Supporting Information for

Antibody-templated assembly of an RNA mimic of Green Fluorescent Protein

Alessandro Bertucci[#], Alessandro Porchetta[#], and Francesco Ricci*

Department of Chemistry, University of Rome Tor Vergata, Via della Ricerca Scientifica 1, 00133, Rome, Italy.

Corresponding Author
francesco.ricci@uniroma2.it

Table of Contents

Experimental Procedures

Materials and Methods

Results and Discussion

Supporting figures

Materials and Methods

Chemicals

Antibodies were purchased from Roche Diagnostic Corporation, Germany (sheep polyclonal anti-digoxigenin anti-Dig, anti-digoxigenin Fab fragments), Sigma-Aldrich, USA (mouse monoclonal anti-2,4-dinitrophenol anti-DNP), Bio-Rad, UK (mouse polyclonal anti-Salmonella), Zeptometrix Corporation, USA (murine monoclonal anti-p17, anti-HIV antibody). All the antibodies were aliquoted in PBS pH 7.4 and stored at 4°C for immediate use or at -20°C for long-term storage. RNAse free water, Potassium Chloride, Magnesium Chloride, and HEPES buffer were purchased from Sigma-Aldrich (USA). RPMI 1640 with L-glutamine was purchased from Lonza Ltd (Basel, Switzerland). Fluorophore 3,5-difluoro-4-hyroxybenzylidene imidazoline (DFHBI) was provided by Tocris Bioscience (Bristol, UK). Unmodified and ligand-tagged RNA oligonucleotides were synthesized and purified (HPLC purification) by IBA GmBH (Gottingen, Germany) and used without further purification. The sequences of the RNA constructs are reported below.

RNA sequences

Spinach aptamer

5'- GACGCGACUGAAUGAAAUGGUGAAGGACGGGUCCAGCUGCUUCGGCAGCUUGUU
GAGUAGAGUGUGAGCUCCGUAACUAGUCGCGUC - 3'

Dig-modified Split-1

5' - DIG - *UUUUUUUUUU*GACGCGACUGAAUGAAAUGGUGAAGGACGGGUCCAGCUGC
UU - 3'

Dig-modified Split-2

5' - CGGCAGCUUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUCGCGUC*UUUUUUUU*
UU - DIG - 3'

DNP-modified Split-1

5'- DNP - *UUUUUUUUUU*GACGCGACUGAAUGAAAUGGUGAAGGACGGGUCCAGCUGC
UU - 3'

DNP-modified Split-2

5' - CGGCAGCUUGUUGAGUAGAGUGUGAGCUCCGUAACUAGUCGCGUC*UUUUUUUU*
UU - DNP - 3'

In italic are reported the 10-uracil-long tails introduced to enhance flexibility of the system and favor the assembly process.

Buffer conditions
All RNA oligomers were stored (100 μM) and used in a buffer containing 40 mM HEPES pH 7.4, 125 mM KCl and 1 mM $MgCl_2$. DFHBI was stored as a 1 mM solution in the same buffer containing 5% DMSO. All antibodies were solubilized in phosphate buffer saline PBS pH 7.4 (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.47 mM KH_2PO_4)

Fluorescence measurements. Fluorescence measurements were carried out on a Cary Eclipse Fluorimeter (Varian), setting excitation wavelength to λ = 460 nm ($slit_{ex}$= 10 nm, $slit_{em}$ = 10 nm) and using quartz cuvettes of reduced volume (100 μL). All measurements were performed at T = 37°C. When collecting fluorescence emission spectra, the RNA strands Split-1 and Split-2 were mixed together to a final concentration of 20 nM each (or other concentrations when stated in the text), the relevant antibody was added at the desired final concentration (in the range 0.1 nM - 100 nM), the mixture was incubated for 10 min at 37°C, and eventually DFHBI was added at a final concentration of 10 μM (unless otherwise noted). The fluorescence emission spectrum of DFHBI alone at the relevant concentration was always acquired and subtracted as reference blank sample. Kinetics measurements were carried out under the same conditions (λ_{exc} = 460 nm, λ_{em} = 500 nm). Fluorescence measurements relative to DFHBI binding (Figure 2D in the main text and Figure S1) were obtained by consecutive additions of the fluorophore (range 0.01 μM - 30 μM) to, respectively, 20 nM solutions of the original Spinach aptamer, Split Spinach (i.e. Split-1 + Split-2), Split Spinach + antibody (20 nM), Split Spinach + anti-Dig Fab Fragment (100 nM).

Data analysis
Data for binding and affinity curves were analyzed in OriginPro (OriginLab™). Binding curves (Fig. 2D and 3C) were obtained by plotting signal gain % (SG%) vs concentration of antibody in solution, and fitting the data with the following Hill-type equation:

$$SG\% = \frac{A * [antibody]^n}{{K_{1/2}}^n + [antibody]^n}$$

Where A is the maximum signal gain ($SG\%_{max}$), n is the Hill coefficient, and $K_{1/2}$ is the equilibrium antibody concentration at half-maximum signal gain.
Signal Gain % is calculated from the enhancement of fluorescence intensity achieved upon the addition of the assembling antibody, relatively to the initial Split Spinach mixture.

Affinity curves for DFHBI (Figure 2B) were obtained by plotting fluorescence intensity vs concentration of the fluorophore, and fitting the data with the following Langmuir-type equation:

$$I_{Fluo} = a + b * \frac{[DHFBI]}{K_d + [DHFBI]}$$

where K_d is the dissociation constant, *a* and *b* are fitting parameters of the Langmuir equation.

Fluorescence Microscopy

Three different solutions were prepared as follows: a) DFHBI (10 μM), b) Split Spinach (20 nM) + DHFBI (10 μM), c) Split Spinach (20 nM) + antibody (20 nM) + DFHBI (10 μM). A single drop (1.5 μL) of each solution was deposited on a black plastic substrate and imaged using an Axio Scope A1 ZEISS microscope. The emitted photons were collected by a 10x air objective (ZEISS) and a monochrome CCD camera (Axiocam 503 mono - ZEISS). The images were analyzed using ZEN 2 lite (ZEISS) software.

HeLa whole cell lysate.

HeLa Whole Cell Lysate was a gift from the Department of Biology of the University of Rome Tor Vergata. Briefly, HeLa cells were cultured until confluence in Dulbecco's modified Eagle's medium (Lonza, BE12-604F) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, 10270-106), and 1% penicillin-streptomycin solution (Lonza, 17-602 E) at 37 °C under 5% CO2. Cells were then lysed in RIPA buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 1% deoxycholate sodium, 0,1% sodium dodecyl sulfate, 5 mM MgCl2) plus protease inhibitor cocktail (Sigma Aldrich, P8340).

Supporting figures

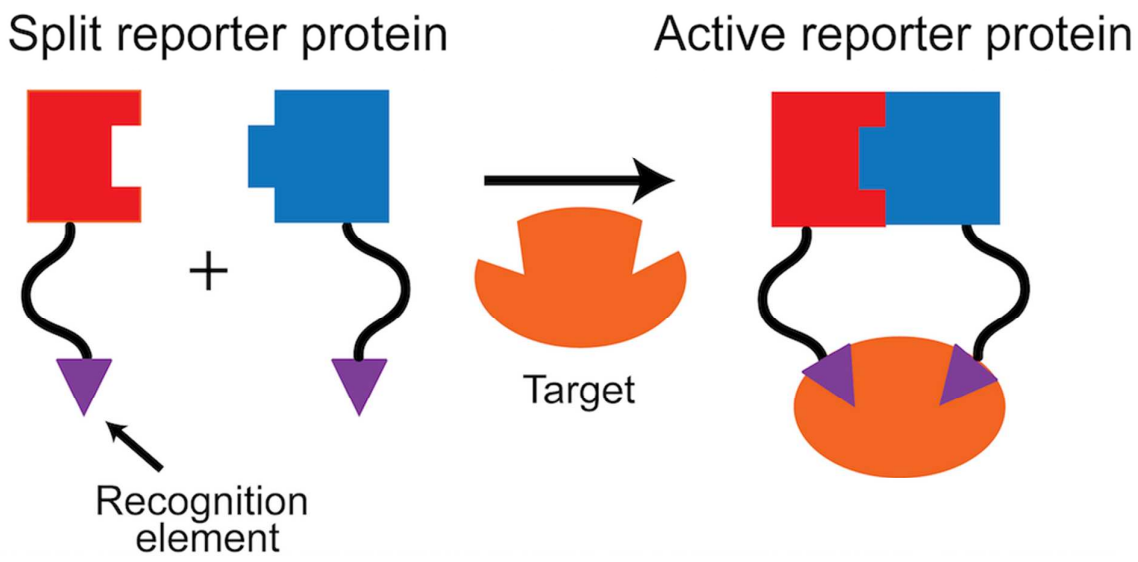

Figure S1. Protein-fragment complementation assays allow for probing biomolecular interactions through the use of a reporter protein split into two pieces, each one tethered to a recognition element. Inspired by this strategy, we have conceived our split Spinach aptamer assay (Fig. 1B).

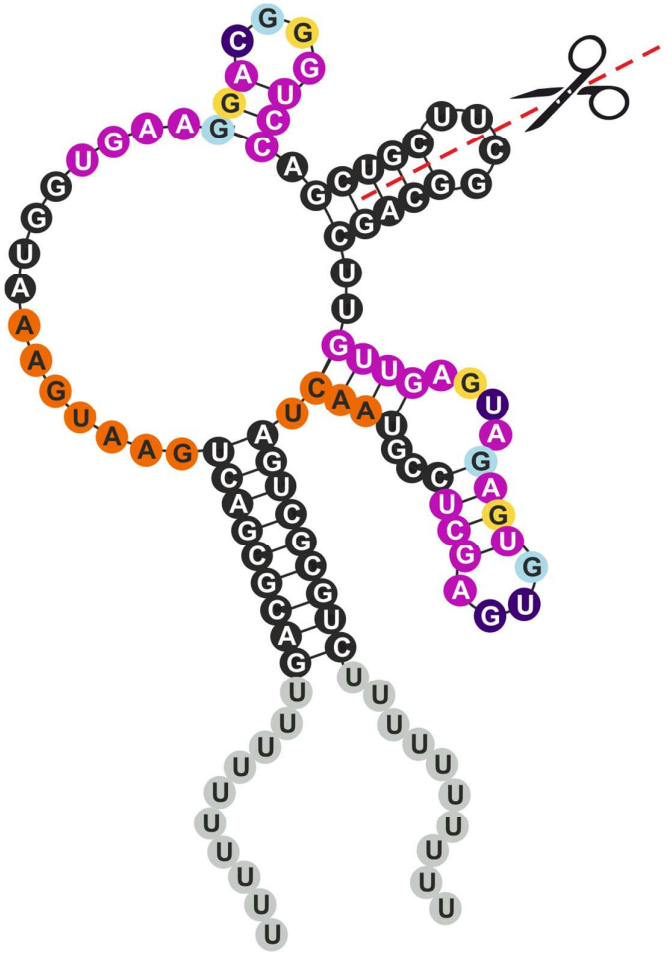

Figure S2. Illustration of the sequence of the split Spinach aptamer used in this work, including additional poly-U tails and highlighting the position of the split point. For a simplified description, we used here the previously proposed *in silico* model of the aptamer. Nevertheless, nucleotides are shown in different color to match with the real base pairing in the crystal structure as reported in *Warner, K. D.; Chen, M. C.; Song, W.; Strack, R. L.; Thorn, A.; Jaffrey, S. R.; Ferré-D'Amaré, A. R.* Nat. Struct. Mol. Biol. ***2014**, 21, 658-663.*

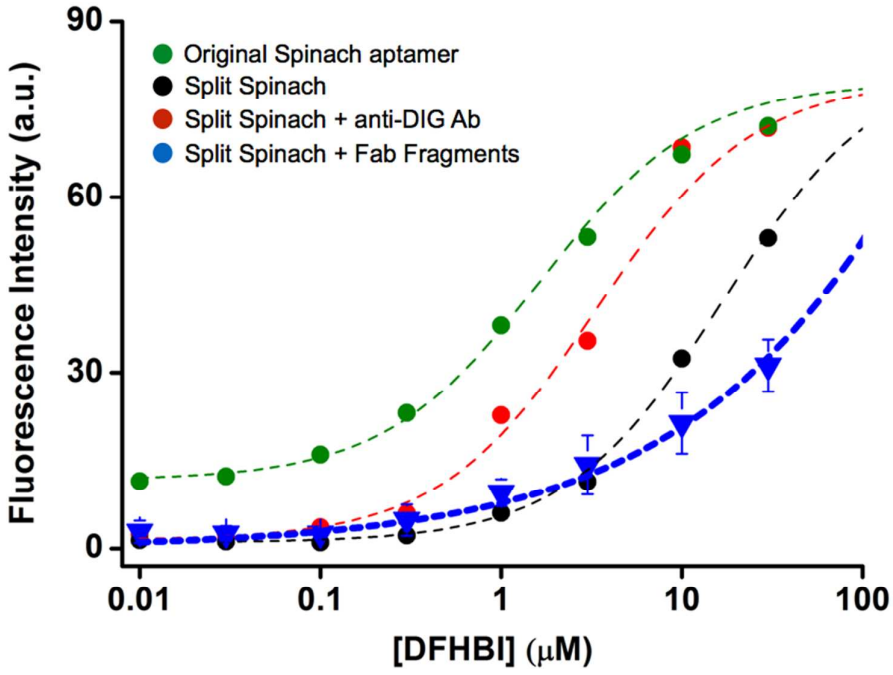

Figure S3. Binding curve for DFHBI of the Split Spinach system in presence of saturating concentration (100 nM) of anti-Dig Fab fragments (blue profile) compared to the other curves reported in Figure 2A. Fab fragment, having a single binding site for digoxigenin, does not trigger any co-localization-induced assembly of the anti-Dig-tagged RNA strands (see red line for anti-Dig antibody). The affinity for DFHBI is even slightly reduced compared to the Split Spinach configuration, which is probably due to steric hindrance affecting the association of the RNA strands bound to the antibody fragments.

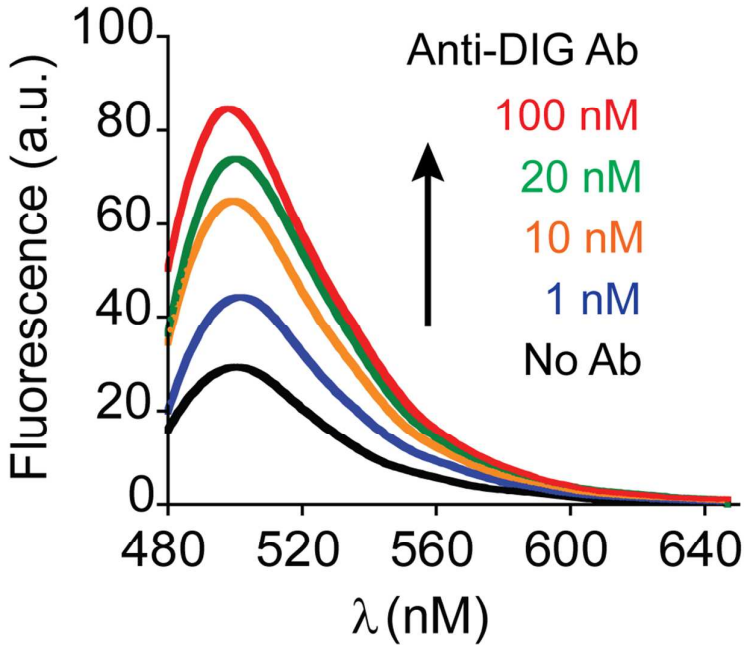

Figure S4. Fluorescence emission spectra of the assembled Spinach-DFHBI complex as a function of the concentration of the Anti-DIG antibody. The experiments are obtained in phosphate buffer saline PBS buffer, pH 7.4, 37 °C, using an equimolar concentration of the split strands ([split 1] = [split 2] = 20 nM) and a concentration of DFHBI of 10 μM. The experimental values represent averages of three separate measurements.

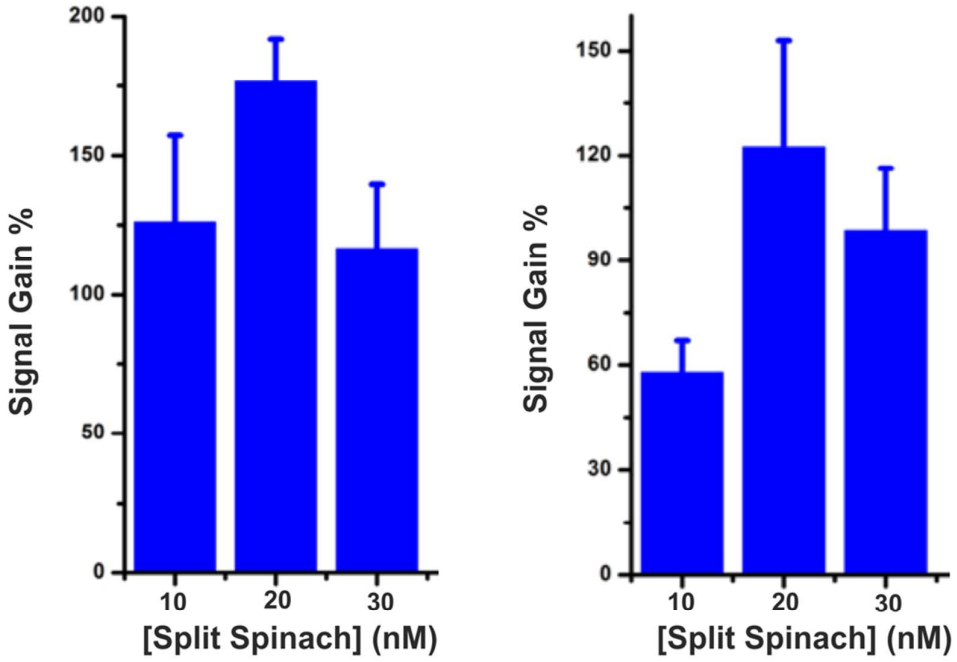

Figure S5. Signal Gain % as function of concentration of split Spinach in presence of respective equimolar concentrations of Anti-Dig antibody, i.e. 10, 20, 30 nM (n = 3). Left) [DFHBI] = 3 μM. Right) [DFHBI] = 10 μM. A concentration of 20 nM of split Spinach offers the best performance in terms of signal gain %, namely the best trade-off whit regards to signal-to-noise ratio (S/N). A higher split Spinach concentration, i.e. 30 nM, leads to a more favorable assembly of the two split halves without the need of templating antibody, which results in a higher noise contribution. The enhancement of fluorescence intensity triggered by the antibody-templated mechanism becomes at this point not as large as to proportionally counterbalance the background, which eventually leads to a reduced S/N compared to that at a lower concentration of 20 nM.

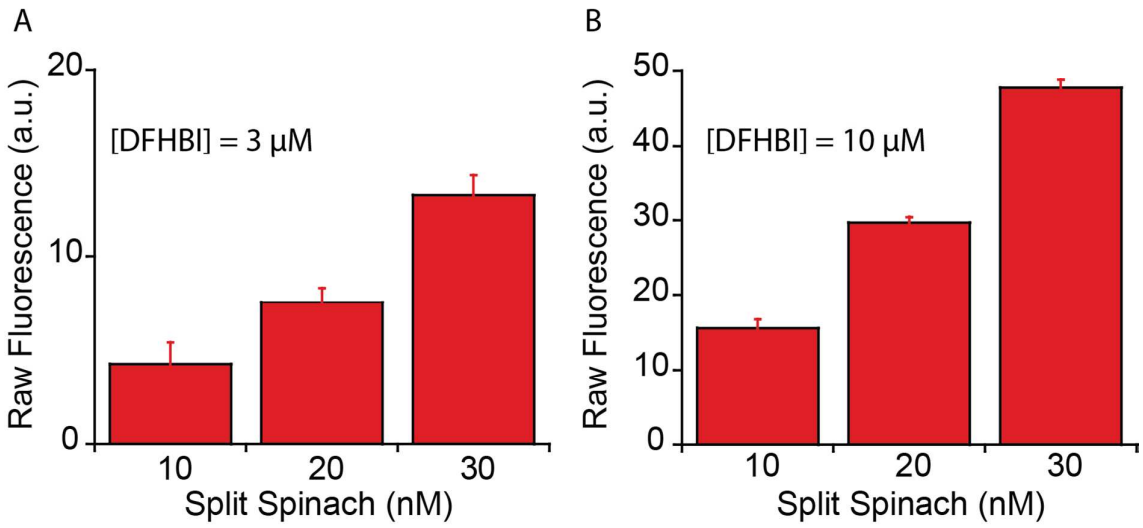

Figure S6. Raw fluorescence intensity of split Spinach in the concentration range 10 – 30 nM, namely background fluorescence. A) DFHBI concentration is 3 µM; B) DFHBI concentration is 10 µM. The high values registered at 30 nM cause the drop of signal gain % shown in Figure S5.

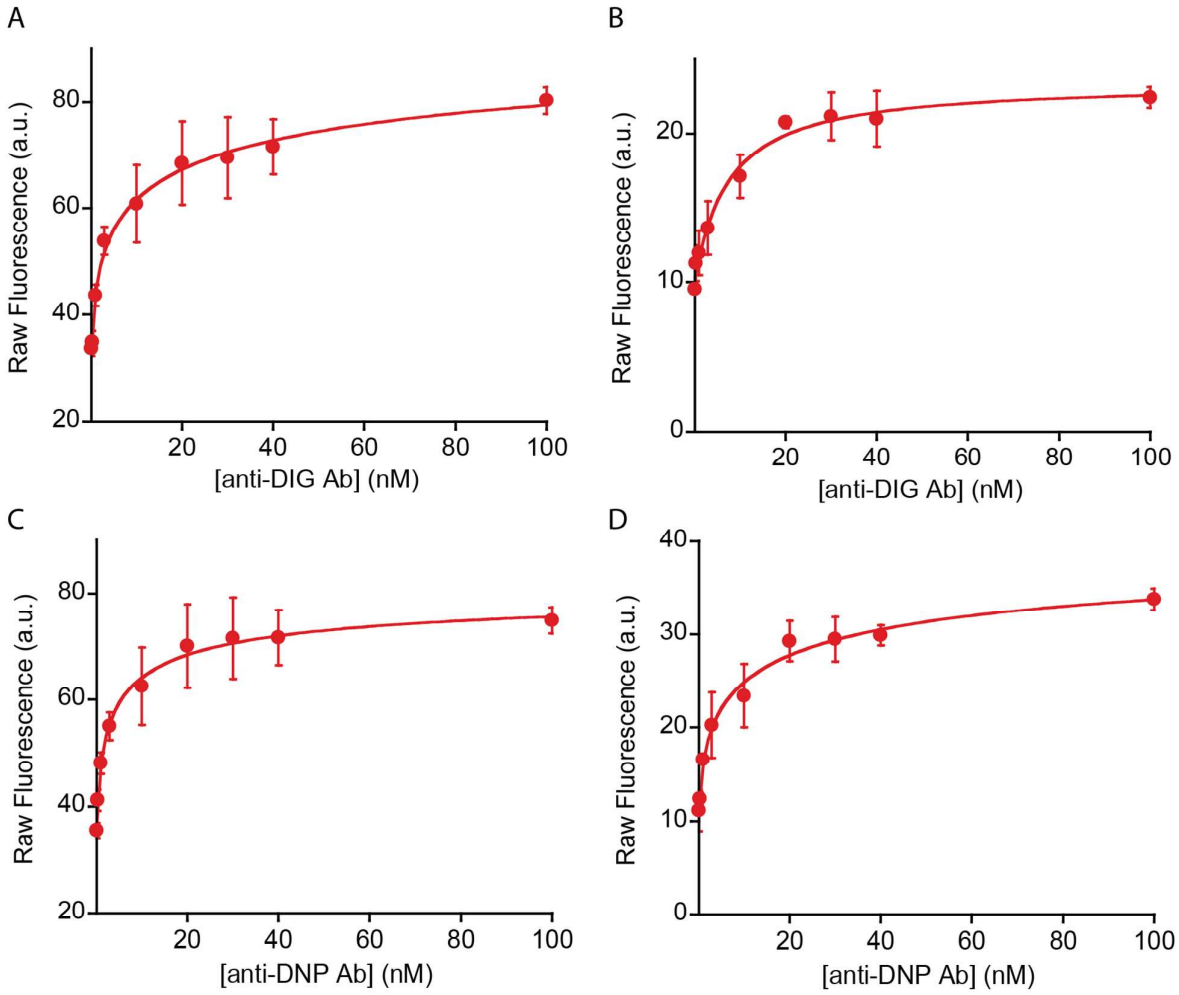

Figure S7. Binding curves for increasing antibody concentrations. Raw fluorescence intensity is reported on the y axis. A) DFHBI 10 μM, Split Spinach 20 nM, Anti-Dig Antibody; B) DFHBI 3 μM, Split Spinach 20 nM, Anti-Dig Antibody, C) DFHBI 10 μM, Split Spinach 20 nM, Anti-DNP Antibody, D) DFHBI 3 μM, Split Spinach 20 nM, Anti-DNP Antibody.