

Supporting Information

Hydrogel embedded quantum dot-transcription factor sensor for quantitative progesterone detection

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2. SI Materials and Methods

2.1. Measurements. ^1H -NMR and ^{13}C -NMR spectra were acquired at 400 MHz using a Varian INOVA-400 spectrometer (Agilent Technologies, Inc., CA) maintained at 25 °C. Scanning electron microscopy (SEM) images were acquired using a Zeiss SUPRA 40VP operating at an accelerating voltage of 3.0 kV. Atomic force microscopy experiments were analyzed using Molecular Force Probe 3D Instrument (MFP-3D, Asylum Research, CA). Rheological measurements were obtained on an AR 1000 rheometer (TA Instruments, DE). Diffusion experiments were analyzed using an FV1000 scanning confocal microscope (Olympus Corporation of the Americas, PA). Fluorescence spectra were measured on a Nanolog spectrofluorometer (HORIBA, Ltd., NJ), equipped with a plate reader. Intensity was recorded in relative fluorescence units, with baseline correction according to manufacturer's instruction.

2.2. Materials. Monomers, 4-arm-PEG-NH₂ (Item # 4arm-PEG-NH2-10K-5g, MW=10 kDa, HCl salt) and NHS-PEG-NHS (Item # SVA-PEG-SVA-3400-5g, MW=3400 Da), were purchased from Laysan Bio (Arab, AL). MatTek 35mm glass bottom petri dish, Invitrogen™ UltraPure™ Salmon Sperm DNA Solution, Slide-A-Lyzer™ MINI Dialysis Device (10K MWCO), Corning™ 384-Well Nonbinding Surface (NBS™) Microplates were purchased from Fisher Scientific (Pittsburgh, PA). Progesterone (PRG), deuterated dimethyl sulfoxide (DMSO-*d*₆), deuterium oxide (D₂O), fluorescein sodium salt, sodium borate, boric acid, HEPES, magnesium chloride, glycerol, Tris hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). Phosphate buffered saline (PBS 1x) was purchased from Life Technologies (Grand Island, NY). Sterol responsive allosteric transcription factor (SRTF1-his₆) was produced from *E. Coli*

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according to a previous procedure,¹ and CdSe/CdS/ZnS core/shell/shell quantum dots (quantum yield = 27 ± 3 % in HEPES) were synthesized according to a previous protocol.¹ Oligonucleotides were purchased from Integrated DNA Technologies, Inc. (Coralville, IA) and were HPLC purified by the manufacturer. Olympus AC240TS-R3 AFM probes were purchased from Bruker Nano Inc. (Camarillo, CA). 1X HEPES is a solution of 25 mM HEPES and 150 mM NaCl, adjusted to pH 7.6. 1X borate buffer is a solution of 100 mM boric acid, 25 mM sodium borate, and 75 mM NaCl, adjusted to pH 8.6.

2.3. Hydrogel Synthesis. Hydrogels were prepared using a modified previous protocol at four different weight percentage.² Variance of weight percentage permitted tunable control of the base polymer network density. To form hydrogel, a solution of 4-arm-PEG-NH₂ in borate buffer at pH 8.6 was mixed with a solution of NHS-PEG-NHS in phosphate buffered saline (PBS 1x) buffer at pH 7.4. The molar ratio of amine to the NHS was 1:1, and the total concentration of the polymer in solution was 5, 10, 25, and 50 wt%, respectively. Structure and purity of polymers were confirmed using ¹H NMR and ¹³C-NMR spectroscopy.

2.4. Nanotopology Characterization. We evaluated the pore size of lyophilized hydrogel through scanning electron microscopy (SEM). The hydrogels were prepared as outlined above, and lyophilized overnight, before being mounted onto stubs, gold coated, and analyzed using a Zeiss SUPRA 40VP scanning electron microscope at an acceleration voltage of 3.0 kV. Pore sizes were measured with ImageJ. We evaluated the morphology of the dried and hydrated hydrogel samples. The atomic force microscopy (AFM) images were taken using Molecular Force Probe 3D Instrument. The

hydrogels were prepared as outlined above. One group of 10 wt% hydrogels were dried under ambient conditions. A rectangular silicon nitride tip on a soft cantilever was chosen to minimize the damage to the samples. The images were collected in tapping mode for dried and hydrated samples. The roughness was calculated from root-mean-square variation of the height (in z-direction) over the area of $5 \times 5 \mu\text{m}^2$ on three different locations.

2.5. Hydrogel Swelling Studies. Cylindrical hydrogel samples (20 mm diameter, 3 mm thickness) were immersed in 50 mL PBS (1x, pH 7.4) for 15 days. At predetermined time point, weights of the hydrogel were measured. The swelling degree was calculated by dividing the weight change of hydrogel after swelling by its weight before swelling.³

2.6. Hydrogel Mechanical Properties. For rheological experiments, a TA Instruments RA 1000 rheometer was used at 20 °C. The hydrogel of 20 mm diameter, 3 mm thickness was synthesized in mold in a moisture chamber at room temperature. The oscillatory stress sweep was performed from 0.1 to 100 Pa at a frequency of 1 Hz. The frequency sweep was measured from 0.1 to 10 Hz at a controlled oscillatory stress of 1 Pa. A normal force of 0.1 N was applied to the hydrogel using 20 mm geometry. The hydrogels were then incubated in PBS (1x, pH 7.4) for 4 days. The oscillatory stress sweep and frequency sweep were performed again after swelling.

2.7. Diffusion Studies. To characterize the diffusion of biomolecules within the hydrogels, we determined the diffusion coefficient of entrapped fluorescein, oligonucleotides, and transcription factor by fluorescence recovery after photobleaching

(FRAP). Hydrogels (0.3 mL) were loaded with 10 μ M fluorescein, 1 μ M Cy5-DNA, 0.25 μ M Cy5-TF, respectively, and imaged at 25 °C on an Olympus FV1000 scanning confocal microscope equipped with a 488 nm and 633 nm laser, for fluorescein and Cy5 excitation, respectively. A second (405 nm) scan head supplied the 5 s bleach pulse. Excitation laser power was set to 5%, and fluorescence images were collected. For image acquisition, 100 time series images were obtained (including 5 pre-bleach images) for each molecule. Diffusion coefficients were calculated by importing images into MATLAB and calculating the average intensity as a function of time within the region of interest (ROI). The ROI average intensity versus time was fit to a Gaussian model (step size = 0.001), according to the equations given by Jain et al.,⁴ to arrive at the diffusion coefficient, D , in cm^2/sec .

Diffusion coefficients of QD and QD-TF were acquired during nonsteady-state diffusion experiments. 1 mL of 100 nM QD or QD-TF were added on top of 100 μ L hydrogel polymerized in MatTek 35mm glass bottom petri dish. The vertical cross section images were taken at 615 nm emission ($\lambda_{\text{exc}} = 405 \text{ nm}$) continuously over a period of 30 min with Olympus FV1000 scanning confocal microscope. The intensity profiles were fit to Fick's second law, to arrive at the diffusion coefficient, D , in cm^2/sec .

2.8. Sensor Assembly in Hydrogel. Based upon our prior experience, we selected a molar ratio of QD/TF/DNA = 1/4/18.¹ 320 μ L of 0.15 μ M QDs in 1X borate buffer were mixed with 320 μ L of 0.6 μ M SRTF1-his₆ in 1X HEPES, at room temperature for 45 min. 320 μ L of 2.7 μ M double-stranded Cy5-labelled DNA (Table S1) in 1X HEPES was added to the mixture. After 30 min, 248 μ L of borate buffer, and 372 μ L of 5X binding buffer (25 mM MgCl_2 , 25% glycerol, and 250 mg/L Invitrogen™

UltraPure™ Salmon Sperm DNA in 0.1 M Tris-HCl) were added and the mixture was incubated for 15 min at room temperature. Solutions of 90 mg 4-arm-PEG-NH₂ and 60 mg NHS-PEG-NHS, respectively, were prepared in 750 μ L mixture solution. Hydrogel formation was carried out by mixing 25 μ L of each macromonomer solution for each sample. Analogously, QD-based FRET system in solution was prepared using the same ratio, without any pegylated macromolecules.

2.9. Repeated Progesterone Sensing. To apply analyte to the sensor, the sensor was transferred to Slide-A-Lyzer™ MINI Dialysis Device and dialyzed against PRG solution overnight (Figure 1A and B). With the presence of PRG, the transcription factor unbinds its cognate DNA site. (Figure 1C and D). The sensor was transferred back to the plate reader to read the sensor output in the presence of PRG. The sensor was then dialyzed against HEPES overnight to remove PRG for another measurement. This cycle was repeated.

2.10. Sensor Evaluation. Fluorescence measurements were recorded for each respective 50 μ L of the sensor (in hydrogel or aqueous solution) in a black 384-well plate on a Nanolog spectrofluorometer), equipped with a plate reader. The fluorescence intensity was monitored from 550 nm to 750 nm (λ_{exc} = 400 nm) with a 450 nm long-pass filter before the emission detector (Figure 1E). The overall fluorescence spectra were deconvolved into two gaussians, one for the FRET donor fluorescence spectra and one for the acceptor, in order to calculate F_A/F_D . The sensor output is the normalized ratio of the areas of acceptor emission over donor emission (F_A/F_D).¹

The sensor output, defined as the ratio of the areas of acceptor emission over donor emission (F_A/F_D), is calculated using the integrals of the peak emissions and

plotted to determine the linear range of the sensors. F_A/F_D ratios are fit to a four-parameter logistic function:

$$S(c)=S_1 + (S_0- S_1)/(1+10^{((\text{LogEC}_{50} - c)*h)}) \quad (1)$$

where S_0 is the intensity of the sensor signal with no analyte present, S_1 is the intensity of the sensor signal at saturating analyte concentration, c is the analyte concentration, EC_{50} is the concentration of analyte that gives half-maximal response, and h is the Hill slope.

The limit of detection (LOD) is defined as the progesterone concentration yielding a signal greater than 3 times the pool standard deviations above background.

The linear range is defined as the range of analyte concentrations for which the biosensor response changes linearly with the concentration.⁵ The interval is determined between the bend points, where the slope of the response changes upon approaching the lower and upper plateau.⁶

2.11. Statistical Analysis. Unless otherwise indicated, data presented were generated from three independent experiments. Error bars represent standard deviation values. Multivariate analysis is performed using one-way ANOVA that is corrected using the Bartlett's variance test, and for multiple comparisons, the Bonferroni multiple-comparison test is used. Statistical analysis is performed using the GraphPad Prism software (version 7.0; GraphPad Software Inc.).

Table S1 Oligonucleotide sequences

#	Function	Sequence	After hybridization
1	Cognate DNA, contains recognition sites	5'-GAA TGA CTA GCC GAT CCG CTA GTT AGGC-3'	1 and 2 form cognate DNA labeled with Cy5
2	Complementary to 1, with Cy5 at both 3' and 5' end	5'-Cy5-GCCT AAC TAG CCG ATC GGC TAG TCA TTC-Cy5-3'	

Table S2 Sensor performance.

	EC₅₀ (nM)	R²	Hill slope	LOD (nM)	Linear range (nM)
Aqueous	292	0.98	-1.14	50	75 to 1132
Gel	223	0.98	-1.48	55	78 to 632

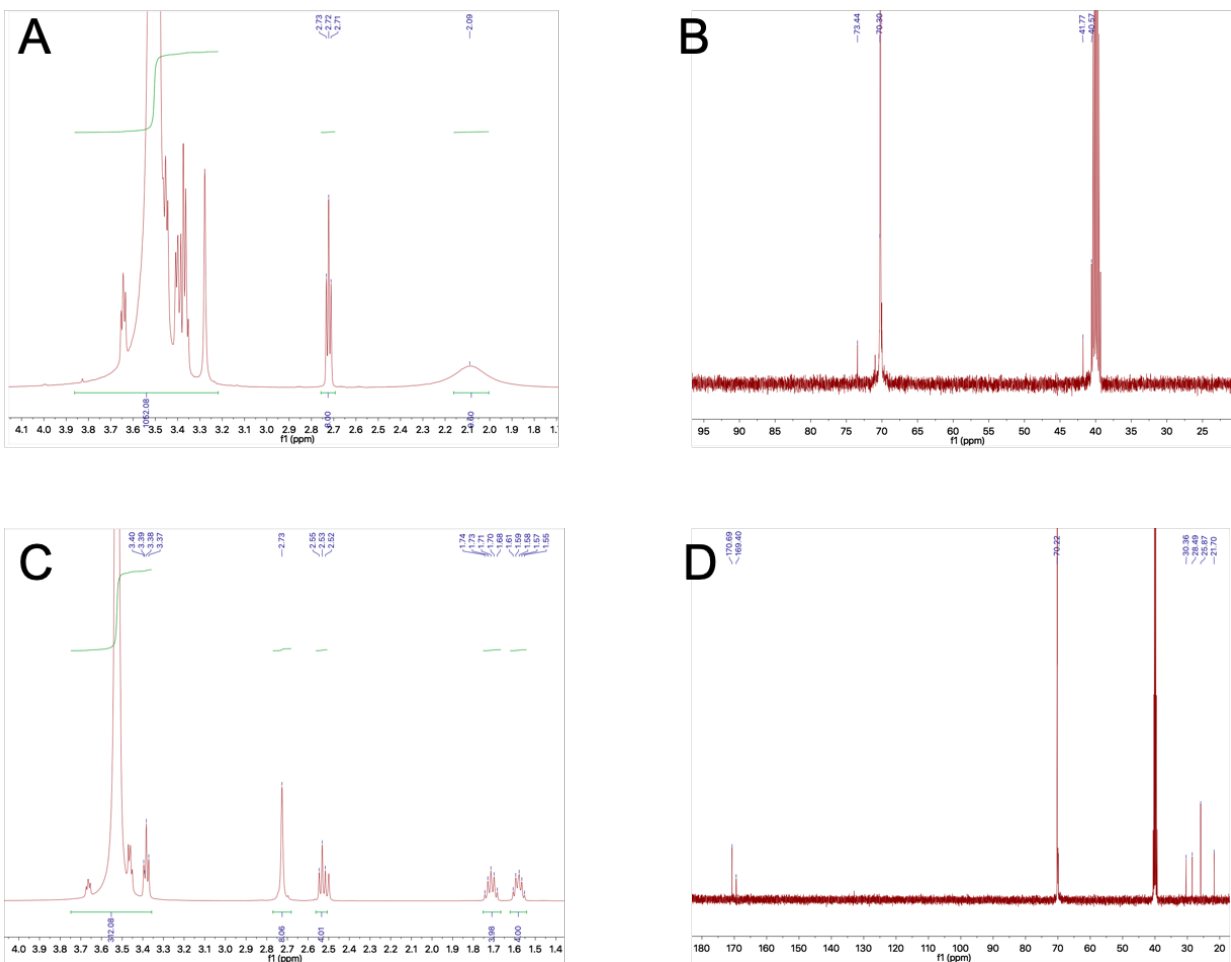


Figure S1. ^1H NMR and ^{13}C NMR of 4-arm-PEG- NH_2 and NHS-PEG-NHS.

(A) ^1H NMR (CDCl_3 , 400 MHz) of 4-arm-PEG- NH_2 : \square 3.86-3.22 (overlap, 1052H), 2.72 (t, $J=5$ Hz, 8H), 2.08 (broad, 8H) ppm.

(B) ^{13}C NMR (DMSO, 400 MHz) of 4-arm-PEG- NH_2 : \square 73.2, 70.2, 45.4, 41.7 ppm.

(C) ^1H NMR (CDCl_3 , 400 MHz) of NHS-PEG-NHS: \square 3.86-3.22 (overlap, $J = 5$ Hz, 312H), 2.73 (s, 8H), 2.53 (t, $J = 10$ Hz, 4H), 1.74-1.68 (tt, $J = 10, 5$ Hz), 1.61-1.55 (tt, $J = 10, 5$ Hz) ppm.

(D) ^{13}C NMR (DMSO, 400 MHz) of NHS-PEG-NHS: \square 170.7, 169.4, 70.2, 30.4, 28.5, 25.9, 21.7 ppm.

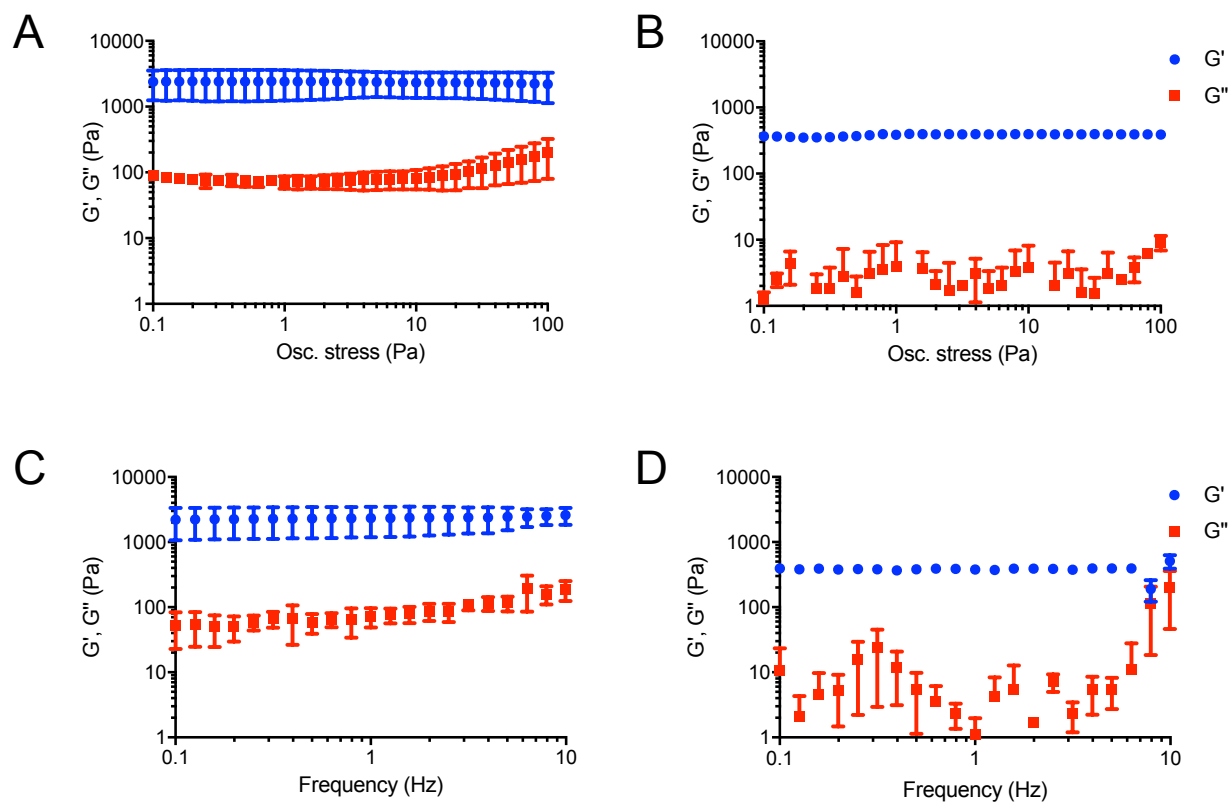


Figure S2. Mechanical properties of 5 % hydrogel before swelling (A and C), and swelled hydrogel (B and D).

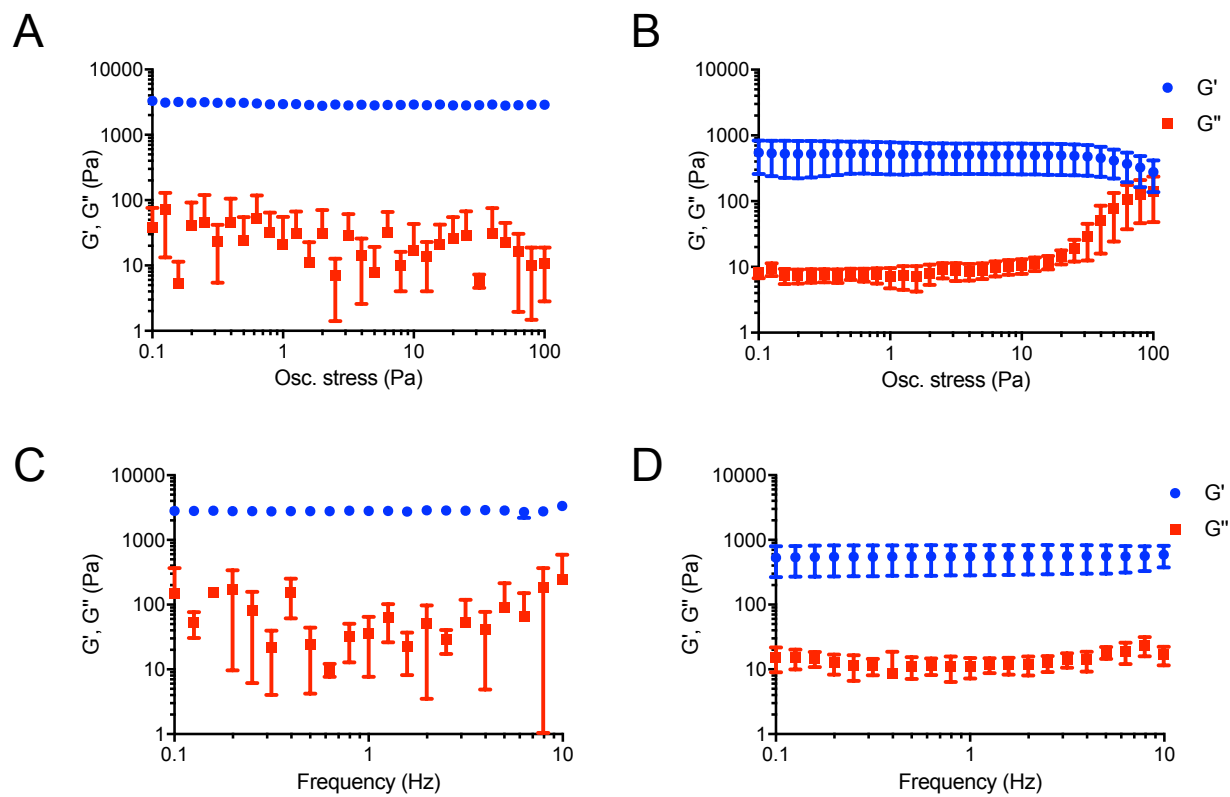


Figure S3. Mechanical properties of 10 % hydrogel before swelling (A and C), and swelled hydrogel (B and D).

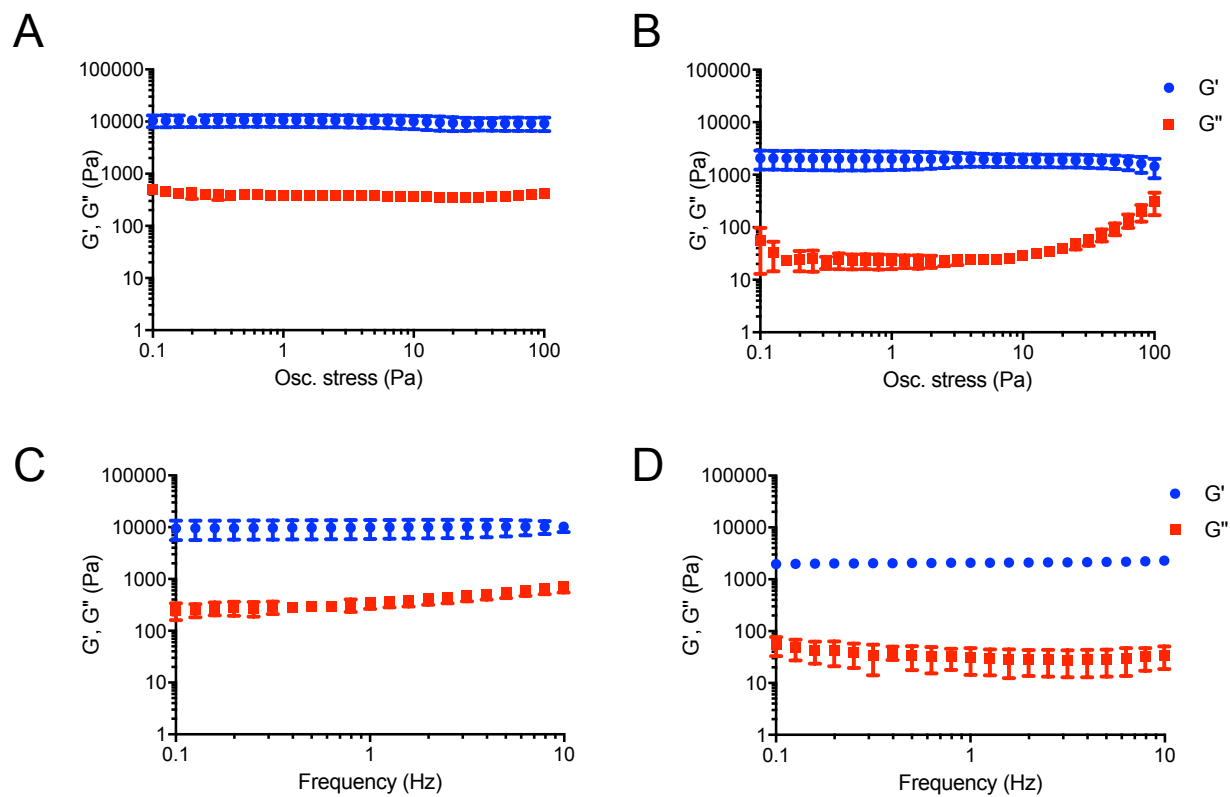


Figure S4. Mechanical properties of 25 % hydrogel before swelling (A and C), and swelled hydrogel (B and D).

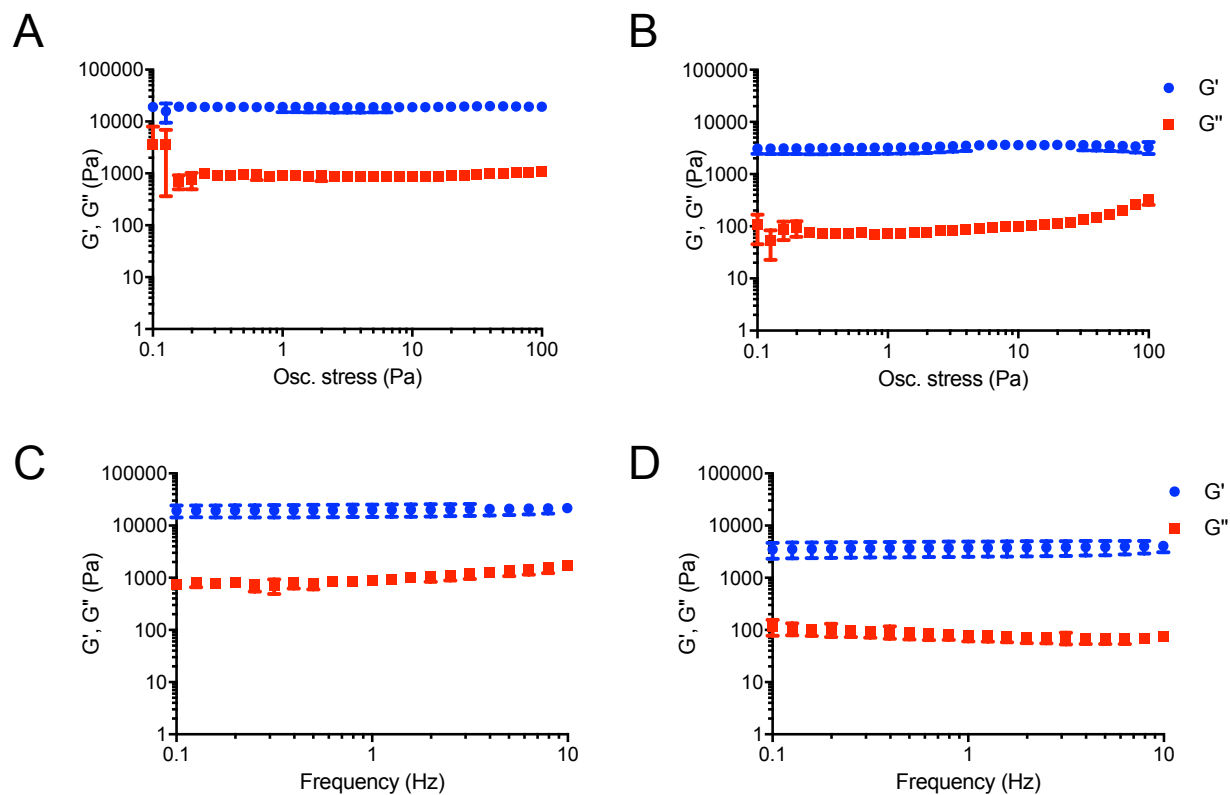


Figure S5. Mechanical properties of 50 % hydrogel before swelling (A and C), and swelled hydrogel (B and D).

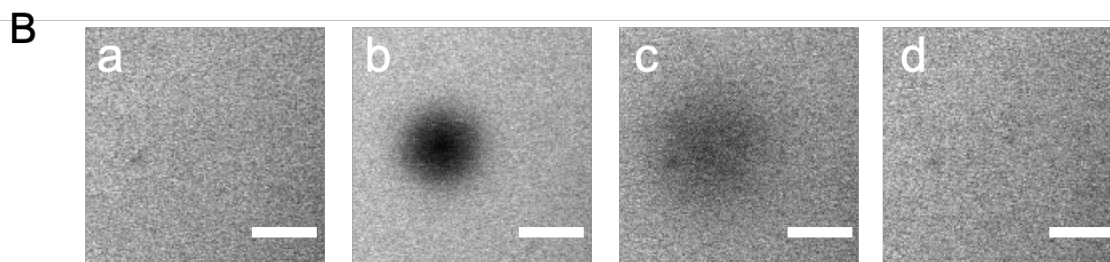
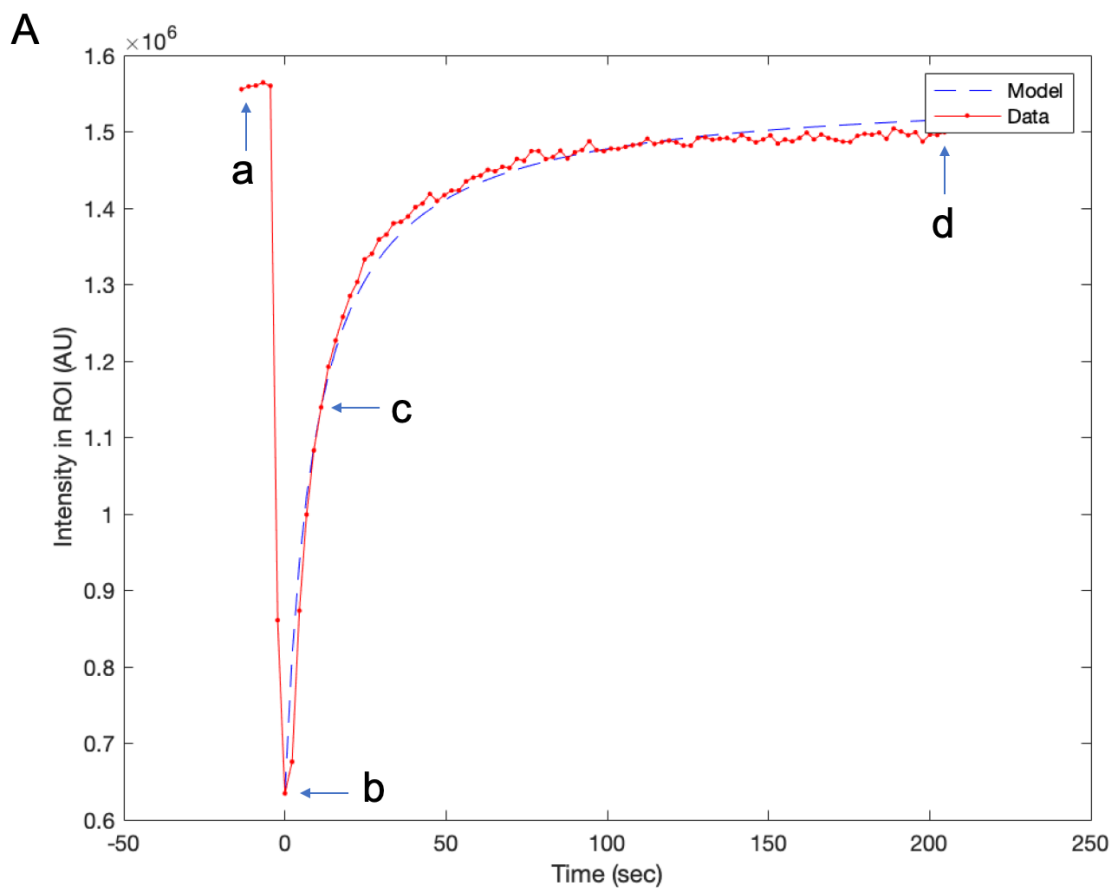


Figure S6. Diffusion coefficient measurement of fluorescein in 10 wt% hydrogel. (A) Fluorescence recovery after photobleaching (FRAP) of fluorescein. (B) FRAP images taken prior to bleaching, just after bleaching, during recovery, and after recovery. Scale bar is 100 μm .

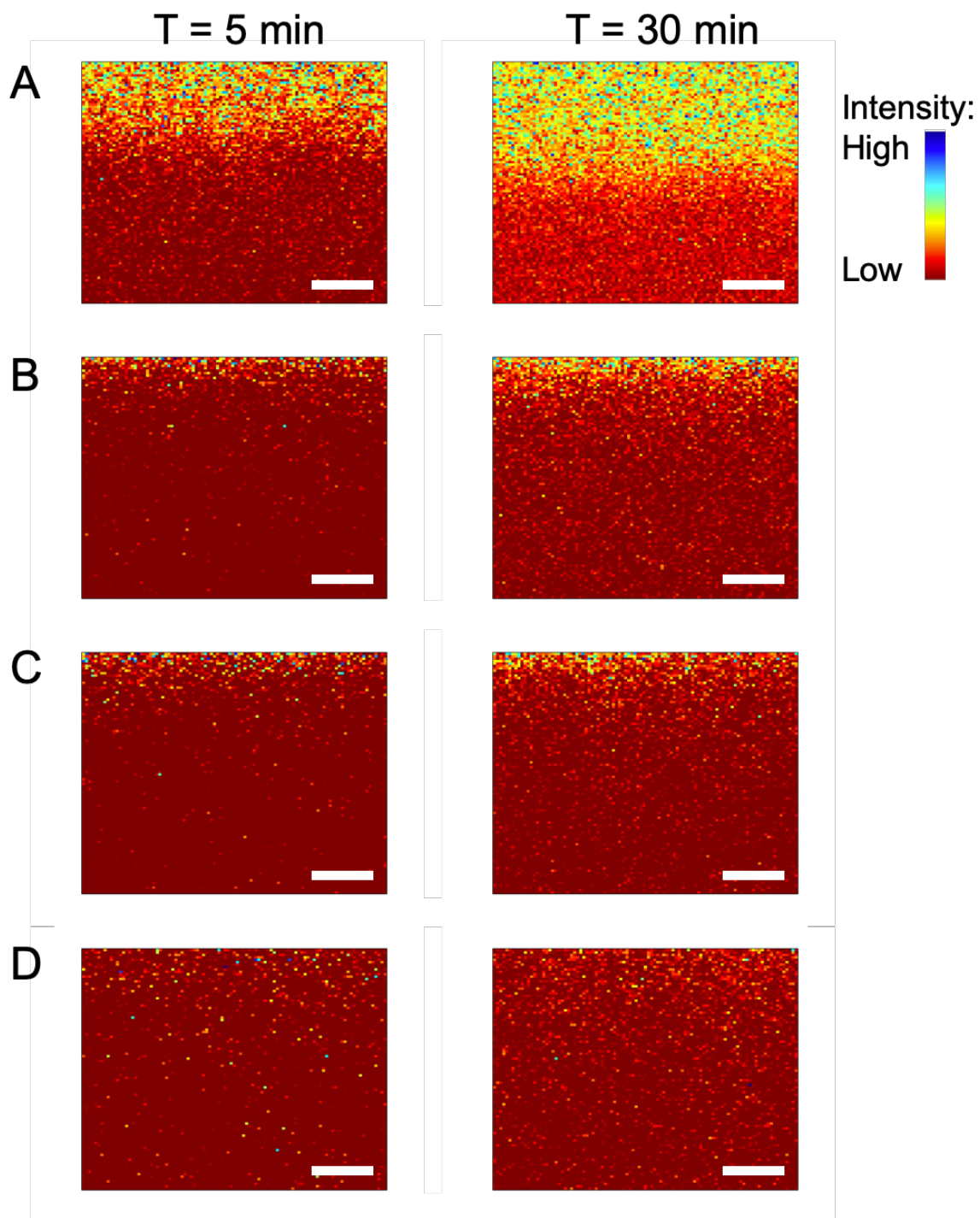


Figure S7. Diffusion of QD in hydrogel. After QD exposure, vertical cross section images at $T = 5$ min and $T = 30$ min in (A) 5 wt%, (B) 10 wt%, (C) 25 wt%, and (D) 50 wt% hydrogel. Scale bar is 50 μm .

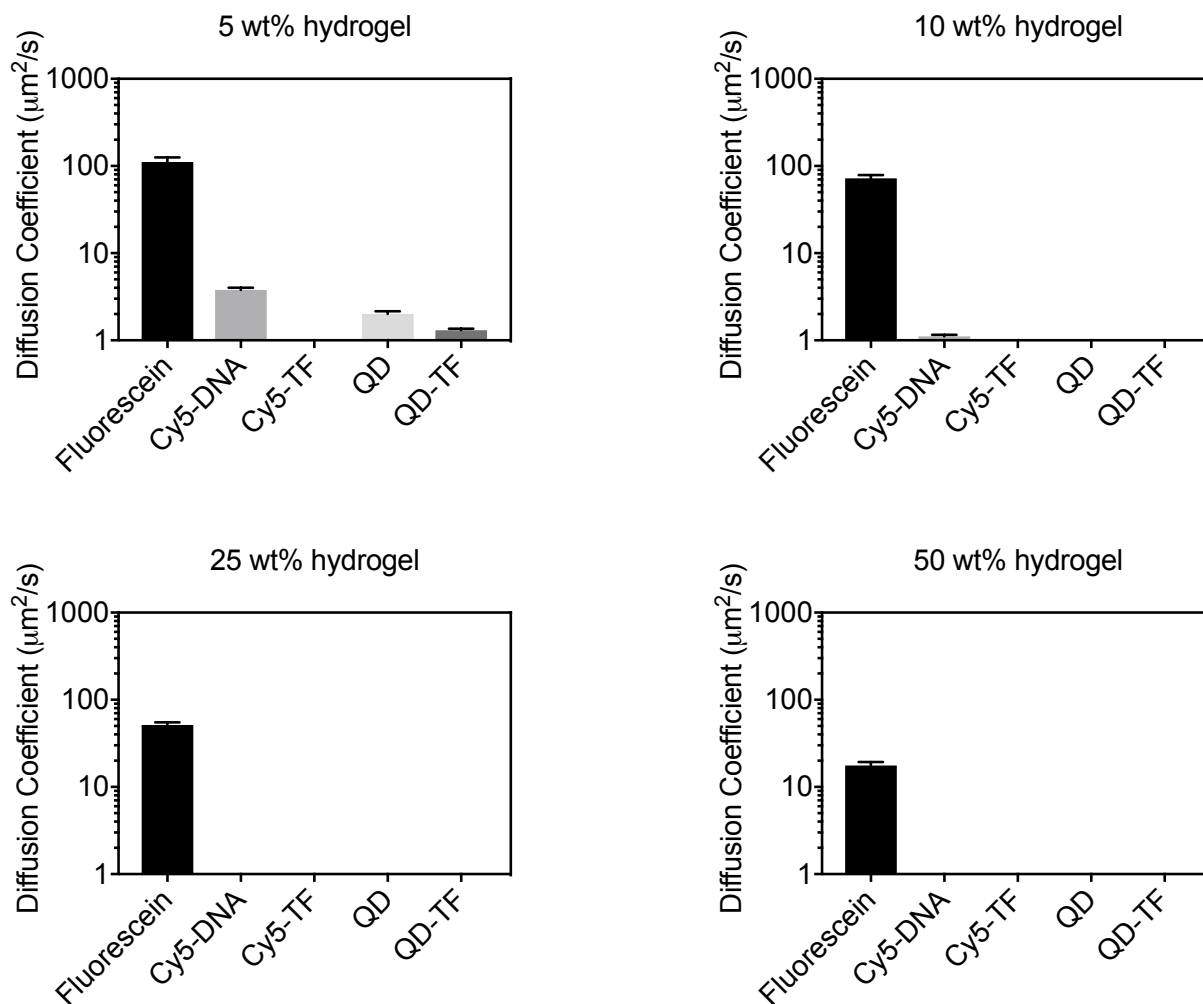


Figure S8. Diffusion coefficients of the species or sensor constituents in different wt% hydrogels.

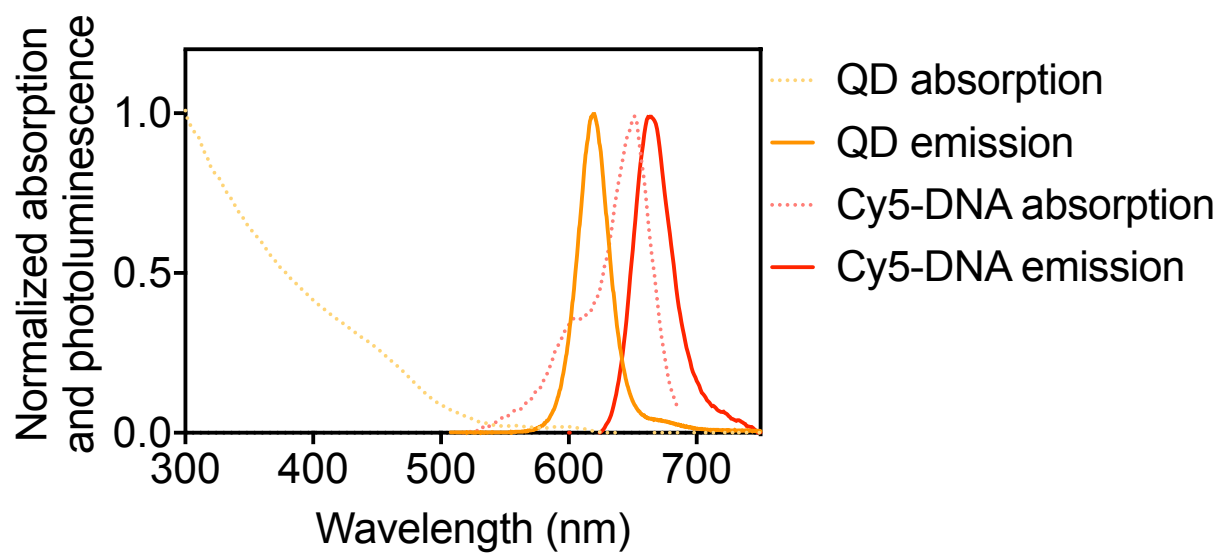


Figure S9. Absorption (dashed) and fluorescent emission (solid) of QDs (yellow, $\lambda_{\text{ex}} = 400$ nm) and Cy5-DNA (red, $\lambda_{\text{ex}} = 590$ nm) in 10 wt % hydrogel.

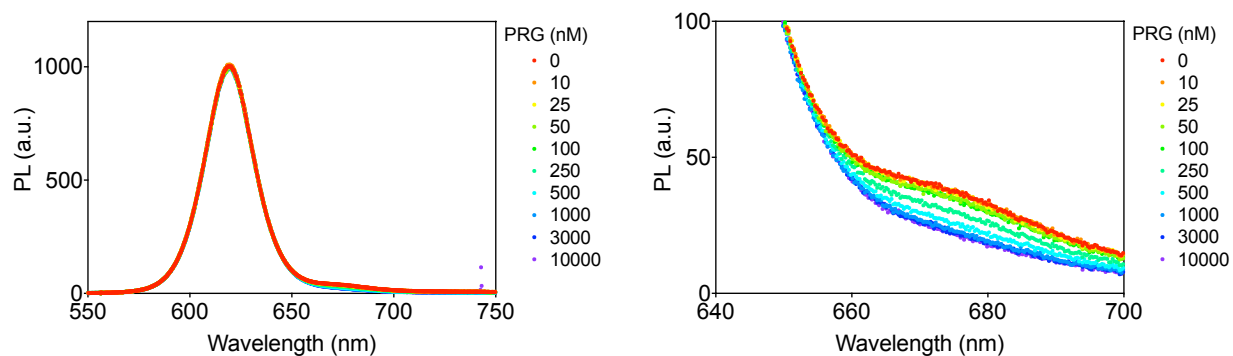


Figure S10. Hydrogel embedded sensor's response to analyte titration. Spectral data for the PRG dose-dependent change in photoluminescence intensity for the sensor. Zoomed figure was shown in the right. After progesterone addition, when TFs are not bound to the DNA, FRET is reduced, leading to increased F_D and decreased F_A .

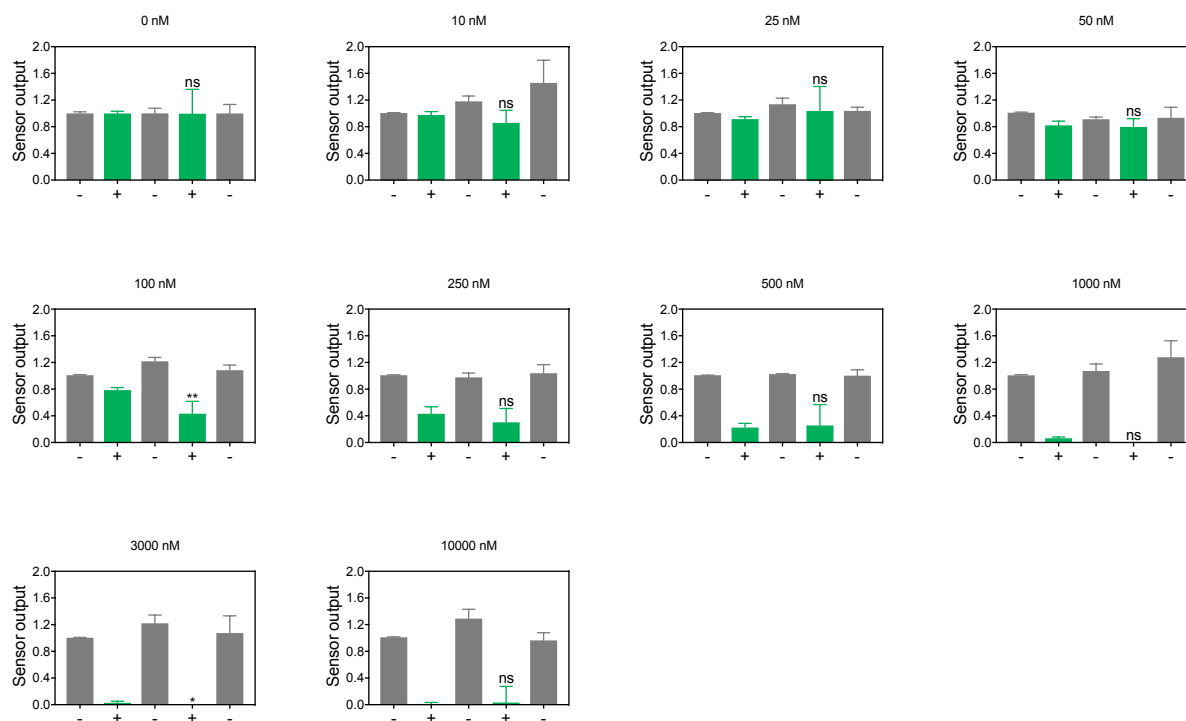


Figure S11. Reversibility of the sensor. Evolution of the sensor output after two cycles of PRG addition (+) and two cycles of PRG removal (-) to the sensor. Each panel corresponds to a specific concentration of PRG added. With PRG addition, F_A/F_D at $[PRG] = 0$ nM is normalized to 1, F_A/F_D at $[PRG] = 10000$ nM is normalized to 0, and linear interpolation is applied for concentrations in between. Without PRG addition, F_A/F_D is scaled, where F_A/F_D at $[PRG] = 0$ nM is normalized to 1. * $P \leq 0.05$. ** $P \leq 0.01$. ns, not significant, compared to respective sensor output in the same condition.

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