Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2014

Electronic Supplementary Information

Supplementary Methods

Cell lines and culture conditions

For cellular studies, three human ovarian tumor cell lines (SKOV3, IGROV-1 and A2780/S), one human cervix carcinoma cell line (HeLa) and one murine monocytic/macrophagic cell line (J774a.1) were used. A2780/S and IGROV-1 cell lines were maintained in RPMI1640 medium, HeLa and J774a.1 cell lines in DMEM medium and SKOV-3 cell line in F-12 medium. All cell lines were supplemented with fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin and maintained under standard culture conditions (37°C, 5% CO_2, 95% air and 100% relative humidity).

Cell growth inhibition studies

The cytotoxic effects of five different GNRs samples (1/9, 1/3, 1, 3 and 9) were evaluated against SKOV3, IGROV-1, A2780/S and HeLa, according to the sulforhodamine B (SRB) assay described by Skehan *et al.*[33]. Exponentially growing cells were inoculated into 96-well microplates at specific plating density/well (range $1 – 5 \times 10^3$) according to the various types of cell lines. After 24 h, the medium was replaced with fresh medium containing PEG-GNRs for exposure times of 72 and 168 h. Gold concentrations in the different suspensions ranged from 0.003 to 100 μM. Cells were then fixed *in situ* by 10% trichloroacetic acid (TCA) and stained by SRB solution at 0.4% (w/v) in 1% acetic acid. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM tris base and its absorbance was read on an

automated plate reader at a wavelength of 540 nm. The IC_{50} gold concentration resulting in a 50% reduction in the net protein content in cells treated with particles as compared to controls was determined after 72 or 168 h particle exposures. The IC_{50} data represent the mean of at least three independent experiments.

Cell viability studies

Exponentially growing cells were inoculated into 96-well microplates and maintained under standard culture conditions for 24 h. Thereafter, the medium was replaced with fresh medium containing different concentrations of PEG-GNRs. After 24 h, the MTT reduction assay described by Mosmann[34] was performed: cells were incubated with a 0.5 mg/ml MTT solution at 37°C for 4 h and then with cell lysis buffer (20% SDS, 50% N,Ndimethylformamide, pH 4.7) for 3 h. The absorbance values of blue formazan were determined at 590 nm by an automated plate reader.

Supplementary data

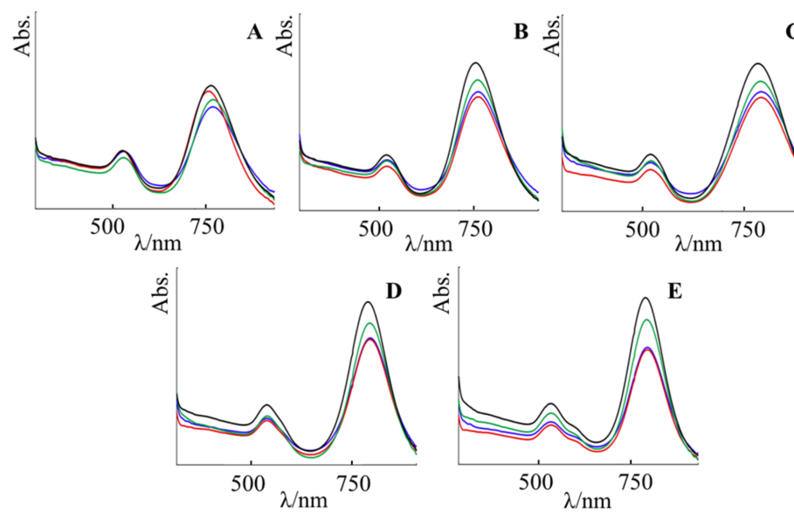

Fig. S1. Stability of PEGylated gold nanorods with lysozyme. Absorption spectra of five sizes of PEG-GNRs in PBS with lysozyme. A) GNRs 1/9; B) 1/3; C) 1; D) 3; E) 9.

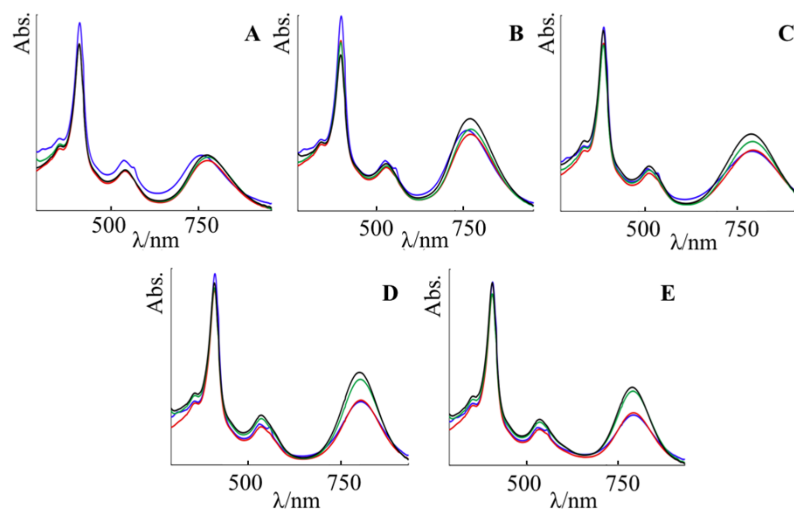

Fig. S2. Stability of PEGylated gold nanorods with cyt c. Absorption spectra of five sizes of PEG-GNRs in PBS with cyt c. A) GNRs 1/9; B) 1/3; C) 1; D) 3; E) 9.

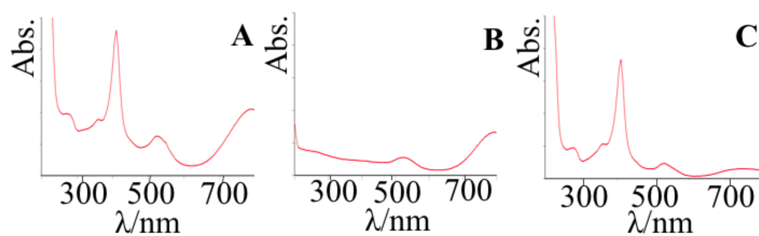

Fig. S3. Interaction of PEGylated gold nanorods with cyt c. Absorption spectra of PEG-GNRs 1 with cyt c incubated for 24 h at 37°C (A) and after centrifugation and washing (B). Supernatant obtained after the first washing cycle (C).

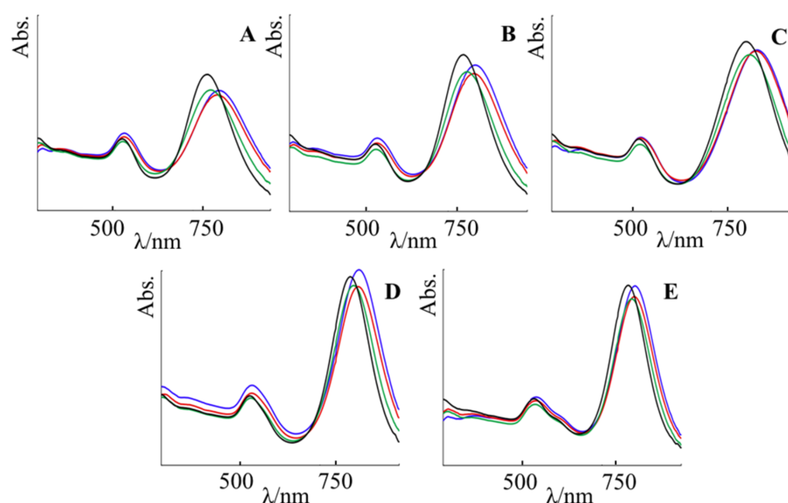

Fig. S4. Stability of PEGylated gold nanorods in RPMI. Light extinction spectra of PEG-GNRs of different average size in RPMI A) GNRs 1/9; B) 1/3; C) 1; D) 3; E) 9. The figure shows spectra recorded at time zero (black line) and after 24h (green line), 72h (red line) and 168h (blue line).

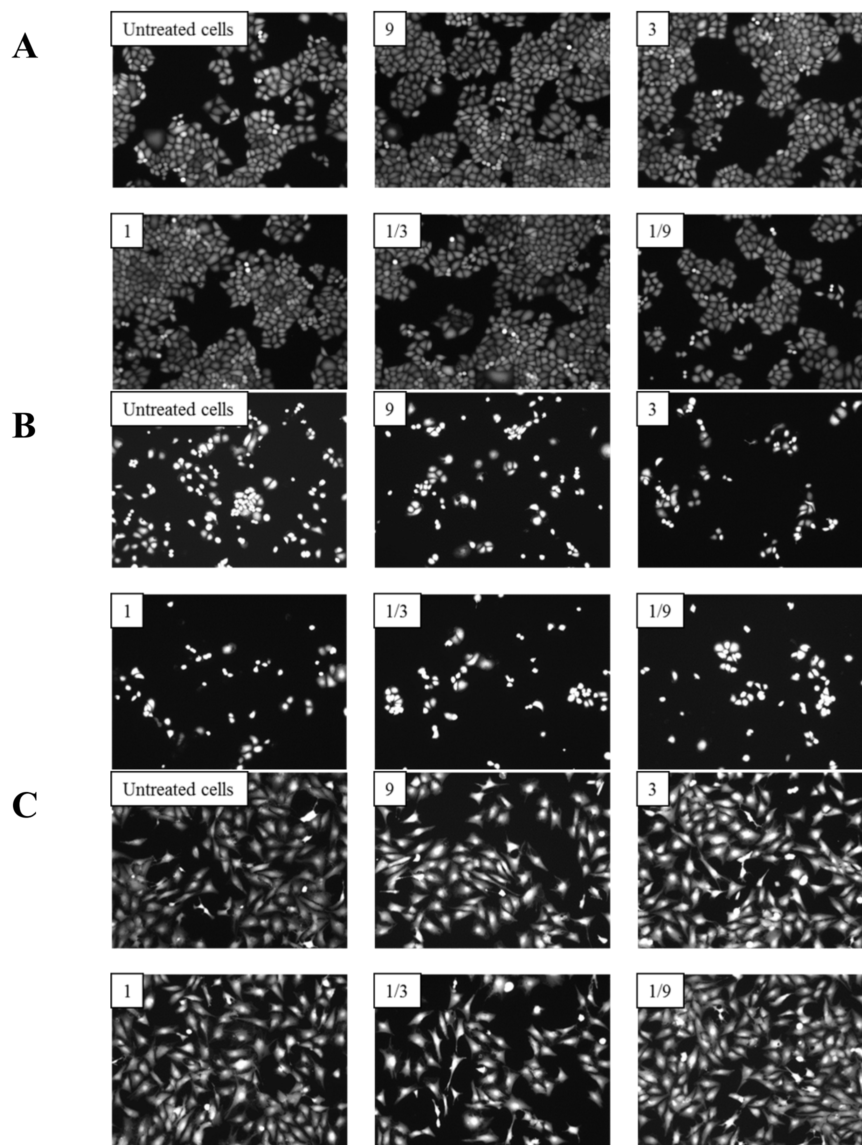

Fig. S5. Membrane integrity. Calcein fluorescence in HeLa (A), IGROV (B) and SKOV3 (C) untreated cells and after treatment with PEG-GNRs of different sizes. All samples exhibit no release of calcein after treatment with GNRs, indicating that the membrane integrity has not been altered.

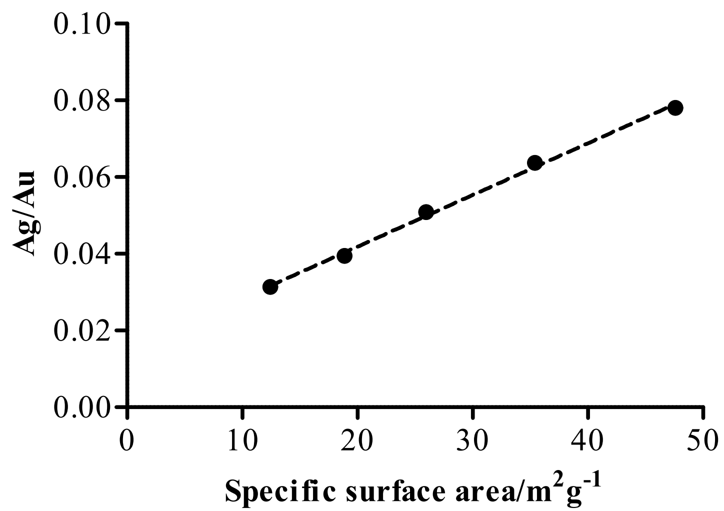

Fig. S6. Particle composition by elemental analysis. Ratio between Ag and Au among the different sizes of PEG-GNRs.