Supplementary Material (ESI) for Dalton Transactions

Electronic Supporting Information

Fabrication of fluorescent silica-Au hybrid nanostructures for targeted imaging of tumor cells

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Experimental Section

Chemicals: All chemicals were analytic grade reagents and used without further purification.

Synthesis of Au nanoparticles: Approximately 5 nm Au nanoparticles were prepared by the citrate reduction of $HAuCl_4$. Briefly, 10 mL of 1 mM aqueous solution of $HAuCl_4$ was mixed with 0.8 mL of 38.8 mM aqueous sodium citrate solution used as stabilizer. 0.3 mL of 100 mM aqueous solution of $NaBH_4$ was then added dropwise under vigorous stirring, giving rise to a red Au hydrosol. The Au hydrosol was only used after aging for 24 h to decompose residual $NaBH_4$.

Synthesis of surface-modified RuBPy-doped SiO_2 nanoparticles: RuBPy-doped SiO_2 NPs were first synthesized according to previously published procedures.[7] Briefly, a water-in-oil microemulsion was prepared by mixing 1.77 mL of Triton X-100, 7.5 mL of cyclohexane, 1.6 mL of *n*-hexanol, 340 μL of water, and 80 μL of 0.1 M RuBPy solutions. After stirring for 1 h, 100 μL of tetraethyl orthosilicate (TEOS) was then added as a precursor for silica formation, followed by the addition

of 65 μL of NH_4OH to initiate the polymerization process. The reaction was allowed to continue for 24 h at room temperature, followed by addition of 50 μL of TEOS and either 50 μL of APTS (for amine modification). The reaction proceeded for another 24 h with stirring. After the reaction was complete, nanoparticles were centrifuged, sonicated, and washed with ethanol and water to remove any surfactant and dye molecules.

Synthesis of $DySiO_2$-$(Au)_n$ nanocomposites: Our hybrid nanoparticles were fabricated through conjugation of these dye doped silica nanoparticles with Au nanoparticles by a simple electrostatic adsorption procedure. In a typical experiment, 3mL of citrate-protected Au colloid suspension was diluted by adding 10 mL of ppH_2O under stirring for 10 minutes. Then the amine modified $DySiO_2$ nanoparticles dissolved in 2 mL ethanol solution were added slowly into the Au colloid solution while stirring rapidly. After continuous stirring for 2 h, the solution was centrifuged and washed with ethanol and water 3 times, respectively. Finally, the collected precipitation was redispersed into 4 mL water (~1 μg mL^{-1}).

Conjugation of $DySiO_2$–$(Au)_n$ nanocomposites with aptamer: The thiol-modified aptamer was attached to the $(Au)_n$-$DySiO_2$ nanocomposites according to the method modified from literature.[14] The oligonucleotides used to functionalize the Au NP were 5′-CAG GCT ACG GCA CGT AGA GCA TCA CCA TGA TCC TG-3′, which were purchased from Sangon Biotech (ShangHai, China). The aptamers were reacted directly with the $(Au)_n$-$DySiO_2$ NPs through attachment of both $HOCH_2(CH_2)_5S$ and oligo-S units onto the Au NPs surface, the bioconjugate is denoted as

''Apt-$(Au)_n$-$DySiO_2$ nanocomposites''. Briefly, a 2.9 mL aliquot of aqueous NP solution was mixed with 29 μL of 2.9 μM thio-aptamer to obtain a final concentration of 1 $\mu g \cdot mL^{-1}$ $(Au)_n$-$DySiO_2$ NPs and 0.1 μM aptamers. After reaction for 24 h at room temperature, the mixture was equilibrated with 0.1% BSA for 60 min at room temperature before conducting two centrifuge/wash cycles (6,000 rpm, 5 min) to remove any excess thiol aptamer. Following the removal of the supernatants, the oily precipitates were resuspended in 0.1% BSA solution and then stored in a refrigerator (4 ^oC). BSA was present to prevent aggregation of the Apt-Au NPs in the serum free media.

Cells incubated in media with Apt-$(Au)_n$-$DySiO_2$ NPs: Human breast cancer cell line, MCF-7 and Human normal cell line, HEK293T were obtained from American Type Culture Collection (ATCC, Rockville, MD) and tested in this study. MCF-7 **and HEK 293T** cells were grown in Minimum essential medium Eagle (Gibco); the media were supplemented with 10% FBS and 0.01 mg/ml bovine insulin (Gibco). Cells were grown at 37 ^oC in a humidified atmosphere with 5% CO_2 (in air). The medium was changed every 3 days. At ca. 80% confluency, the cells were trypsinized with trypsin/EDTA solution (0.25% trypsin, 1 mM EDTA) for 5– 10 min at 37 ^oC to detach the cells for reseeding and growing in the new culture dishes at a density of ca.10^5 cells per culture dish. After 24 h, the growth medium was removed and the cells were washed three times with phosphate-buffered saline (PBS) solution. The cells were incubated in serum-free media (1.0 mL) containing 1 $\mu g \cdot ml^{-1}$ Apt-$(Au)_n$-$DySiO_2$ NPs for 12 h to minimize nonspecific adsorption of serum proteins

onto the particle surfaces.

Cell fluorescence staining: Cells were collected by centrifugation at 200×g for 5 minutes, and then washed twice with PBS. Stained cells suspension were placed on a clean microscope slide and covered with a cover-slip. Cells were viewed and counted using an Olympus BX-51 optical system microscope (Tokyo, Japan) at 400× magnification with a green filter. Pictures were taken with an Olympus digital camera DP70. We note that the definition is sharper by eyes through the microscope than in the photo. Tests were done in triplicate, counting a minimum of 300 total cells from at least three random microscope fields each.

Cell viability assay: MCF-7 **and HEK 293T** cells was dispensed into a 96-well flat-bottom microtiter plate (~10,000 cells/well) and allowed to attach overnight using the MEM medium with 10% FBS. The MTT assay was carried out as per manufacturer's instructions (PROMEGA). It is based on the absorbance of formazan (produced by the cleavage of MTT by dehydrogenases in living cells), the amount of which is directly proportional to the number of live cells. In brief, after 24 and 48 h treatment with the SiO_2-coated QRs, media was changed and 150 μL of MTT reagent was added to each well and well-mixed. The absorbance of the mixtures at 570 nm was measured. The cell viability was calculated as the ratio of the absorbance of the sample well to that of the control well and expressed as a percentage. Tests were performed in quadruplicate. Each point represents the mean (SD bars) of replicates from one representative experiment.