Electronic Supplementary Material (ESI) for ChemComm.
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Electronic Supporting Information

Supported core-shell nanobiosensors for quantitative fluorescence imaging of extracellular pH

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MATERIALS AND METHODS

Chemical and reagents. Sodium citrate tribasic dihydrate (≥99.0% ACS grade), fluorescein 5(6)-isothiocyanate (FiTC; 90%), triethylamine (≥99.5%), (3-aminopropyl)triethoxysilane (APTES; ≥98%), ammonium hydroxide solution (28-30% NH_3), and L-ascorbic acid (HAsc; ≥99.0%) were purchased from Sigma-Aldrich. Silver nitrate (99.9995%) was obtained from Strem Chemicals, triethoxysilane eosin-5-isothiocyanate (EiTC) from Marker Gene Technologies, (TEOS; 99.9%) from Alfa Aesar, and anhydrous *N*,*N*-dimethylformamide (DMF; 99.8%) from EMD Chemicals, *O*-(propargyloxy)-*N*-(triethoxysilylpropyl)-carbamate (PTSC; 90%) was purchased from Gelest, 11-azidoundecyltrimethoxysilane (AUTS; ≥95%) from SiKÉMA, copper sulfate pentahydrate (≥98.5%) from VWR International. Buffers were prepared with potassium phosphate monobasic (≥99.0%, Anachemia) and precise volumes of sodium hydroxide (97.0% ACS, BDH) aqueous solution. Unless otherwise specified, every chemical reagent was used without further purification. Ultrapure water (18.2 MΩ) was used in all experiments unless specified and anhydrous ethanol, obtained from Commercial Alcohols, was used as a solvent. All glassware for nanoparticles synthesis was conditioned with concentrated nitric acid, and then rinsed thoroughly with water.

Synthesis of silver nanoparticles. The procedure to prepare silver cores follows a scaled-up reduction methodology derived from various works cited in literature.[1-3] 114 mg of sodium citrate tribasic dihydrate was dissolved in 500 mL of water (0.77 mM) and heated to the boiling point under vigorous stirring. Afterward, 90 mg of silver nitrate in 500 µL of water is quickly added to the mixture, and kept at this temperature during 50 minutes before gradually cooling under moderate agitation. Total volume is adjusted to 350 mL with deionised water.

Preparation of fluorescent silica shell ($Ag@SiO_2$-fluorophore). Condensation of a silica shell on the silver core was achieved using experimental conditions adapted from Blaaderen et *al*.[4] A fluorescent silane precursor was prepared from the fluorophore molecule, a step adapted from our previous works.[3, 5-7] For example, 2.2 mg of FiTC was added to 114 µL of DMF, 1.6 µL of triethylamine and 1.6 µL of APTES, and left to agitate for 2h – thus obtaining a 50 mM solution of the desired molecule. This mixture was then diluted to a volume of 13.5 mL with anhydrous ethanol.

A 100 mL volume of the suspension of Ag nanoparticles was diluted in 600 mL of ethanol, and 14 mL of 10 mM TEOS/EtOH and 8 mL of ammonium hydroxide were added in order to form a thin silica layer. After 15 minutes, a new addition of 4.4 mL of the FiTC-APS precursor solution previously described was added and left to react for 20-24h at room temperature, and the resulting suspensions were washed in ethanol and centrifuged three times (11,000 RCF, 20 minutes). The enhancement of fluorescence by the plasmonic core was determined by comparing the emission intensity before and after dissolution of the silver cores with dilute nitric acid (Figure S5).

"Click" grafting of metal@SiO_2 NPs on silica surfaces. Surface activation of 24×30 mm silica coverslips was obtained by immersion in Piranha 3 H_2SO_4 : 1 H_2O_2 (30%) for 15 minutes. After thorough rinsing with water and conditioning in ethanol, the substrates were deposited individually in polypropylene Petri 100×100 mm dishes and put in contact with 2 mM of a silane/EtOH solution under moderate agitation for 3 hours. Following the reaction, they were rinsed and stored in ethanol. Functionalization of $Ag@SiO_2$-fluorophore nanoparticles with the coupling agent was done in 1mM silane/EtOH for 4 hours, before being centrifuged three times (11 000 RCF, 15 minutes) and dispersed in ethanol. In new Petri dishes, 10 mL of core-shell particle suspension, 4 mL of freshly prepared 5 mM copper sulfate solution, and 1 mL of 5 mM ascorbic acid in 80%

ethanol-water solution were mixed and left to react under agitation for various durations, and then rinsed several times in ethanol and water in a sonication bath.

Cardiac fibroblast culture. Human (WT) and mouse (P2Y2R) valve interstitial cells were isolated by collagenase digestion (Jackson Laboratory, USA). Aortic valves from mice were dissected under stereo microscope (Zeiss, ON, Canada) and pooled together (n = 10) in order to start culture. Cells were incubated with Dubelcco's Modified Eagle Medium (DMEM) and 10% fetal bovine serum (FBS), 1% sodium pyruvate, and 1% glutamate. Culture medium used during *in vitro* analysis was conventional ringer buffer, containing 5 mM glucose, 5 mM potassium gluconate, 1 mM calcium gluconate, 1 mM magnesium sulfate, 2.5 mM sodium phosphate monobasic, 25 mM sodium bicarbonate, 10 mM HEPES and 140 mM sodium gluconate. Typical pH values for this solution were around 7.40.

Characterization methods. Nanoparticle suspensions were analysed by UV-visible spectrophotometry (Cary 50), transmission electronic microscopy (Tecnai G2 Spirit Biotwin), and scanning electron microscopy (Quanta 3D, FEI). Characterization of the functionalised and grafted surfaces was achieved by UV-visible spectroscopy, fluorescence microscopy (BX-53, Olympus) and by spectrofluorimetry (Jobin Yvon Fluorolog 3-22 equipped with a cooled PMT R928 detector, Horiba). Microfluidic flow cells were custom-made from the NPs-grafted coverslip and a clean microscope slide – glued so as to set them at a controlled distance from each other – as well as two injection needles used as inlet and outlet ports. An epifluorescence microscope (Eclipse TE2000-5, Nikon) equipped with an enclosed chamber for controlled 37°C temperature and 5% CO_2 concentration was used for measurements on the live cell cultures.

The measurement of extracellular pH by flow cytometry[8] used an appropriate volume of potassium phosphate buffer for each pH, with an addition of nigericin (a proton uniporter blocker) for a final concentration of 10 μM. Fibroblasts were made to fluoresce by treatment with 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein) (BCECF), suspended in the buffering solutions described above and kept on ice during 1 minute, and finally measured using emission channels at 530 nm (BCECF) and 600 nm (ratiometric correction). Typical extracellular values for extracellular pH in cells over-expressing CA12 proteins ranged from 5.9 to 6.5, while unmodified control cells retained the medium value of 7.3.

References

1. Z. Bai, R. Chen, P. Si, Y. Huang, H. Sun and D.-H. Kim, *ACS Appl. Mater. Interfaces*, 2013, **5**, 5856.
2. W. Deng, D. Jin, K. Drozdowicz-Tomsia, J. Yuan, J. Wu and E. M. Goldys, *Adv. Mater.*, 2011, **23**, 4649.
3. M. L. Viger, D. Brouard and D. Boudreau, *J. Phys. Chem. C*, 2011, **115**, 2974.
4. O. G. Tovmchenko, C. Graf, D. J. van den Heuvel, A. van Blaaderen and H. C. Gerritsen, *Adv. Mater.*, 2006, **18**, 91.
5. M. L. Viger, L. S. Live, O. D. Therrien and D. Boudreau, *Plasmonics*, 2008, **3**, 33.
6. L. Rainville, M.-C. Dorais and D. Boudreau, *RSC Adv.*, 2013, **3**, 13953.
7. D. Brouard, O. Ratelle, A. G. Bracamonte, M. St-Louis and D. Boudreau, *Anal. Methods.*, 2013, **5**, 6896.
8. P. Franck, N. Petitipain, M. Cherlet, M. Dardennes, F. Maachi, B. Schutz, L. Poisson and P. Nabet, P., *J. Biotechnol.*, 1996, **46**, 187.

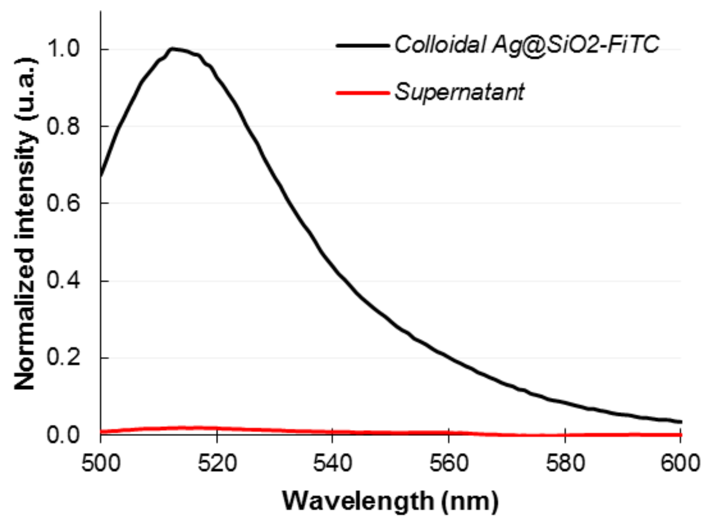

Figure S1: Fluorescence signal from FiTC from core-shell Ag@SiO_2-FiTC NPs suspended in phosphate buffer (pH = 6.8) and from the supernatant after 16 h (centrifugation: 15 min at 18 000 RCF).

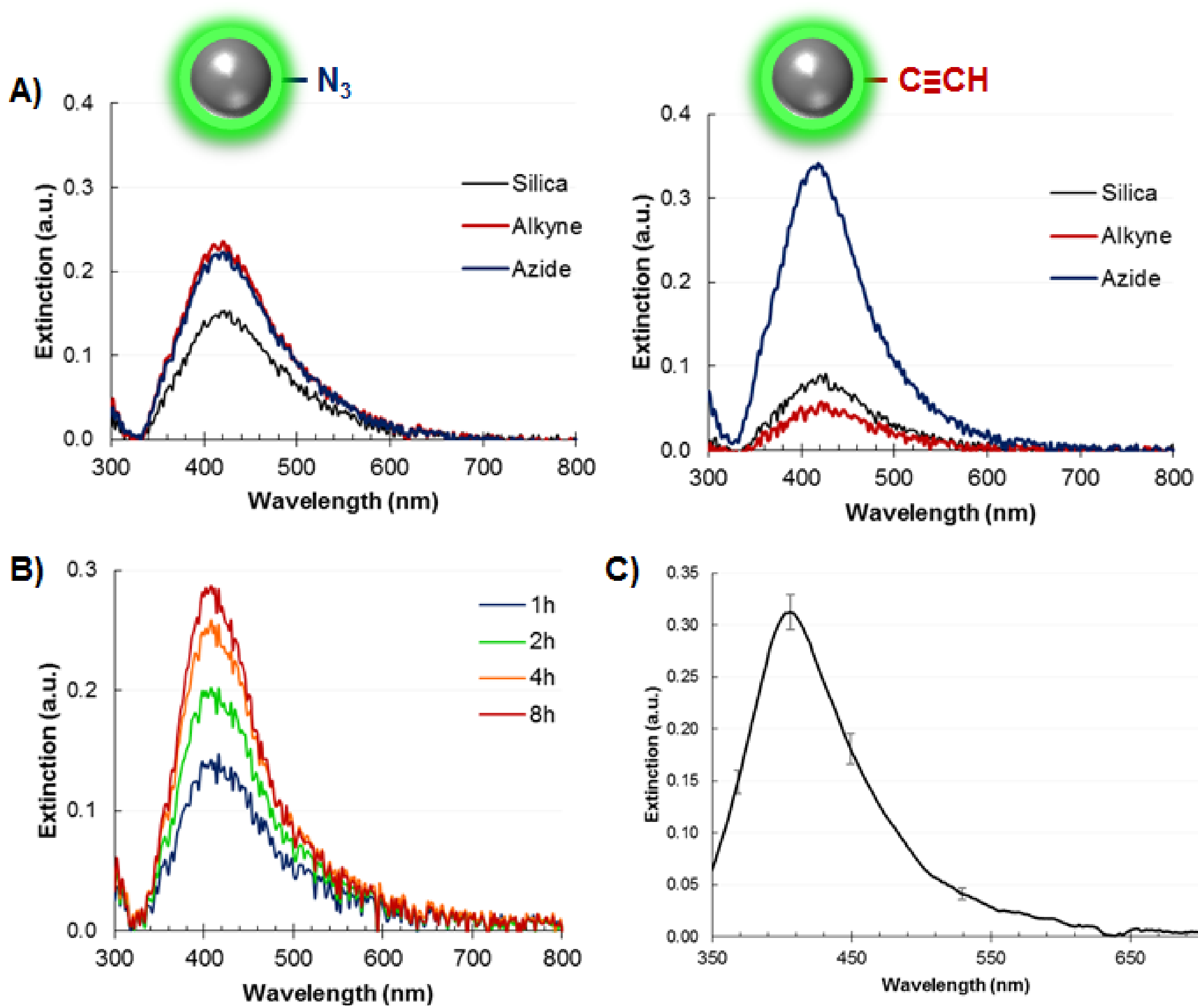

Figure S2: A) Optical density of Ag@SiO_2-grafted surfaces with permutation of coupling agents.
B) Grafting efficiency for different reaction times.
C) Reproducibility of extinction spectrum from 3 coverslips grafted with Ag@SiO_2 NPs from distinct synthesis batches. Error bars show standard deviation (n=3).

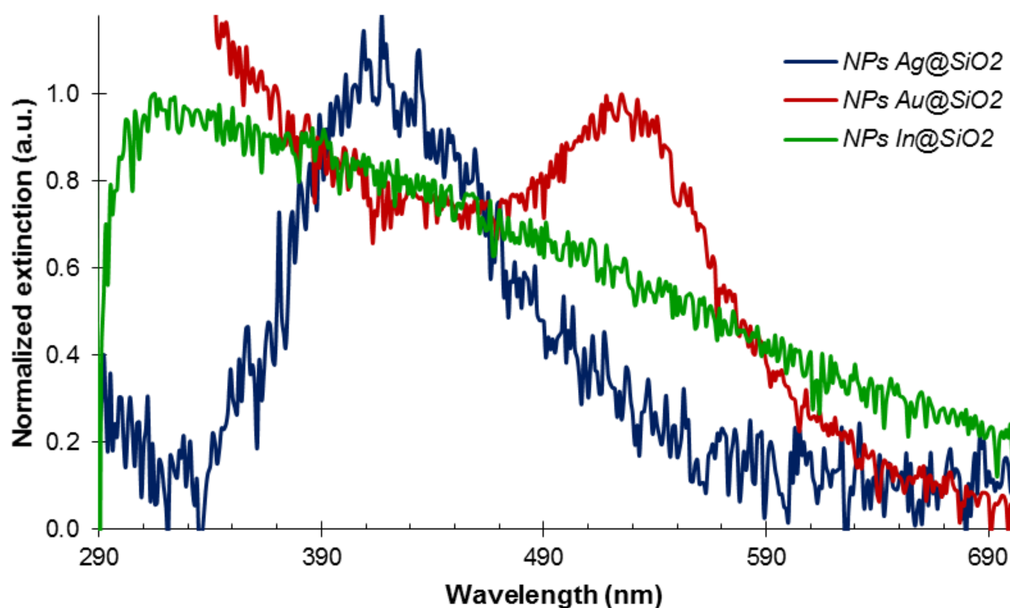

Figure S3: Extinction spectra of different types of plasmonic core-shell nanoparticles on silica coverslips.

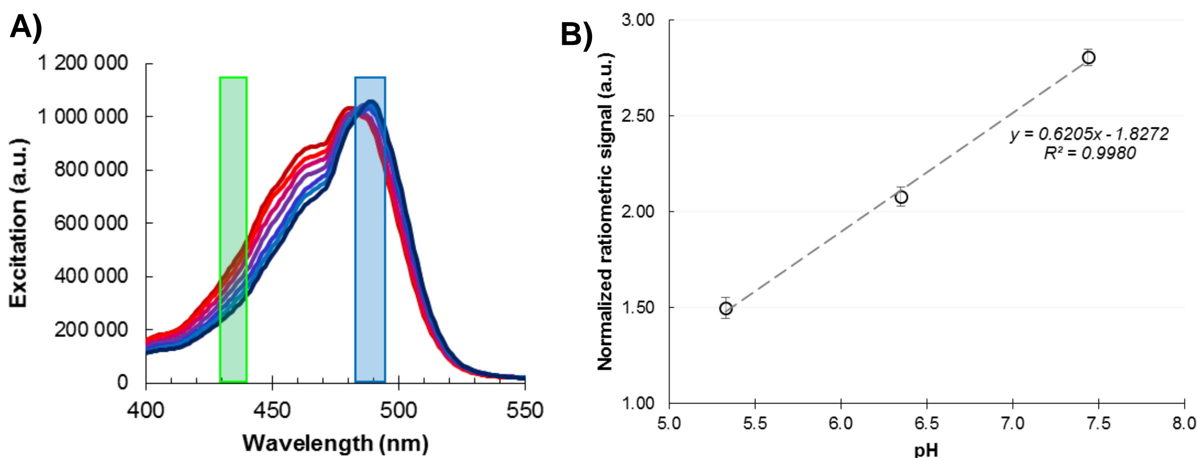

Figure S4: A) Illustration of the excitation bandwidths (λ_{exc} = 436 ± 5 nm and 489 ± 6 nm) selected by two filter cubes used on a fluorescence microscope for ratiometric measurements with the Ag@SiO_2-FiTC surfaces. B) Calibration curve obtained with fluorescence imaging of pH sensing substrate (λ_{em} = 536 ± 20 nm) in cell culture media (pH adjusted with addition of dilute nitric acid).

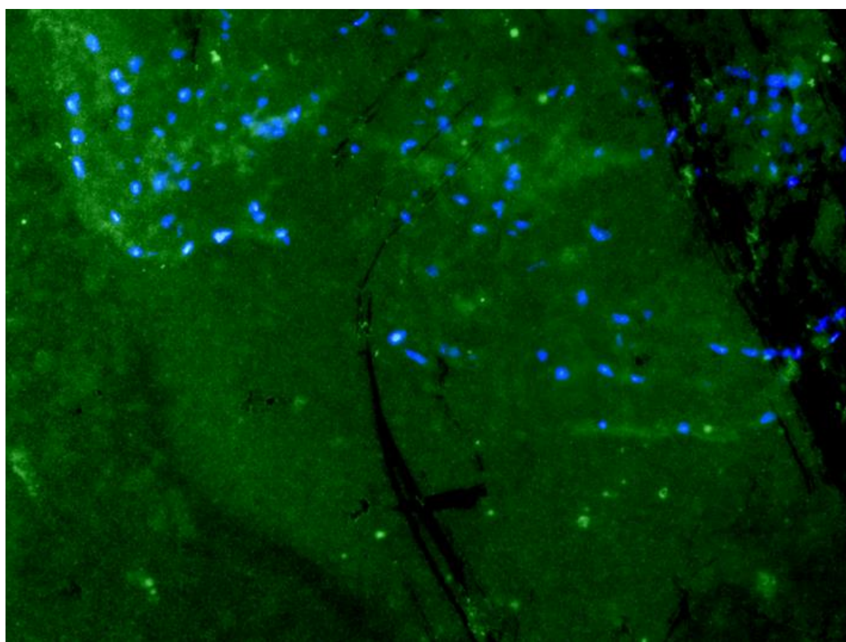

Figure S5: Epifluorescence microphotograph of the fluorescent surface (FiTC, green) and cell nuclei (DAPI, blue) taken with a 40X objective.

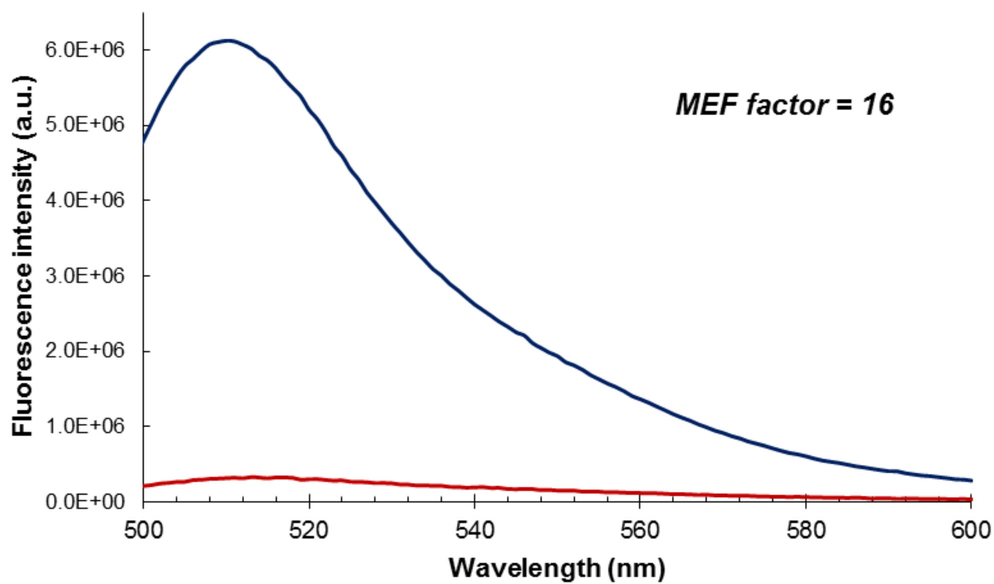

Figure S6: Fluorescence intensity from Ag@SiO_2-FiTC core-shell particles grafted on surfaces before (blue) and after (red) etching of the metal cores. The enhancement factor was calculated as the ratio of intensities at both maxima.