Supporting Information

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SI Text

Consider the capture efficiency of ligand molecules by the nanoswitches. Here, we describe how almost all ligand molecules will be bound to the nanoswitch at low ligand concentrations when the dissociation constant of the more strongly binding antibody K_{D1} is lower than the concentration of nanoswitch $[N]$ and the dissociation constant of the more weakly binding antibody K_{D2} is much lower than the local concentration between the two binding sites on the nanoswitch $[C]$. When the ligand is at a much lower concentration than the nanoswitch, we can assume that the change in free nanoswitch concentration and the proportion of nanoswitches that bind to two ligands are both insignificant. With $[L]$ as the ligand concentration and $[N]$ as the nanoswitch concentration, the proportion of looped nanoswitch $[N_{loop}]$ is given by the following:

$$[N_{loop}] = \frac{[C][L][N]}{K_{D1}K_{D2} + K_{D2}[N] + K_{D1}[N] + [C][N]}.$$

Here, K_{D1} is the dissociation constant of the more strongly binding sandwiching antibody. If we add the assumptions that the concentration of the nanoswitch $[N]$ is much greater than the dissociation constant of the more strongly binding antibody and the local concentration $[C]$ is much greater than the dissociation constant of the more weakly binding antibody, then this expression reduces to $[N_{loop}] \approx [L][1 - (K_{D2}/[C])(1 + K_{D1}/[N])] \approx [L][1 - K_{D2}/[C]]$. For our system, we have the parameters K_{D1} = 10 pM and K_{D2} = 100 pM. Additionally, the local concentration $[C]$ is ≈30 nM (11) and the nanoswitch concentration $[N]$ is 0.7 nM, resulting in almost all ligand captured by looped nanoswitch with $[N_{loop}] \approx 0.999[L]$.

Here, we demonstrate how a nanoswitch can capture more ligand than just using a capture antibody. When capturing ligand with an antibody instead of a nanoswitch, the proportion of antibody–ligand complex is given by the following:

$$[A \cdot L] = \frac{[A][L]}{K_D + [A]}.$$

Therefore, if we assume that for the nanoswitch both antibodies have similar affinities, the ratio between capture from a nanoswitch to capture from single antibodies is given by the following:

$$\frac{\text{Cap}_{\text{nanoswitch}}}{\text{Cap}_{\text{single}}} = \frac{[C](K_D + [A])}{K_D^2 + 2K_D[A] + [C][A]}.$$

This quantity is greater than 1 when $[C] > K_D + 2[A]$, which generally holds true since the local concentration $[C]$ is ≈30 nM (11). As an example, if we used antibodies that had K_D = 1 nM, then nanoswitches would capture 2.2 times than just using capture antibodies.

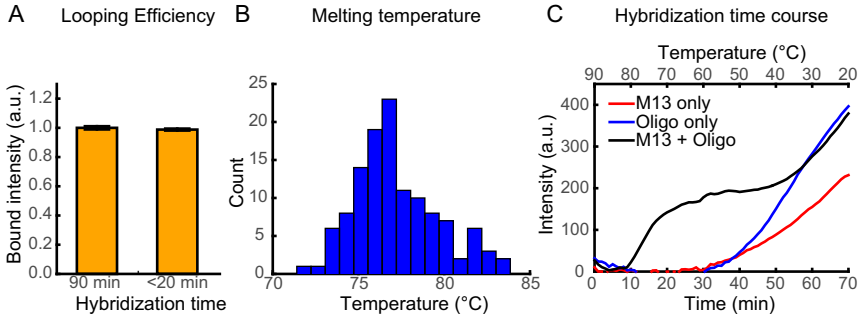

Fig. S1. (*A*) Bound intensity for long (90-min) and short (<20-min) nanoswitch construction times for 200 pM PSA. Hybridization conditions are described in *Methods*, and error bars are the SEM over four replicates. (*B*) Distribution of melting temperatures in the hybridization buffer (50 mM NaCl, 10 mM $MgCl_2$) for all 121 tiling oligonucleotides computed by the OligoAnalyzer Tool (IDT). (*C*) Measurement of annealing using SYBR Green I during a normal 90-min hybridization time course stepping down 1° per minute for only the M13 scaffold, only the tiling oligonucleotides, and both the M13 and tiling oligonucleotides.

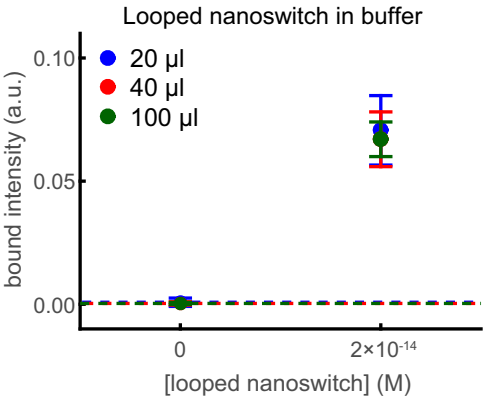

Fig. S2. Detection of looped DNA nanoswitch in buffer. Average bound intensity per lane as a function of concentration in buffer. Results are for 20 µL of sample run in one gel lane (blue), 40 µL of sample run across two gel lanes (red), or 100 µL of sample run across multiple gel lanes (green). Error bars are the SD over eight replicates, with the background given by the dashed line.

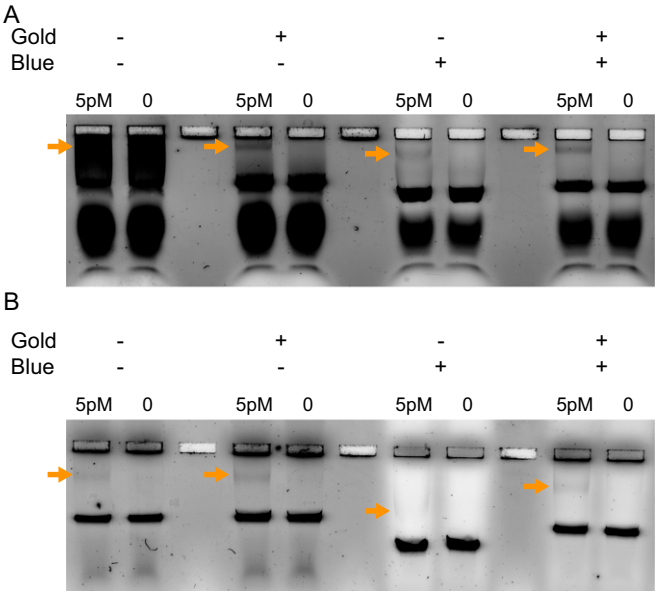

Fig. S3. Use of dyes to enable entry of nanoswitches into the agarose gel for separation. Gel electrophoresis results of 5 or 0 pM of PSA in (*A*) 20% FBS or (*B*) 20% bovine urine in the absence of any dyes, only SYBR Gold, only Coomassie Brilliant Blue G-250, or both SYBR Gold and Coomassie Brilliant Blue G-250. The location of the looped bands are specified by the orange arrows.

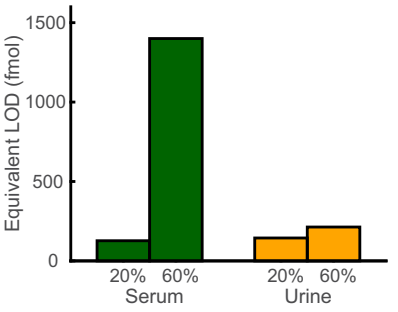

Fig. S4. Equivalent limit of detection (LOD) as a function of the percentage of biological material in the sample. The equivalent LOD is the LOD in 12 µL of undiluted sample. For a sample diluted to 20%, this means that the signal is averaged over three different lanes, while for 60% this is only one lane. The LOD was determined by extrapolating the concentration from the signal equal to background signal plus 3 SD of the background signal.

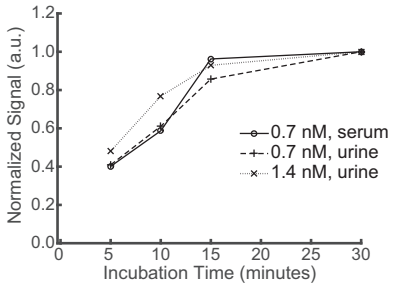

Fig. S5. Normalized signal resulting from different incubation times for 5 pM human PSA in 20% serum or 20% urine for the nanoswitches diluted to an incubation concentration of 0.7 or 1.4 nM.

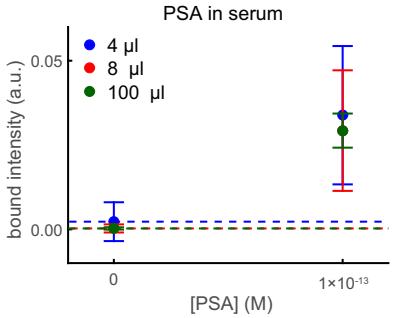

Fig. S6. Detection of PSA in 20% serum using a lower nanoswitch concentration of 400 pM. Average bound intensity per lane as a function of concentration for PSA in 20% serum. Results are for 4 µL of serum (i.e., 20 µL of diluated sample) run in one gel lane (blue), 8 µL of serum run across two gel lanes (red), or 100 µL of serum run across multiple gel lanes. Error bars are the SD, with the background given by the dashed line for four replicates at 100 fM and 20 replicates at 0 fM.

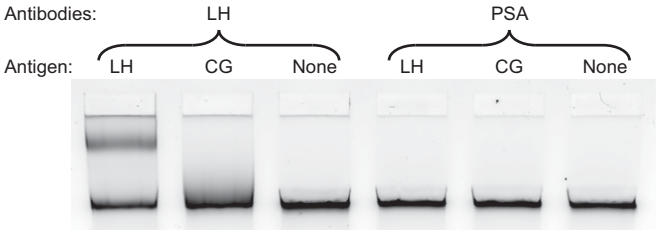

Fig. S7. Representative gel image of NLISA using antibodies against LH or PSA to assay 1 nM LH, 1 nM CG, and a control lane lacking any target in 20% FBS. Looping or smearing is not observed when using antibodies against PSA since the nanoswitch does not bind either ligand.

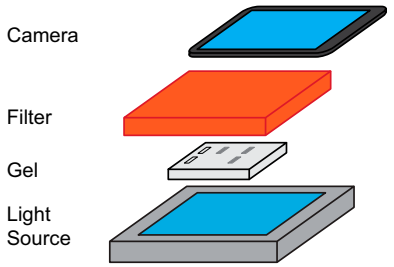

Fig. S8. Schematic representation of the mobile imaging platform, with Safe Imager 2.0 Blue Light Transilluminator light source, agarose gel, Safe Imager filter, and iPad camera. A Styrofoam box was used to block ambient light.

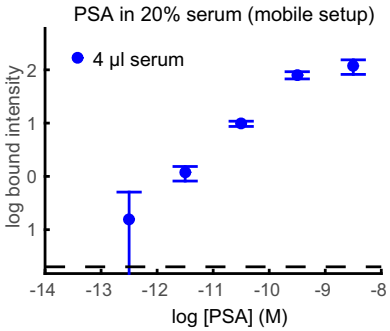

Fig. S9. Quantification of PSA in 20% FBS at concentrations from 6×10^{-14} to 6×10^{-10} M for gel images taken with the mobile imaging platform. Here, the LOD is 79 pM. Error bars are the SD, with the background given by the dashed line.

Table S1. Breakdown of the cost per sample

Reagent	Source	Cost per sample
SYBR gold	Thermo Fisher Scientific	$0.02
UltraPure Agarose	Thermo Fisher Scientific	$0.01
10× TBE	Bio-Rad	$0.01
Antibodies	Biospacific	$0.02
Protein lobind plate	Sigma	$0.02
Total		$0.08