Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

Electronic Supporting Information

One step synthesis of polymer core-shell particles with carboxylated Ruthenium complex: potential tool for biomedical applications

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The supporting information contains four sub-sections:

ESI-1 Characterization of the ruthenium complex

Comparative analysis between ^{1}H NMR spectra of tris-chelated complex of Ru(II), obtained using a Bruker Avance II 250 MHz spectrometer in D_2O

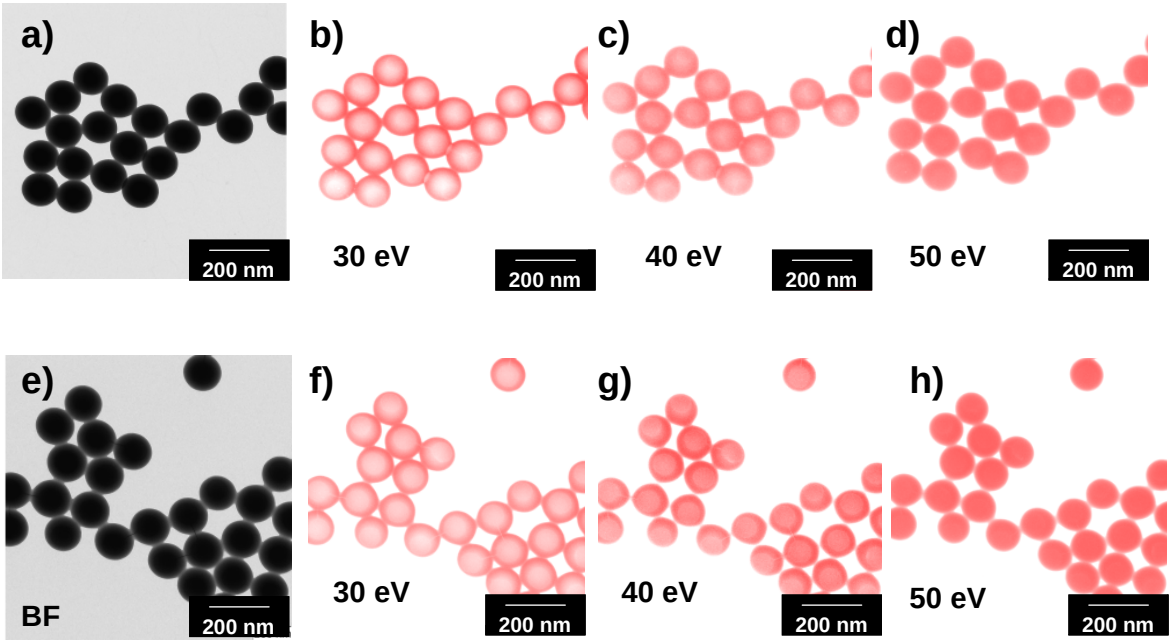
	Literature [1]	*Synthesis*
C-H (1)	7,93 (d)	7,58 (d)
C-H (2)	7,73 (d)	7,67 (d)
C-H (3)	8,94 (s)	8,79 (s)

(1) Nazeeruddin, M. K.; Kalyanasundaran, K.; Grätzel, M.; Sullivan, B. P. and Morris, K. (1998) One-Pot Synthesis of Tetrahydronium Tris(4,4′-Dicarboxylato-2,2′-Bipyridine)-Ruthenium(II) Dihydrate. *Inorganic Syntheses. Volume 32:* Transition metal complexes and precursors (Darensbourg, M.Y. (Ed.) pp 181-186, Chapter 3, John Wiley & Sons, Inc., New York.

ESI-2 Parameters used in the confocal microscope to analyze cells and serum spot.

Material	Dye	Excitation and emission wavelength (nm)	Laser	Filter configuration
Nucleus	Hoechst 33342	350/460	Argon (488)	HFT KP700/514, mirror, mirror, BP 435-485
Membrane	DilC	549/565	543	NT80/20/543, BG39, LP560
Particles (cells)	Ruthenium complex	470/630	Argon (488)	HFT488/543, mirror, BG39, KP685
Particles (serum spots)	Ruthenium complex	470/630	Argon (488)	HFT488, mirror, NFT545, KP685

ESI-3 TEM bright-field and low energy loss (30 to 50 eV) images for PSHEMA particles without (a-d) and with ruthenium complex (e-h).



ESI-4 Application of Ru-PSHEMA particles for conjugation with biomolecules using biotin-streptavidin interaction.

BSA coated PSHEMA particles were conjugated to streptavidin by adding 0.2 mg of protein to 400 µL of an aqueous particle dispersion at 3 mg/mL. The suspension was stirred for 30 minutes at RT. Subsequently, 1.5 mg of EDC was added and mixed by vortexing and then adjusted to pH 6.5. The dispersion was kept on a shaker for 3 h at RT. To separate the protein-labeled particles from unbound proteins and EDC molecules, the suspension was centrifuged at 5000 rpm for 5 min at RT and washed twice with phosphate buffer 0.01 mol/L, pH 7.6, for 10 min at 4°C. Streptavidin particles were used to analyze biotin immobilized onto a glass slide using fluorescence microscopy. Amine coated slides were functionalized with albumin by adding a solution containing 5% of BSA and 100 mg of EDC in 4 mL of phosphate buffer 0.01 mol/L, pH 6, to the glass slide. The system was incubated in a humidity chamber for 1 h. Then, the glass slide was three times washed with phosphate buffer 0.01 mol/L, pH 7.4, and dried at RT. Biotin-NHS was diluted in dimethylformamide for 1 mmol/L and dilutions from 10^{-4} to 10^{-10} mol/L were prepared using phosphate buffer 0.01 mol/L, pH 7.4. 2 µL spots from each biotin solution were deposited on the albumin coated glass slide and kept in a humidity chamber for 1h at RT. A solution of streptavidin monoclonal antibody 0.01 mg/mL containing 0.2 mg of EDC was deposited on the albumin coated glass slide and was used as positive control. After 1 h, the glass slide was three times washed with phosphate buffer 0.01 mol/L, pH 7.4, and dried at RT. A dispersion of streptavidin coated particles 1% (w/v) in phosphate buffer 0.01 mol/L, pH 7.4, was deposited onto the glass slide, which has then kept in the humidity chamber for 20 minutes. After washing with water, the glass slide was dried and kept in the dark until analysis.

Biotin-streptavidin interaction was chosen as model system due to its extensive use to bind molecules such as antibodies and nucleic acids to the detection system, like fluorescent particles. Therefore, streptavidin was covalently bound to the PSHEMA particles. Bovine serum albumin was firstly immobilized on the particle surface as spacer for the conjugations to avoid conformation changes on the protein and to provide better steric accessibility to the forthcoming interaction. An amine coated glass slide was functionalized with biotin spots from 1 mmol/L to 0.1 nmol/L and a representative scheme of the functionalized glass slide is shown in Figure ESI-4A.

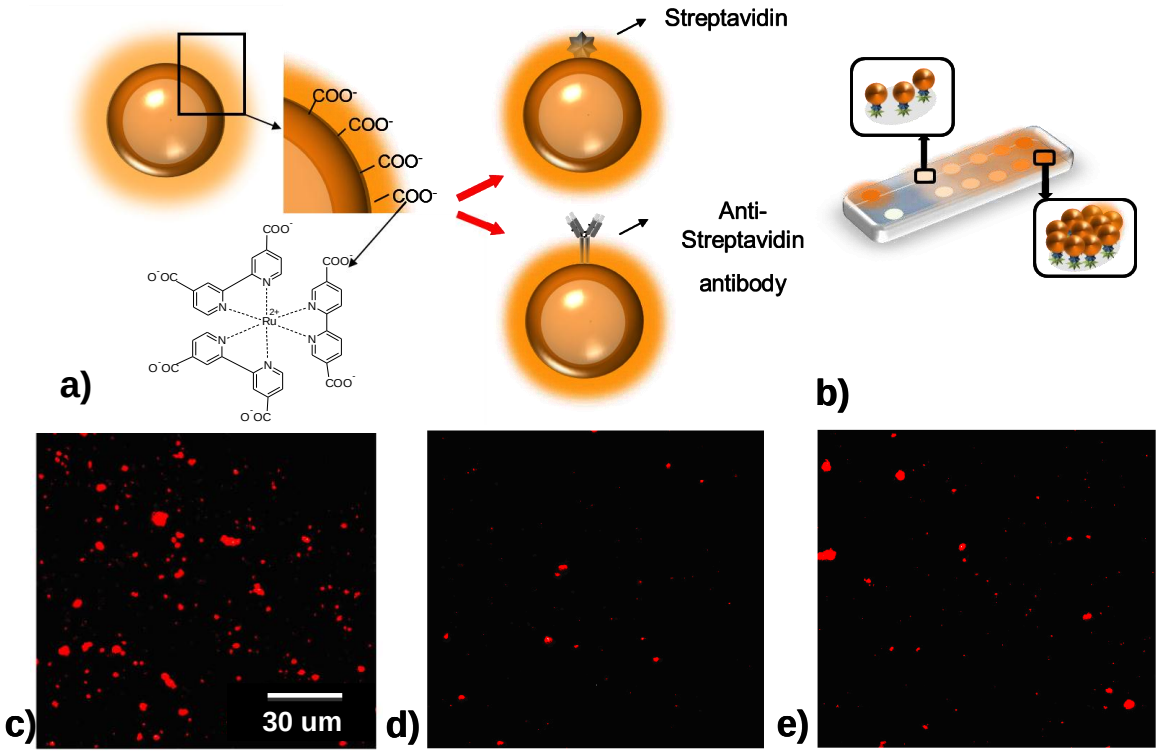


Figure ESI-4A. Scheme of particle structure (a) showing carboxylate groups used for bioconjugation with proteins and antibodies. Bioassay (b) based on streptavidin coated Ru-PSHEMA particles and biotin functionaliyzed albumin glass slide. Digital fluorescence images of three spots: (c) 1 mmol/L biotin, (d) 0.01 µmol/L biotin and (e) positive control consisting of anti-streptavidin antibody immobilized on the glass slide, 10 µg/mL. The images were taken through a 40 x microscope objective.

Fluorescent particles attached to biotin-modified glass slides are clearly confirmed by the density of fluorescent dots in Figure 1c-e. The density of fluorescent particles per spot increased with the increase of biotin concentration immobilized on the glass slide. After 10 nmol L^{-1}, the particle density is similar to the observed in the negative control, caused by non-specific adsorption of particles on the glass slide. Positive control formed by anti-streptavidin antibody clearly shows high number of attached particles in contrast to the negligible number observed in negative control. These findings demonstrate that the test succeeds and that PSHEMA particles are successfully applied to bioconjugation. An image series is presented in the Figure ESI-4B.

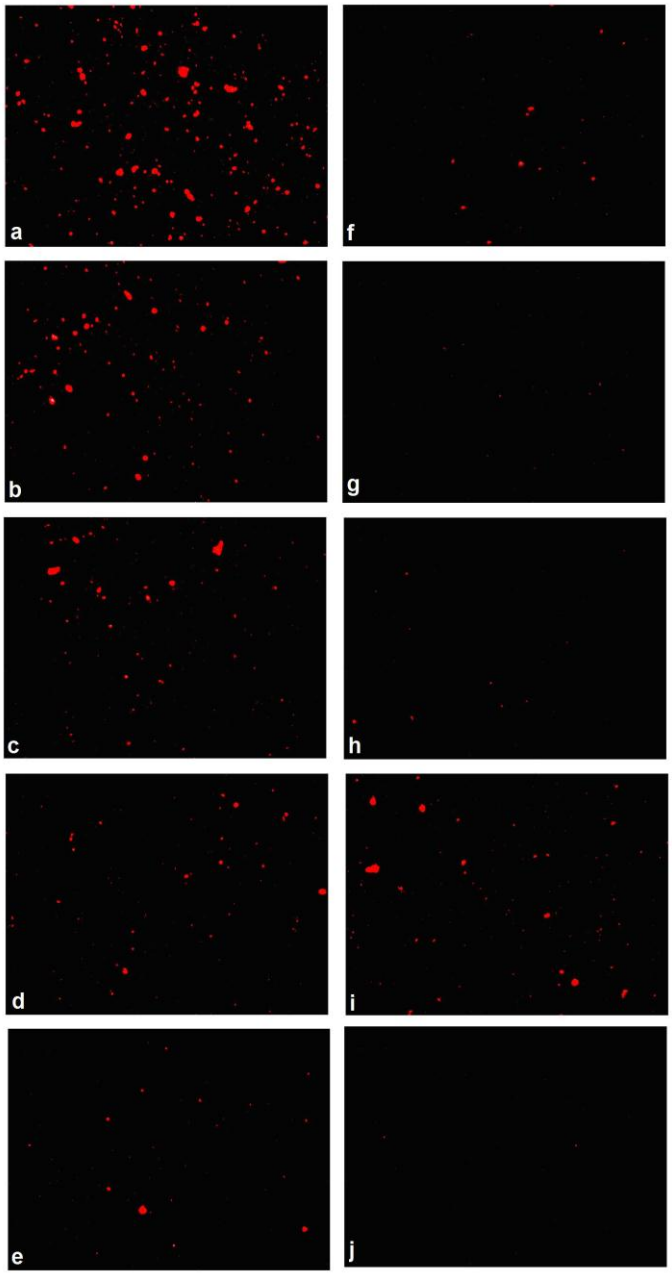
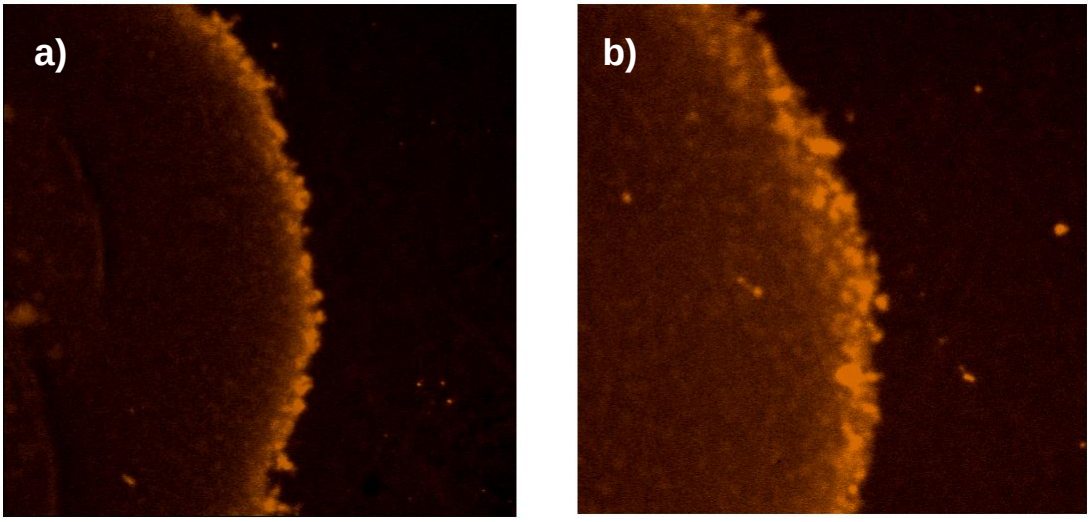
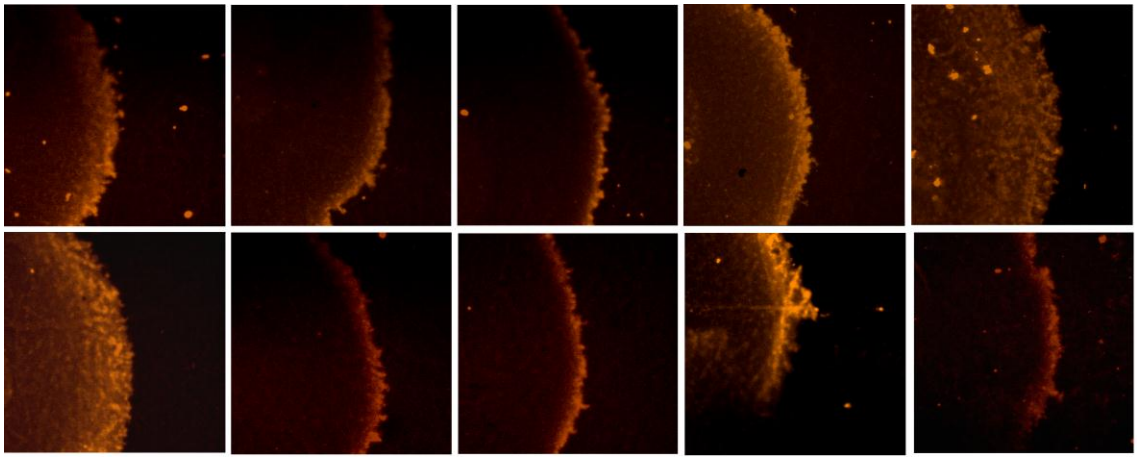


Figure ESI-4B. Digital fluorescence image of the bioassay on glass slide based on biotin-streptavidin interaction. The concentrations of biotin are (a) 1 mmol/L, (b) 0.1 mmol/L, (c) 0.01 mmol/L, (d) 1 µmol/L, (e) 0.1 µmol/L, (f) 0.01 µmol/L, (g) 1 nmol/L, 0.1 nmol/L, (h) 0.01 nmol/L (e) positive control consisting of anti-streptavidin antibody immobilized on the glass slide, 10 µg/mL and (j) negative control formed by amine coated glass slide. The images were taken through a 40 x microscope objective.

ESI-5 Digital fluorescence images of spots of serum samples after interacting with PSHEMA-Ru particles coated with anti-NS1 protein antibodies through (a) absorption and (b) covalent coupling. (Magnification: 100x)



ESI-6 Digital fluorescence images of spots of ten serum samples after interacting with PSHEMA-Ru particles covalently coated with anti-NS1 protein antibodies. (Magnification: 100x)



ESI-7 Digital fluorescence image of fibroblast cells without PSHEMA particles used as control. (Magnification: 600x)