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Experimental

Bioconjugation

Anti LFA-1 FITC (20 μL, 0.5 mg mL^{-1}, BD Biosciences) was added to a solution of the SERRS active linker[1] (10 μL, 1 mM) in HEPES buffer, pH 7.6 (50 μL, 300 mM) containing EDC (10 μL, 2 mg mL^{-1}, Aldrich) and sulfoNHS (10 μL, 2 mg mL^{-1}, Apollo Scientific). 5 replicates samples were prepared. The reaction was allowed to proceed overnight at 10 °C. The samples were centrifuged three times in spin columns (MWCO 10,000, Millipore) and the residue was added to 1 mL citrate-reduced Au[2] (4.5 nM) and 1 mL citrate-reduced Ag[3] (0.4 nM). Following 16 hours, the nanoparticle conjugates were centrifuged twice (5000 rpm, 20 mins) and resuspended in phosphate buffer, pH 7.6 (10 mM). The samples were analysed by UV-Vis spectroscopy to determine the nanoparticle concentration using molar extinction coefficients for Au and Ag of 2.7×10^8 M^{-1} cm^{-1} and 2.87×10^{10} M^{-1} cm^{-1} respectively. [4, 5]

FAM labelled 5'-thiol DNA: 5' thiol $(A)_{10}$ CGC ATT CAG GAT FAM 3' (1×10^{-8} moles) was added to 10 resplicate samples of 1 mL citrate-reduced Au (17 nM). Phosphate buffer (pH 7.6, 60 mM) was added to a final concentration of 10mM and NaCl (2 M) was added over a period of 48 hours to a final concentration of 0.1 M. Following this, the nanoparticle conjugates were centrifuged twice (7000 rpm, 20 mins). 5 samples were resuspended in 0.3 M NaCl and phosphate buffer (pH 7.6, 10mM) for treatment with DTT. The remaining 5 samples were resuspended in DNase I reaction buffer (10mM Tris-HCl, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, pH 7.6, New England BioLabs) for treatment with DNAse I. The samples were analysed by UV-vis spectroscopy to determine the nanoparticle concentration using a molar extinction coefficient for Au of 2.7×10^8 M^{-1} cm^{-1}.

Quantitation by fluorescence spectroscopy

DTT (250 mM final concentration, Aldrich) or trypsin (final concentration 0.2 mg mL^{-1}, Sigma) was added to the antibody-nanoparticle conjugates. DTT (100 mM final concentration) or DNAse I (final concentration 125 units mL^{-1}, New England BioLabs) was added to the DNA-nanoparticle conjugates. The samples were left for 16 hours at 37 ^oC and then centrifuged. The supernatant was extracted and analysed by fluorescence spectroscopy using an excitation wavelength of 495 nm. The concentration of FITC or FAM label in the supernatant was determined using a calibration graph of standard solutions. The number of biomolecules per nanoparticle was calculated by evaluation of the FITC concentration against the nanoparticle concentration (determined by UV-Vis spectroscopy).

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