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Supporting Information

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A Förster Resonance Energy Transfer-Based Ratiometric Sensor with the Allosteric Transcription Factor TetR

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Thuy T. Nguyen,[a] Margaret Chern,[b] R C. Baer,[c] James Galagan,[a,c,d] Allison M. Dennis*[a,b]

Table S1. Synthetic DNA sequences for TetR binding.

Name[a]	Sequence[b]
tetO	5'-GTCA **TCCCTATCATTGATAGAGA** TACTG-3'
tetO'	3'-**C**AGT AGGGATAGTAACTATCTCT ATGA**C**-5'
scram	5'-TCGT GAAACCGAGCGAGGGACAC GCACA-3'
scram'	3'-**A**GCA CTTTGGCTCGCTCCCTGTG CGTG**T**-5'

[a] *Scram* is abbreviated for scrambled sequence; the apostrophe indicates the reverse strand.

[b] The *tetO* binding sequence is the center region shown in blue;[1] nucleotides labeled with Cy5 dye are labeled in red and underlined.

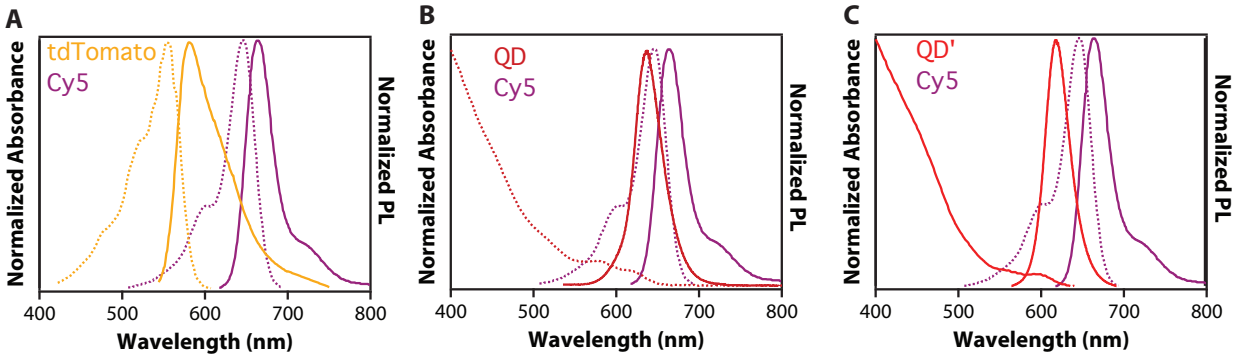

Figure S1. Normalized absorption (dashed lines) and emission (solid lines) spectra of the donors and acceptors used in the TF-based sensors. (**A**) tdTomato-TetR^C + Cy5-DNA. (**B**) QD-TetR^C + Cy5-DNA. (**C**) QD'-TetR^D + Cy5-DNA.

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Table S2. FRET Parameters.

	Donor	QY (%)[a]	*J* (x10^{16} M^{-1} cm^{-1} nm^4)[b]	R_0 (nm)[c]	E_{max}[e]	K_D (nM)[e]	h[e]
tdTomato-TetR^C	tetR-tdTomato	69.0	1.34	7.43	0.34 ± 0.04	99 ± 32	1.0 ± 0.5
QD-TetR^C	QD CdSe/4CdS/2ZnS	23.4	2.69	6.96	0.68 ± 0.01	240 ± 40	2.3 ± 0.2
QD'-TetR^D [d]	QD' CdSe/6CdS/2ZnS	17.0	2.02	6.29	N/A	N/A	N/A

[a] Quantum yield (QY) of tdTomato from literature.[2]

[b] All three sensors utilized Cy5 as the acceptor dye; the Cy5 molar extinction coefficient of 250,000 M^{-1} cm^{-1} was used for each of the overlap integral (*J*) calculations.

[c] R_0: Förster distance, or the calculated donor-acceptor distance at which a 1:1 donor:acceptor system using these fluorophores would exhibit 50% FRET efficiency.

[d] QD'-TetR^D sensor utilizes a different batch of QDs than the first QD-based sensor. The optical properties are similar, but not identical to the first batch of QDs.

[e] Data from Figures 2 and S3 fit to a modified Hill equation (Equation S3). The lack of a plateau in the QD'-TetR^D sensor made for a non-physiological fit.

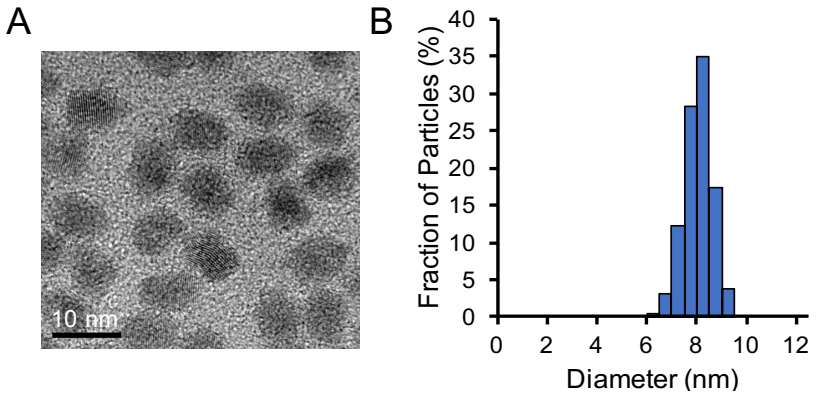

Figure S2. Characterization of the QDs used in the TetR^C-QD sensor. (**A**) TEM image of QDs. (**B**) Histogram of QD sizing based on TEM images.

Table S3. Summary of Sensor Metrics based on Hill function fits

Sensor	$TetR^C$-FP	$TetR^C$-QD	$TetR^D$-QD'
EC_{50} (nM)[a]	205 ± 7	729 ± 7	118 ± 3
h[a]	1.8 ± 0.2	3.2 ± 0.1	2.0 ± 0.1
LOD (nM)[b]	42	214	17
Linear Range / Width (nM)[c]	87 - 481 394	452 - 1174 722	54 - 255 201

[a] From data in Figures 3 and S3c fit to Equation S4. [b] Nominal analyte concentration where fit curve crosses 3σ of S_0, where F_A/F_D is at a maximum. [c] Determined from calculation of the bend points on the curve, as previously described.[3]

Table S4. Binding affinities of TetR variants for *TetO* determined by Biolayer Interferometry (BLI).

	K_D (10^{-10} M)	K_{on} (10^3 $M^{-1}s^{-1}$)	K_{off} (10^{-5} s^{-1})
$TetR^C$	0.830 ± 2.0	73.4 ± 2.2	0.610 ± 1.4
$TetR^D$	7.32 ± 0.78	184 ± 6.5	13.4 ± 1.4

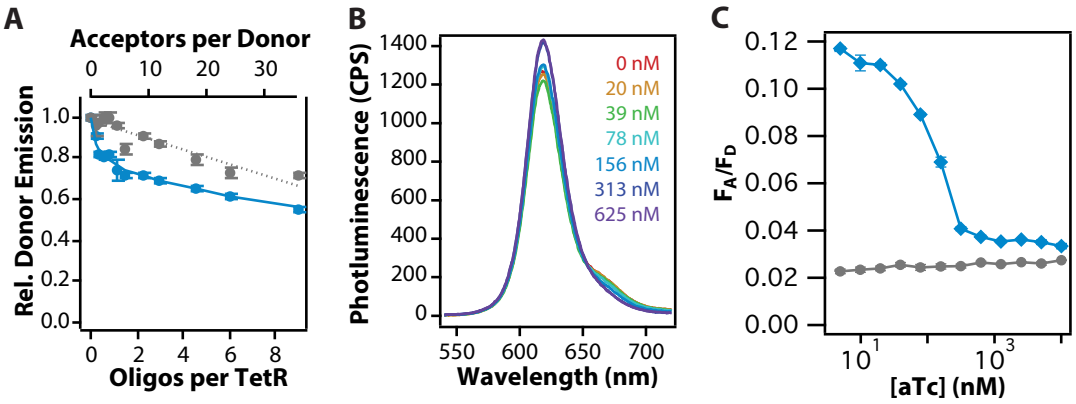

Figure S3. $TetR^D$-QD sensor results. (**A**) Relative donor emission as a function of the number of Cy5-labeled DNA acceptors titrated to the QD-$TetR^D$ donor. (**B**) Representative spectral data for the aTc dose-dependent change in emission for the sensor comprising QD-$TetR^D$ + Cy5-DNA. Spectra are background subtracted to eliminate the effects of direct acceptor excitation. A selection of the analyte concentrations is plotted for visual clarity. (**C**) Ratio of acceptor fluorescence intensity to donor fluorescence intensity as a function of aTc concentration for sensor comprising QD-$TetR^D$ + Cy5-DNA, using a 1:4:18 ratio of QD:TF:DNA and QD concentration of 50 nM. Data are mean ± standard deviation of n=3.

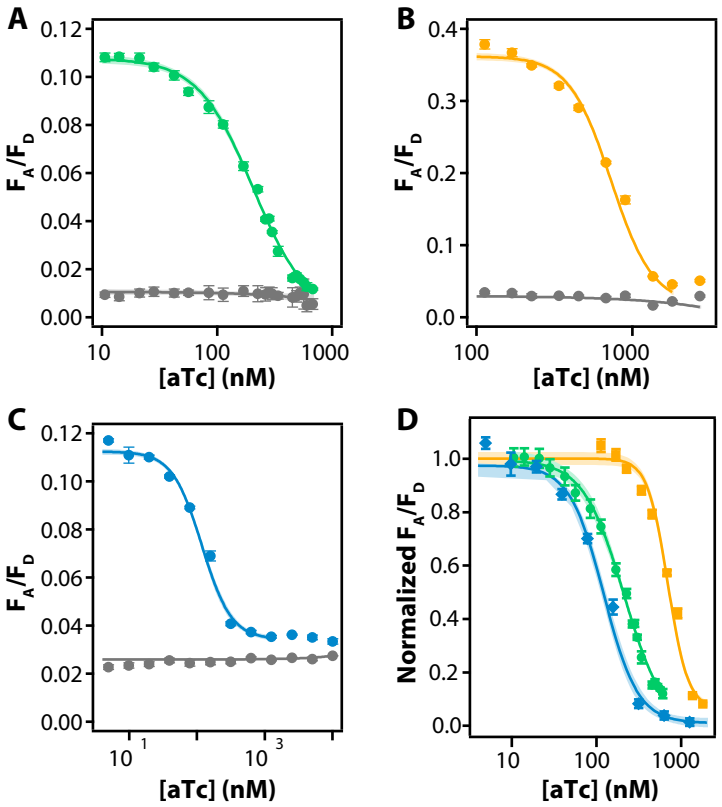

Figure S4. Sensor responses fit to Hill functions. Ratio of acceptor fluorescence intensity to donor fluorescence intensity as a function of aTc concentration for sensor comprising **(A)** tdTomato-TetR^C + Cy5-DNA at a 1:1:3 ratio of tdTomato:TF:DNA, **(B)** QD-TetR^C + Cy5-DNA at a 1:4:18 ratio of QD:TF:DNA, or **(C)** QD-TetR^D + Cy5-DNA at a 1:4:18 ratio of QD:TF:DNA. tdTomato (200 nM) and QD (50 nM) concentrations were selected to maintain a constant aTF concentration at 200 nM. Data are mean ± standard deviation of n=3. Plots fit to a Hill equation, including the [aTc] = 0 nM point by setting its x-axis value as a low, non-zero concentration (i.e., 100-fold lower than the lowest concentration included in the titration). **(D)** Normalized data and fits from the 3 plots superimposed. The 95% confidence bands displayed as lighter shaded regions in all plots.

Experimental Materials and Methods

Materials

Quantum Dot Synthesis. Cadmium oxide (CdO; 99.95%, Alfa Aesar), sulfur (99.95%, ACROS Organics), and 1-octadecene (ODE; 90%, ACROS Organics) were used as purchased from Fisher Scientific. Zinc acetate (99.99%), selenium (99.99%; pellets), oleic acid (OA; 90%), oleylamine (80%–90%), trioctylphosphine (TOP; 97%), and trioctylphosphine oxide (TOPO; *ReagentPlus®,* 99%) were used as purchased from Sigma-Aldrich. HPLC- grade solvents including hexanes (Fisher Scientific), methanol (Honeywell), chloroform (J.T. Baker), and ethanol (Sigma- Aldrich) were bought and used without further purification.

CL4 Ligand Synthesis. DL-Thioctic acid (≥98%; ACROS Organics), 1,1'-carbonyldiimidazole (CDI; 97%, ACROS Organics), methyl acrylate (≥99%, ACROS Organics), sodium borohydride (NaBr4), and silica gel sorbent (230-400 mesh, grade 60) were purchased from Fisher Scientific. Ethylenediamine (≥99%), lithium

hydroxide (LiOH; ≥98%), hydrochloric acid (HCl; 37%), and sodium hydroxide (NaOH; 50% in H2O) were purchased from Sigma-Aldrich.

Protein Expression and Purification. NEB® 5-alpha (Cat# C2987I) and BL21(DE3) (Cat# C2527I) competent *Escherichia coli* cells were purchased from New England Biolabs and used to replicate and express plasmids, respectively. LB broth (Lennox; powder), kanamycin sulfate (mixture of Kanamycin A (main component) and Kanamycin B and C; powder), isopropyl b-D-thiogalactopyranoside (IPTG; ≥99%), phosphate buffered saline, pH 7.4 (PBS; packets), and lysozyme from chicken egg white (~7000 U/mg; powder(crystalline)) were purchased from Sigma-Aldrich. Dextrose (granules (crystalline)), Halt™ protease inhibitor cocktails (100X) were purchased from Fisher Scientific. 1,4-Dithiothreitol (DTT; >99% (protease-free)) was bought and used as is from Gold Biotechnology (St. Louis, MO). Nickel-nitrilotriacetic acid (Ni-NTA) agarose resin and Strep-tactin® Superflow Plus resin were purchased from Qiagen (Germantown, MD) for affinity-tag chromatography purification of proteins. Strep-tactin® Purification Buffer Set was purchased from IBA (Gottingen, Germany). Sodium phosphate (NaH_2PO_4; ≥98%, monobasic monohydrate), sodium chloride (NaCl; BioXtra, ≥99.5%), and imidazole (≥99%) were purchased from Sigma for buffer preparation used with Ni-NTA agarose resin.

Acrylamide/Bis-Acrylamide (37.5:1) 40% (w/v) solution (BioBasic, ON, Canada), glycine (≥99%, Sigma-Aldrich), N,N,N',N'-tetramethylethylenediamine (TEMED; ~99%, Sigma-Aldrich), ammonium persulfate (APS; ≥98%, Sigma-Aldrich), SDS-PAGE protein standards, broad range (unstained, Bio-Rad), tris(hydroxymethyl) aminomethane (ultra-pure, Research Products International (RPI)), sodium dodecylsulfate (SDS; powder, RPI), bromophenol blue (Sigma-Aldrich), 2-mercaptoethanol (BME; ≥99%, Sigma-Aldrich), glycerol (≥99.5%, Fisher Scientific), Coomassie Brillant Blue G (250, Sigma-Aldrich), and acetic acid (glacial, J.T.Baker) were purchased for SDS-PAGE protein molecular weight verification. Protein assay kit II (Bradford reagent) was purchased from Bio-Rad for protein quantification.

FRET assays. Bovine serum albumin (BSA; DNase- and protease-free, Fisher Scientific), tris-hydrochloride (Tris-HCl; ≥99%, Promega), magnesium chloride hexahydrate ($MgCl_2$; ≥99%, Sigma-Aldrich), salmon sperm DNA solution (UtraPure™, Invitrogen), and anhydrotetracycline hydrochloride (aTc; Alfa Aesar) were used as purchased.

Methods

Quantum Dot Preparation. CdSe cores were nucleated using a modification of a previously described protocol.[4] Briefly, 1 g TOPO, 8 ml ODE, and 1.9 ml 0.2 M $Cd(OA)_2$ (1:4) were added to a 100 ml round bottom flask and degassed at room temperature for 30 mins. The flask was heated to 80°C and degassed for another 30 mins. The temperature was raised to 300°C under argon atmosphere and a pre-mixed solution of 0.4 ml 1 M TOP:Se, 3 ml oleylamine, and 1 ml ODE was immediately injected into the flask. After 3 mins, the flask was removed from the heating mantle and cooled to room temperature on a cork ring. Once cooled, the raw QD core solution was transferred into an argon atmosphere glovebox and precipitated using a mixture of methanol and ethanol. After centrifugation, CdSe cores were resuspended in hexanes and stored at 4ºC under air-free conditions for future use.

Four or six atomic layers of a CdS shell were deposited on top of the CdSe cores using a previously described modified successive ion layer adsorption and reaction (SILAR) method[4-6] to produce two batches of core/shell QDs, indicated as QD and QD', respectively. For shell deposition, 5 ml ODE and 5 ml oleylamine were added to a 100 ml round bottom flask and degassed for 30 mins at room temperature followed by 30 mins at 80°C. Two hundred nmols of CdSe cores in hexanes were injected into the reaction flask and degassed for another 30 mins at 80°C. After the core solution was heated to 160°C, enough

cadmium oleate to coat the CdSe cores with a single atomic monolayer of material was added in the form of 0.2 M $Cd(OA)_2$ (1:4 Cd:OA) in ODE. The reaction was maintained at 160°C for 1 hour before the temperature was raised to 240°C, where it was held for 1.5 hrs. The same amount of sulfur was added in the form of 0.2 M sulfur dissolved in ODE and the reaction annealed for 1 hour. All subsequent injections and anneals were performed at 240°C with Cd and S anneals of 2.5 and 1 hours, respectively. An additional 2 layers of ZnS was added on top of the CdSe/CdS QDs to passivate the surface. The same SILAR method was used as above, but with 0.2 M $Zn(OA)_2$ (1:4 Zn: OA) as the cation precursor and 1 hour anneal times for both Zn and S additions.

QDs were precipitated using a mixture of methanol and ethanol and resuspended in chloroform. TEM images were taken on a JOEL 2100 and images analyzed to determine size distribution. QDs were transferred to water in a biphasic ligand exchange reaction using a zwitterionic ligand CL4 as previously described.[5, 7] Dynamic light scattering was used to determine hydrodynamic diameter on a Brookhaven 90plus Nano-particle Sizer. The number weighted hydrodynamic diameter is reported. Quantum yield measurements were taken using the six-inch Quanti-phi integrating sphere attachment on a Horiba Nanolog (Horiba Jobin Yvon).

Plasmid Preparation. The MoClo modular cloning system[8, 9] was used to generate a tdTomato-$TetR^C$ expression vector, which included a terminal StrepII tag for affinity purification. All PCRs were purified using Qiagen Qiaquick PCR Purification columns. Plasmids were grown in *E. coli* Top10 and purified using Qiagen QIAprep Spin Miniprep Kit columns. Level 0 plasmids were grown on LB + ampicillin agar or in LB + ampicillin broth. Level 1 plasmids were grown on LB + kanamycin agar or in LB + kanamycin broth. Agar plates were grown overnight at 37°C, while liquid cultures were grown at 37°C overnight with shaking at approximately 200 rpm.

To make a MoClo-compatible IPTG inducible promoter, oligos synT7_AB F and synT7_AB R were annealed in 1X Annealing Buffer (10 mM Tris, 50 mM NaCl, pH 7.5) and cloned into plasmid DVA_AB using a simultaneous BbsI (NEB) digestion and T4 Ligase (NEB) ligation to make plasmid T7_AB. To separate the TetR domains from tdTomato, we used an alpha-helical linker with sequence AEAAAKEAAAKA[10] coded in the MoClo fusion site M. Subcloning vectors DVA_CM and DVA_MD were made by using primer pairs pri_SpeI_C_LacZ & pri_LacZa_M_SpeI and pri_SpeI_M_LacZ & pri_LacZa_D_SpeI, respectively, to amplify the LacZ fragment from DVA_AB. Fragments and destination vector DVA were digested with SpeI (NEB), then ligated using T4 Ligase.

TetR_CM was cloned using primer pair pri_BbsI_C_TetR & pri_TetR_M_BbsI and inserted into DVA_CM using simultaneous BbsI digest and T4 ligation. tdTomato_MD was cloned in four pieces to remove internal BbsI sites. Fragment 1 was PCRed using primers pri_BbsI_M_tdTomato & pri_tdTmA_int_R, fragment 2 using pri_tdTmA_int_F & pri_tdTomatoA linker, fragment 3 using pri_tdTomatoB linker & pri_tdTB_int_R, and fragment 4 using pri_tdTmB_int_F & pri_tdTomatoSTII_D_BbsI. Purified PCRs were ligated into DVA_MD as above.

Level 1 expression vector pT7TetR-tdTomato-STII was constructed using level 0 plasmids DVA_AE, T7_AB, B0034m_BC, TetR_CM, tdTomato_MD, and B0015_DE using simultaneous BsaI (NEB) and T4 ligase reaction.

Table S4. Oligo Sequence Used in Cloning.

Oligo	Sequence
synT7_AB F	tgaagacttGGAGtaatacgactcactatagggagaTACTaagtcttca
synT7_AB R	tgaagacttAGTAtctccctatagtgagtcgtattaCTCCaagtcttca
Pri_SpeI_C_LacZ	agactagtgggtctcaAATGatgtcttctgcaccatatgcggtgtg
pri_LacZa_M_SpeI	ctactagtaggtctctTCTTacgtcttccccgcgcg
Pri_SpeI_M_LacZ	agactagtgggtctcaAAGAatgtcttctgcaccatatgcggtgtg
pri_LacZa_D_SpeI	ctactagtaggtctctACCTacgtcttccccgcgcg
pri_BbsI_C_TetR	tgaagacttAATGtctcgtttagataaaagtaaagtgattaacagcg
pri_TetR_M_BbsI	tgaagacttTCTTtagcagcagcttcagcagacccactttcacatttaagttgtttttctaatc
pri_BbsI_M_tdTomato	tgaagacttAAGAagctgctgctaaagctatggtgag
pri_tdTmA_int_R	tgaagacttcttttttctgcattacggggccgtcg
pri_tdTmA_int_F	tgaagacttaaagaccatgggctgggagg
pri_tdTomatoA linker	tgaagacttCATGttgttgtcctcggaggaggc
pri_tdTomatoB linker	tgaagacttCATGgccgtcatcaaagagttcatgc
pri_tdTB_int_R	tgaagacttggttttcttctgcattacggggccg
pri_tdTmB_int_F	tgaagacttaaccatgggctgggaggc
pri_tdTomatoSTII_D_BbsI	tgaagacttACCTctatttttcaaactgcggatggctccagccgcccttgtacagctcgtccatgccg

Protein Expression and Purification. *E.coli* BL21(DE3) were transformed with plasmids. Cells were grown at 37ºC in LB broth supplemented to a final concentration of 0.4% glucose and 33 µg/ml kanamycin. Protein expression was induced at an OD600 0.5 - 1 by adding IPTG to a final concentration of 1 mM followed by expression at 30ºC for 4 - 16 hours. Cells were harvested by centrifugation, redispersed in 10 mM PBS, 1 mM DTT, 1X Halt protease inhibitor cocktail, and stored at -80ºC until purification. For purification, 1 mg of lysozyme was added for every ml of thawed whole cell lysate and incubated for 1 hour at 4°C.

Soluble proteins were obtained by centrifugation at 4ºC for 30 mins at 18,000 rpm. Cleared cell lysates were purified using a Strep-tactin column for the tdTomato-TetR^{C} (FP-TetR) fusion protein and a Ni-NTA column for TetR-6His. Fractions were collected and analyzed using a 12% SDS-PAGE gel and fractions containing FP-TF were pooled. The pooled fractions were concentrated and buffer exchanged into tris-buffered saline (TBS) via 10 kDa centrifugal filters (Amicon). Concentrations were determined using a Bradford assay as well as UV spectroscopy using the molar extinction coefficient of tdTomato (138,000 M^{-1} cm^{-1}).

Biolayer Interferometry (BLI)

Single stranded reverse and biotinylated forward DNA ordered from Integrated DNA Technologies (IDT) was annealed in 1X Annealing Buffer (10 mM Tris, 50 mM NaCl, pH 7.5) and heated to 95°C for five minutes, then allowed to cool slowly to room temperature by either removing the heat block from the heating element and placing on the benchtop, or by decreasing temperature in a thermal cycler in 5C° increments.

BLI was performed on a ForteBio Octet RED96 using Streptavidin (SA) coated sensor tips (ForteBio). After baselining SA tips in 1X BLI buffer (28 mM Tris, 5 mM MgCl, 25 mM NaCl, 4.25% Glycerol, 1.67 mg/mL BSA, pH 8.0) for 60 seconds, tips were dipped in BLI buffer containing 250 nM double stranded oligo and DNA was allowed to bind the tip, discontinuing while binding remained linear, usually at around 0.1 nm and 60-90 seconds. DNA-bound tips were baselined again in BLI buffer for 60 seconds, then dipped in varying concentrations of TF in BLI buffer and left to bind until equilibrium was reached. Disassociation data was acquired by dipping the TF:DNA bound tips into BLI buffer and allowing the complex to dissociate. Data from a DNA coated tip un-exposed to TF was subtracted from each curve to control for any DNA dissociation from the sensor. Curves were normalized to the final five seconds of baselining and Savitsky-Golay filtered to remove noise. Data were fit to a mass transport model using Forte'Bio's Data Analysis Software.

To acquire small-molecule induced dissociation data, tips were coated in DNA as above, then dipped in solution containing 200 nM TF until binding reached equilibrium. Sensors were then dipped in buffer containing various concentrations of aTc as well as 1% ethanol (no aTc control). Data were normalized as above, with an additional rescaling of the data to set the the binding at the initiation of dissociation to 1.

***DNA Hybridization*.** The synthetic 28bp *tetO*-containing oligonucleotide and its complement with modified 5'- and 3'-Cy5 were purchased from Integrated DNA Technologies (IDT) and hybridized to generate double-stranded fluorescent oligos. Equimolar amounts of each oligonucleotide were mixed with 1X nuclease-free duplex buffer (30 mM Hepes pH 7.5, 100 mM KAc, IDT), heated to 95ºC for 2 mins, and cooled to room temperature wrapped in aluminum foil to prevent photobleaching of the Cy5 dye.

FRET assays. The FRET response of each sensor as a function of the donor-acceptor ratio was tested by titrating the acceptor to a fixed concentration of donor. Briefly, the sensors were prepared in a solution of TBS + 0.2% (w/v) BSA and 1X binding buffer (20 mM Tris-HCl, 5 mM $MgCl_2$, 5% glycerol, 50 ng/µl salmon sperm DNA). For the tdTomato-$TetR^C$ sensor, the final concentration of FP-$TetR^C$ was kept constant at 200 nM, while Cy5-modified DNA were titrated to yield donor-acceptor ratios ranging from 0 – 9. The sensor solution was pipetted into wells of a black, non-binding 384-well plate (Corning) with a final volume of 60 µl. For triplicate measurements, each of the above solutions were prepared with a final volume of 180 µl in microcentrifuge tubes and pipetted into 3 separate wells of 60 µl each.

For the QD-based sensors, QDs and tetR-6His were mixed at a 1:4 ratio with a final QD concentration of 50 nM for self-assembly of the tetR-6His to the surface of the QDs.[11] The QD-TetR conjugates were incubated with varying concentrations of the Cy5-modified DNA to yield donor-acceptor ratios ranging from 0 – 9.

Emission spectra were taken with the MicroMax plate reader attachment on a Horiba Nanolog fluorimeter with excitation of tdTomato-$TetR^C$ at 500 nm, and excitation of QDs at 400 nm with a slit width of 2 nm and 3 s integration time per well. Negative controls were prepared as described above using a Cy5-modified scrambled DNA sequence as the acceptor to account for collisional quenching of the donor.

The analyte response curves of each sensor were obtained as described above, but with the titration of aTc. For the tdTomato-$TetR^C$ sensor, the ratio of tetR-tdTomato and Cy5-modified DNA were kept constant at 1:3. aTc was added such that the final concentrations of the components were 200 nM TetR-tdTomato, 600 nM Cy5-modified DNA, and 0 – 675 nM aTc. For the QD-based sensors, a 1:4:18 ratio of QDs, TetR-

6His (TetR^C or TetR^D), and Cy5-modified DNA was kept constant. Final assay concentrations were 50 nM QDs, 200 nM TetR-6His, 900 nM Cy5-modified DNA, and 0 – 2700 nM aTc.

Calculating FRET parameters. The overlap integral, *J*, describes the spectral overlap of the donor emission and acceptor absorbance according to the following equation:[12, 13]

$$\mathbf{J} = \int \overline{\mathbf{F_D}}\,(\boldsymbol{\lambda})\boldsymbol{\varepsilon}_{\mathbf{A}}(\boldsymbol{\lambda})\boldsymbol{\lambda}^{\mathbf{4}}\mathbf{d}\boldsymbol{\lambda}, \qquad (\mathbf{S1})$$

where $\overline{\mathbf{F_D}}(\boldsymbol{\lambda})$ is the normalized donor emission spectrum and $\boldsymbol{\varepsilon}_{\mathbf{A}}(\boldsymbol{\lambda})$ is the molar extiniction coefficient of the acceptor as a function of wavelength $\boldsymbol{\lambda}$. The Förster distance $\mathbf{R_0}$ is defined as the donor-acceptor distance at which 50% FRET efficiency is observed as described by:[12, 13]

$$\mathbf{R}_0^6 = (\mathbf{0.02108})\boldsymbol{\kappa}^2\mathbf{Q_D}\frac{\mathbf{J}}{\boldsymbol{\eta}^4}, \qquad (\mathbf{S2})$$

where $\boldsymbol{\kappa}^2$ is the dipole orientation factor, which is set to 2/3 under the assumption of random dipole orientation, $\mathbf{Q_D}$ is the donor quantum yield, and $\boldsymbol{\eta}$ is the solvent refractive index.

In order to estimate the degree of FRET signal of the sensors, the relative donor emission was plotted with respect to acceptor titration and fitted to a modified Hill equation:[14, 15]

$$\frac{\mathbf{F_{DA}}}{\mathbf{F_D}} = \mathbf{1} - \mathbf{E_{max}}\left[\frac{\mathbf{1}}{\mathbf{1} + \left(\frac{\mathbf{K_D}}{\mathbf{c}}\right)^{\mathbf{h}}}\right], \qquad (\mathbf{S3})$$

where $\mathbf{F_{DA}}$ is the fluorescence intensity of the donor in the presence of acceptor, $\mathbf{F_D}$ is the fluorescence intensity of the donor in the absence of acceptor, $\mathbf{E_{max}}$ is the maximum FRET efficiency, $\mathbf{c}$ is the concentration of acceptor, $\mathbf{h}$ is the Hill coefficient, and $\mathbf{K_D}$ is the acceptor concentration at which there is 50% donor quenching. The Hill fit parameters are reported in Table S2.

Raw spectral data were background subtracted for direct acceptor excitation and the donor and acceptor emission peak-fitted using OriginPro.

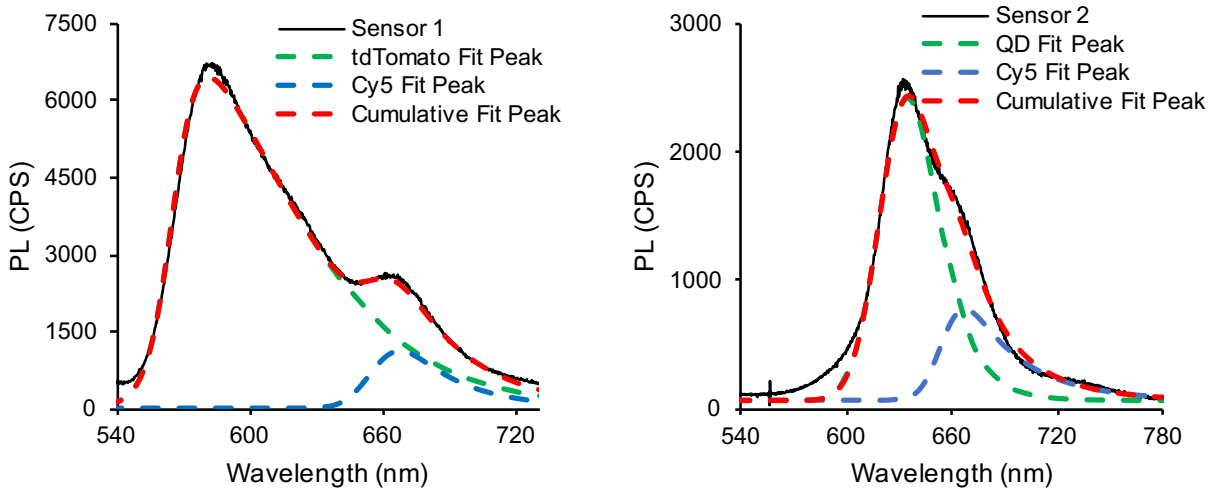

Figure S5. Peak fitting results from Origin Pro of (**A**) tdTomato-TetRC + Cy5-DNA fitted with a sum of an asymmetric sigmodal and an exponentially modified Gaussian, and (**B**) QD-TetRC + Cy5-DNA fitted with a sum of two exponentially modified Gaussians.

The sensor output, defined as the ratio of the areas of acceptor emission over donor emission ($\mathbf{F_A/F_D}$), was calculated using the integrals of the peak emissions.

To compare the outputs of the sensors, F_A/F_D ratios were fit to the Hill equation:

$$\boldsymbol{S(c) = S_1 + \frac{(S_0 - S_1)}{1 + \left(\frac{EC_{50}}{c}\right)^{h}}}, \qquad \mathbf{(S4)}$$

where $\boldsymbol{S_0}$ is the intensity of the sensor signal with no analyte present, $\boldsymbol{S_1}$ is the intensity of the sensor signal at saturating analyte concentration, $\boldsymbol{c}$ is the analyte concentration, $\boldsymbol{EC_{50}}$ is the concentration at which there is 50% signal (*aka* the effective concentration), and **h** is the Hill coefficient, which indicates cooperative binding. Where normalized data is presented, errors were propagated mathematically with an assumption of no covariance.

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