Supporting Information

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SI Materials and Methods

All reagent-grade chemicals, including cocaine, mercury chloride, doxorubicin, sodium phosphate monobasic, and sodium phosphate dibasic (doxorubicin from LC Laboratories; all others from Sigma-Aldrich) were used as received. DNA molecules modified with a carboxyfluorescein (FAM) and a black-hole-quencher-1 (BHQ) were used as purchased [16-base linker and 36-base linker Hg(II) constructs from IBA-Solutions for Life Sciences; all others from BioSearch Tech]. The sequences are as follows:

Cooperative Hg(II)-binding aptamer:

5′ (FAM)-GTCCTG-$(AC)_n$-CTGGTC-(BHQ), n = 3, 8, 12, 18, 25;

Single-site Hg(II)-binding aptamer:

5′ (FAM)-GCCCTG-$(AC)_{25}$-CTGGCC-(BHQ);

Cooperative doxorubicin-binding aptamer:

5′ (FAM)-$(ACCATCTGTGTAAGG)_2$-T_n-$(GGTAAGGGGTGGT)_2$-(BHQ), n = 30, 50;

Single-site doxorubicin-binding aptamer:

5′ (FAM)-ACCATCTGTGTAAGGGGTAAGGGGTGGT-(BHQ);

Cooperative cocaine-binding aptamer:

5′ (FAM)-AGACAAGGAAAATTTAGACAAGGAAAA-T_{50}-TCCTTCAATGAAGTGGGTCGTTTCCTTCAATGAAGT-GGGTCG-(BHQ);

Single-site cocaine-binding aptamer:

5′ (FAM)-GGGAGACAAGGAAAATCCTTCAATGAAGT-GGGTCGACA-(BHQ).

We obtained all fluorescence measurements using a Cary Eclipse Fluorimeter (Varian) with excitation at 485 (±5) nm and acquisition at 515 (±10) nm. We measured the mercury-binding and doxorubicin-binding receptors at 30 °C and the cocaine-binding receptors at 37 °C in 37.5 mM sodium chloride, 12.5 mM sodium phosphate buffer, pH 7. We obtained binding curves by adding sequentially increasing target concentrations to a stock solution of the receptors at 20 nM. For the Hg^{2+}- and doxorubicin-binding receptors, we titrated in solutions containing the target molecule but lacking the aptamer; we then adjusted the observed signal to account for the resultant dilution of the aptamer. For the cocaine-binding aptamer, we titrated in a solution of cocaine also containing the aptamer at 20 nM, thus keeping the aptamer concentration constant throughout the experiments. We thermally equilibrated all samples for at least 15 min before taking the first measurement. To ensure that the binding equilibrium had been reached before measurement, we obtained kinetic traces after the addition of each new target concentration (e.g., Fig. S2). The data used in the equilibrium plots (Figs. 3–5) represent the average of 10 fluorescence measurements collected at least 2 min after these additions. Hill coefficients were determined by fitting these data using GraphPad Prism plotting software. Error bars represent 95% confidence intervals based on SEs derived from these fits.

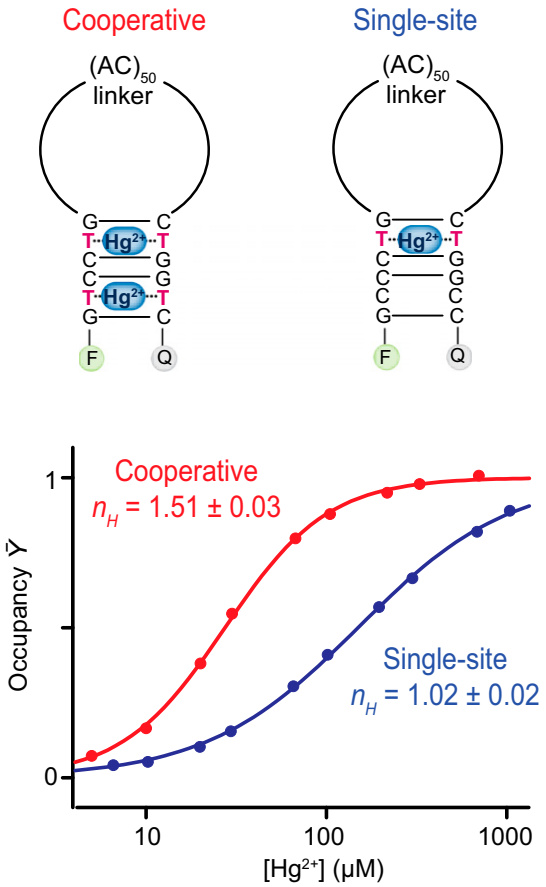

Fig. S1. Our cooperative 50-base loop Hg^{2+}-binding aptamer achieves a Hill coefficient of 1.51 ± 0.03, which represents considerable cooperativity. In contrast, the single-site analog of this aptamer, in which one of the Hg^{2+}-binding thymine–thymine mismatches is replaced with a nonbinding cytosine–cytosine mismatch, is noncooperative and exhibits a Hill coefficient of just 1.02 ± 0.02.

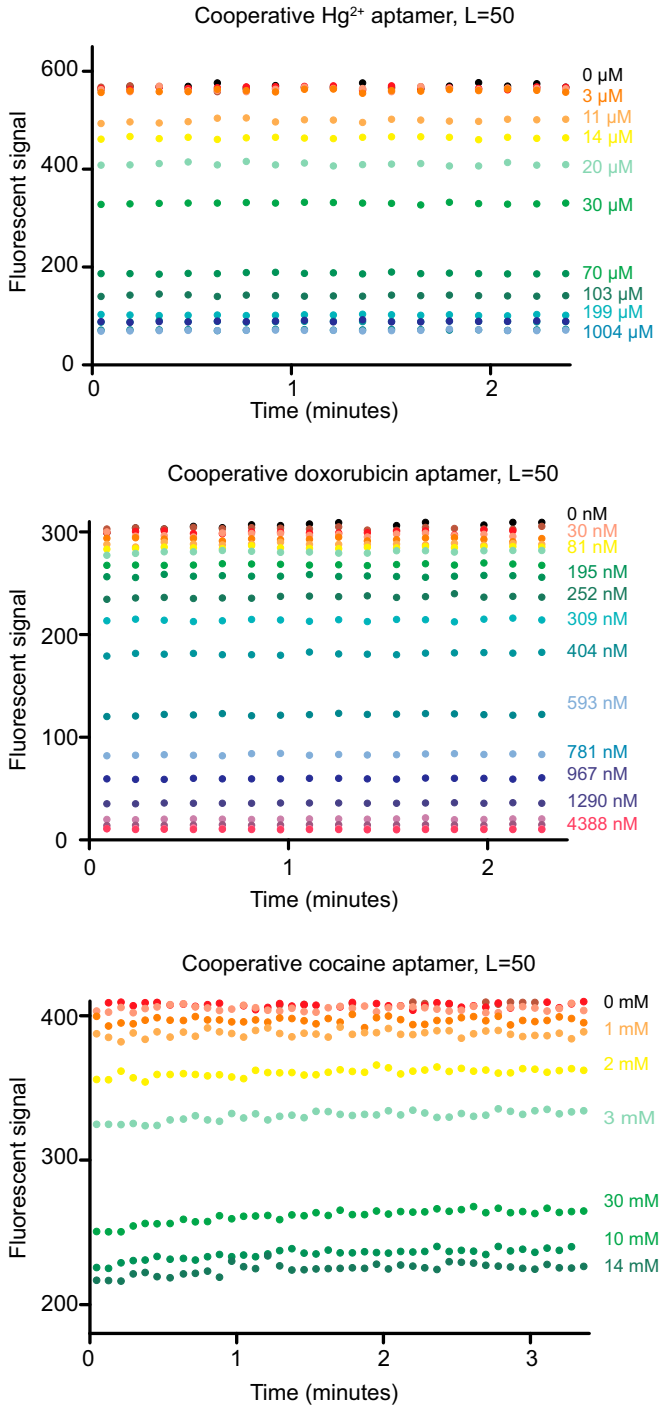

Fig. S2. To confidently ascertain that a receptor is cooperative, it is crucial that all measurements be conducted in the equilibration limit. Specifically, because the approach to equilibrium is slowest at the lowest target concentrations and accelerates at higher target concentrations (thus artificially underpopulating the receptor at low target concentrations relative to what would be seen at equilibrium), data collected before full equilibration can generate cooperative-like binding curves even in the absence of real cooperativity. To ensure that our samples were always fully equilibrated, we measured the fluorescence over time when we successively added target molecule to solution. For example, for our 50-base linker Hg^{2+} (*Top*) and doxorubicin (*Middle*) constructs, the aptamers reach equilibrium within the 30- to 60-s mixing and transfer dead time of our experiments before the initiation of measurements. Note that, in these plots, raw fluorescence has been normalized to account for the (modest, <2% per titration point) decrease in probe concentration due to the addition of target molecule solution with each point. The 50-base linker cocaine construct (*Bottom*) equilibrates somewhat more slowly, but nevertheless easily reaches equilibrium before the measurements reported in Fig. 5 (which were collected after the 2-min mark). Note that, in contrast to our other constructs, to study this construct we started with the free aptamer at 20 nM and added to this increasing aliquots of 20 nM aptamer plus cocaine, thus keeping to total aptamer concentration constant. The slight increase in signal that is seen after addition thus reflects the (relatively slow) off rate of the aptamer–cocaine complex.