Supporting Information for:

Direct Visualization of Electrophoretic Mobility Shift Assays Using Nanoparticle-Aptamer Conjugates

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Movie 1

Real time detection of NP-EMSA. Photographs of NP-EMSA gel were taken at 1 min interval for up to 30 min and compiled. Dotted lines serve as a horizontal guide to aid observation of the band shift.

Materials and Methods

Aptamers. RNA aptamers were purchased from Integrated DNA Technologies, Inc. (Coralville, IA). The RNA aptamer was end labeled with either Cy5 or biotin and stocks of aptamers were reconstituted with DEPC-treated water (Applied Biosystems/Ambion, Foster City, CA) and stored at -20°C until use. The sequences for the RNA aptamers are:

5'Biotin-RNA: 5'-**biotin**-GCC UGU AAG GUG GUC GGU GUG GCG AGU GUG UUA GGA GAG AUU GC-3'

3'Cy5-RNA: 5'-GCC UGU AAG GUG GUC GGU GUG GCG AGU GUG UUA GGA GAG AUU GC-**Cy5**-3'

Generation of monomeric CRP. Purified human CRP was from Sigma (St. Louis, MO) and polyoclonal anti-CRP antibody was from Calbiochem (EMD Biosciences, San Diego, CA). All other chemicals were from Sigma and used as received unless otherwise specified. Monomeric CRP was prepared by heating pCRP in an SDS containing buffer (20 mM HEPES, 140 mM NaCl, and 0.1% SDS) for 1 h at 85°C. Total protein

concentration after modification was determined by the bicinchoninic acid method (Pierce BCA Protein Assay Kit, Thermo Scientific) and the purity of monomeric CRP was confirmed by SDS-PAGE showing the presence of only one 23 kDa monomeric CRP band and no obvious proteolytic degradation during treatment (Fig S3).

Gold nanoparticle synthesis. AuNPs were synthesized by boiling 0.25 mM $HAuCl_4$ and adding 1 mM trisodium citrate dihydrate to reduce the gold.[1] The average diameter of the synthesized AuNPs was 18 ± 2.7 nm determined by dynamic light scattering (DLS) and atomic force microscopy (AFM), with a λ_{max} of 520 nm (OD = 1.07) by UV/Vis (Fig. S1). A 1 mL aliquot of the citrate-capped AuNP was incubated with 0.05% Tween-20 for 30 min to stabilize the AuNP before streptavidin in PBS (50 μg/mL final concentration) was added to the AuNPs. Following 2 h of streptavidin incubation, the AuNPs were pelleted at 21,000 *g* for 30 min and the supernatant was removed. The pellet was then resuspended in 500 μL phosphate buffer (0.39 mM Na_2PO_4, 0.61 mM $NaHPO_4$, pH 7.2) and 1 μM of CRP-aptamer containing a 5' biotin modification added to the streptavidin-conjugated AuNP and incubated for 30 min. The mixture was subsequently centrifuged at 21,000 *g* for 30 min to remove unbound biotin-RNA and the pellet was resuspended in 500 μL phosphate buffer.

Theoretical calculation of adsorbed streptavidin on AuNP. The surface area of one streptavidin molecule[2] was estimated to be ~25nm^2 and the surface area of an 18nm spherical AuNP is 1018 nm^2. Therefore, a maximum of 40 molecules of streptavidin can occupy the surface of the AuNP (Table S1). Using an extinction coefficient of 7.81×10^8 $M^{-1}cm^{-1}$ for the 18nm AuNP [3], the concentration of the prepared AuNP was 1.48×10^9 M. The number of molecules of AuNP, streptavidin and biotin-RNA are given in Table S1.

Maxiumum number of CRP on one ON-NP. If each streptavidin binds one biotin-RNA, and if one mCRP interacts with one biotin-RNA the amount of CRP needed to saturate all biotin-RNA on the ON-NP in a 500 μL sample volume would be $3.6x10^{13}$ molecules of mCRP (Table S2). Therefore the concentration of CRP used is near the saturation limit supporting the observed LOD of 6 μg/mL mCRP.

Flocculation Assay. To ensure that the AuNP was fully coated with streptavidin, a flocculation assay using NaCl was performed. Tween-20 treated AuNPs were incubated with increasing concentration of streptavidin for 2h, pelleted at 21,000 *g* for 30 min to remove excess streptavidin and resuspended in 10mM phosphate buffer. The absorbance spectrum of each sample were taken before and 1h after the addition of NaCl (400 mM final concentration) on a UV-vis on a Perkin Elmer LS55 UV/vis spectrophotometer. The change in absorbance for each streptavidin concentrations (at λ_{max} = 520 nm) from three independent experiments was acquired (Fig. S2).

AFM sample preparation and imaging. A 10 μL aliquot of AuNPs were added to aminoprophyltriethoxysilane-modified mica and incubated at room temperature for 10 min. The mica was thoroughly rinsed with DI water (18.1MΩ, Millipore, MA) and dried under a gentle stream of nitrogen gas. AFM imaging was carried out in air using a tapping mode AFM (Asylum Research MFP-3D). Topographic AFM images were acquired using soft silicon AFM tips (Budget Sensors, model Tap 190AL, f_o = 150 kHz, k = 5 N/m) at a scan rate of 1 Hz and a 512 x 512 resolution. The AFM image was subjected to 3rd order polynomial flattening before particle height analysis. The average particle height obtained from the AFM image was 17.5 ± 3.8 nm, n = 50.

Table S1. Theoretical estimation of biomolecules adsorbed on an 18nm-sized AuNP.

Sample	Concentration ($x10^{-9}$ M)	Theoretical # of molecules ($x\ 10^{13}$)	Actual # of molecules ($x\ 10^{13}$)
AuNP	1.48	n/a	n/a
Streptavidin	719	3.56	4.33
Biotin-RNA	1000	3.56	6.02

Table S2. Estimation of ON-NP to mCRP mole ratio. Mole ratio of ON-NP to mCRP was estimated based on the assumption that one molecule of mCRP binds to one molecule of biotin-RNA on the ON-NP. An estimated 40 molecules of biotin-RNA per ON-NP was assumed, which corresponded to $3.56x10^{13}$ molecules of biotin-RNA in the 500μL ON-NP sample volume.

mCRP (μg/mL)	# of molecules ($x10^{13}$)	Mole ratio of ON-NP to mCRP
1.2	0.3	11.8
2.4	0.6	5.9
3.6	0.9	3.9
4.8	1.2	3.0
6.0	1.5	2.4
7.2	1.8	2.0
8.4	2.1	1.7
9.6	2.4	1.5
10.8	2.7	1.3
12.0	3.0	1.2
13.2	3.3	1.1
14.4	3.6	1.0
15.6	3.9	0.9
16.8	4.2	0.8
18.0	4.5	0.8

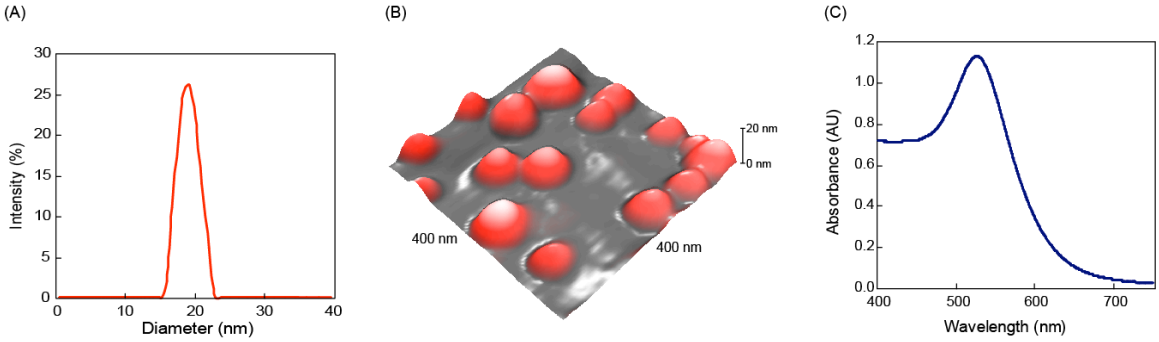

Figure S1. Characterization of the synthesized AuNP by (A) DLS, (B) AFM, and (C) UV/Vis. The average size of the AuNP was 18 ± 2.7 nm by DLS and 17.5 ± 3.8 nm by AFM. The absorbance was 1.07 measured at λ_{max} = 520 nm.

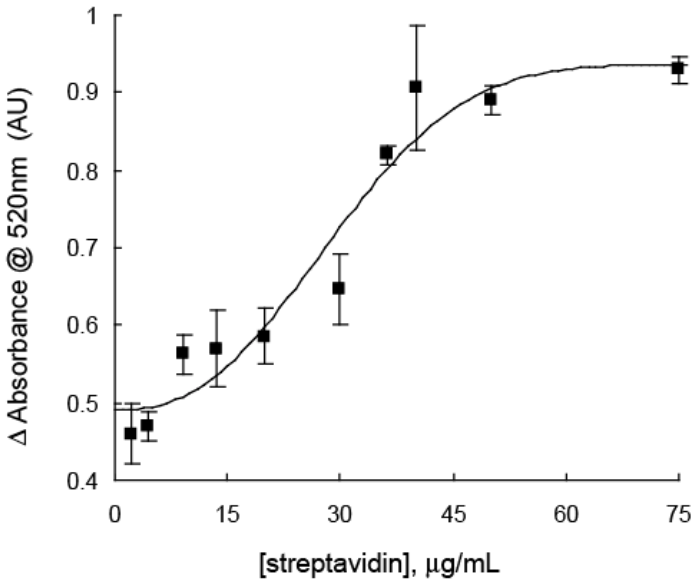

Figure S2. Flocculation assay of streptavidin-coated AuNPs. Flocculation of streptavidin-coated AuNP was induced by the addition of NaCl (400 mM final concentration) and the change in absorbance was measured after 1 h. Data represents mean ± SD from 3 independent experiments.

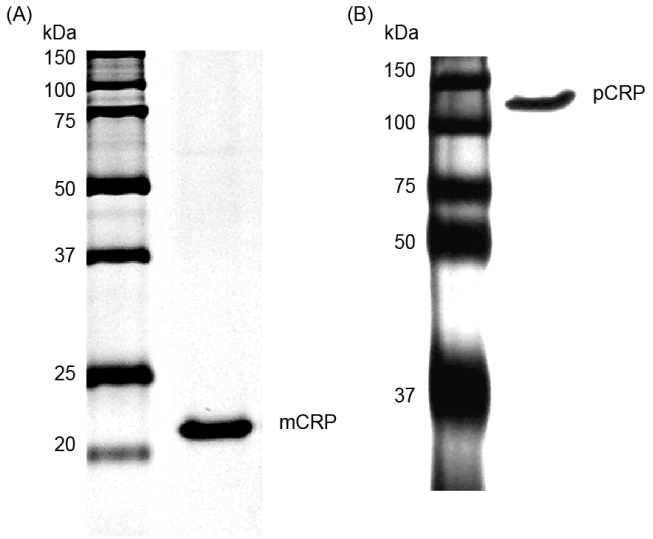

Figure S3. CRP isoforms evaluated by PAGE followed by silver staining. (A) mCRP was resolved on a 12% SDS PAGE, and (B) pCRP was resolved on a 10% native PAGE. PAGE showed the expected molecular weights of 23kDa and 120 kDa for mCRP and pCRP, respectively.

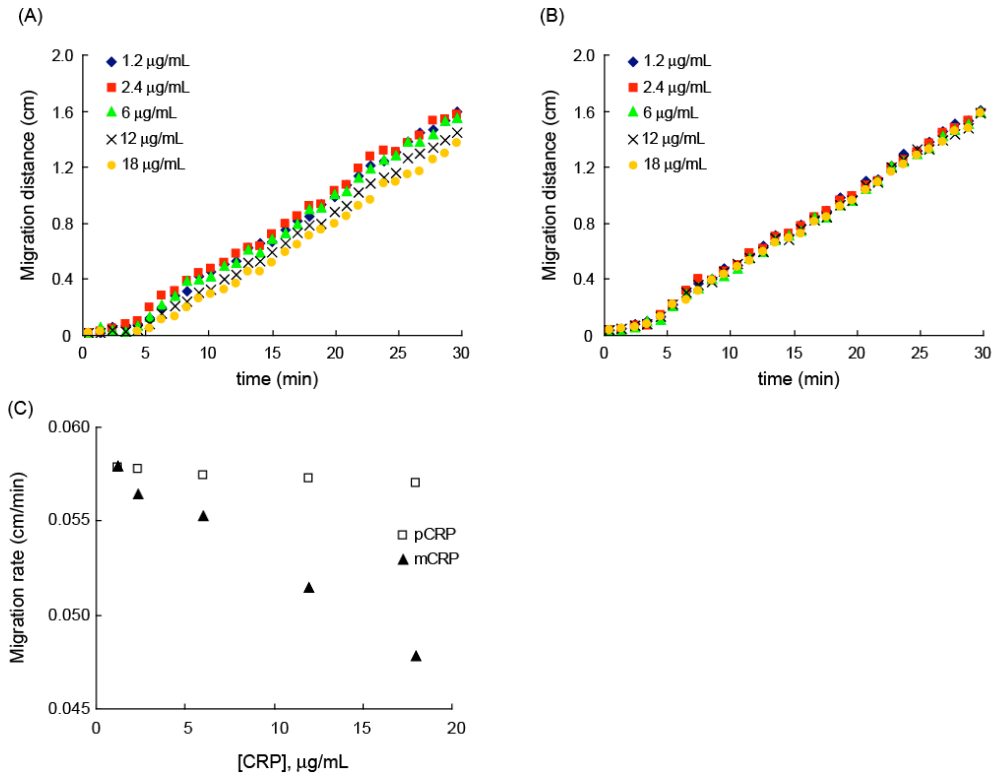

Figure S4. Time-course migration profiles of ON-NPs. (A) ON-NP with mCRP and (B) ON-NP with pCRP. (C) The migration rates or slopes of the ON-NP/CRP at each concentration were fit using linear regression.

References

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