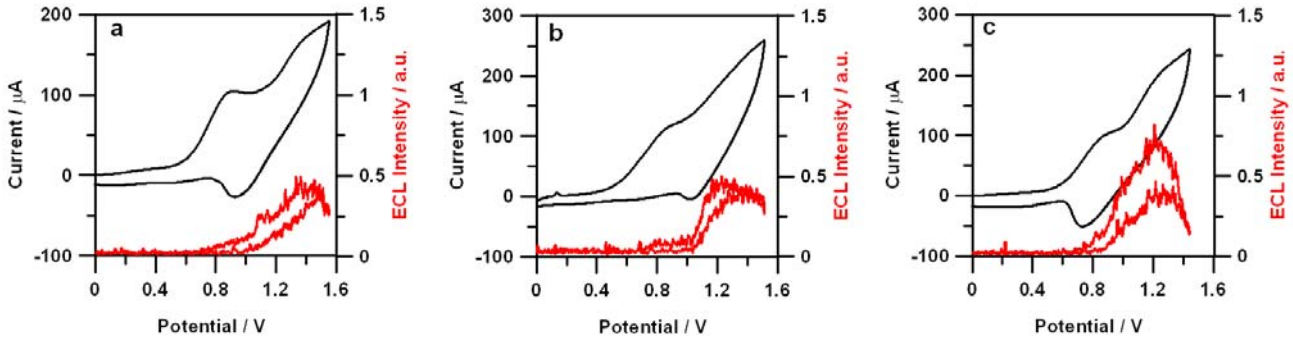


Figure S12. Cyclic voltammetry and I_{ECL}-potential curves of 100 µM PB solutions of (a) **R@NP** (b) **DPA@NP** (c) **DPA+R@NP** scan rate 0.1 Vs^{-1}, PMT bias 750 V. (E vs SCE).

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2. S. Zanarini, M. Felici, G. Valenti, M. Marcaccio, L. Prodi, S. Bonacchi, P. Contreras-Carballada, R. M. Williams, M. C. Feiters, R. J. M. Nolte, L. De Cola and F. Paolucci, *Chem. Eur. J.* 2011, **17**, 4640.