Supporting Information

Hybrid Assembly of DNA-Coated Gold Nanoparticles with Water Soluble Conjugated Polymers for Studying Protein-DNA Interaction and Ligand Inhibition

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Testing the hydrophobic interaction of ACP-430 and dsDNA

Both dsDNA and the ACP-430 are negatively charged, thus electrostatic interaction cannot occur between them. To explain the observed initial quenching of the ACP after incubation with dsDNA-AuNP, hydrophobic interaction is proposed. The interaction was tested by exposing the dsDNA-AuNP and the ACP-430 mixture to solvent with different polarity, of which the percentage of the organic solvent is increased incrementally by adding DMSO. As the polarity decreases with amount of DMSO, the hydrophobic interaction becomes less favorable. Hence, less ACP molecules are associated with dsDNA-AuNP entity and more ACP molecules prefer to stay in solution freely and thereby emission intensity steadily increasing (Figure S1)

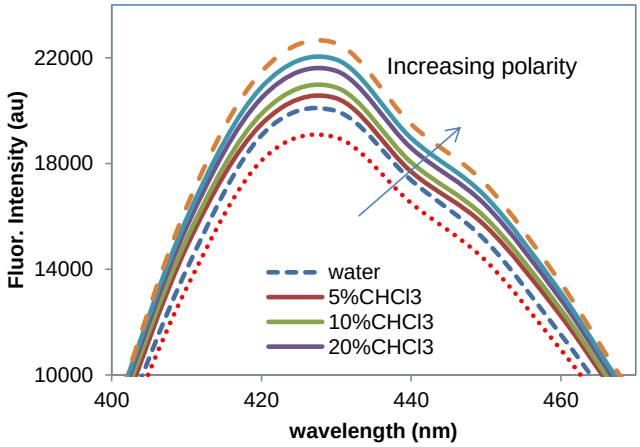

Figure S1. Fluorescence of ACP-430 is progressively recovered to the state of free CP (dashed line) as the solvent decreases in polarity. Enhanced quenching is observed while polarity is increased in 10%vol NaCl (dotted line). Study is conducted without any protein added.

Fluorescence of CPs in the presence of dsDNA or protein, but not AuNPs

Energy or electron transfer could possibly take place between CPs and dsDNA/protein. For confirming that the interaction, if any, between CPs and dsDNA/protein does not result in appreciable quenching, control experiments were performed to test CPE's emission in the presence of DNA or protein without AuNPs. 50 nM of dsDNA or 250 nM protein were added into 100 nM CPEs in PBS buffer, the emission spectra of ACP-430 and CCP-410 is measured (Fig. S2).

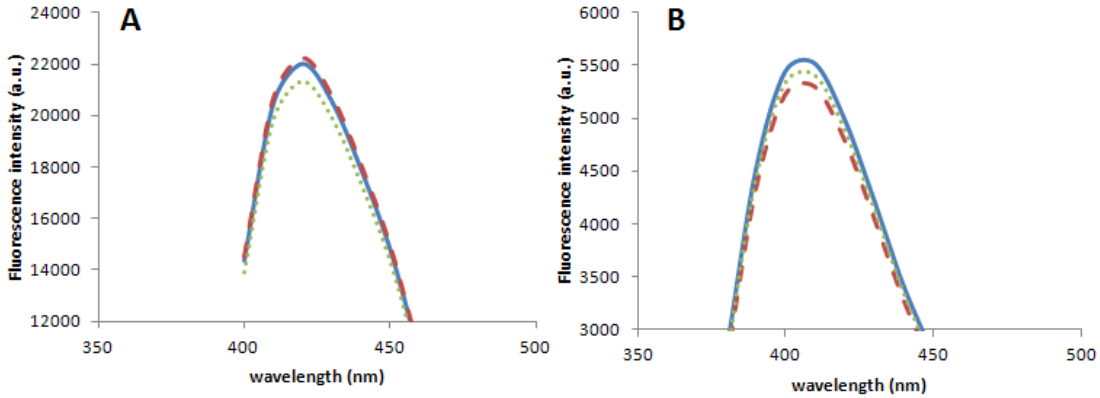

Figure S2. (A) ACP-430 and (B) CCP-410 fluorescence spectra at free state (solid line), incubated with DNA (dashed line) and incubated with protein (dotted line). The concentration of the CPE is 100 nM, and protein and DNA are 250 nM and 50 nM, respectively.

Determination of FoxA1 affinity for DNA probes via fluorescence anisotropy assay

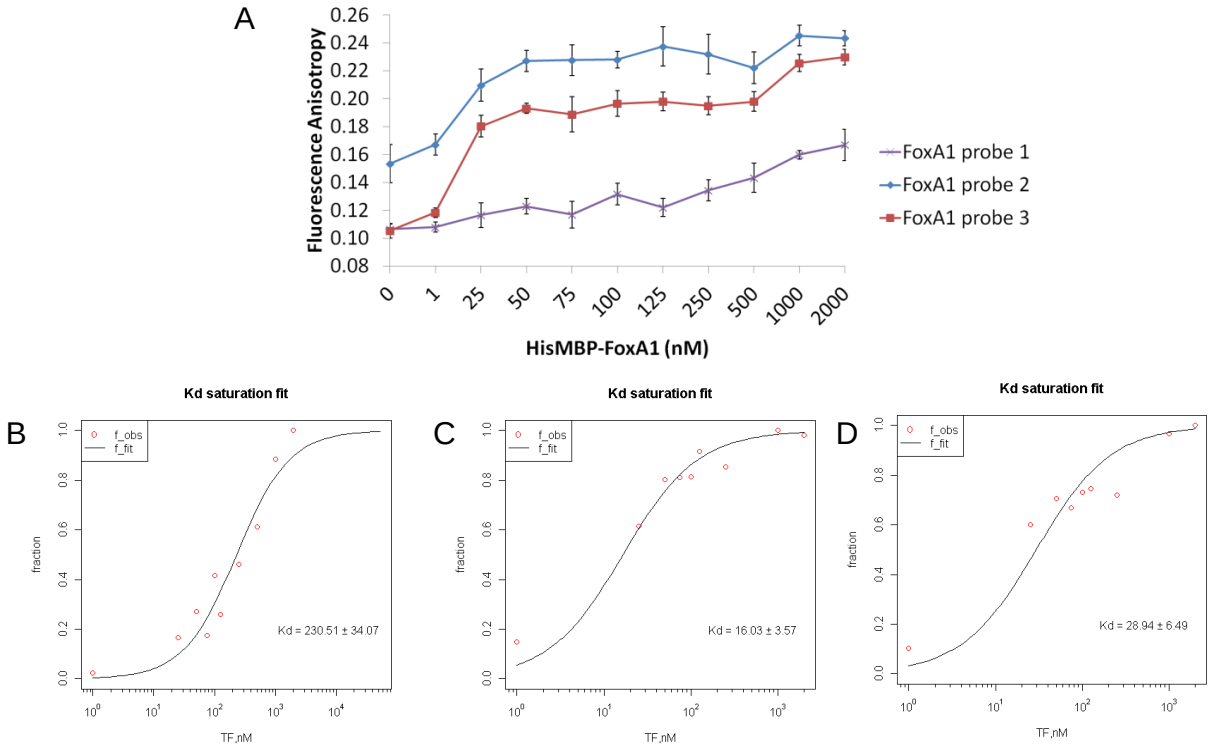

Figure S3. (A) Binding profile of FoxA1 to 2 nM FAM fluorescence labelled probes 1-3. FoxA1-DNA complex formation is detected by increased fluorescence anisotropy with increasing concentrations of FoxA1. Data represent the average of 3 independent measurements.

Average Kd plots that were used to determine the dissociation constant of (B) probe 1, (C) probe 2, (D) probe 3, were shown using the protein-DNA binding data obtained from (A).

Detecting Ap2γ-DNA interactions using CCP-410/AuNPs and ACP-430/AuNPs hybrid sensors.

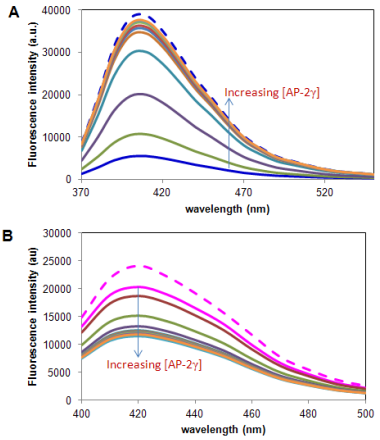

Fig. S4 (A) light-on and (B) light-off measurement of AP-2γ –wtR3 DNA binding using CCP-410/(wtR3)-AuNP and ACP-430/(wtR3)-AuNP hybrid assembles, respectively.

Quantification of Ap2γ-DNA binding constant (K_d) and stoichiometry (n) through titration

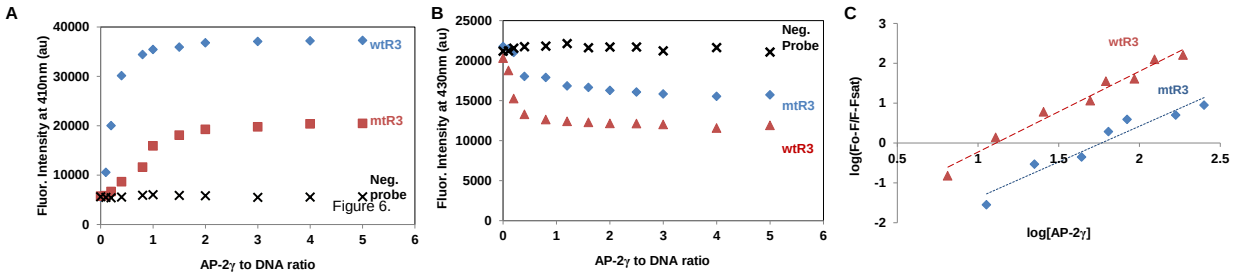

Figure 6.

Figure S5. Titration plot of fluorescent intensity of (A) CCP-410 and (B) ACP-430 for AP-2γ binding to DNA (wtR3 or mtR3)-AuNP conjugates. (C) Log fluorescence intensity plot as function of log [AP-2γ] measured with ACP-430/AuNPs hybrid assembly.

Purification of HisMBP-FoxA1 protein. Full-length FoxA1 cDNA was cloned into a HisMBP bacterial expression vector (Figure S6A). HisMBP-tagged FoxA1 fusion protein was expressed in BL21 (DE3) cells and purified using nickel affinity chromatography followed by ion exchange chromatography (Figure S6B). The overall purity of the protein after nickel affinity and ion exchange chromatography was approximately 80% (Figure S6C).

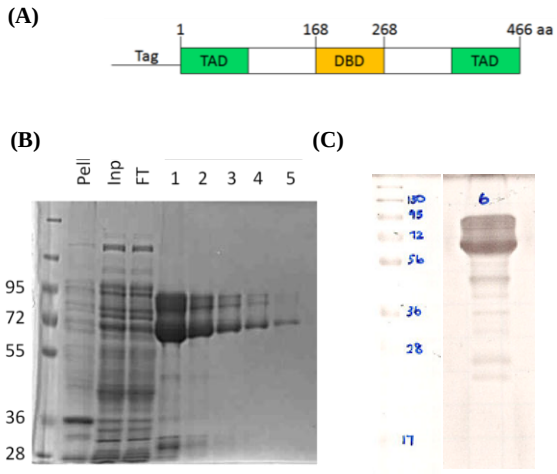

Figure S6. (A) HisMBP bacterial expression vector. (B) HisMBP-tagged FoxA1 fusion protein gel. (C) The overall purity of the protein after nickel affinity and ion exchange chromatography.

Quantification of dsDNA coverage on dsDNA-AuNP conjugates using dye (thiazole orange) intercalation method. Initially, excess amount of thiol labeled ssDNA (150 nM) was added to 5 nM AuNPs to ensure sufficient coverage on the surface of AuNPs. To quantify the amount of dsDNA conjugated to AuNPs (ssDNA conjugated followed by on-particle annealing of the antisense strands), each aliquot (25 uL) of DNA-AuNP mixture went through three rounds of centrifugation and rinsing with 5 mM HEPES buffer (pH 7.4). The dsDNA-AuNP conjugate was redispersed in 100 uL HEPES buffer. 1 mM KCN was added to dissolve AuNPs completely and release attached dsDNA. The concentration of released dsDNA in supernatant was quantified with thiazole orange (TO) dye, which emits at 530 nm. Fluorescence intensity from TO proportional to the amount of dsDNA since TO only gives appreciable emission as it intercalates into the dsDNA. The fluorescence was measured with Nanodrop 3300 (Thermo Scientific). Firstly a series of known concentration of dsDNA was used to generate calibration curve (Figure S7). Then the samples were measured and quantified accordingly.

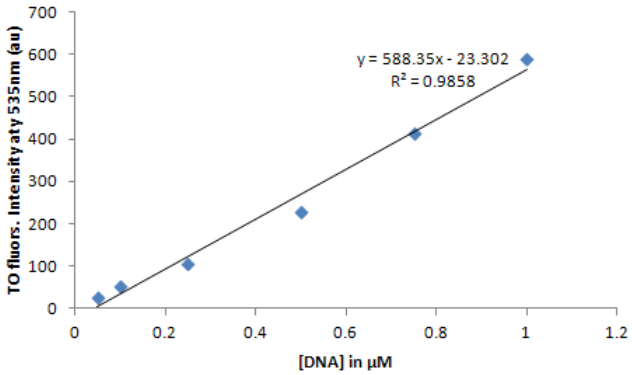

Figure S7. Calibration curve of Thiazole Orange (staining) with known concentration of dsDNA.