Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Controlled assembly of peptide-functionalized gold nanoparticles for label-free detection of blood coagulation Factor XIII activity

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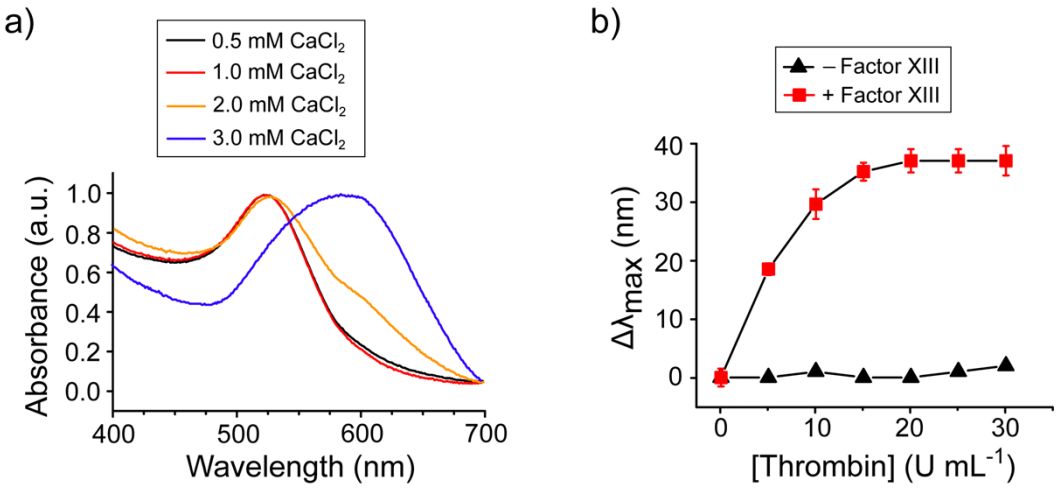

Fig. S1. a) UV-Vis absorption spectra of CALNNGQG- and PLL-capped AuNPs in PBS buffer pH 7.4 upon introduction of various concentrations of $CaCl_2$. 1 mM was determined to be the maximum concentration of $CaCl_2$ that can be used without affecting the stability of the peptide-functionalized AuNPs prior to performing the assay. b) Red-shift of the LSPR peak of a population of CALNNGQG- and PLL-capped AuNPs in PBS buffer pH 7.4 in the presence of 1 mM $CaCl_2$ and 0.5 U mL^{-1} Factor XIII as a function of concentration of thrombin, demonstrating the dependence of Factor XIII activity on the concentration of thrombin. At least 20 U mL^{-1} of thrombin was required to produce maximal and complete activation of Factor XIII to obtain the highest LSPR peak shift for the AuNP aggregation-based assay.

EXPERIMENTAL SECTION

Materials. Wang resin (100 – 200 mesh, 0.66 mmol g^{-1}) was purchased from Novabiochem (UK). Fluorenylmethoxycarbonyl (Fmoc)-protected amino acids, dimethylformamide (DMF), 20% piperidine in DMF, dichloromethane (DCM), *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU), *N*,*N*-diisopropylethylamine (DIEA), and acetonitrile (ACN) were purchased from AGTC Bioproducts (UK). Ninhydrin test kit was purchased from AnaSpec Inc. (USA). Gold nanoparticles (15 nm in diameter) were obtained from BBI Solutions (UK). Factor XIII was purchased from Haematologic Technologies Inc. (USA). Trifluoroacetic acid (TFA), triisopropylsilane (TIS), dithiothreitol (DTT), poly(L-lysine) (PLL, M_w 4 – 15 kDa), thrombin, calcium chloride ($CaCl_2$), tris(2-carboxyethyl)phosphine (TCEP), human serum albumin (HSA), lysozyme, and phospholipase A_2 from *naja mossambica mossambica* were obtained from Sigma-Aldrich.

Peptide synthesis. Peptides CALNNGQG, CALNNGKG, and CALNN were synthesized manually using a standard solid phase Fmoc chemistry protocol. For each coupling, the Fmoc protecting group was removed with 20% piperidine in DMF, followed by washing with DMF and DCM. 4 molar equivalent of Fmoc-protected amino acid, 3.95 molar equivalent of HBTU, and 6 molar equivalent of DIEA in DMF were added to the resin and the reaction was allowed to proceed for 2 h before washing the resin thoroughly with DMF and DCM. Couplings were monitored using ninhydrin test. After synthesis, peptides were cleaved in a solution of 95% TFA, 2.5% TIS, 2.5% H_2O, with 2.5% w/v DTT for 4 h. TFA was removed using rotary evaporation and the peptides were precipitated in cold diethyl ether and dried under vacuum. The products were then redissolved in deionized water and lyophilized and the crude peptides were purified by reverse-phase high performance liquid chromatography (HPLC) (Shimadzu, Japan) with a linear gradient of H_2O/ACN solution containing 0.1% TFA. The Phenomenex C_{18} Gemini NX column was 150 × 21.2 mm and had a 5 μm pore size and 100Å particle size. Molecular weight and purity of the peptides were determined by mass spectrometry and HPLC. The peptides were stored at -20°C until further use.

Functionalization of gold nanoparticles. A suspension of 1.4×10^{12} gold nanoparticles per mL (0.24 nM) in H_2O were mixed with peptides (1 mM) or PLL (0.5 mg mL^{-1}). Reactions were allowed to proceed overnight and the particles were repeatedly centrifuged (20000 *g*, 20 min, 4°C) to remove excess peptide or PLL. The functionalized gold nanoparticles were resuspended in PBS buffer (10 mM phosphate, 2.7 mM KCl, 137 mM NaCl, pH 7.4).

Colorimetric detection of Factor XIII. All assays were carried out in 96-well plates at room temperature. Factor XIII at the desired concentrations was added to functionalized gold nanoparticles (0.1 nM) in PBS buffer pH 7.4 containing thrombin (20 U mL^{-1}), $CaCl_2$ (1 mM), TCEP (0.1 mM), and HSA (1 mg mL^{-1}). The reaction was followed over time and UV-Vis absorption spectra were recorded with a SpectraMax M5 microplate reader (Molecular Devices, USA). Control experiments were performed using lysozyme (5 nM) or phospholipase A_2 (5 nM).

Zeta potential measurements. Zeta potentials of peptide-functionalized gold nanoparticles were measured using a Zetasizer Nano ZS (Malvern, UK).

Transmission electron microscopy (TEM) characterization. Gold nanoparticle samples (5 µL) were adsorbed onto 300 mesh carbon-coated copper grids (Electron Microscopy Science, USA). The grids were then washed three times with deionized water and allowed to dry. TEM images were acquired using a JEOL 2010 FEG-TEM (JEOL, Japan) operated at 200 kV.