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Tuneable Plasmonic Gold Dendrimer Nanochains for Sensitive Disease Detection.

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Chemicals and materials

20nM AuNP (EM.GC20) were purchased from BBI Solutions (UK). PAMAM G5 dendrimer were purchased form Sigma-Aldrich (UK). Two monoclonal anti-p24 antibodies comprised the immunosandwich assay for the detection of p24: Capricorn HIV-1/2 (Capricorn Products, USA) and 183-H12-5C (3537, AIDS Reagent Program, NIBSC, UK). A goat anti-mouse polyclonal antibody (ab6789, Abcam, UK) was used as the control antibody in the lateral flow strip. Recombinant p24 protein was purchased from Aalto Bio Reagents, Ireland. The remaining chemical reagents were purchased from Sigma –Aldrich, UK.

General methods and characterization

UV-Vis spectra were collected from a SpectraMax i3x multimode microplate reader (Molecular Devices Ltd, UK). TEM images were collected from FEI TITAN 80/300 transmission electron microscope operating at 200 kV. The hydrodynamic diameters of AuNP-PAMAM aggregates dissolved in water were evaluated by dynamic light scattering (DLS). DLS data were collected by using a Malvern DLS apparatus (Nano-ZS), Malvern Instruments Ltd.

Aggregation of 20 nm AuNP with PAMAM-G5 dendrimers

30 μL of aqueous dilutions of PAMAM-G5, (40 to 200 nM), was added and shaken immediately over 300 μL of commercial aqueous suspension of 20 nm AuNP (1.2 nM in number of particles). As a control, 30 μL of double-distilled 18.2MΩ water was added to 300 μL of commercial aqueous suspension of 20 nm AuNP, to a final volume of 330 μL. They were designated as Gold-Dendrimer-nanoChain-X (GDnC-X), where X represents the number of equivalents of PAMAM dendrimer added relative to the number of equivalents of AuNP present during the aggregation procedure. The colour of

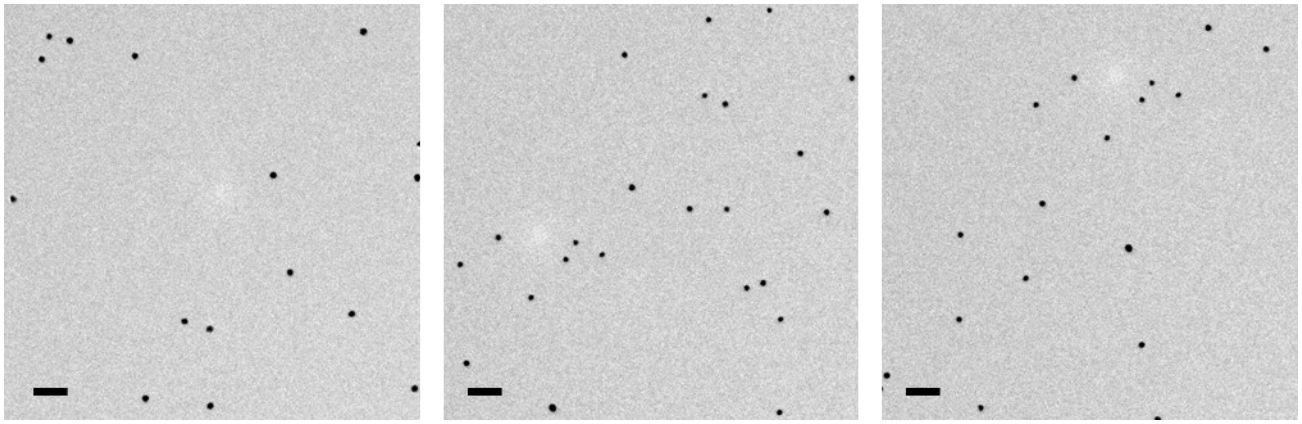
the suspension changes immediately from ruby red to purple-blue colour due to the formation of chainlike aggregates.

UV-vis, DLS and TEM characterization of GDnC-X

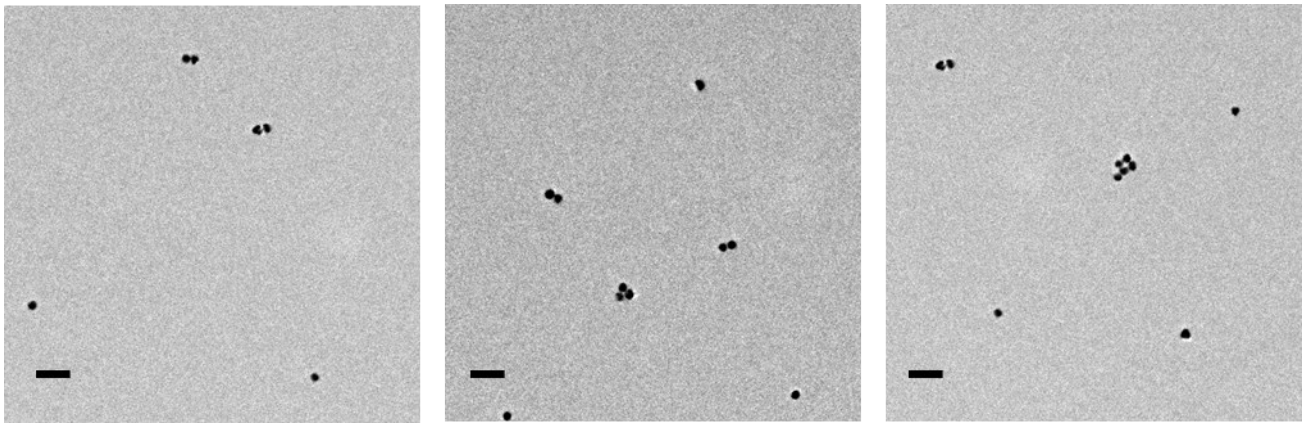
UV-vis spectra were recorded using a volume of 200 μL of the 330 μL GDnC-X obtained. The spectra were collected over 400 to 900 nm. For DLS and ELS analyses, freshly GDnC-X samples were diluted 10 times with bidistilled water. The samples for inspection by TEM were carefully dropped over a carbon coated copper mesh grid prior to slow evaporation.

TEM Images

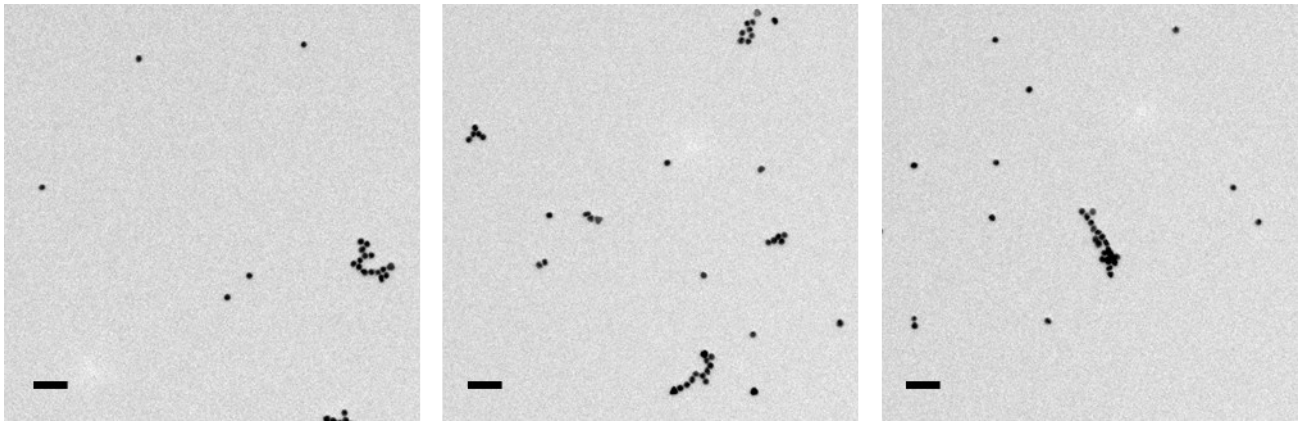
GDnC-0



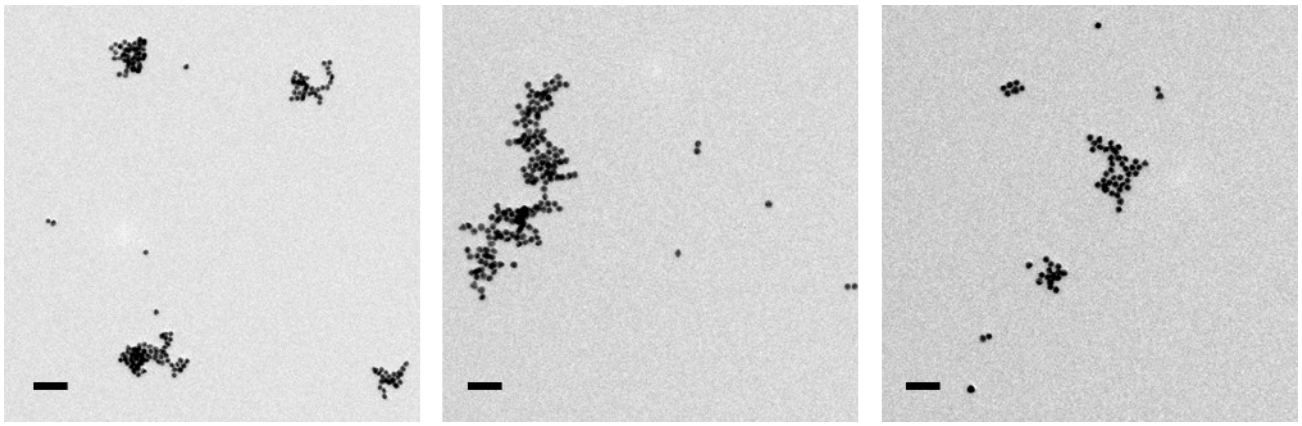
GDnC-3.3



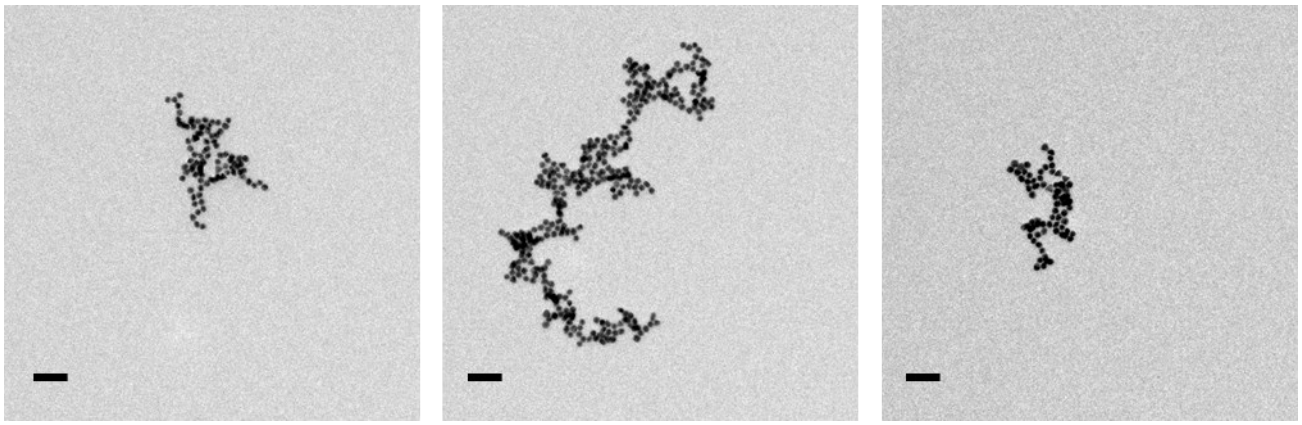
GDnC-6.6



GDnC-10.0



GDnC-13.3



SIFig1. Representative set of pictures taken for each GDnC-X. The black horizontal scale bar represents 100 nm.

Preparation of AuNP-Ab conjugates and AuNP-PAMAM-Ab conjugates

A previous protocol for coupling an antibody to 20 nm AuNPs was adapted[i]. Around 5 samples of 330 μL suspension of GDnC-X were combined to a final volume of 1.5 mL. 4 μL of antibody at 1 mg/mL was added to them and incubated for 30 m at 25 ºC. Subsequently 100 μL 1% BSA (in H_2O) was added, then the mixture incubated for another 30 m. Lastly, the solution was separated by centrifugation at 14000 rpm (12200 g) 4 ºC for 20 m, and the precipitated GDnC-X -Ab conjugates were redispersed in 300 μL of 5% BSA in PBS (pH 7.4).

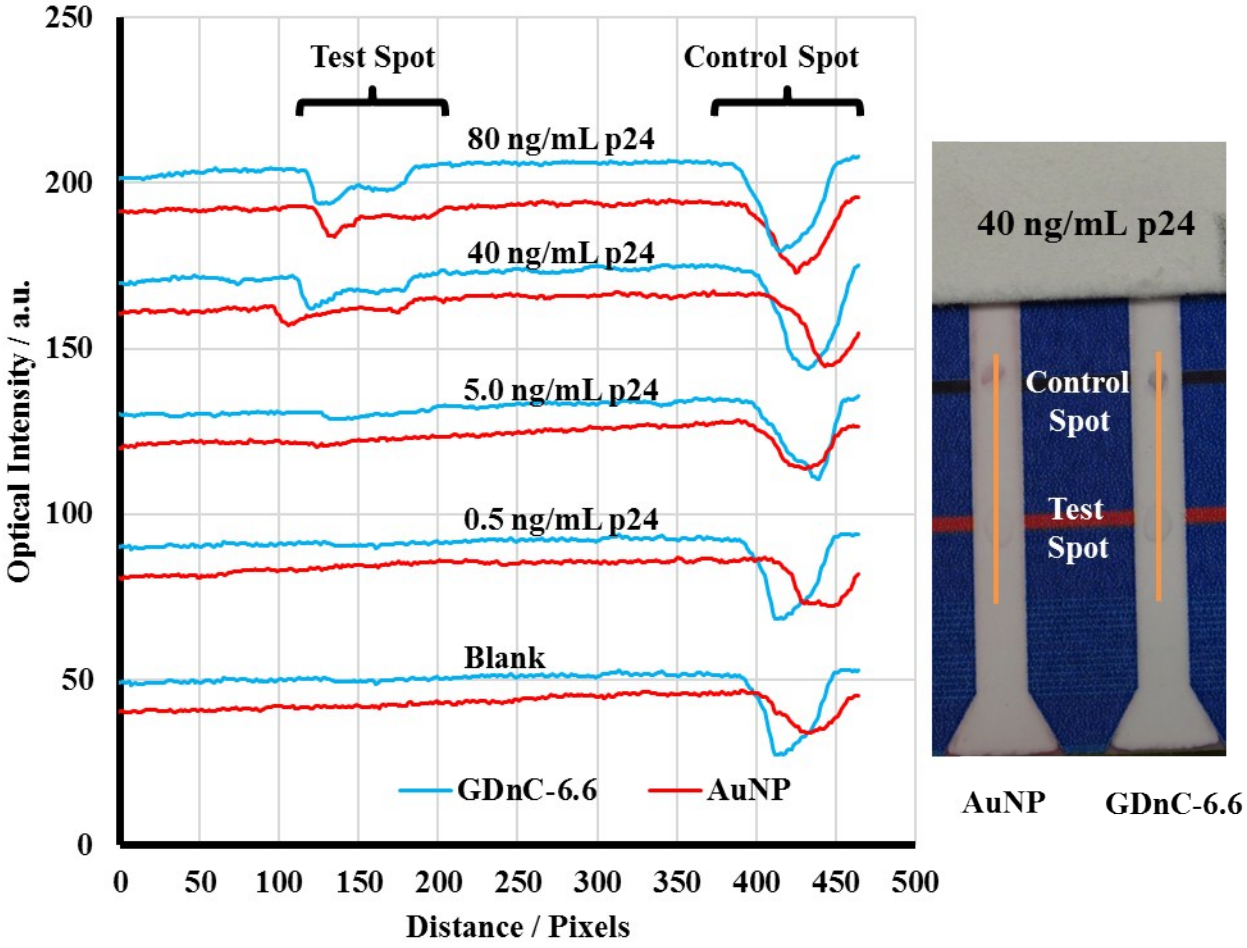
Preparation of lateral flow immunoasaay test strip

The LFIA test strip consists of two components: a nitrocellulose membrane and an absorbent pad. Firstly, wax channels (3 mm width) were printed over the nitrocellulose membrane. Secondly, the printed membrane was heated at 100 ºC for 10 s to effect melting and matrix penetration, generating hydrophobic barriers that constrain the flow. Subsequently, nitrocellulose membrane and absorbent pad were assembled on a plastic adhesive baking layer. The test zone and control zone on the nitrocellulose membrane were prepared by dispensing 0.3 μL of Capricorn HIV-1/2 (1 mg/mL) and 0.3 μL goat anti-mouse antibody (1 mg/mL) respectively by hand. The distance between the test zone and the control zone was approximately 6 mm. Finally, the membrane was dried at room temperature for 2 h.

LFIA assay procedure

The procedure of microfluidic test (lateral flow) was as follows: a) 40 μL GDnC-X-Ab conjugates in 5% BSA in PBS (pH 7.4), were mixed with 40 μL p24 solution in 5% BSA PBS-0.1% TWEEN (pH 7.4), giving a final volume of 80 μL. b) 15 μL of the pre-mixed p24 and GDC-X-Ab were deposited on the sample pad place and allowed to migrate along the strip toward the absorption pad. c) After 10-15 min, pictures of the sample pads were taken using a Canon G15 camera in Automatic Mode. d) The intensity of the colour at the test zone could be quantitative analysed with ImageJ and Mathematica 10.0 software. Pixel intensities of the images were analysed using both the green channel of the RGB colour-space, and they grey-value (grey = (red + green + blue)/3). Following this conversion, ImageJ was used to extract an intensity profile along the strip, averaging across the width of the strip to reduce noise. These intensity plots were imported into Mathematica, where a low-pass filter was applied to further reduce noise, followed by fitting and subtracting a linear regression to remove lighting artefacts and normalise the baseline. Finally, the peak height at the test line was extracted,

corrected by substation of the blank sample results to all the points and plotted against the concentration of p24. The peak height is proportional to the number of p24 molecules immobilised. The points were fitted with a Langmuir adsorption isotherm model. The control zone should always show a positive signal (red control line), indicating that the test is working properly.



SIFig2. Digital photographic image of a LFIA and intensity analysis of line sections along the long axis of the test (orange line) for different p24 concentrations (blank, 0.5, 5.0 and 40 ng/mL). 40, 80, 120, 160 and 180 values were added to each line section pair of p24 concentrations in order to plot all of them in the same chart. The red lines correspond to results from a traditional AuNP assay and blue lines correspond to GDnC-6.6 assays.

[i] Parolo, C.; de la Escosura-Muñiz, A.; Polo, E.; Grazú, V.; de la Fuente, J. M.; Merkoçi, A. *ACS Appl. Mater. Interfaces* **2013**, 5 (21), 10753.