Supplementary Material (ESI) for Chemical Communications


# Supporting Information

## Silver nanocluster aptamers: *In situ* generation of intrinsically fluorescent recognition ligands for protein detection

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**S1. Sequence of aptamer-AgNC chimera DNA:**
5′- AGTCCGTGGTAGGGCAGGTTGGGGTGACTAAAAACCCTTAATCCCC-3′
Sequence of DNA chimera [thrombin aptamer (blue) and nanocluster templating sequence (red)].

5′-GAGAGAGAGAGCCCAGTTCGGAGGGGGGG **AAAAA**CCCTTAATCCCC-3'
Sequence of random (control) DNA chimera.

**S2. Synthesis of aptamer-AgNCs:** Briefly, 3 μM DNA chimera or control DNA (Integrated DNA Technologies, HPLC purified) and 18 μM $AgNO_3$ (Sigma Aldrich) were sequentially added and mixed with sodium phosphate buffer (20 mM, pH 6.6), and the reaction mixture was incubated at room temperature, in the dark, for 20 minutes. 18 μM $NaBH_4$ (Sigma Aldrich) was added and the reaction mixture was incubated at room temperature, in the dark, for one hour. Following reduction of $Ag^+$ ions, highly fluorescent aptamer-AgNCs were produced with fluorescence emission at 700 nm. The pH of the solution was raised to approximately pH 7 by addition of NaOH. NaCl (50 mM final concentration) was then added to the aptamer-AgNCs

**S3. Protein - aptamer-AgNC binding:**
Protein binding-induced fluorescence quenching was tested by incubating aliquots of the aptamer-AgNCs with proteins as described in the article text. Fluorescence spectra were collected using a Cary Eclipse Fluorescence spectrophotometer. Protein binding was directly monitored using gel-shift analysis. Following incubation of the aptamer-AgNCs with proteins (above), 10 uL of each reaction was run on an agarose gel (2%, Metaphor® agarose, with and without ethidium bromide) using sodium phosphate buffer (20 mM pH ~7.0, 50 mM NaCl) as running buffer. The migrations of the aptamer-AgNCs and protein-aptamer-AgNC complexes were observed either by ethidium bromide staining (Figure S1) or native AgNC fluorescence (Figure 2b) using a Gel Imager (Syngene Bio Imaging). As seen in Figure S1 (ethidium bromide staining) the aptamer-AgNC (lane 3) is shown to migrate equivalently to the aptamer-nanocluster DNA chimera (lane 1). However, the observed migration for both is reduced upon thrombin binding (lanes 2 and 4), showing that the thrombin protein is able to bind the aptamer-nanocluster chimera DNA as well as the aptamer-AgNC. In contrast to this specific binding, no gel shift, and hence no protein binding, was observed for aptamer-AgNC incubated with the nonspecific proteins (lanes 5, 6 and 7). Similarly, we found that the native fluorescence of AgNCs (Figure 2b; fluorescence imaging) is quenched only in lane 2, which contained thrombin protein, but no quenching of fluorescence is observed in lanes 3, 4, and 5 with the nonspecific proteins. Both of these experiments prove that thrombin protein can bind its aptamer sequence in the presence of $Ag^+$ ions and AgNCs, and that quenching of fluorescence occurs only upon binding of thrombin protein with its aptamer. Moreover, these experiments confirm the specificity of thrombin aptamer for its target protein.

**S4. Protein detection limit and dynamic range determination:**
Briefly, aptamer-AgNCs were synthesized as before, but with 15 μM DNA chimera and a concominent molar increase for all other consituents. This reaction was diluted to a final aptamer-AgNC concentration of 10 nM by addition of sodium phosphate buffer (20 mM, pH ~7) and NaCl (final concentration 20 mM). The aptamer-AgNCs solution was divided into equal aliquots and different concentrations of thrombin protein were added to each. The total volume of each reaction was adjusted by addition of sodium phosphate buffer (20 mM, pH ~7). Thrombin concentration varied from 0, 0.5, 1, 10, 50, 100, 500, 1000, and 1500 nM. Three replicates for each thrombin concentration were tested, with the exception of 1500 nM. The samples were incubated at 32 °C for 5 minutes and then at room temperature for 50 minutes, followed by storage at -20 °C. Samples were thawed prior to collecting fluorescence measurements. Fluorescence measurements were made using a spectrofluorometer (Fluorolog®-3). The values reported are the averages for the triplicate analysis with standard deviations.

The detection limit was calculated, following the IUPAC criterion, as the concentration of the thrombin protein that resulted in a change in fluorescence (quenching), which was three times the standard error of the control (AgNCs without thrombin protein). Application of this criterion resulted in a detection limit of 1 nM, which was experimentally confirmed.

**S5. FCS and TCSPC studies**
Aptamer-AgNCs concentration and single-AgNC brightness were determined by fluorescence correlation spectroscopy (FCS), with fluorescence lifetime measured by time-correlated single photon counting (TCSPC). FCS was performed on an inverted

microscope (IX71, Olympus) using an oil immersion microscope objective (UPlanSApo 100×/1.40, Olympus) and 635 nm pulsed excitation at 40 MHz repetition rate (LDH-P-635, PicoQuant). The power density used was ~ 1 kW/cm$^2$. A 488/632 dual-band dichroic (Chroma), a HQ700/75 band pass filter (Chroma), and a 75 µm diameter pinhole were used to spectrally and spatially filter the emission collected by the objective. A single-photon counting avalanche photodiode (SPCM-AQR14, Perkin-Elmer) was used to collect the fluorescence emission. Collected photons were autocorrelated in a hardware correlator (ALV 5000E/FAST, ALV GmbH). For TCPSC, photon arrival times with respect to the laser pulse were recorded using a PicoHarp from Picoquant.

The autocorrelation functions were fit to a 2D model with one triplet state described in Equation (1) and elsewhere.[1-4]

$$G(\tau) - 1 = \frac{1}{N}\left(1 + \frac{\tau}{\tau_d}\right)^{-1}\left(\frac{1 - F + F \cdot \exp\left(-\frac{\tau}{\tau_r}\right)}{1 - F}\right) \qquad (1)$$

Where $G$ is the autocorrelation function and $\tau$ is the lag time. $N$ is the average number of the fluorescent molecules residing in the detection volume. $\tau_d$ and $\tau_r$ are the characteristic diffusion time and blinking time constants, respectively. $F$ is the effective fraction of molecules in non-fluorescent dark states. The dimensions of the FCS detection volume were measured using a standard (TetraSpeckTM blue/green/orange/dark red beads from Invitrogen) with predetermined hydrodynamic diameter (110 nm). The half radius of the detection volumes ($\omega_0$) was estimated to be 0.39 µm. The average diffusion times, $\tau_d$, from autocorrelation analysis were 520 µs for aptamer-AgNCs alone and > 600 µs for aptamer-AgNCs bound with a thrombin. The average brightness (e.g. count rate) per fluorescent aptamer-AgNC was obtained by dividing the bulk fluorescence (e.g. total count rate) on the single-photon counting detector by the average occupancy in the detection volume ($N$ in Equation (1)). Representative autocorrelation curves for samples of AgNCs before and after thrombin additions are shown in Figure S7(A). The concentration of fluorescent aptamer-AgNCs was found significantly reduced upon thrombin binding.

The fluorescence lifetime data were fit using one-exponential model convolved with a Gaussian approximation of a measured instrument response function (IRF). All data fitting was done using the Levenberg-Marquardt least-squares method in Igor Pro (WaveMetrics). Fluorescence lifetime data of aptamer-AgNCs is shown in Figure S7(B).

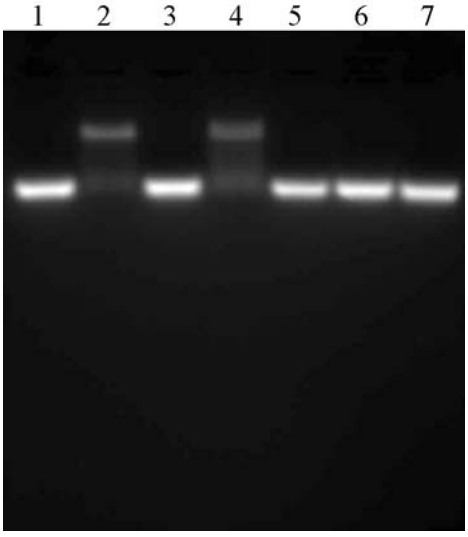


**Figure S1. Gel-shift analysis of specific and nonspecific protein binding to aptamer-AgNCs, as visualized by ethidium bromide staining.** Lane 1: aptamer-nanocluster DNA chimera; lane 2: chimera with thrombin; lane 3: aptamer-AgNCs; lane 4: aptamer-AgNCs with thrombin; lane 5: aptamer-AgNCs with streptavidin; lane 6: aptamer-AgNCs with PDGF; lane 7: aptamer-AgNCs with BSA. 2% (Metaphor® agarose) gel stained with ethidium bromide (DNA is visualized). A similar gel is shown in Figure 1C of the manuscript, however that gel was imaged by intrinsic AgNC fluorescence.

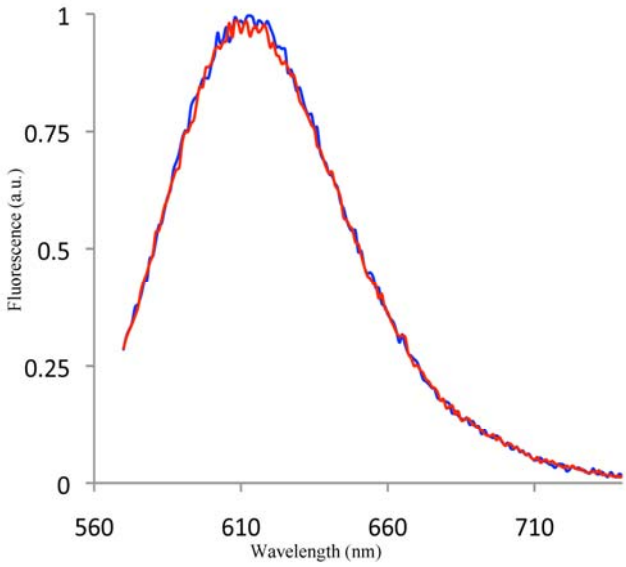


**Figure S2. Fluorescence emission spectra of silver nanoclusters generated using the control sequence, in the presence and absence of thrombin protein.** Blue line: Silver nanoclusters templated on random DNA sequence in the absence of thrombin protein (3μM random DNA strand, 50 mM NaCl, sodium phosphate buffer: pH-7, 20 mM); Red line: Silver nanoclusters templated on random sequence in the presence of thrombin protein (3μM random DNA strand, 50 mM NaCl, 3 μM thrombin protein 3 μM, Phosphate buffer: pH-7, 20 mM).

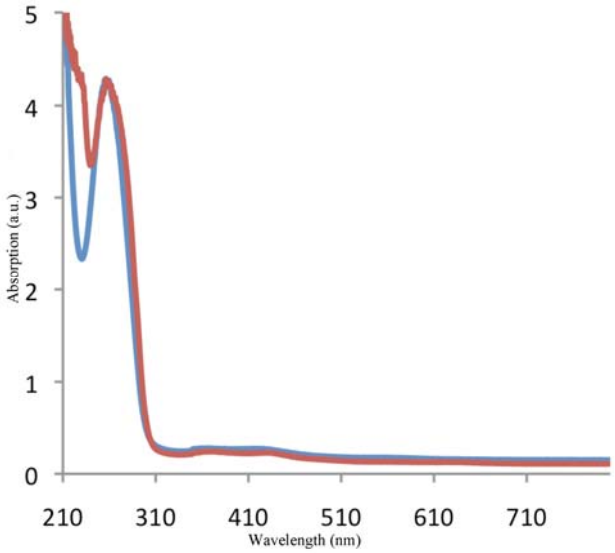


**Figure S3. UV-Vis spectra of aptamer-AgNCs before and after the addition of thrombin protein.** Blue line: aptamer-AgNCs in sodium phosphate buffer (20 mM pH ~7.0, 50 mM NaCl); red line: aptamer-AgNCs after addition of thrombin (20 mM pH ~7.0, 50 mM NaCl). No new absorption peak was observed, showing that no large Ag nanoparticles are formed.

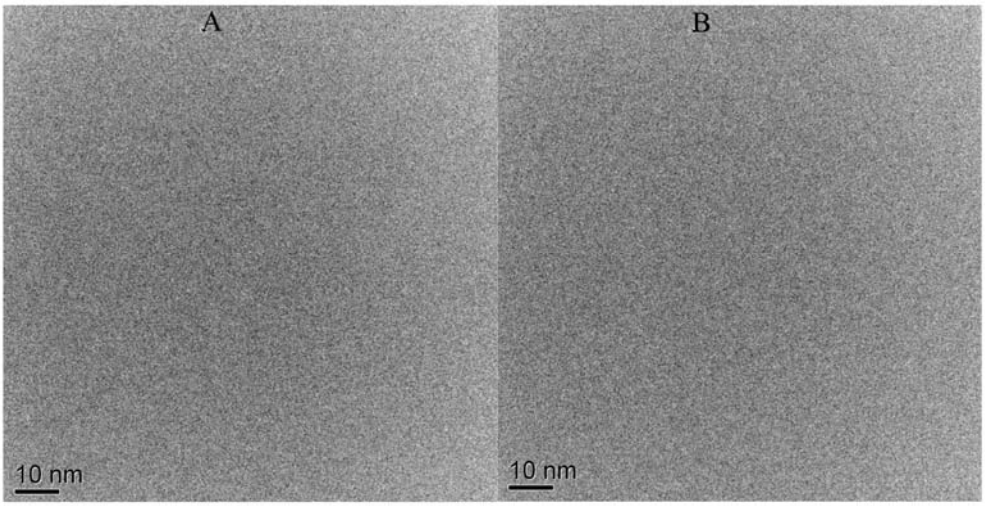


**Figure S4. TEM images taken before (A) and after addition of thrombin protein (B).** No Ag nanoparticles were observed.

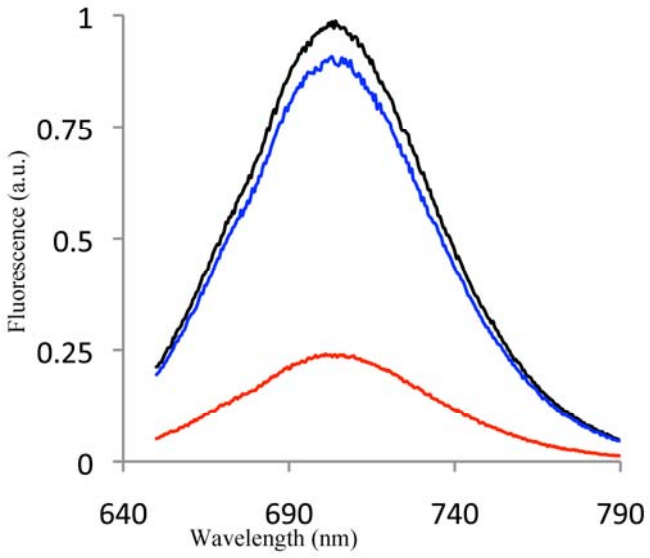


**Figure S5. Fluorescence emission spectra showing the effect of native and denatured thrombin protein on fluorescence quenching.** Black line: aptamer-AgNCs; Blue line: aptamer-AgNCs + denatured thrombin protein; Red line: aptamer-AgNCs + native thrombin protein. Thrombin protein was thermally denatured.[5] Here concentration of thrombin protein and aptamer-AgNCs is in 1:1 ratio (3 μM).

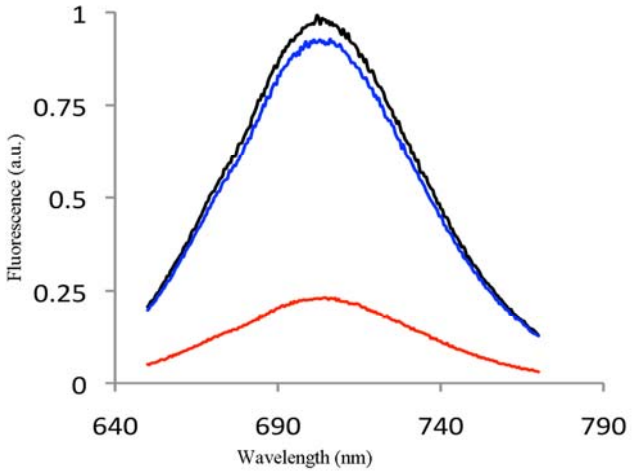


**Figure S6. Competitive assay to study the effect of thrombin protein on fluorescence quenching.** Black line: aptamer-AgNCs; Blue line: aptamer-AgNCs added to a solution of Th protein that was precomplexed with an excess of DNA aptamer (3:1 aptamer to thrombin); Red line: aptamer-AgNCs with thrombin protein.

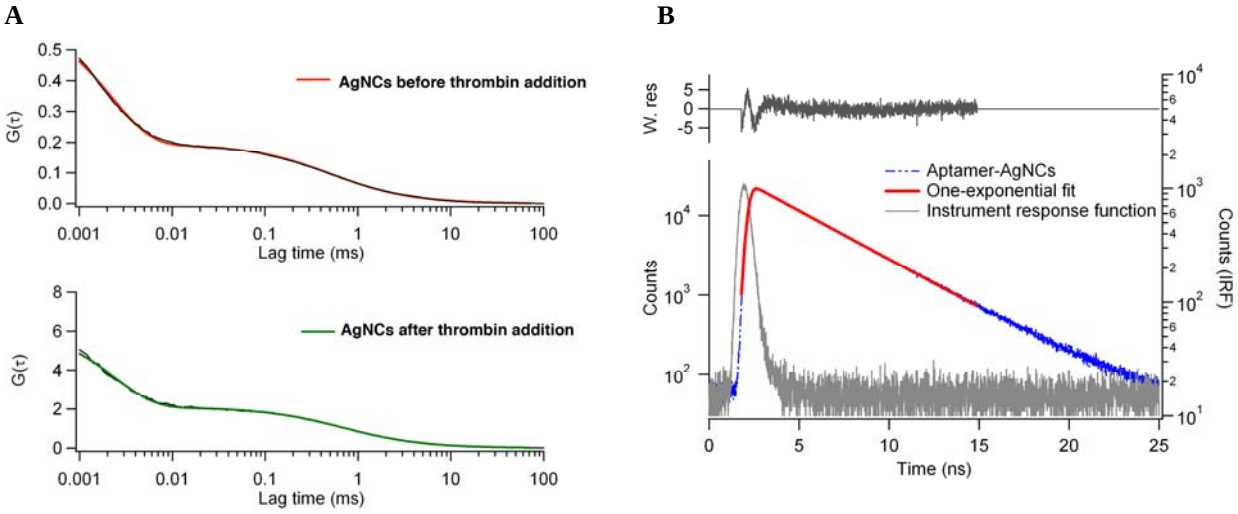


**Figure S7. Single-molecule analysis of aptamer-AgNCs: (A) Fluorescence correlation spectroscopy (FCS) analysis and (B) Time-correlated single photon counting (TCSPC) analysis.** In FCS analysis, the average occupancy of fluorescent aptamer-AgNCs in the detection volume (*N* in Equation (1)) significantly reduced upon thrombin binding. In TCSPC analysis, the fluorescence lifetime of aptamer-AgNCs was estimated to be 3.55 ns and found unchanged upon thrombin binding (Table 1). Reduced $\chi^2$ of the fit: 1.83.

**References:**

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