SUPPORTING INFORMATION:

A Rational Approach to Optimizing Conformation-Switching Aptamers for Biosensing Applications

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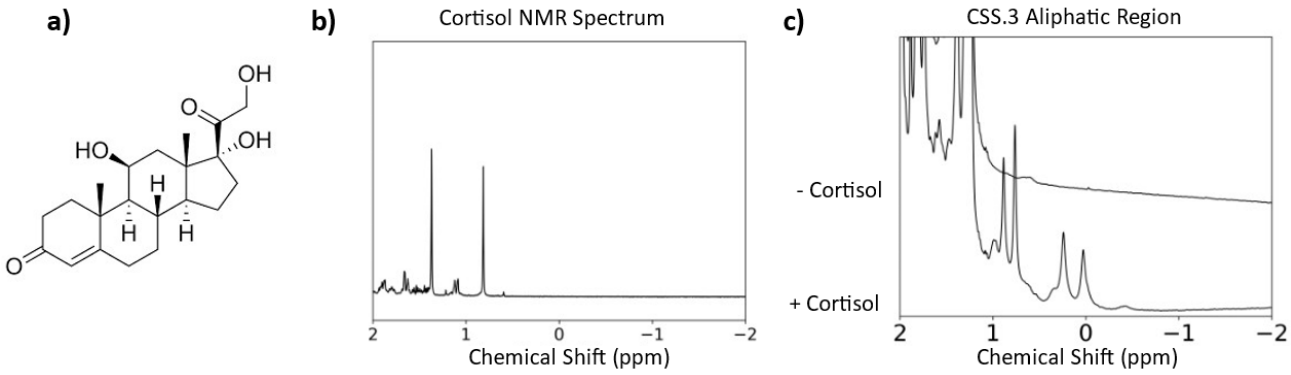
S1. Components of Simulated Interstitial Fluid (sISF).

Reagent	Concentration
$CaCl_2$	2.5 mM
Glucose $C_6H_{12}O_6$	5.5 mM
HEPES	10 mM
KCl	3.5 mM
$MgSO_4$	0.7 mM
NaCl	123 mM
NaH_2PO_4 (Sodium phosphate Monobasic)	1.5 mM
Saccharose/Sucrose $C_{12}H_{22}O_{11}$	7.4 mM
BSA (optional)	0.3 mM

S2. Summary of Cortisol Binding Aptamers. CSS.3 demonstrated best binding affinity in literature and when using bio-layer interferometry. Binding was measured on the BLI in binding buffer at 30°C.

Aptamer Name	Sequence + Primers 5'->3'	Published K_D (uM) *strand displacement	Measured K_D (uM) *BLI
CSS.1	CTC TCG GGA CGA C GC CCG CAT GTT CCA TGG ATA GTC TTG ACT A GTC GTC CC	2.5	1.5
CSS.2	CTC TCG GGA CGA C TA GCG TAT GCG CCA GAA GTA TAC GAG GAT A GTC GTC CC	3.2	2.8
CSS.3	CTC TCG GGA CGA C GC CAG AAG TTT ACG AGG ATA TGG TAA CAT A GTC GTC CC	1.6	0.24

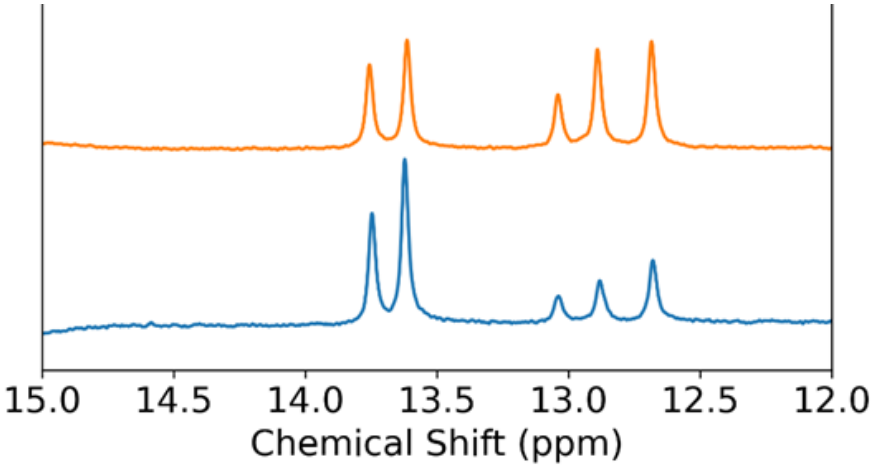
S3. a) Structure of cortisol and b) NMR spectrum of cortisol in sISF showing the presence of two distinct methyl peaks at 0.75 ppm and 1.31 ppm. c) Aliphatic region of CSS.3 NMR spectrum in the absence and presence of cortisol showing shifted peaks from cortisol methyl protons at 0.12 and 0.26 ppm which suggest cortisol is binding to the face of the DNA.



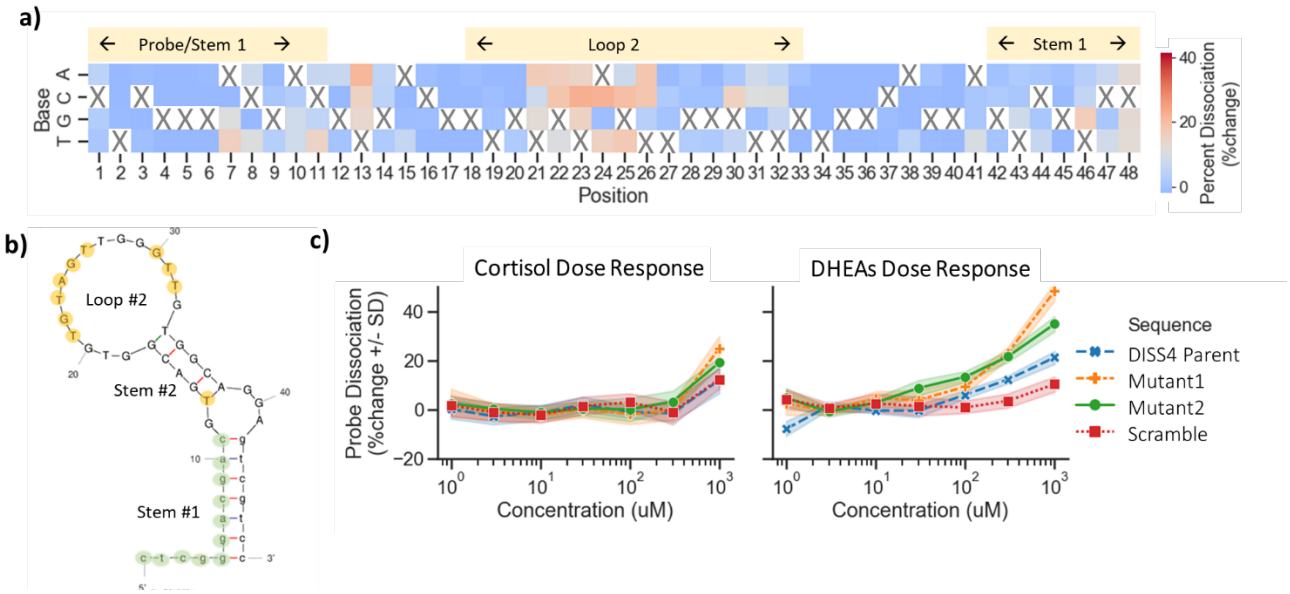
S4. Sequences of CSS.3 truncated aptamers

Aptamer	# base pairs in Stem #1	Sequence (5' -> 3')
CSS.3	8	CTCTCGGGACGACGCCAGAAGTTTACGAGGATATGGTAACATAGTCGTCCC
_cut1	7	GGACGACGCCAGAAGTTTACGAGGATATGGTAACATAGTCGTCC
_cut2	6	GGACGACGCCAGAAGTTTACGAGGATATGGTAACATAGTCGTC
_cut3	5	GGACGACGCCAGAAGTTTACGAGGATATGGTAACATAGTCGT
_cut4	4	GGACGACGCCAGAAGTTTACGAGGATATGGTAACATAGTCG
_cut5	3	GGACGACGCCAGAAGTTTACGAGGATATGGTAACATAGTC

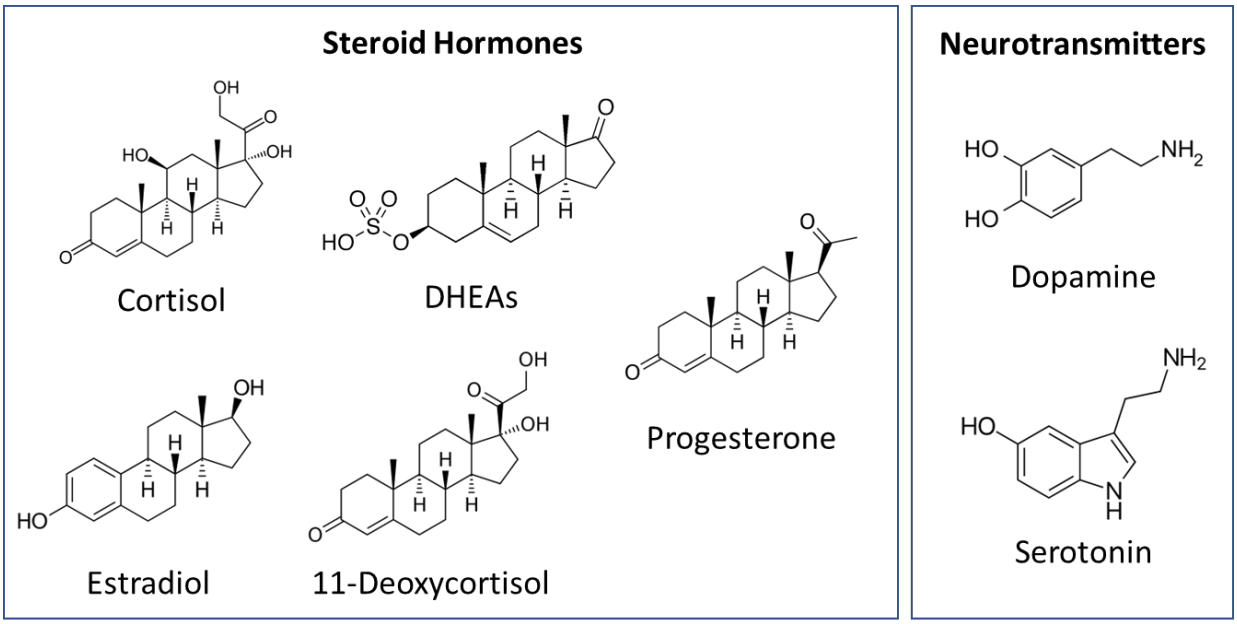
S5. We found no difference in NMR peak intensity between spectra of DNA duplex (CGCGAATTCGCG) generated in PBS (blue) compared to sISF (orange). Six imino peaks are expected due to symmetry of the duplex, however, the terminal imino peak (G12) is not observed due to rapid exchange.



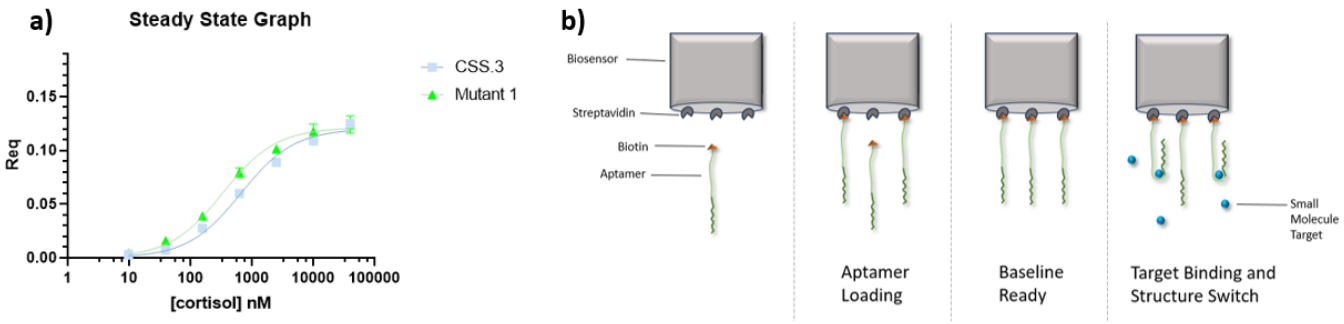
S6. Microarray-based screening of DISS.4 aptamer identified by Yang, et al. (31). a) All single point mutations of the DISS.4 parent aptamer were screened against 300 uM DHEAs. DHEAs binding is color coded such that orange = improved binding, blue = worse binding, and grey = no change. Nucleotides in positions 21-26 were most favorable for mutation. b) Mapping of functional mutations (highlighted in yellow) to the MFold-predicted DISS.4 parent 2D structure identifies loop #2 as permissive to mutation. Optimized probe hybridization region highlighted in green. c) DHEAs dose response analysis identified two DISS.4 mutants with higher sensitivity to DHEAs compared to DISS.4 parent sequence and a negative control scramble sequence. All sequences were non-responsive to cortisol.



S7. Structures of cortisol and structurally-similar counter targets used for screening



S8. A.) BLI cortisol dose response data for CSS.3 and Mutant 1 aptamers in sISF. Aptamers were run vs. cortisol in a spectrum of concentrations ranging from a 9.77 nM to 4000 nM with a 4x Fold dilution scheme. The graph shows Req vs concentration with standard variation, calculated for each data point individually from 3 replicates. The placement of the SSG trace, denoting the dose response of the Mutant 1 aptamer (Light green), further to the left shows a lower kD, and therefore higher affinity. B.) Schematic representation of the BLI protocol used to test DNA aptamers for direct binding without the use of a complementary strand used in experiments described in Fig. 2 in the main text.



S9. Cortisol LOD for Mutant 1 and CSS.3 calculated based on the low-concentration region of the dose response data shown in S8.