Supplementary Material (ESI) for Chemical Communications

Silica Nanoparticle Assisted DNA Assays for Optimal Signal Amplification of Conjugated Polymer Based Fluorescent Sensors

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General. The UV-Vis absorption spectra were recorded on a Shimadzu UV-1700 spectrometer. Fluorescence was measured using a Perkin Elmer LS-55 equipped with a xenon lamp excitation source and a Hamamatsu (Japan) 928 PMT, using 90 degree angle detection for solution samples. The NPs are imaged using a field emission scanning electron microscope (FE-SEM JEOL JSM-6700 F).

Materials. DNA oligonucleotides are ordered from Research Biolabs, Singapore. Tetraethoxysilane (98%, Fluka), ethanol (99.9%, Merck), ammonia solution (30%Wt, SINO Chemical Company), 3-aminopropyl triethoxysilane (99%, Aldrich), sodium chloride (99.5%, Merck), Tween 20 (Aldrich), acetonitrile (anhydrous 99.8+%, Alfa Aesar), 2,4,6-trichloro-1,3,5-triazine (98%, Alfa Aesar), boric acid (99+%，Alfa Aesar), sodium tetraborate decahydrate (99+%，Alfa Aesar), and 10× phosphate buffer saline (ultrapure grade, 1st BASE) are commercial products and are used as received without further purification. MilliQ Water (18.2 Ω) was used to prepare all the buffers. The polymer was synthesized according to the procedures reported previously. [1]

Synthesis and modification of silica nanoparticles.

TEOS (1.5 mL) dissolved in ethanol (15.24 mL) was added rapidly to a mixture of ethanol (6.37 mL), MilliQ water (10.05 mL) and ammonia (0.32 mL, 30%). The mixture was stirred vigorously at room temperature for 8 h to obtain the seed silica NPs with ~55 nm in diameter. The seed NP suspension (4.5 mL) was further diluted with ethanol (5.08 mL), followed by addition of ammonia (0.27 mL, 30%). Another 1.84 mL of TEOS in ethanol was added dropwise into the NP suspension and the mixture was stirred for 6 h. The obtained NPs were centrifuged and washed with ethanol (3×),

followed by washing with MilliQ water (6×). The purified NPs were dispersed in MilliQ water and stored at 4˚C for further use.

3-amino-propyl-trimethoxysilane (140 µL) was added to NPs (6.4 mg) in ethanol (1 mL). The mixture was stirred at room temperature for 2 h. It was followed by heating at 50 ˚C for additional 1 h. After centrifugation and washing with ethanol (8×) and acetonitrile (5×), the NPs were further reacted with 2,4,6-trichloro-1,3,5-triazine (38.4 mg) in acetonitrile (1 mL) at room temperature for 2 h.[2] The surface activated NPs were centrifuged and washed with acetonitrile (3×), ethanol (1×), MilliQ water (4×), and borate buffer (3×).

5'-amine modified oligonucleotide (5'-NH_2-AGCACCCACATAGTCAAGAT-3', 25 µL of 100 µM) was added to the activated NPs (3.2 mg) in borate buffer (85µL, pH = 8.5), and the mixture was slightly votexed at room temperature for 16 h. The obtained NPs were centrifuged and washed with MilliQ water (3×) to remove the free DNA. The supernatant was collected for UV/Vis absorbance measurement.

Hybridization of DNA-immobilized NPs.

The probe immobilized NPs were washed twice with PBST buffer (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate and 0.1% Tween 20) before hybridization. Different amount of FITC labeled oligonucleotide was mixed with NP (1.6 mg) in PBST buffer and the total volume was adjusted to 100 uL. The mixture was shaken at room temperature for 2 h. The solution was centrifuged and the residue was thoroughly washed with PBST buffer (5×). Finally the nanoparticles were dispersed in ~100 µL PBST buffer. The supernatant was kept for fluorescence analysis.

Base mismatch detection with stringent salt washing.

NP (0.2 mg) dispersed in PBST buffer (85 μL) was mixed with each oligonucleotide (DNA_T, the random sequence and the sequence with two base mismatches, 15 μL of 100 μM) and the resulting mixtures were shaken at room temperature for 2 h. The mixture was centrifuged and the residue was thoroughly washed with PBST buffer (4×). The collected NPs were further washed with a diluted PBST buffer (10 mM NaCl in 10 mM phosphate buffer, 0.1% Tween 20) for additional four times before FRET test.

FRET Experiments and Analysis:

To study the effect of DNA strands/NP on FRET, the hybridized NPs (0.2 mg) were dispersed in PBST buffer (2 mL) in a quartz cuvette. Before CCP addition, the solution fluorescence was measured upon excitation at 490 nm. Different volumes of CCP at a [RU] = 100 μM (38 μL for $7DNA_T$/NP, 40 μL for $24DNA_T$/NP and 42 μL for $56DNA_T$/NP to maintain a charge ratio of +/- close to 3) were then added to each NP solution. The emission spectra were collected upon excitation of the CCP at 370 nm. Fl emission was also monitored upon excitation at 490 nm in the presence of CCP. For all the Fl emission spectra, the background emission and the polymer emission tail were subtracted.

To study the FRET for free $dsDNA_T$ in solution, the hybridized DNA_T (1×10^{-9} M, which corresponds to the same [Fl] of a $7DNA_T$/NP solution (0.2 mg/2 mL)) in 2 mL of PBST buffer was added in a quartz cuvette. Fl emission was first measured upon excitation at 490 nm in the absence of the CCP. After addition 3.5 μL of CCP ([RU] = 20 μM, an amount corresponds to a charge ratio +/- of 3), the solution fluorescence was

measured by excitation at 370 nm and 490 nm, respectively. The corresponding Fl emission spectra are shown in Figure S-1.

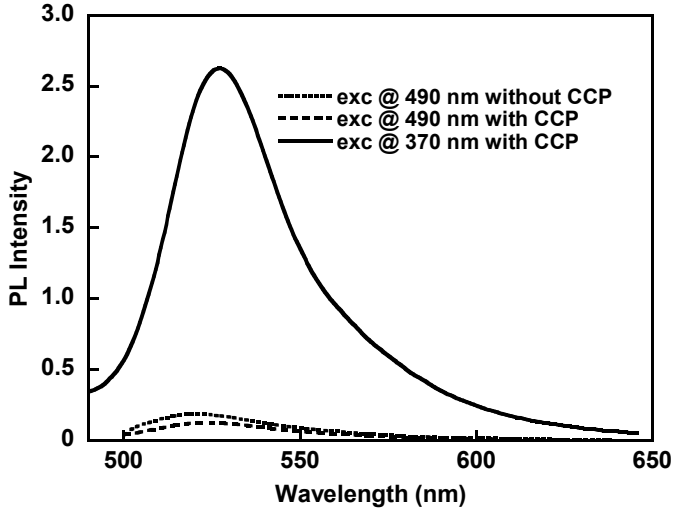

Figure S-1: Fl emission spectra of free $dsDNA_T$ in the presence (exc @ 370 nm) and absence (exc @ 490 nm) of CCP. $[dsDNA_T] = 1\times10^{-9}$ M. 3.5 μL CCP with a [RU] = 20 μM.

To study the selectivity of NP based DNA detection, 0.2 mg of NPs (after hybridization and stringent washing steps) was dispersed in 2 mL of PBST buffer in a quartz cuvette. 3 μL of CCP with a [RU] = 100 μM was then added. Fl emission was measured upon excitation of each NP solution at 370 nm.

We used the fluorescence change of the DNA_T solution before and after hybridization with NP removal to determine the number of DNA_T molecules on each NP. The emission spectra change for the DNA_T solutions before and after hybridization with NP removal for $7DNA_T$/NP is shown as Figure S-2.

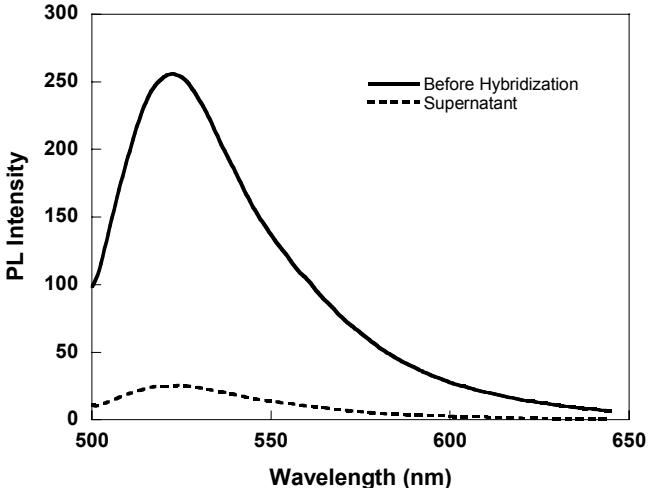

Figure S-2. The emission spectra of DNA_T solution (0.18 μM) before hybridization and the supernatant (after hybridization and NP removal) with NPs (0.2 mg/2mL).

To calculate the signal amplification provided by the NP-based assays, the integrated fluorescence signals for hybridized NPs and hybridized NPs + CCP are shown in Table 1.

Table 1. The integrated fluorescence signals for hybridized NPs and hybridized NPs + CCP

Integrated Area for (490-630 nm)	7 DNA_T/NP	24 DNA_T/NP	56 DNA_T/NP
Hybridized NPs (direct excitation @490 nm)	7.15	36.43	106.59
Hybridized NPs + CCP (FRET excitation@370nm)	827.64	2823.13	4135.20
Signal Amplification Fold	116	78	39

[1] Liu, B; Bazan, G. C. *J. Am. Chem. Soc.* **2006**, *128*, 1188.

[2] Steinberg, G.; Stromsborg K.; Thomas, L.; Barker, D.; Zhao, C. *Biopolymers* **2004**, *73*, 597.