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Supporting Information

Ruthenium(II) Complex-Photosensitized Multifunctionalized Porous Silicon Nanoparticles for Two-Photon Near-Infrared Light Responsive Imaging and Photodynamic Cancer Therapy

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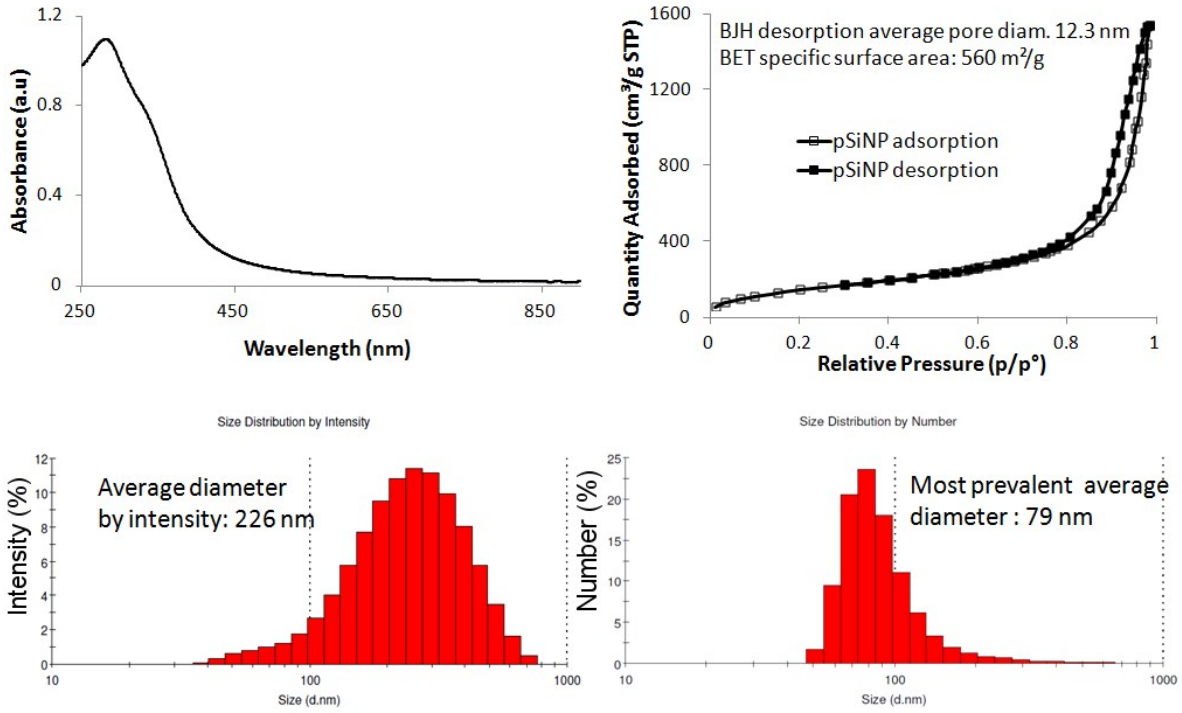


Figure S1: Characterization of the pSiNP. a) UV-vis spectroscopy b)TEM c) Size distribution by intensity of dispersed light d) Size distribution by number of particles

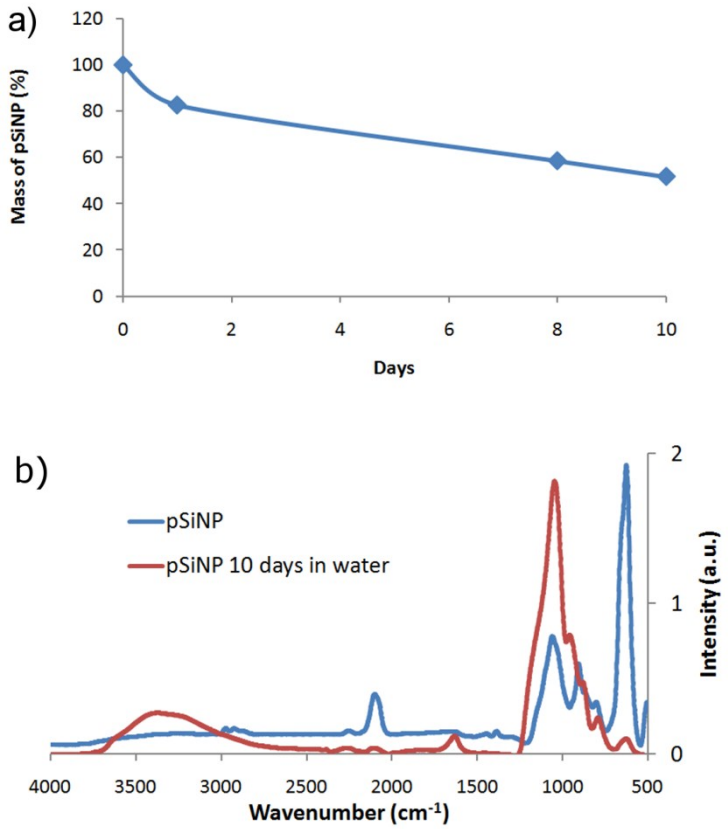


Figure S2. a) Spontaneous degradation of pSiNP upon stirring in deionized water; b) DRIFT spectra of pSiNP before and after 10 days of stirring in water

For the experiment, the four samples containing the same mass of pSiNP was dispersed in 2 mL of water and the mass was measured at designated time points (0, 1h, 8h and 10h) of stirring in water at room temperature. The material was centrifuged, washed with ethanol and ether, and dried before the weight measurements.

Mass of pSiNP decreases in pure water over 10 days due to slow degradation and confirms the advantage of pSiNP in biodegradability over analogous silica based nanomaterials, which are stable in aqueous solutions. Loss of Si-H and predominance of Si-O upon stirring in water is evident on DRIFT spectra

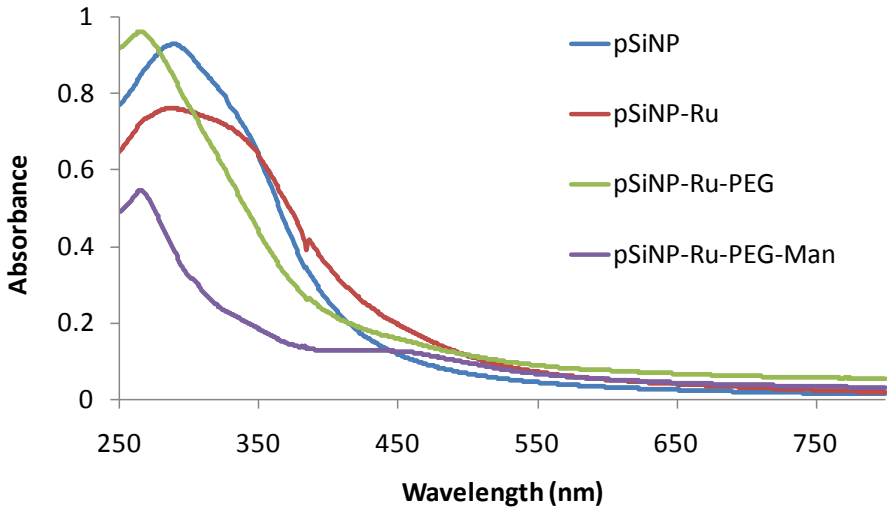


Figure S3. UV/VIS spectra of the ethanolic suspension of the prepared materials. Increase in the absorption around 450 nm (most indicative for pSiNP-Ru-PEG-Man) points to the presence of the Ru(II) complex.

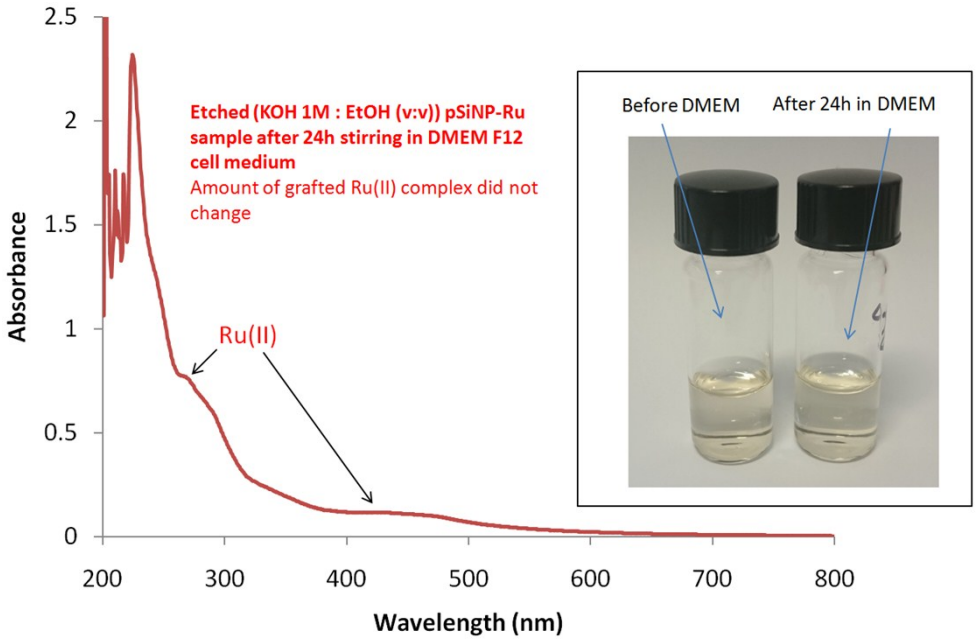


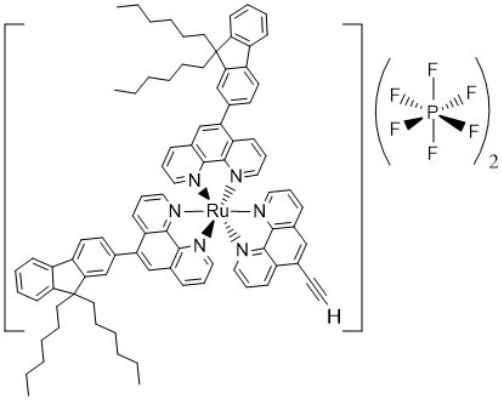
Figure S4. Determination of the leakage of Ru(II) photosensitizer. After 24 h of stirring in DMEM medium no release of Ru(II) complex was observed. After complete etching of the silicon nanocarrier by stirring in 1M KOH : EtOH (1:1) the same amount of Ru(II) complex was determined for the material before and after stirring in DMEM.

Experimental Section

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC FT NMR spectrometer (at 250.1 MHz for ^{1}H and 62.9 MHz for ^{13}C). Elemental analysis was carried out on a Perkin-Elmer 2400. UV-vis absorption measurments were performed using a lambda 35 Perkin Elmer spectrometer. Infrared spectra were recorded on Nicolet IS5 spectrometer with the ATR ID5 module. Transmission electron micrographs (TEM) were obtained on JEOL 1200 EXII instrument. The specific surface areas and pore size distribution of the samples were estimated using nitrogen adsorption–desorption isotherms with Micrometrics ASAP 2020 instrument. Before the sorption measurement, samples were degassed at 80 °C for 6 h under reduced pressure. The specific surface area of sample (S_{BET}) was calculated according to the Brunauer, Emmett, Teller (BET) method from the linear part of the nitrogen adsorption isotherm at low relative pressure, the volume of the mesopores and pore size distribution were analyzed according to the Barrett, Joyner and Halenda (BJH) method. The DLS and zeta potential measurements of the materials were performed on a Malvern nanozetasizer in diluted ethanol solution.

Ligands 5-dihexylfluorene-1,10-phenanthroline ligand **(5-Fluo-Phen)**[1] and 5-ethynyl-1,10-phenanthroline (**5-E-Phen**),[2] were prepared according to already described procedures.

I. Synthesis of the ruthenium(II) complexes $Ru(5\text{-}Fluo\text{-}Phen)_2(5\text{-}E\text{-}Phen)](PF_6)_2$



$Ru(5\text{-}Fluo\text{-}Phen)_2(5\text{-}E\text{-}Phen)](PF_6)_2$

[1] C. Girardot, G. Lemercier, J.-C. Mulatier, J. Chauvin, P. L. Baldeck, C. Andraud, *Dalton Trans.,* **2007**, *31*, 3421-3426.
[2] E. Sakuda, Y. Ando, A. Ito, N. Kitamura, *Inorg. Chem*. **2011**, *50*, 1603-1613.

Ru(5-Fluo-Phen)$_2$Cl$_2$. 128 mg of $RuCl_3 \cdot 3H_2O$ (0.49 mmol, 1 eq.) and 207 mg of LiCl (4.9 mmol, 10 eq.) were dissolved in 2 mL DMF, under argon. A DMF solution (3 mL) of 500 mg of **5-Fluo-Phen** (0.98 mmol, 2 eq.) was added dropwise, and the mixture was refluxed for 3.5 hours. 50 mL of an NH_4PF_6 saturated aqueous solution was then added to the resulting solution at room temperature. The precipitate was collected by filtration, dissolved in dichloromethane and the resulting organic phase washed three times with water and dried over $MgSO_4$. The drying agent was filtered off and the solvent was evaporated to yield 400 mg of a dark violet solid. Yield: 65%. Anal. calcd for $RuC_{74}H_{80}N_4Cl_2$, 3 H_2O: C, 71,0; H, 6,9; N, 4.5. Found: C, 70.5; H, 6.6; N, 4.5%.

Ru(5-Fluo-Phen)$_2$(5-E-Phen)](PF$_6$)$_2$. The ligand **5-E-Phen** (16 mg, 0.085 mmol) and complex **Ru(5-Fluo-Phen)$_2$Cl$_2$** (110 mg, 1 eq.) were dissolved, under argon, in 4 mL of DMF. The mixture was heated at reflux over night. Saturated aqueous solution of NH_4PF_6 was then added to the resulting solution at room temperature. The precipitate was collected by filtration, washed two times with water and one time with hexane. 150 mg of a dark red powder was obtained with a quantitative yield. ^{1}H NMR (250.13 MHz, $CDCl_3$) δ(ppm): 8.8 (3H, s_{large}), 8.6–8.1 (14H, m), 8.0–7.6 (9H, m), 7.5 (3H, s_{large}), 7.4 (6H, s), 3.5 (1H, m), 2.3–1.5 (8H, m), 1.0 (24H, m), 0.75 (20H, m). Anal. cacld for $RuC_{88}H_{88}N_6P_2F_{12}$, 1 H_2O: C, 64.5; H, 5.5; N, 5.1. Found: C, 64.4; H, 5.5; N, 4.9.

Preparation of pSiNPs

Boron-doped p^{++}-type Si (0.8-1.2 mΩ.cm resistivity, <100> orientation) from Siltronix (France) was electrochemically etched in a 3:1 (v:v) solution of aqueous 48% hydrofluoric acid (HF):absolute ethanol (Sigma-Aldrich). Etching was performed in a Teflon cell with a platinum ring counter electrode. A constant current of 180 mA·cm^{-2} was applied for 300 s,

and then the sample was rinsed 3 times with ethanol. The porous layer was then removed from the substrate by application of a constant current of 4 $mA.cm^{-2}$ for 250 s in an electrolyte solution containing 1:20 (v:v) aqueous 48% hydrofluoric acid:absolute ethanol. After 3 rinses with ethanol, the porous layer was placed in ethanol in a glass vial. After degassing the sample for 20 min under a nitrogen stream, the porous silicon film was fractured by ultrasonication during 16 h. The largest particles were then removed by spinning them down by centrifugation at 3,000 rpm for 2 min (Minispin, Eppendorf). In order to remove the smallest fragments, the solution was finally centrifuged at 14,000 rpm for 30 min (centrifuge Eppendorf 5804) and the pellet containing pSiNP was redispersed in absolute ethanol.

Quantification of $[Ru(5\text{-}Fluo\text{-}Phen)_2(5\text{-}E\text{-}Phen)]^{2+}$ on the materials

The amounts of complex grafted on the pSiNP-based materials were determined by etching the known masses of materials in 2ml of a solution mixture containing 1 M potassium hydroxide in water and ethanol (1:1) and the absorbance of the solution was recorded between 225 and 325 nm. Calibration curve was also constructed by dissolving the known amounts of the starting complex in 2 ml of 1 M KOH:EtOH solution and the absorbance values were measured at 269 and 453 nm.

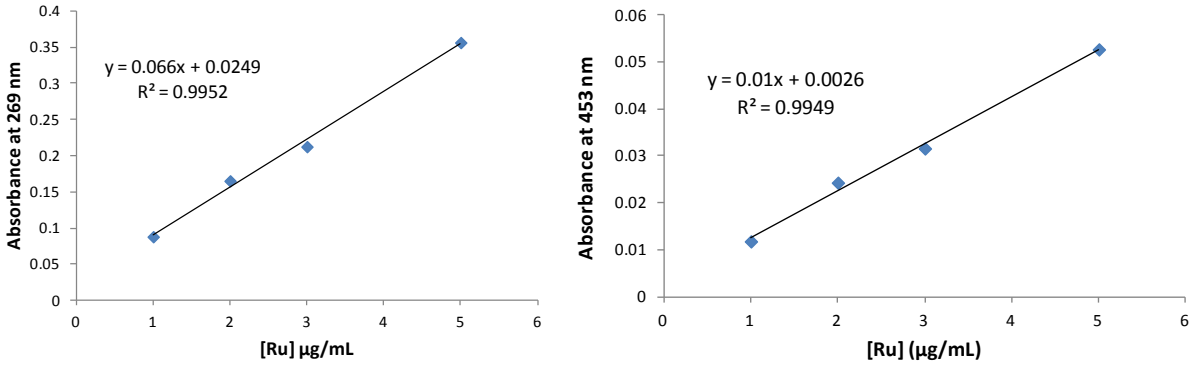


Figure S5. Calibration curves for determination of concentration of $[Ru(5\text{-}Fluo\text{-}Phen)_2(5\text{-}E\text{-}Phen)]^{2+}$ = [Ru] in the synthesized materials.

Quantification of mannose ethyl squarate on pSiNP-Ru-PEG-Man materials

The amount of mannose ethyl squarate grafted on the pSiNP-Ru-PEG-Man material was determined by adjustment of previously published procedure.[3] Known mass of pSiNP-Ru-PEG-Man material was dissolved in 0.5 ml of 1 M potassium hydroxide aqueous solution over night. The base was then neutralized with sulfuric acid (0.5 mL, 0.5M) and 25 µl of 80% solution of phenol in water was added. Concentrated sulfuric acid was then added (2.5 mL) and the sample was left standing at room temperature for 10 min. After that period the sample was shaken and incubated at 30˚C for 20 min. Absorbance of the sample was then measured at 489 nm while solution of the corresponding amount of pSiNP-Ru-PEG material was used as the blank, after performing the same steps as for pSiNP-Ru-PEG-Man material (etching, neutralization, addition of phenol/water, incubation). Calibration curve was also constructed by dissolving the known amounts of mannose ethyl squarate and preparation of the samples in the same manner.

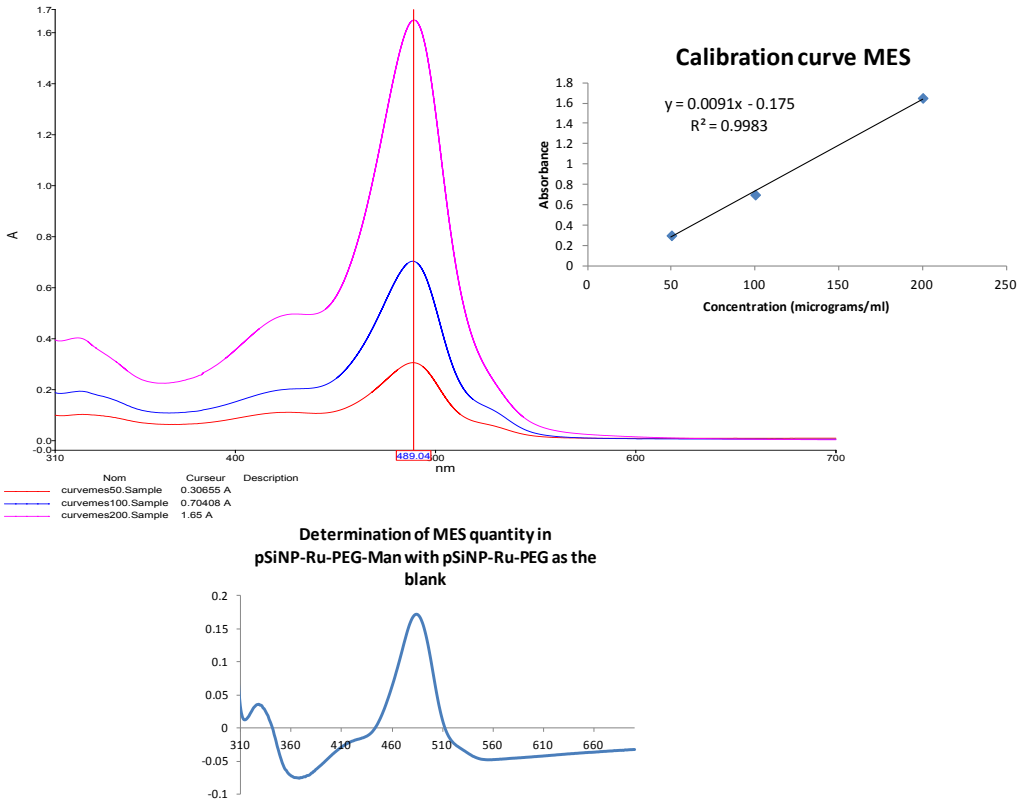
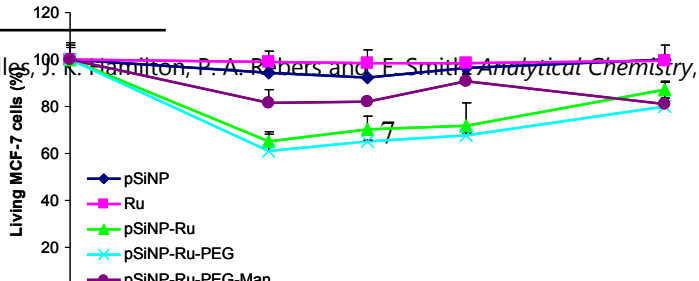


Figure S6. Quantification of mannose ethyl squarate (MES) in pSiNP-Ru-PEG-Man



[3] M. DuBois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, *Analytical Chemistry*, 1956, **28**, 350-356.

Figure S7. Cytotoxic study of the nanomaterials on MCF-7 cells. Data are mean ± standard deviation of three experiments.

For cytotoxic studies MCF-7 cells were seeded into 96-well plates in 200 μL culture medium and allowed to grow for 24 h. The ethanolic suspensions of the materials were added to the wells containing seeded cells in DMEM medium in order to achieve the final treatment concentrations. Control cells were treated with the same volume of ethanol in order to account for ethanol-caused cell death. Increasing concentrations of nanomaterials were **incubated in culture medium of cells during 72 h**. Then, a MTT assay was performed to evaluate the toxicity. Briefly, cells were incubated for 4 h with 0.5 mg/mL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Promega) in media. The MTT/media solution was then removed and the precipitated crystals were dissolved in EtOH/DMSO (1:1). The solution absorbance was read at 540 nm.

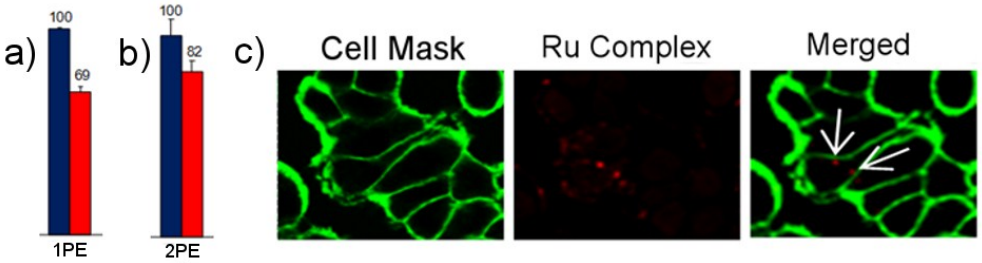


Figure S8. MCF-7 cell culture studies with $[Ru(5\text{-Fluo-Phen})_2(5\text{-E-Phen})](PF_6)_2$**:** a) 1PE PDT, b) 2PE PDT, c) Multiphoton imaging