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Supporting Information

DNA Strand Displacement Reaction for Programmable Release of biomolecules

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Materials and Methods

Integrated DNA Technology (IDT) synthesized all DNA strands. Human thyroglobulin was purchased from Sigma-Aldrich. The monoclonal anti-thyroglobulin antibody (clone number 5E6) and the FAM-labeled monoclonal anti-thyroglobulin secondary antibody (clone number 6F9) were from HyTest. NeutrAvidin was obtained from Pierce. Silica microparticles functionalized with carboxyl groups (2 μm mean diameter) were from Bang Laboratories. 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were from Sigma-Aldrich. Sulfosuccinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (Sulfo-SMCC) was from GBiosciences. 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer, Phosphate buffer Saline (PBS), and Saline Sodium Citrate (SSC) buffer were made in the lab and their pH values were adjusted to 6.0, 7.2, and 7.4, respectively. The 10 mM HEPES buffer containing 150 mM NaCl and 0.005% P20 (HBS-P) at pH 7.4 already prepared, degassed, and filtered was from Biacore GE Healthcare. The HPN buffer was prepared by increasing the NaCl concentration of HBS-P to 500 mM. All DNA hybridizations were done at room temperature (21-24 °C). The ultrafiltration devices and Glen Gel-Pak DNA desalting columns were bought from Millipore and Glen Research, respectively. The NanoDrop ND-1000 UV-Vis spectrometer, Photon Technology International (PTI) fluorometer, Biacore X SPR and Applied Biosystems Voyager MALDI-TOF-MS instruments were used. The CM5 Chips were from Biacore (now GE Healthcare). The BCA kit was from Pierce.

DNA Sequence design

Four DNA sequences were designed using the online packages DINAMelt (http://mfold.rna.albany.edu/?q=DINAMelt/software) and OligoAnalyzer 3.1 (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/). All sequences were checked for their melting points to make sure they were all above 45 °C. The 5'-biotinylated probe strand, β, was made of 46 nucleotides; 13 of which at the 3' end complementary to the first 13 nucleotides of the capture strand, α, at its 5' end. The last 10 nucleotides at the 5' end of β acted as a spacer stretch for the better folding of duplexes. Two displacing "fuel" strands, β' and α', were also designed to release the captured α strand. B' was composed of 20 nucleotides and a toehold of 7 nucleotides at the 5' end to form a 20 base pair duplex with the probe on the beads. The α' fuel strand had 25 bases complementary to α leaving a toehold of 12 nucleobases on α and 5 bases as a short spacer between the functional parts of α and the antibody conjugated to it at

the 5' end. The sequences were optimized to be free of any significant secondary structure at room temperature (on DINAMelt) and any cross-hybridization involving a stretch of more than 4 nucleotides (OligoAnalyzer 3.1). All toeholds were larger than 7 nucleotides to assure a fast SDR kinetics. The sequences are shown below:

α-30: 5-/Thiol or FAM/ TGA GAG ATA GAA TGA GAG GGA GGT GGC GGA -3

α'-25: 5-TCC GCC ACC TCC CTC TCA TTC TAT C -3

β-46: 5-/Biotin/ TCA CAC ACT ATC ACC ACT TTC TTC CAT CCT CAC TCC GCC ACC TCC C -3

Ctrl-46: 5-/Biotin/ ACA CAC ACA CAC ACA CAC ACA ACA CAC ACA CAC ACA CAA CAC TAC T -3

β'-20: 5-GGG AGG TGG CGG AGT GAG GA -3

Immobilization of the DNA probes on silica beads

Each step described here involving a reaction or process on beads starts with re-suspending the beads after addition of buffers/reagents using a benchtop vortex and ends with spinning down the beads at 10,000 g for 3 min to remove the supernatant using a micropipette. The 2.0 μm silica microparticles already grafted with linkers containing carboxyl groups at their ends were from Bang Laboratories. For a typical NeutrAvidin immobilization,[1] 30 mg beads were washed with 1.0 mL MilliQ water, and soaked in 1.0 mL MES buffer (pH= 6.0) for overnight. The beads were then centrifuged down again, the supernatant was discarded, 1.0 mL fresh MES was added and the suspension was sonicated for 90 min. 500 μL freshly prepared EDC (0.4M) was mixed with 500 μL freshly prepared NHS (0.4M) and the mixture was added immediately to the beads. After 10 minutes the beads were washed with 1.0 mL MES6.0 buffer quickly. 750 μL neutravidin (0.4 mg/mL) in MES6.0 buffer was added to the beads and incubated with them for 8 hours on vortex. To quench any unreacted activated NHS ester, 1.0 mL Ethanolamine 1.0 M (pH= 8.5) was used for 30 min on vortex. Two more washes each time with 1.0 mL 2.5x SSC for 1 min on vortex made the beads ready for the immobilization of the probes. 1.20 mL DNA probes (either β-46 or Ctrl-46 biotinylated at their 5' ends, 2.5 μM) in 2.5x SSC buffer were mixed with the beads for 16 hours on vortex. Two washes each time with 1.0 mL HBS-P buffer for 1 min on vortex were done at the end and finally, the bead suspensions were divided into 20 equal portions of each 1.5 mg.

Estimation of β probe density on beads

Surface density of the primary antibody could be tuned by changing the surface loadings of neutravidin, β-biotin, or α-TgAb conjugate. Since binding of the biotinylated β probes is the critical step in functionalization of the surface with DNA probes, estimation of the magnitude of β probe immobilization gives a good evaluation of the level of surface modification. The average β-biotin loading was estimated by taking measurements of β-biotin concentrations before and after mixing with a known amount of beads. Based on that, the solution concentration of neutravidin for immobilization was optimized at a fixed concentration of β-biotin. A typical estimation of β probe density on 46.5 mg carboxylated silica beads already functionalized with neutravidin (according to the experimental procedure described above) is as follows:

[β-46 before mixing with beads]= 36.7 ng/μL, volume= 1850 μL

[β-46 after mixing with beads]= 2.4 ng/μL

β-46 loading: (36.7-2.4) ng/μL × 1850 μL= 63552 ng ÷ 14070.3 ng/nmole= 4516.7 pmole ÷ 46.5 mg beads= 97.1 pmole/mg beads

Using the simple estimation above, the batch-to-batch reproducibility of β probe density was assured. To further assure reproducibility in β probe immobilization, a 1.5 mg portion of each batch of the β-functionalized beads was tested for SDR-based release of α-FAM resulting in a graph similar to Fig. 1 (the experimental procedure is described later under the section “SDR-based release of α-FAM”). The β probe density was chosen such that the signal intensity of the α-FAM release is strong enough to generate a large α-FAM concentration change during the capture step.

Optimization of fuel strand concentration on surface plasmon resonance (SPR)

The CM5 SPR chips have carboxyl functional groups on their surface. The EDC/NHS standard chemistry was used to immobilize neutravidin on the chips. Equal volumes of 11.5 mg/mL NHS and 75.0 mg/mL EDC both in MES buffer were mixed immediately upon preparation. The mixture of EDC/NHS mentioned above was injected (70 μL at 10 μL/min). Neutravidin in acetate buffer (1.0 mg/mL, pH= 4.5) was reacted with the activated surface (70 μL at 10

μL/min). Finally, the chip surface was passivated by injection (70 μL at 10 μL/min) of 1.0 M ethanolamine hydrochloride in water (pH= 8.5). Three injections (each 10 μL at 10 μL/min) of the regeneration solution (50 mM NaOH in 1.0 M NaCl) assured all the non-specifically adsorbed neutravidin was removed. The biotinylated β probe (1.0 μM) in 5x SSC buffer was then immobilized (50 μL at 10 μL/min) followed by five more regeneration cycles (each 10 μL at 10 μL/min) to condition the surface and obtain a reproducible baseline level.

The correlation between α' SDR yields and concentration of the α'-25 fuel strand was examined. For each data point shown in Fig. S1, SDR with α' was done and its yield was obtained by dividing the signal loss after injection of α'-25 by the signal gained in the capture of α-30. In Fig. S1, the concentration of α'-25 was changed while all other parameters such as the flow rates for α-30 and α'-25 injections (2 μL/min), hybridization times (3 min and 2 min for α-30 and α'-25, respectively), and concentration of α-30 (1.5 μM) were kept constant. All yields on the vertical axis were normalized to the yield for [α'-25]= 1.4 μM, where the α'-25/α-30 concentration ratio is close to 1. As seen in Fig. S1, increasing the fuel to capture stand concentration ratios to about 2 and 9 increases the α' SDR yield by 2% and 4%, respectively. This data indicates that a 5x concentration excess of the fuel strand provides a reliable and consistent release.

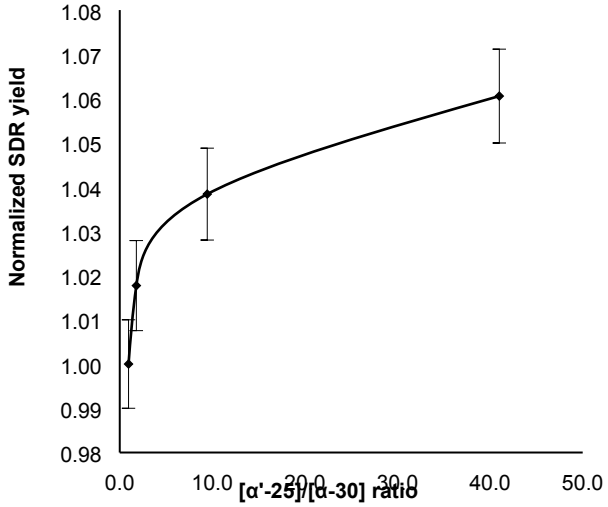

Fig. S1: The α' SDR yield improves at higher concentrations of the fuel strand. The yield improves much slower when the fuel/capture strand concentration ratio becomes larger than 2.

Synthesis of the conjugate

Sulfo-SMCC was chosen as the small molecule linker between the DNA single strand and the anti-thyroglobulin primary antibody (TgAb). Sulfo-SMCC reacts with its NHS ester end to the amino groups of the amino acid side-chains of the antibody and through its maleimide functionality to the thiolated α-30 capture strand. Hence, the synthesis is accomplished in three steps. The 3-step synthesis was optimized to obtain a DNA:antibody ratio around 1.

1) Sulfo-SMCC attachment to TgAb

The kinetics of reaction depends on the concentrations of reagents, the molar ratio, and the reaction time. The procedure should be customized for each antibody, as they might be very different in reactivity as a result of their sources, clonality, post-translational modifications and hosts. The recipe presented here was compiled based on previously reported[2] procedures and was specifically optimized for TgAb.

For a typical reaction, 100 μg TgAb (clone # 5E6) was used. The antibody is supplied in PBS buffer containing 0.1% sodium azide as a preservative. Since an azide could act as a nucleophile, it is necessary to remove it before the reaction. Three filtrations and buffer exchanges with PBS pH 7.2 on an Amicon ultrafiltration device (MWCO 50 kDa) at 14000 g (for 3 min each time) removed the azides efficiently. Sulfo-SMCC was dissolved in PBS pH 7.2 to yield a concentration of 1.0 mg/mL. 100 μL TgAb (1.0 mg/mL, 666.7 pmole) was then reacted to the 8x molar excess of Sulfo-SMCC (5.33 nmole, 1.0 mg/mL) for 30 min on vortex. After 30 min, the reaction mixture was centrifuged at 21000 g for 2 min to make sure no precipitation has occurred. Ultrafiltration and buffer exchange with PBE for three times (at 14000 g, 3min each time) using an Amicon filter (MWCO 50 kDa) removed the unreacted/ hydrolyzed Sulfo-SMCC and small-molecule by-products. The functionalized TgAb was quantified by a NanoDrop UV-Vis spectrometer.

2) Disulfide bond reduction of α-30 capture strand

The thiolated α-30 capture strand from IDT (α-30-thiol) bears a disulfide bond at its 5' end with a C6 linker. Thus, it is necessary to reduce that bond to obtain nucleophilic thiol groups. The DTT reduction procedure could be carried out using 10-300 mM DTT at pH 8.0 to 8.5 for 30 min.[3] It should be noted that pK_a for the thiol group is about 8.3 and therefore, pH of PBE used in this work was set to 8.3. 250.0 μL α-30-thiol (10.0 μM) in PBE pH 8.3 was used to dissolve

11.6 mg DTT (making the final concentration of 300 mM DTT) and the reaction was run at room temperature on vortex for 4 hours. A Glen Gel-Pak gravity column for DNA desalting was used to purify the reduced α-30-thiol and remove the small molecule by-products and unreacted DTT based on size exclusion chromatography. The reduced α-30-thiol collected from the column was quantified by NanoDrop and used immediately in the next reaction to minimize the chance of re-oxidation.

3) Conjugation of the reduced α-30-Thiol strand to the Maleimide group of TgAb-SulfoSMCC

To obtain a DNA:Ab ratio of around 1.0 in the product, an experimental ratio of 1.2:1.0 DNA:Ab was established. Different optimization attempts also indicated that the initial concentrations of TgAb-SulfoSMCC and the reduced α-30-thiol should be approximately 1.0 μM each. In a typical reaction, 144 μL TgAb-SulfoSMCC (0.84 mg/mL, 805 pmole) was diluted to 644 μL using PBE buffer pH 7.2 and was added to it 212 μL reduced α-30-thiol (4.56 μM, 967 pmole) in PBE buffer pH 7.2. The reaction time was 30 min on vortex and centrifugation at 21000 g for 2 min at the end showed no precipitation. The unreacted reduced α-30-thiol was removed using an Amicon ultrafiltration device (MWCO 50 kDa) by three filtrations (at 14000 g each time for 3 min) and buffer exchange with HBS-P buffer containing 500 mM NaCl (HPN). The concentration of α-TgAb conjugate was measured by UV-Vis spectrometry and the DNA:Ab ratio was calculated to be 0.98 using the molar extinction coefficients of TgAb and α-30-Thiol at 260 nm and 280 nm. The BCA protein assay for a number of conjugated products made in different batches was in a very good agreement with the values calculated from the UV-Vis measurements.

Characterization of the conjugate by Surface Plasmon Resonance (SPR) measurements

To assure that the conjugates are comprised of the α-30 capture strand covalently attached to a still functional TgAb, interaction analyses were performed on a Biacore X SPR instrument. A CM5 Biacore chip was functionalized with neutravidin using the standard EDC/NHS chemistry[4] and the biotinylated β-46 probes were immobilized on the chip. The surface was first passivated by injecting BSA 0.1% in HBS-P buffer and then the α-TgAb conjugate (91.5 μg/mL with respect to TgAb) was injected to the surface of the chip to immobilize the conjugate via duplex formation between the α-30 capture strand of the conjugate and the immobilized β probes on the chip. The Human Thyroglobulin (Tg) antigen (225 μg/mL) was then injected followed by the

injection of the secondary antibody, TgAb-FAM, (180 μg/mL) to form the sandwich complex on the chip. The complex was released after injection of the fuel strand α'-25 (5.0 μM). It should be noted that α'-25 was used here because its sequence is orthogonal to the β probes on the surface and will not bind to surface to generate any signal. As a result of this, the signal loss observed upon injection of α'-25 is entirely due to the DNA strand displacement reaction. The α' SDR yield was obtained by dividing the signal loss after injection of α'-25 by the sum of signals (total capture) gained in the capture of the α-TgAb conjugate, Tg, and TgAb-FAM. The same experiment was repeated without the antigen (Tg) injection step to examine the non-specific adsorption of TgAb-FAM and also the SDR yield of the α-TgAb conjugate alone. The SPR data in resonance units (RU) is shown in details in Table S1:

Step	Sample run		Control run	
	Time (sec)	Signal change	Time (sec)	Signal change
BSA (1.0 mg/mL)	900	- 5	5406	7
α-TgAb conjugate (91.5 μg/mL)	1563	2902	6092	2941
BSA (1.0 mg/mL)	2031	- 9	6755	- 9
Tg (225 μg/mL)	2665	1535		No injection
BSA (1.0 mg/mL)	3103	- 9		No injection
TgAb-FAM (180 μg/mL)	3788	410	7382	- 1
α'-25 fuel (5.0 μM)	4403	-2334	8120	- 2575
Total Capture		4824		2938
α'-25 SDR Yield (%)		48.4		87.6

Table S1: SPR Signal level changes in resonance unit (RU) were calculated by subtracting the signal level before the injection from the one after the injection where the signal is fully stable. A negative value for the signal level change means the signal dropped by that amount. All injections were at 5.0 μL/min and the injection volume of 15.0 μL. The times columns correspond to the X axis of the sensogram shown in Fig. S2.

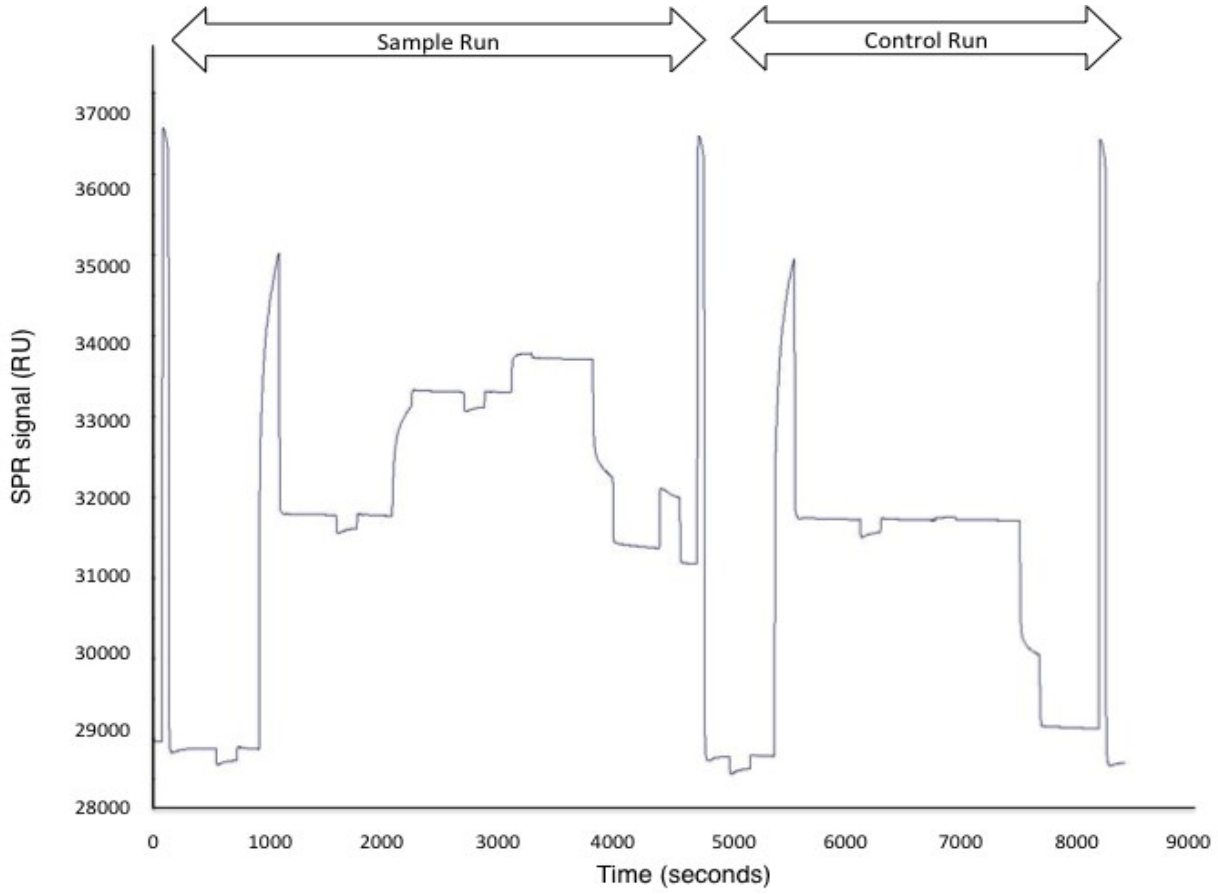

Fig. S2: The SPR sensogram based on which Table S1 was composed. To find which peak relates to which step use the time signature (in seconds) reported in the "time" columns of Table S1. All the peaks before 5000 seconds are related to the sample experiment while the peaks after 5000 seconds (on the time axis) are related to the control experiment, in which no antigen (Tg) was injected.

In a separate control experiment, the α-30-SH capture strand and the unconjugated TgAb were mixed together and were then, injected over the chip. Incubation of the antigen, Tg, in the next step produced no signal confirming that the covalent attachment of the capture strand, α-30, to TgAb is crucial to the successful immobilization of TgAb on the chip.

SDR-based release of α-FAM

Each step described from this point on starts with re-suspending the silica beads functionalized with β probes in the reaction solution, vortexing for a specified period of time, and centrifugation of the suspension at 10000 g for 3 min to remove the supernatant. As a summary of the information below, Fig. S3 includes the capture and release profiles used to build Fig. 2. Fig. S4 examines the linear ranges of capture and release profiles shown in Fig. 3. Fig. S5 shows the calibration curves of Fig. 4 before normalization with respect to the conjugate

concentrations. Fig. S6 is the residual percentage plot of Fig. 4 comparing how close the results in buffer and serum are. It was found in Fig. S7 that α'-25 fuel strand was also very efficient in releasing the FIA sandwich complex at a relatively high Tg concentration (1.0 μg/mL) in buffer. Fig. 5 confirmed the same results could be obtained for a small concentration of Tg (62.5 ng/mL) in bovine serum.

To pick a concentration that falls in the linear range of α-FAM, a standard curve was obtained using different dilutions of the α-FAM stock ($y= 2180.4x + 15344$, $R^2= 0.998$, [α-FAM]= 3.9-500 nM). A concentration of 500 nM was taken for the experiment. The calibration curve was used to estimate concentrations of α-FAM in the supernatants and thus calculate the captured and released amounts in pmoles.

1.5 mg β-functionalized beads were sonicated in 300 μL HPN buffer (pH=7.4) for 45 min and were incubated with 150.0 μL α-FAM (500 nM) in HPN for 5 min. The beads were washed twice with 150.0 μL HPN buffer each time for 1 min. The third wash was also with 150.0 μL HPN but for 15 min. Finally, the release step included incubation with 150.0 μL β'-20 fuel strand (2.50 μM) in HPN for 15 min. All supernatants were saved after each step for fluorescent measurements. A control experiment was done at the same time on 1.5 mg silica microparticles functionalized with Ctrl-46, a DNA probe that has no stretch complementary to α-FAM.

SDR-based FIA on beads

Each step again starts with adding a given reagent to 1.5 mg beads, re-suspending and incubating for a period time on vortex, and centrifugation at 10,000 g for 3 min to remove the supernatant before going to the next step. The general procedure is as follows: 1.5 mg beads were suspended in 300 μL HPN buffer and sonicated for 60 min followed by incubation in 2.0 mg/mL BSA in HPN for 5 min. Resuspension of the beads in 100.0 μL α-TgAb conjugate (concentration varies with the type of experiments) in HPN for 15 min puts a layer of primary TgAb on the surface of the beads via DNA-directed immobilization. After two washes with 150.0 μL HBS-P buffer for 3 min and another 5 min incubation with 150.0 μL 2.0 mg/mL BSA in HBS-P buffer, 150.0 μL Tg (concentration depends on the experiment) in HBS-P buffer containing 2.0 mg/mL BSA was added and incubated for 30 min. The two washes and BSA incubation steps were repeated as described above. 150.0 μL TgAb-FAM (9.0 μg/mL) in HBS-P buffer containing 2.0 mg/mL BSA was then reacted for 30 min to form the sandwich complex. The supernatants of this step were saved for fluorescence measurements to obtain the capture signal. The first

wash with 150.0 μL HBS-P was only for 1 min and was repeated once more. Incubation with 150.0 μL BSA 2.0 mg/mL in HBS-P for 45 min completed the wash steps between the capture and release. The release step was accomplished by resuspending the beads in 150.0 μL β'-20 fuel strand (5.0 μM) in HBS-P buffer containing 2.0 mg/mL BSA for 45 min. The supernatant of this step was also saved to measure the release signal.

Optimization of α-TgAb conjugate concentration

The general procedure was adopted with the following changes: the volume at the conjugate incubation step was reduced from 100.0 μL to 50.0 μL and five different concentrations were used. For each concentration of the conjugate, 50.0 μL Tg (100 μg/mL) in HBS-P buffer containing 2.0 mg/mL BSA was used. For each concentration of the conjugate a replicate with the Ctrl probe-functionalized beads was done at the same time. The capture signal of the Ctrl beads was subtracted from the capture signal of its corresponding experiment to account for the capture resulted from the non-specific adsorptions. The yield in percentage was the release signal divided by the Ctrl-subtracted capture signal at each conjugate concentration. As seen in Fig. S3, the capture and release signals show a linear response to the conjugate concentration in the range of 5-40 μg/mL.

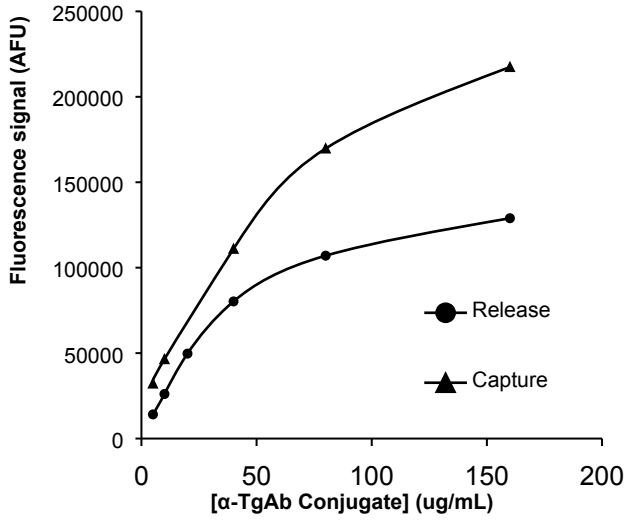

Fig. S3: Capture and release profiles as functions of [α-TgAb conjugate] were used to calculate the SDR yields in Fig. 2.

Estimation of the SDR yields at a fixed conjugate concentration

The general procedure was adopted with the following changes: the conjugate concentration of 40.0 µg/mL was used for all experiments. 100.0 µL Tg at six different concentrations were used to build the capture and release profiles of Fig. 3. The linear segments of the mentioned profiles are examined closely in Fig. S4.

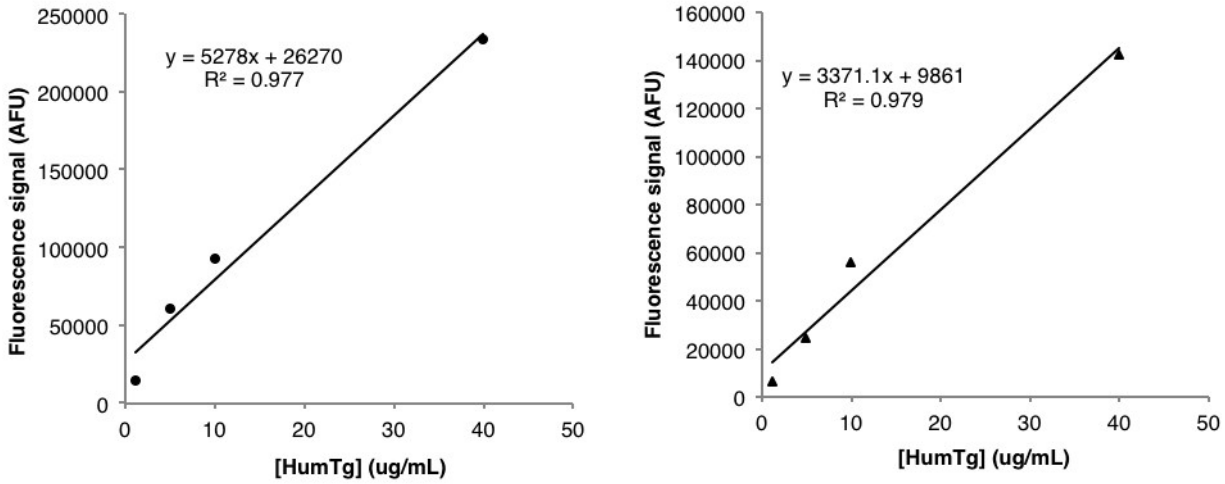

Fig. S4: The linear ranges of capture (left) and release (right) profiles shown in Fig. 3 and also the regression coefficients suggest that lower conjugate and Tg concentrations might improve the linearity of the assay.

The linearity of the capture and release profiles (Figure S4) is much worse than the one for TgAb-FAM calibration curve (y= 117.8x + 20679, R^2= 0.9999, [TgAb-FAM]= 70.3-4500 ng/mL). This is evident from comparing linear regression coefficients. Linearity depends on many factors including the non-specific adsorptions and how strong the multivalent antigen-antibody binding is across the concentration range examined, among the others. Both mentioned factors would improve at the lower Tg and conjugate concentrations.

Comparison of Calibration curves for Tg in serum versus Tg in buffer

The general procedure was followed with different concentrations of Tg in buffer and in bovine serum with the following changes: the conjugate concentrations of 20.0 µg/mL and 22.6 µg/mL were used for the buffer and bovine serum experiments, respectively. 150.0 µL Tg at six different concentrations were used to build the calibration curves. The second and third washes after TgAb-FAM incubation were done for 45 min and 15 min, respectively. Of particular interest is the improvement observed with linearity in terms of the regression coefficients (compare Fig. S4 and Fig. S5) when a much lower concentration range (62.5-2000 ng/mL) for Tg was examined

at a much smaller conjugate concentration. The calibration curve for TgAb-FAM under the same experimental conditions was perfectly linear (y= 6731.5x + 3185.1, R^2= 0.9999, [TgAb-FAM]= 2.3-150 ng/mL).

Fig. S5 shows Fig. 4 before normalization with respect to the conjugate concentrations. As seen in the release profile of Fig. S3, the release curve has a linear behavior around the conjugate concentration of 20 μg/mL. Thus, the slight increase in loading concentration of the conjugate is reflected as a proportional increase in the release signal intensity.

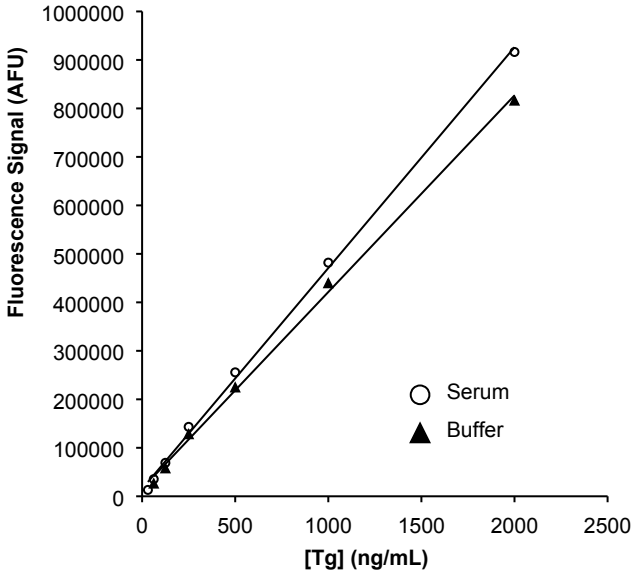

Fig. S5: The calibration curves in buffer and serum before normalization with respect to the conjugate concentrations.

The two calibration curves in Fig. S5 could not be directly compared since the loading concentrations of the α-TgAb conjugate have been different in serum (22.6 μg/mL) and buffer (20.0 μg/mL). However, the conjugate concentration range of 20-22.6 μg/mL lies in the linear range of release signal (Fig. S3). The vertical axis of dataset in Fig. S5 was therefore normalized with regard to the conjugate concentration to obtain Fig. 4. Fig. S6 presents the residual percentages at each Tg concentration in Fig. 4. The small residual percentages (in Fig. S6) for the intermediate and high concentrations of Tg (in Fig. 4) indicate a very similar assay performance in the serum and buffer. The residual percentage for the normalized signals at each Tg concentration was calculated as follows:

$$\text{Residual \%} = ((\text{Signal}_{\text{serum}} / \text{Signal}_{\text{buffer}}) - 1) \times 100$$

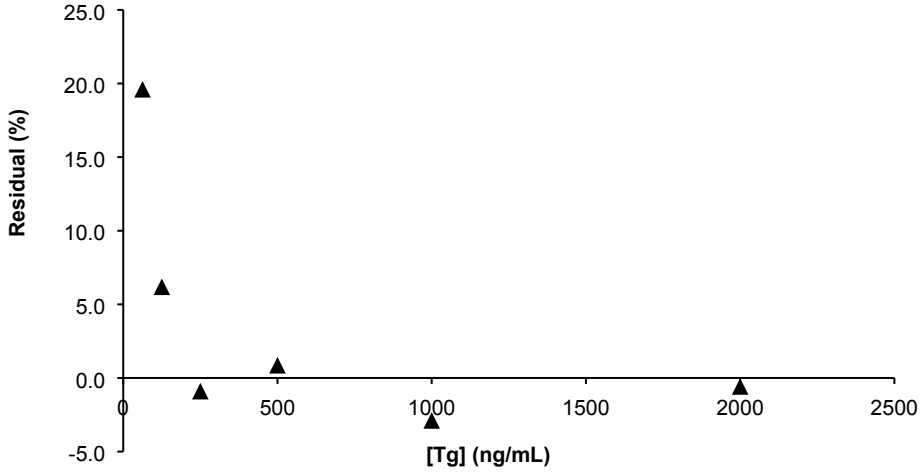

Fig. S6: The residual plot for the comparison of signal difference at each concentration of Tg shown in Fig. 4. As expected, the residual percentages are very small at the intermediate and high concentrations of Tg and become more conspicuous at the two most dilute Tg concentrations.

SDR-mediated release using different fuel strands

Although all results shown so far were obtained using the β'-20 fuel strand, both SDR formats were found to work with almost similar yields as indicated in Fig. S7. Following the general procedure for the SDR-mediated Tg FIA on beads and using 100.0 μL conjugate (20 μg/mL) in HPN and 150.0 μL Tg (1.0 μg/mL) in HBS-P, three different release experiments were conducted using α'-25, β'-20, and α'-25+β'-20 as fuel strands (all at 5.0 μM). It should be noted that α'-25 and β'-20 have 13 complementary base pairs and form a partial duplex as soon as they are mixed together. Nonetheless, the release efficiency for the mixture remains almost the same as the ones for the each fuel strand alone.

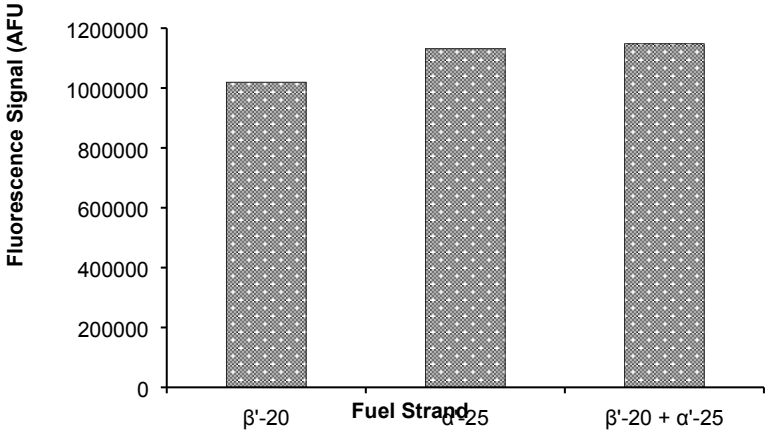

Fig. S7: Both SDR formats will bring about very efficient releases at [Tg]= 1.0 μg/mL in buffer.

When the same experiments were repeated with Tg in the bovine serum at a much lower concentration of 62.5 μg/mL similar results were obtained (Fig. 5).

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