Supporting Information

Neutral Dye Doped Silica Nanoparticles for Electrogenerated Chemiluminescence signal amplification

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Summary:

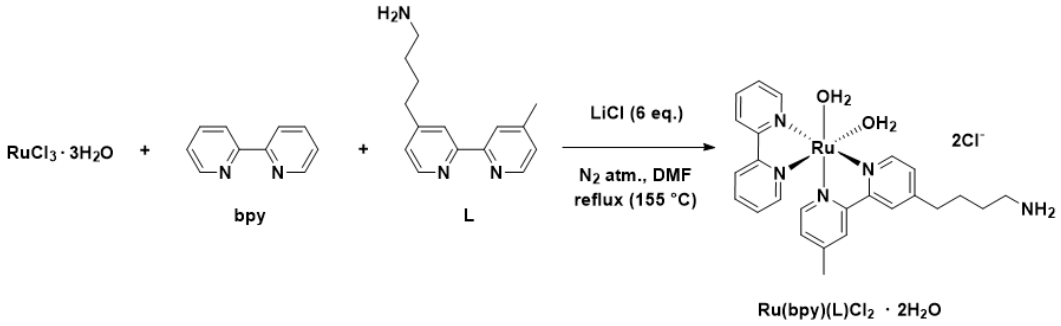
S1. SYNTHESIS AND CHARACTERIZATION

Materials:

All reagents and solvents were used as received without further purification. Dimethylformamide (DMF, ≥ 99.8%), Ethylene glycol (≥ 99%), Dimethyl Sulfoxide (DMSO, ≥ 99.5%), Dichloromethane (DCM, ≥ 99.8%), Acetonitrile (ACN, 99%), Diethyl Ether (Et_2O, ≥ 99.7%), Tetraethyl orthosilicate (TEOS, 99.99%), Chlorotrimethylsilane (TMSCl, ≥ 98%), 3-(Triethoxysilyl)propyl isocyanate (TEPI, 95%), Acetic acid (AcOH, ≥ 99.7%), Hydrochloric acid (HCl, 37%), Triethyl-amine (TEA, ≥ 99.5%), N,N-Diisopropylethylamine (DIPEA 99.5%), 2-(dibutylamino)ethanol (DBAE, 99%), Acetone, Hexane, Ethanol (EtOH) and Ethylene glycol (99.8%) Pluronic F127, were purchased from Sigma-Aldrich.

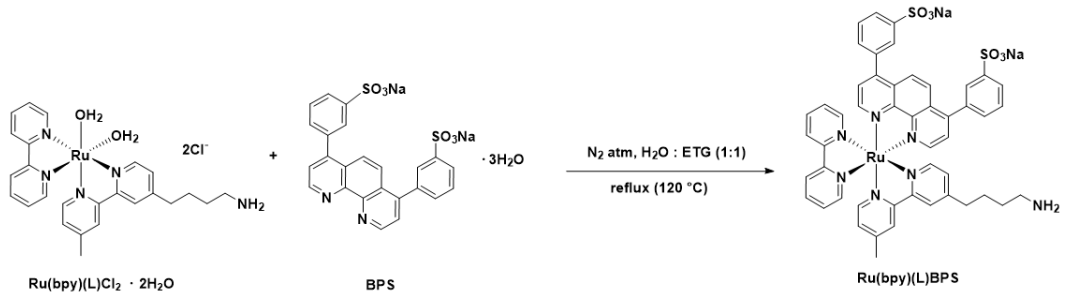
$RuCl_3 \cdot 3H_2O$, Bathophenanthrolinedisulfonic acid disodium salt trihydrate ($Na_2BPS \cdot 3H_2O$, 98%), 2,2′-bipyridine (bpy, ≥ 99%), Lithium chloride (LiCl, ≥ 99 %), Sodium chloride (NaCl, ≥ 99.5%), Sodium sulfate (Na_2SO_4 ≥ 99.0%), Potassium chloride (KCl ≥ 99.0%), sodium phosphate monobasic dihydrate ($NaH_2PO_4 \cdot 2H_2O$ ≥ 99.0%), sodium phosphate dibasic (Na_2HPO_4 ≥ 99.5%), were purchased from Fluka.

Scheme S1: Synthesis of Ru(bpy)(L)BPS:



Step 1: Synthesis of $Ru(bpy)(L)Cl_2 \cdot 2H_2O$

Under inert nitrogen atmosphere a mixture of $RuCl_3 \cdot 3H_2O$ (162.52 mg, 0.622 mmol, 1 eq.), ligand L, 4-(4'-methyl-[2,2'-bipyridin]-4-yl)butan-1-amine, (150 mg, 0.622 mmol, 1 eq.), 2,2'-bipyridine (bpy) (97.07 mg, 0.622 mmol, 1 eq.) and LiCl (158.13 mg, 3.73 mmol, 6 eq.) were dissolved into DMF (∼ 3.0 mL) under magnetic stirring and then refluxed at 155 °C overnight. The reaction was then cooled at room temperature and 4 mL of acetone was added to it. The flask was kept in the fridge to promote the precipitation. The black-brown precipitate obtained was then separated by vacuum filtration and washed with three aliquots of 5 mL of acetone, then three of 5 mL of water and finally with three aliquots of 5 mL of hexane. At the end, the clean precipitate was dried under high vacuum. The final yield calculated was 144 mg, (38.2%).



Step 2. Synthesis of Ru(bpy)(L)BPS

Under inert nitrogen atmosphere the complex Ru(bpy)(L)Cl_2·2 H_2O (100 mg, 0.165 mmol, 1 eq.) and the ligand BPS bathophenanthroline disulfonic acid disodium salt trihydrate (97.44 mg, 0.165 mmol, 1 eq.) were dissolved in 3 ml of a degassed mixture of water and ethylene glycol (1:1) under magnetic stirring. The mixture was then refluxed at 120°C overnight. The reaction was then cooled at room temperature and 4 mL of acetone was added to the solution to promote the precipitation. The brown precipitate obtained was then separated by vacuum filtration and washed with three aliquots of 5 mL of acetone and finally with three aliquots of 5 mL of water. At the end, the clean precipitate was dried under high vacuum. The final yield calculated was 120 mg, (70.5%).

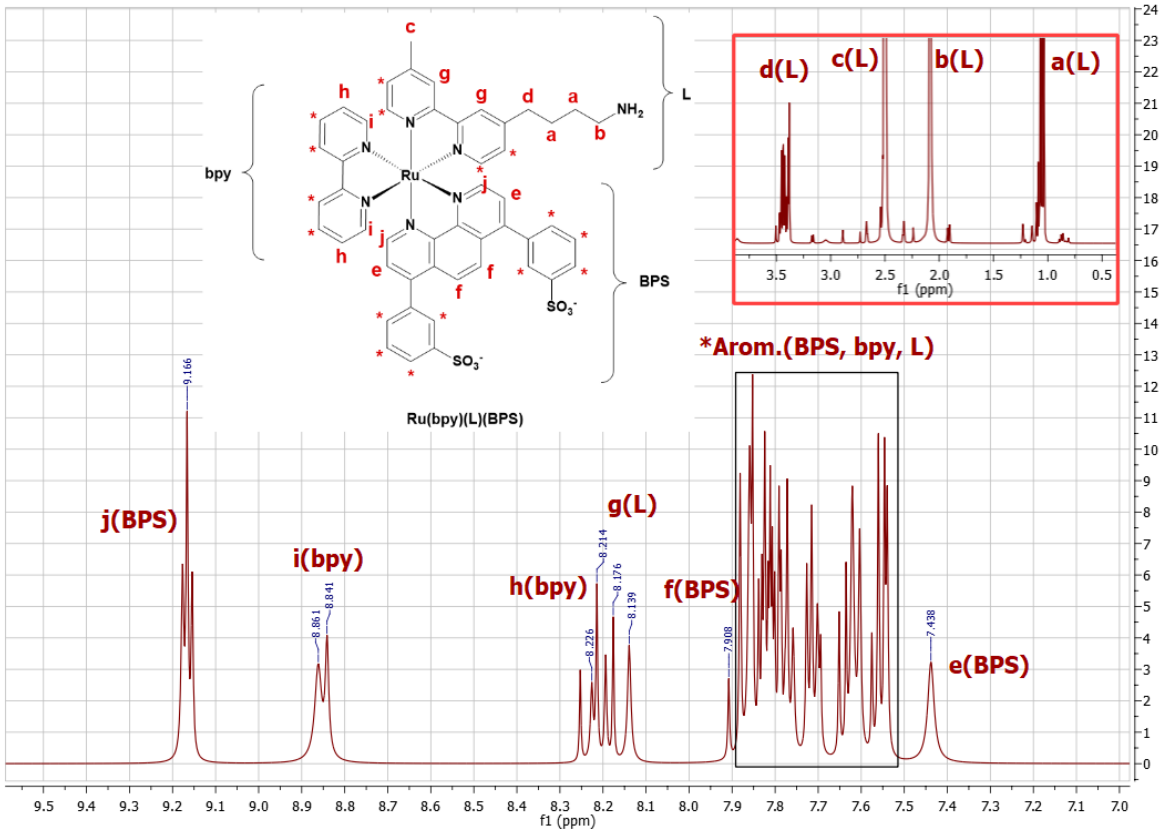
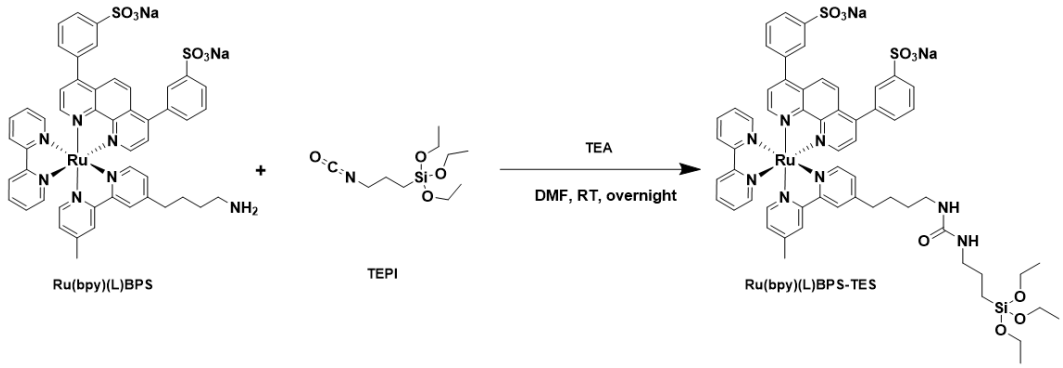
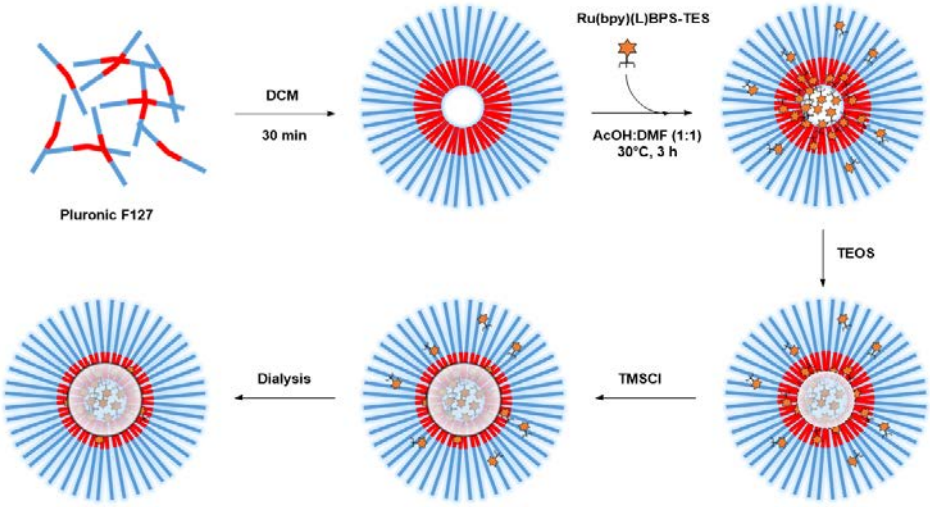


Figure S1: ^{1}H-NMR spectrum of Ru(bpy)(L)BPS (δ, ppm, $(CD_3)_2SO$, 400 MHz), showing the aromatic region in which, several characteristic signals of each ligand can be identified. The inset in red is showing the aliphatic part instead, completely belonging to the ligand L.



Scheme S2: Synthesis of Ru(bpy)(L)BPS-TES

The complex Ru(bpy)(L)BPS (x mol%, 1 eq.) was inserted in a 4 mL glass scintillation vial and dissolved in DMF (800 µL) under magnetic stirring for 30 minutes. The amount of Ru(bpy)(L)BPS required to reach the desired doping level, was calculated based on the moles of TEOS reported in the **Table S1**. Triethylamine (TEA) (1.1 eq.) and 3-(Triethoxysilyl)propyl isocyanate (TEPI) (1.2 eq.) were then added into the vial and the stirring was kept overnight at 30 °C. The reaction mixture was then injected directly during the nanoparticles synthesis.



Scheme S3: Preparation of covalently doped Ru(bpy)(L)BPS-TES core-shell silica-PEG nanoparticles. [Ru@NPs]

Pluronic F127 (100 mg) and NaCl (67 mg) were inserted in a 4 mL glass scintillation vial and dissolved in DCM (1.5 mL) under magnetic stirring for around 30 minutes. The solvent was then removed with rotavapor at room temperature and the sample dried under high vacuum. The white solid was then redispersed in 1M acetic acid solution (AcOH) (800 µL) by stirring it at 30 °C and after complete solubilization, the solution of complex Ru(bpy(L)BPS-TES was injected directly in the same glass vial. The mixture was stirred for another 3 hours at 30 °C. Tetraethylorthosilicate (TEOS) (175 µL, 0.784 mmol) was then added to the resulting aqueous homogeneous solution and the day after, trimethylsilylchloride (TMSCl) (10 µL) was finally added and the mixture was kept overnight at 30 °C, always under magnetic stirring. The dialysis purification steps were carried out versus water under gentle stirring with regenerated cellulose dialysis tubing (Sigma, mol wt. cutoff > 12 kDa, avg. diameter 33 mm). The whole amount of Ru@NPs solution (1.6 mL) was inserted in the tube and after the dialysis diluted to a total volume of 20 mL with water. The final concentration of the **Ru@NPs** solution was measured considering the volume after dialysis. The solution was first centrifugated for 10 minutes at 8000 rpm, and then the supernatant solution was filtered first with a 0.45 µm RC, then with 0.20 µm RC filters. The exact amount of reagents used for each sample preparation is shown in **Table S1** below.

Sample	% doping	Ru(bpy)(L)BPS-TES	Pluronic F127	NaCl	DMF	AcOH (1M)	TEOS	TMSCl
		[µmol]	[mg]	[mg]	[µL]	[µL]	[µL]	[µL]
Ru@NP3	**0.05**	0.39	100	67	800	800	175	10
Ru@NP8	**0.5**	3.92	100	67	800	800	175	10
Ru@NP11	**0.8**	6.27	100	67	800	800	175	10

Ru@NP12	**1.0**	7.82	100	67	800	800	175	10
Ru@NP19	**1.6**	12.54	100	67	800	800	175	10
Ru@NP21	**2.4**	18.81	100	67	800	800	175	10

Table S1: A set of DDSNPs samples synthesized with different doping of Ru(bpy)(L)BPS along with the amounts of reagents and solvents used in each synthetic procedure.

S2. PHOTOPHYSICAL CHARACTERIZATION

Quartz cuvettes were used for both absorbance and emission measurements (optical path length of 1 cm). UV−Vis absorption spectra were recorded at 25 °C by means of PerkinElmer Lambda 45 spectrophotometer. The fluorescence spectra were recorded with a PerkinElmer 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 = 410 nm). Corrected fluorescence emission and excitation spectra (450 W Xe lamp) were obtained with the same instrument equipped with both a Hamamatsu R928P photomultiplier tube (for the 500−850 nm spectral range). Nanoparticle solutions were diluted with milli-Q water. Luminescence quantum yields (uncertainty ±15%) were recorded on air-equilibrated solutions using $Ru(bpy)_3^{2+}$ as reference dye. (QY = 0.018 in aerated acetonitrile solution). When necessary, deoxygenated samples were prepared by flowing N_2 through the solutions housed in a customized airtight quartz cuvette equipped with a closure cap.

Sample	**N° dyes/NP**	**Aerated Solution**			**Deaerated Solution**		
		Emission Intensity (Integrated)	**Φ [%]**	**Life time τ in µs**	**Emission Intensity (Integrated)**	**Φ [%]**	**Life time τ in µs**
$[Ru(bpy)_3]^{2+}$	/	**$3.560 \cdot 10^6$**	**1.8**	0.17	**$6.983 \cdot 10^6$**	**9.5**	0.82
RuBPS@NP-3	3	$4.842 \cdot 10^6$	**2.4**	0.53	$9.421 \cdot 10^6$	**12.6**	1.37
RuBPS@NP-8	8	$7.291 \cdot 10^6$	**3.6**	0.70	$1.422 \cdot 10^7$	**19.0**	1.29
RuBPS@NP-11	11	$6.892 \cdot 10^6$	**3.4**	0.74	$1.366 \cdot 10^7$	**18.3**	1.34
RuBPS@NP-12	12	$7.055 \cdot 10^6$	**3.5**	0.81	$1.291 \cdot 10^7$	**17.3**	1.50
RuBPS@NP-19	19	$7.534 \cdot 10^6$	**3.8**	0.79	$1.484 \cdot 10^7$	**19.9**	1.56

RuBPS@NP-21	21	$7.205 \cdot 10^6$	**3.6**	0.76	$1.275 \cdot 10^7$	**17.1**	1.46

Table S2: Integrated area from emission curves and corresponding quantum yield Φ for DDSNPs series in aerated and deaerated solutions. The luminous efficiency has been calculated based on the relative $[Ru(bpy)_3]^{2+}$ reference parameters. Instrumental conditions: λ_{exc} = 410 nm; scan range = 500 - 800 nm; slit = 4 nm; step = 1 nm; dwell time = 0.10; n° of scans = 1. for dye/NP ratio have been calculated based on the $[Ru(bpy)_3]^{2+}$ reference and its molar absorptivity ε = 14600 M^{-1} cm^{-1} at 452 nm.

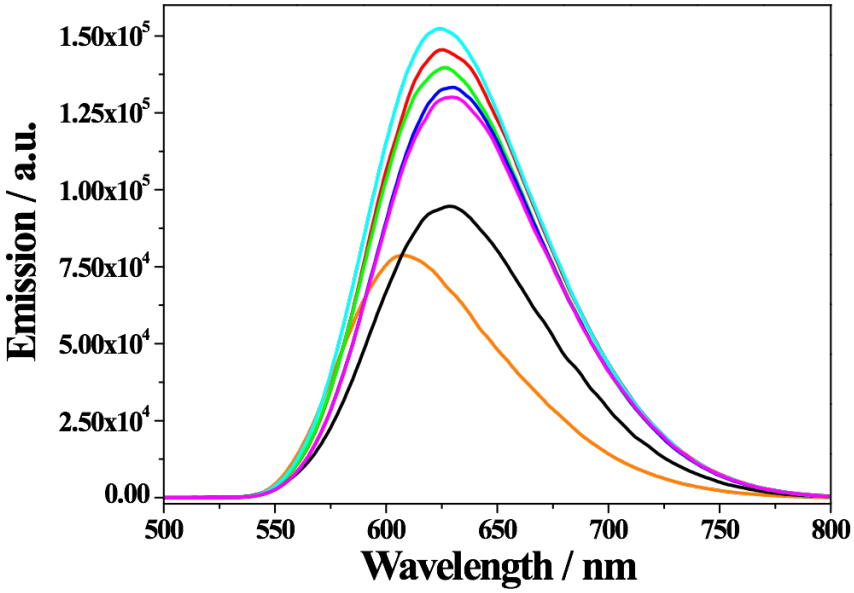


Figure S2: Emission spectra in deaerated solution for the DDSNPs samples (**Ru@NP3**, black; **Ru@NP8**, red; **Ru@NP11**, green; **Ru@NP12**, blue; **Ru@NP19**, cyan; **Ru@NP21**, magenta) in water. Reference used: $[Ru(bpy)_3]^{2+}$ in ACN (orange line). Instrumental conditions: λ_{exc} = 410 nm; scan range = 500 −800 nm; slit = 5 nm; step = 1 nm. All these samples have shown the same absorbance intensity at their maximum.

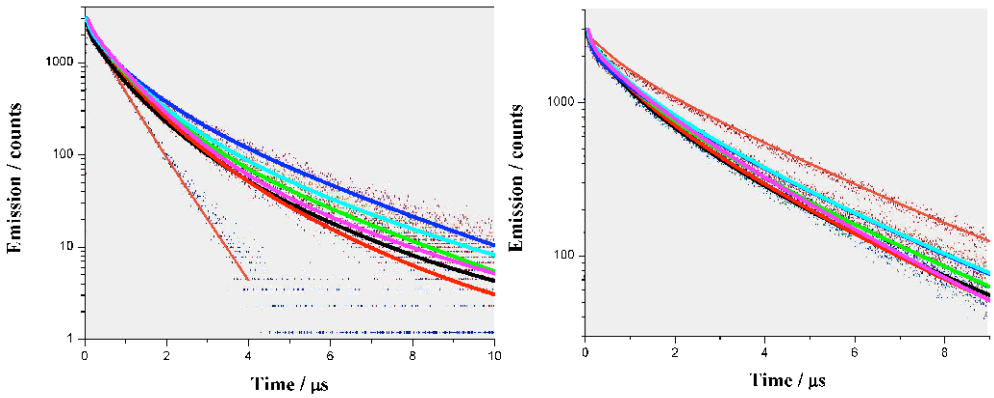


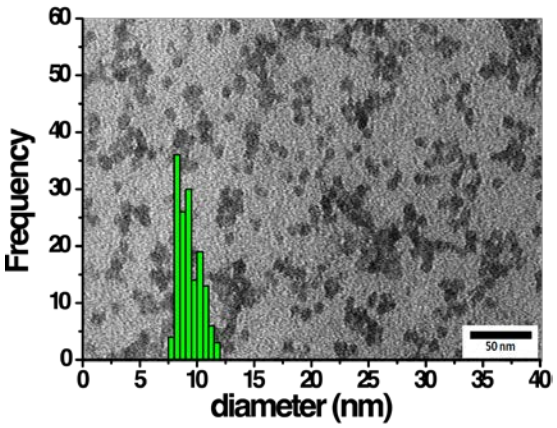
Figure S3: Intensity vs. time graphs (dots) and calculated fittings (lines) showing exponential decays of the complexes studied in solutions and inside the silica NPs in aerated (left) and deaerated (right) conditions (dots on the background). All the calculated fitting (straight lines) have a χ^2 value around 1. Colour legend: Ru(bpy)(L)BPS, orange; Ru@NP3, black; Ru@NP8, red; Ru@NP11, green; Ru@NP12, blue; Ru@NP19, cyan; Ru@NP21, magenta. Instrumental conditions: λexc = 410 nm; λems = 630 nm; time range = 10 ¬μs; peak counts = 3000; channels = 1024; number of components = 4. The lifetimes are resumed in table S2.

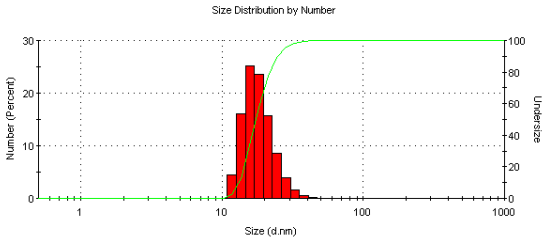
S3. TEM AND DLS ANALYSIS

TEM images of DDSNPs were obtained with a Philips CM 100 microscope, operating at 80 kV, and using 3.05 mm copper grids (Formvar support film, 400 mesh). Two aliquots of 2 µL of nanoparticles solution first diluted with milli-Q water (1:50) were dropped on the grid, waiting for the solvent to evaporate before the second deposition. The grid was then dried under vacuum. The TEM images showing the denser silica cores were analysed with ImageJ software, considering around a few hundred nanoparticles. The obtained histogram was fitted according to Gaussian distribution, obtaining the average diameter for the silica nanoparticles core completed with calculated standard deviation and PDI.

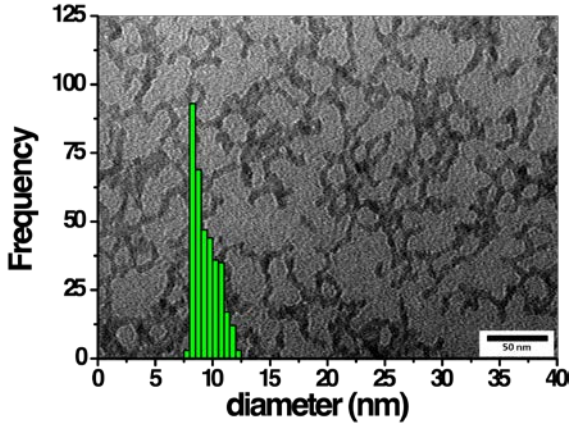
Hydrodynamic diameter (d_H) distributions of nanoparticles were obtained in water at 25 °C with a Malvern Nano ZS DLS instrument equipped with a 633 nm laser diode. Samples were first treated with 0.45 µm and 0.20 µm RC filters and then housed in disposable polystyrene cuvettes of 1 cm optical path length. The width of DLS hydrodynamic diameter distribution is indicated by PDI (Polydispersion Index). In the case of monomodal distribution (Gaussian), calculated by means of cumulated analysis, PDI = $(\sigma/Z_{avg})^2$, where σ is the width of the distribution and Z_{avg} is the average diameter of the particles population, respectively.

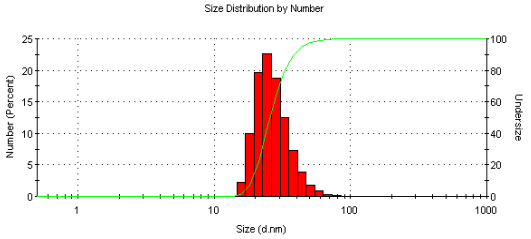
Ru@NP3



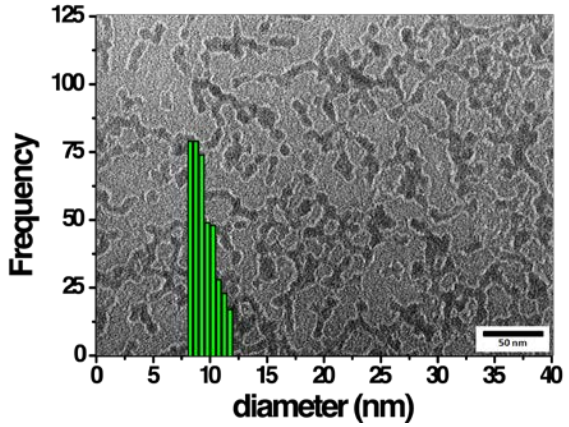


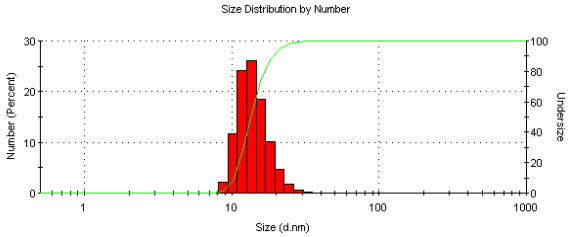
Ru@NP8



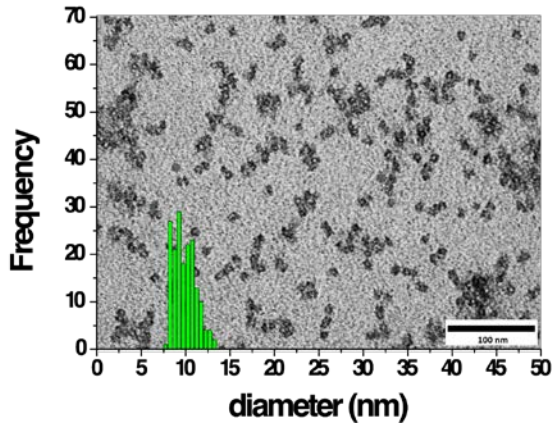


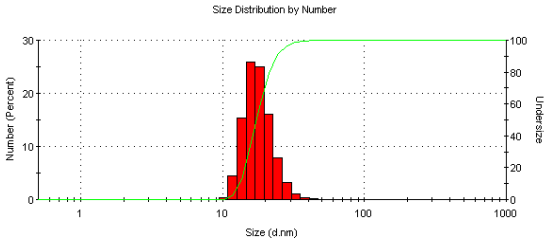
Ru@NP11



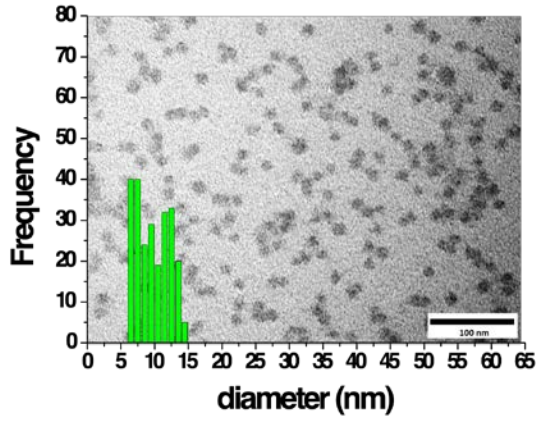


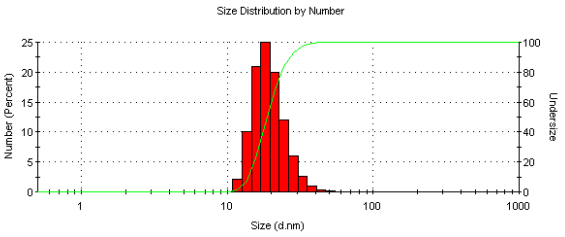
Ru@NP12





Ru@NP19





Ru@NP21

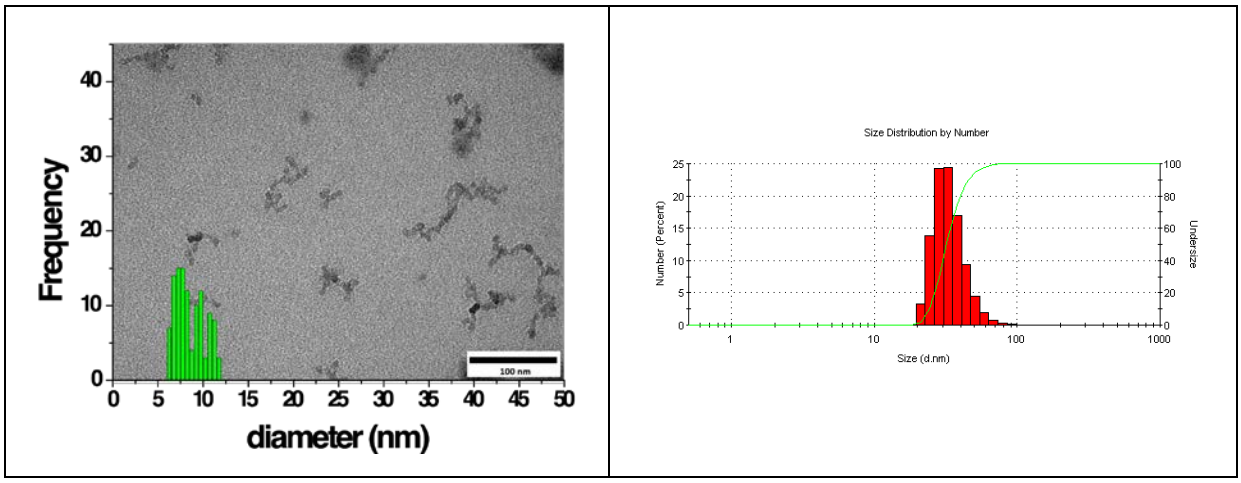


Figure S4: TEM image (left) and silica core diameter distribution (right) of DDSNPs at variable doping levels.

Sample	% mol dye vs. mol TEOS [%]	$d_{core} \pm \sigma$ [nm]	$d_H \pm \sigma$ (PdI) [nm]	ζ-Potential ± σ
Ru@NP3	0.05	9 ± 0.8	20 ± 0.4 (0.4)	-4.2 ± 1.8
Ru@NP8	0.5	9 ± 0.2	25 ± 0.5 (0.3)	-3.5 ± 0.5
Ru@NP11	0.8	9 ± 0.7	24 ± 0.7 (0.3)	-4.6 ± 0.5
Ru@NP12	1.0	10 ± 1.5	21 ± 0.5 (0.3)	-4.3 ± 0.5
Ru@NP19	1.6	7 ± 0.6	20 ± 1.3 (0.5)	-5.4 ± 0.5
Ru@NP21	2.4	8 ± 0.6	26 ± 0.5 (0.3)	-2.2 ± 0.1

Table S3: Doping degree, hydrodynamic diameter and ζ-Potential of the **Ru@NPs** samples.

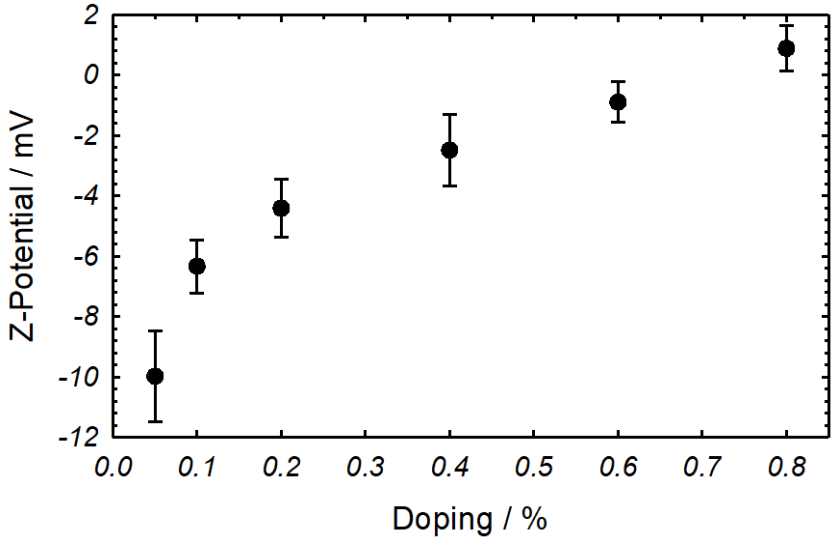


Figure S5: ζ-Potential of **RuBPY@NPs** vs. doping percentage. Conditions: [RuBPY@NPs] = 2µM, [KCl] = 1mM; [phosphate buffer] = 1mM, pH = 7.0, T = 25°C (all samples were filtered with a 0.2 µm RC syringe filter).

ζ-Potential experiments

ζ-Potential values of nanoparticles were determined using a Malvern Nano ZS instrument. Samples were housed in disposable polycarbonate folded capillary cell (DTS1070, 750 µL, 4 mm optical path length). Electrophoretic determination of ζ-Potential was made under Smoluchowski approximation in aqueous media at moderate electrolyte concentration. Measurements conditions: ζ-Potential ± SD (n = 4), [NPs] = 2 µM, [PB] = 1 mM, [KCl] = 1 mM, pH = 7.4, T = 25 °C, samples filtered with a 0.20 µm RC syringe filter before analysis.

S4. ELECTROCHEMILUMINESCENCE STUDY

ECL and electrochemical measurements were carried out with an AUTOLAB electrochemical station (Ecochemie, Mod. PGSTAT 30). Nanoparticles suspension was diluted with a phosphate buffer (PB, pH = 7.4). For ECL generation, 30 mM 2-(dibutylamino)ethanol (DBAE) was added as oxidative coreactant. ECL was obtained in single oxidative steps (sweep steps) by generating the oxidized forms of the amine according to known heterogeneous ECL mechanism. The working electrode consisted of a platinum side-oriented 2 mm diameter disk sealed in glass, while the counter electrode was a platinum spiral and the reference electrode was an Ag/AgCl (3M) electrode.

The ECL signal generated by performing the potential step program was measure with a photomultiplier tube (PMT, Hamamatsu 4220p) placed, at a constant distance, in front of the working electrode and inside a homemade dark box. A voltage in the range 750 V was supplied to the PMT. The light/current/voltage curves were recorded by collecting the pre-amplified PMT output signal (by an ultralow noise Acton research model 181) with the second input channel of the ADC module of the AUTOLAB instrument.

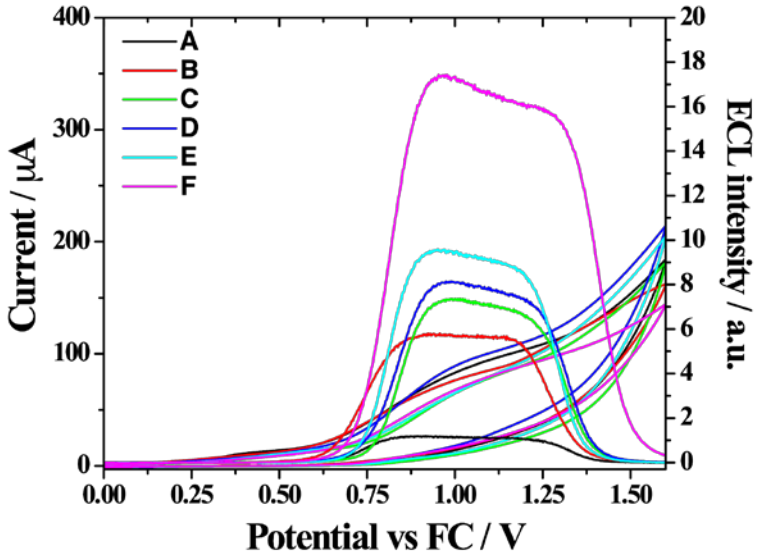


Figure S6: Cyclic voltammogram (CV) and ECL intensity of DDSNPs (5 µM) with different doping percentage **Ru@NP3**, black; **Ru@NP8**, red; **Ru@NP11**, green; **Ru@NP12**, blue; **Ru@NP19**, cyan; **Ru@NP21**, magenta, in the presence of DBAE (coreactant, 30 mM) and PB (pH = 7.4, 100 mM) at

the platinum electrode. Instrumental conditions: scan rate = 0.1 $V \cdot s^{-1}$; PMT bias = 750 V; voltage scan between 0 V and +2.0 V vs. SCE reference electrode.