Supporting Information

Temporal Control of Aptamer Biosensors Using Covalent Self-Caging to Shift Equilibrium

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General

All DNA was purchased from the University of Utah DNA/Peptide Synthesis Core Facility and purified on denaturing PAGE prior to use. Unless otherwise noted, all absorbance and fluorescence measurements were recorded using a Biotek Synergy Mx microplate reader. Milli-Q water was obtained from Millipore Simplicity UV water purification system. All experiments were carried out in a dark room with red light to avoid light exposure to the DNA.

Table S1. DNA sequences

Name	Sequence (5'-3')
TA-0S	BHQ1-CACATCAAT/PC spacer/FAM/ATTGATGTGGTGTGTGAGTGCGGTGCCC
TA-1S	BHQ1-CACATCAAT/PC spacer/PEG_3/FAM/ATTGATGTGGTGTGTGAGTGCGGTGCCC
TA-2S	BHQ1-CACATCAAT/PC spacer/PEG_3/PEG_3/FAM/ATTGATGTGGTGTGTGAGTGCGGTGCCC
TA-3S	BHQ1-CACATCAAT/PC spacer/PEG_3/PEG_3/PEG_3/FAM/ATTGATGTGGTGTGTGAGTGCGGTGCCC
OA-1S	GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA/FAM/PEG_3/PC spacer/TGTCCGAT/BHQ1
OA-CS	TGTCCGAT/BHQ1

Modifiers Used for DNA Synthesis

Fluorescein (FAM), photocleavable spacer (PC spacer), and triethylene glycol (PEG_3) spacer units were installed using phosphoramidites from Glen Research. Black Hole Quencher 1 (BHQ1) on L-tyrosinamide (L-Tym) biosensor sequences was installed using phosphoramidites from Glen Research; BHQ1 on the OTA biosensor sequences was installed using CPG cartridges from Glen Research.

Preparation of Biosensor Stocks Solutions

For experiments using the L-Tym biosensor, all samples were prepared in a buffer containing 10 mM Tris-HCl, 100 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.5. For experiments using the OTA biosensor, all samples were prepared in a buffer containing 10 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, pH 8.5. All biosensor stock solutions were incubated at 90 ^oC for 5 min followed by rapid cooling. Prior to use, the solution was allowed to warm to room temperature.

Hairpin Biosensor Cleavage

100 μL of uncleaved hairpin biosensors were transferred into the wells of a Corning 96-well flat bottom polystyrene plate. The 96-well plate was placed on a 15 cm tall white card box for UV

irradiation. All UV irradiation was carried out with 365 nm high intensity light on a Maestrogen UltraBright UV Transilluminator. Samples were irradiated with UV light for varying amounts of time, but all samples were incubated for at least 20 minutes prior to any fluorescence measurements or analysis by denaturing PAGE.

Denaturing PAGE

Cleaved and uncleaved hairpin biosensors were diluted to 2 μM with buffer and diluted to 1 μM with equal volume of RNA loading dye (2X) purchased from NEB. Samples were loaded onto 8% denaturing polyacrylamide gels and run for 30-40 min at 260 V to separate cleaved from uncleaved fragments. The gels were then stained with SYBR Green II solution for 10 min followed by fluorescence imaging on a Typhoon FLA-7000 laser scanner.

For gel purification, the same procedure was carried out, but the uncleaved fragments on the gels were cut out and chopped into pieces. The gel pieces were then soaked in crush and soak buffer (0.5 M ammonium acetate, 1 mM EDTA, pH 8.0) at -80 ºC for 15 min followed by 2 hour incubation at 90 ºC.

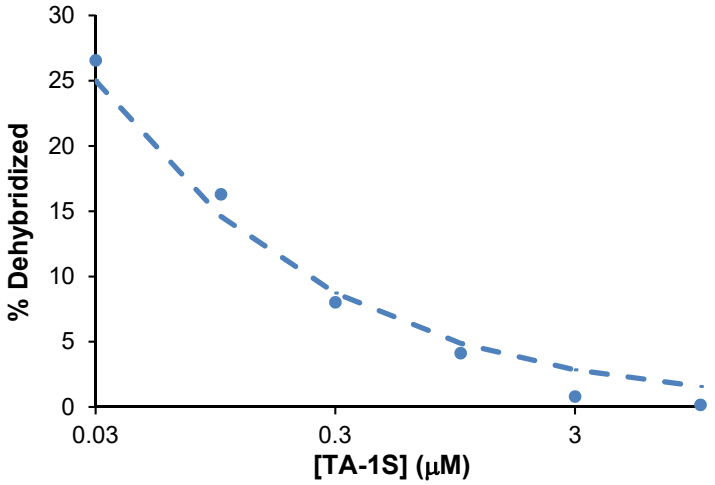
Optimization of Biosensor Concentration

For optimization of biosensor concentration, solutions having varying concentrations of the L-Tym biosensor (0.1, 0.3, 1, and 3 μM) were prepared and cleaved for 40 min by UV irradiation. Then, the samples were scanned for fluorescence intensity using excitation/emission wavelengths of 490/520 nm. Fluorescence intensity values for 0% and 100% dehybridization controls were obtained using the uncleaved sensor and FAM-labeled aptamer, respectively. This enabled calculation of % dehybridized for the cleaved sensors using equation 1

$$\%\, dehybridized = \left(\frac{F - F_{min}}{F_{max} - F_{min}}\right) x\ 100 \qquad (1)$$

in which F is the fluorescence intensity of the cleaved sensor, F_{min} is the fluorescence intensity of the uncleaved sensor (0% dehybridized), and F_{max} is the fluorescence intensity of the FAM-labeled aptamer (100% dehybridized). Figure S1 shows a plot of the % dehybridized versus concentration for biosensor TA-1S.

Figure S1. Percentage of L-Tym biosensors dehybridized as a function of concentration. Dashed line shows fit to calculated equilibrium constant.



The % dehybridized was then converted into actual concentrations of aptamer (Apt), complementary strand (CS) and aptamer-complementary strand duplex (Apt-CS) using equations 2 and 3

$$[Apt] = [CS] = \frac{[Apt]_i \, x \, \% \, dehybridized}{100} \quad (2)$$

$$[Apt - CS] = [Apt]_i - [Apt] \quad (3)$$

where $[Apt]_i$ is the initial concentration of biosensor. Using these concentration values, the K_D value was calculated for each biosensor concentration using equation 4.

$$K_D = \frac{[Apt][CS]}{[Apt{-}CS]} \quad (4)$$

Using these equations, we obtained an independent K_D value for each biosensor concentration. For the 3 μM biosensor solution, the % dehybridized is too small (< 2%) to measure with high accuracy, which leads to large error in the calculation of K_D. Thus, we utilized the three remaining data points for our overall K_D calculation. These data are shown in Table S1, and averaging of these values provides a K_D of 2.5 ± 0.7 nM.

Table S2. K_D values independently calculated from experiments with varying L-Tym biosensor concentrations.

$[Apt]_i$ (μM)	K_D (nM)
0.030	2.9
0.10	3.2
0.30	2.1
1.0	1.8

Using this equation and the K_D value, we calculated that 95% of the aptamer strands would be hybridized to a complementary strand at a concentration of 1 μM. We reasoned that this approximate level of hybridization would be suitable for our biosensing assay, as the majority of biosensors are assembled, minimizing background and maximizing potential signal gain.

We repeated this experiment for the OA-1S biosensor, but now including 2 additional equivalents of OA-CS complementary strand to mimic the ratio used for the dose-responsive biosensing experiments in the manuscript. From these data, we calculate a K_D of 550 ± 140 pM.

Figure S2. Percentage of OTA biosensors dehybridized as a function of concentration. Dashed line shows fit to calculated equilibrium constant.

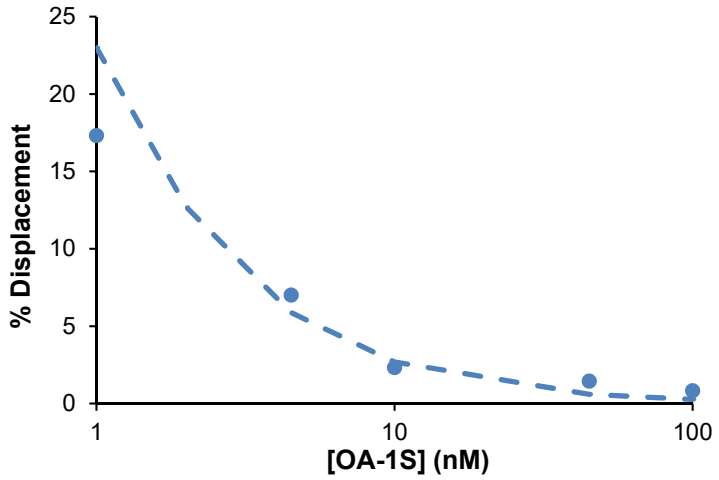


Table S3. K_D values independently calculated from experiments with varying OTA biosensor concentrations.

$[Apt]_i$ (nM)	K_D (pM)
1.0	450
4.5	700
10	480

T_m Measurements

Cleaved and uncleaved hairpin biosensors were diluted to 1 μM and annealed under the conditions described above. The samples were transferred to a MMC-1600 8 multi-cell (cell path length = 1 cm) for UV measurements using Shimadzu UV-1800 UV-VIS spectrophotometer. UV absorbance at 260 nm was recorded and corrected using the absorbance at 380 nm. The data were recorded at a rate of 0.5 ºC/min, in 0.5 ºC intervals. The T_m values were then determined by taking the first derivative of the melting curves using Origin 9.1 software. The final T_m values are an average of three independent trials, and error bars represent the standard deviation.

Dose-Responsive Fluorescence Measurements

In a Corning 384-well flat bottom polystyrene plate, varying concentrations of L-Tym stock solutions were combined with cleaved or uncleaved hairpin biosensor stock solutions (final concentration = 1 μM) described above to give a final volume of 50 μL. The plates were covered and incubated at 25 ºC for 20 min. Then, the samples were scanned for fluorescence intensity using excitation/emission wavelengths of 490/520 nm. Fluorescence values were standardized using a control solution containing only fluorophore-labeled aptamer strand in the same concentration. The percent displacement (*%D*) was calculated using equation 5:

$$\%D = \left(\frac{F-F_0}{F_m-F_0}\right) \times 100 \quad (5)$$

in which *F* is the measured fluorescence, F_o is the fluorescence of the uncleaved biosensor in the absence of ligand, and F_m is the fluorescence of the fluorophore-labeled aptamer strand. The final data are an average of three independent trials, and error bars represent the standard deviation.

For experiments using OTA biosensor, the same procedure was carried out but a Corning 96-well flat bottom polystyrene plate was used and the final OTA biosensor concentration was 45 nM.

Displacement Kinetics

In a Corning 96-well flat bottom polystyrene plate, L-Tym or OTA stock solutions were combined with uncleaved hairpin biosensor stock solution to give a 100 μL sample. Fluorescence intensities were measured immediately after each UV irradiation on a Maestrogen UltraBright UV Transilluminator (365 nm light, high intensity). Similarly, the percent displacement (*%D*) was calculated using equation 5. The final data are an average of three independent trials, and error bars represent the standard deviation.

Continuous Monitoring of Cleaved Biosensors

Cleaved and uncleaved biosensors were transferred into the wells of a Corning 96-well flat bottom polystyrene plate. In the absence of target, the fluorescence intensities of both biosensors were monitored for 20 minutes at 1 minute intervals using excitation/emission wavelengths of 490/520 nm. Then, L-Tym or OTA stock solutions in varying concentrations were added to the biosensors followed by fluorescence monitoring for another 20 minutes at 1 minute intervals. The percent displacement (%D) was calculated using equation 5.

Figure S3. Percentage displacement as a function of time for L-Tym biosensor (top) and OTA biosensor (bottom). Target molecule was added at 20 minutes.

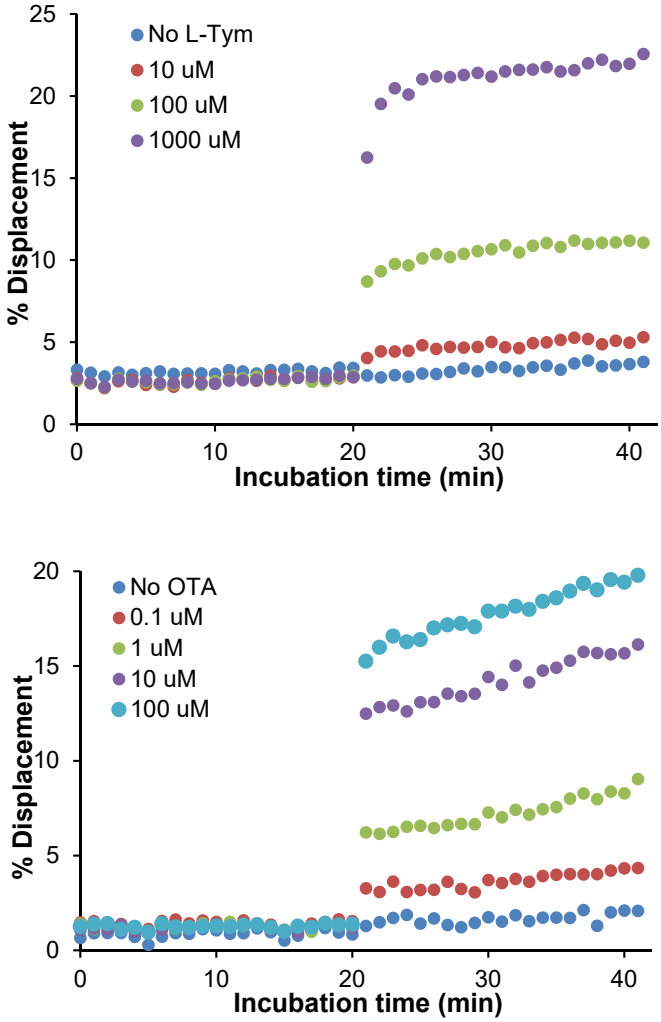


Figure S4. Cleavage kinetics of L-Tym biosensor from PAGE analysis. The data were fit to a first kinetic model to determine the rate constant (k) and half-life ($t_{1/2}$). Errors represent the standard deviation of three independent trials.

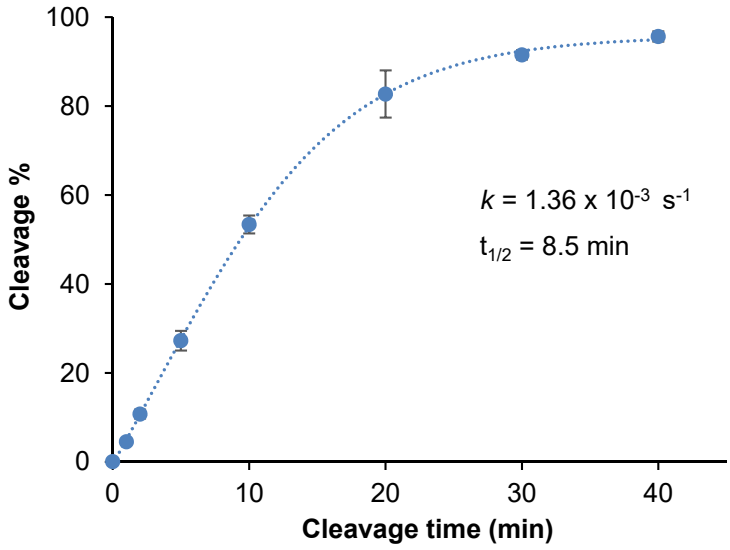
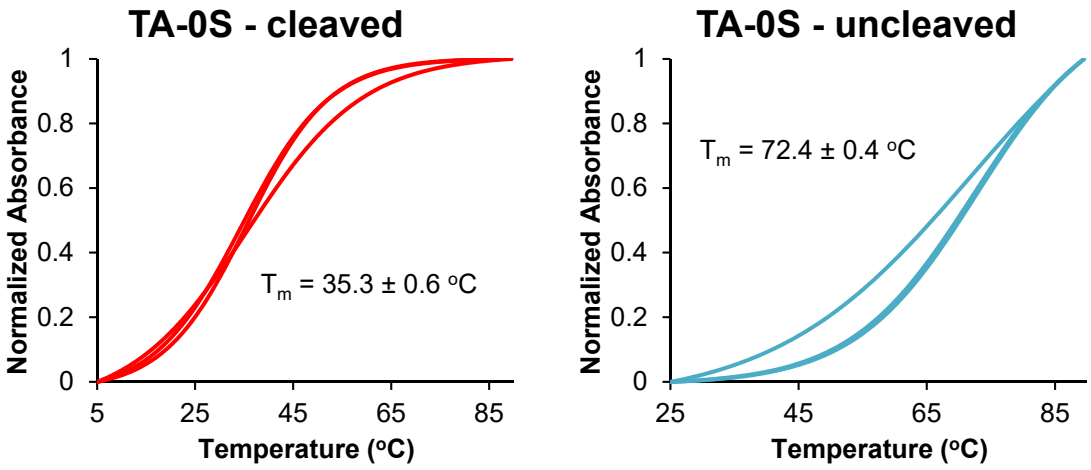
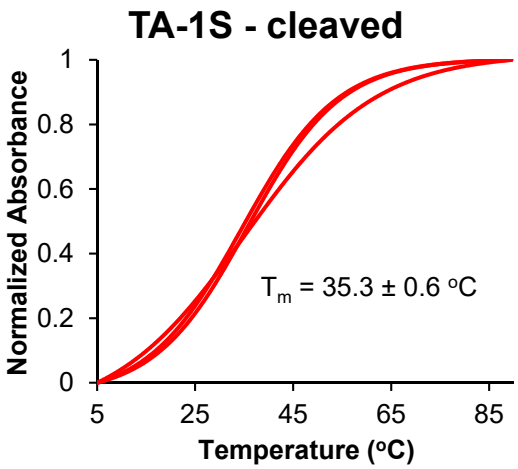
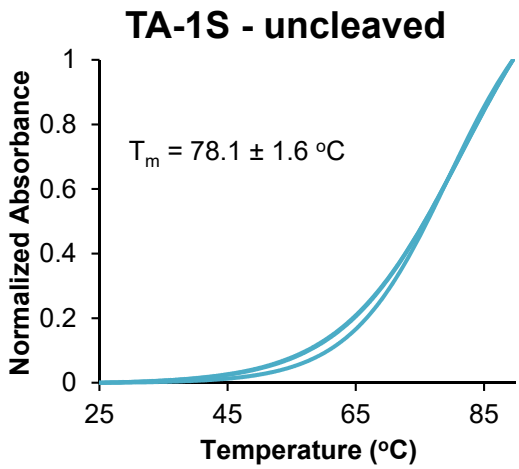
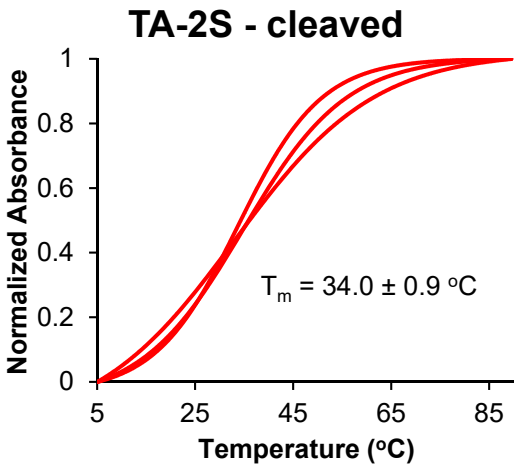


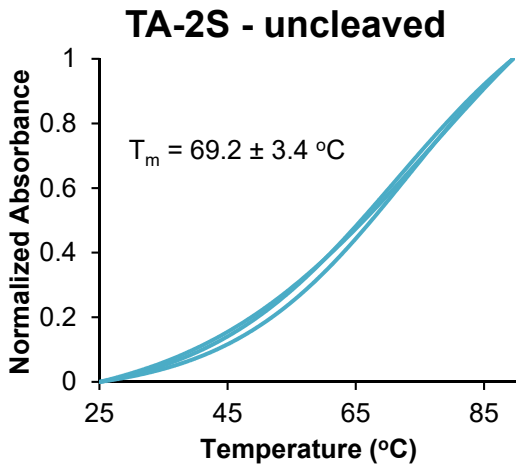
Figure S5. Melting curves for uncleaved and cleaved L-Tym biosensors. Errors represent the standard deviation of three independent trials.

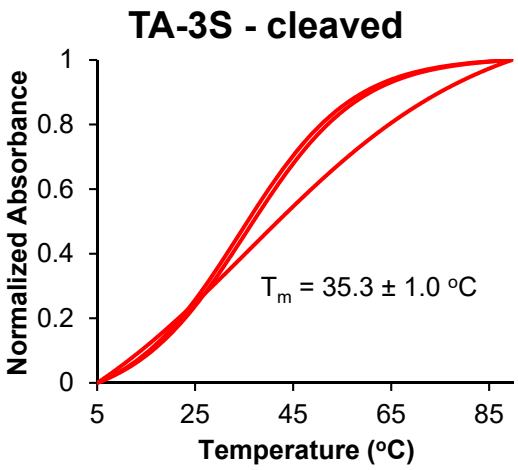



TA-1S - cleaved
Normalized Absorbance
1
0.8
0.6
0.4
0.2
0
5
25
45
65
85
Temperature (°C)
Tm = 35.3 ± 0.6 °C


TA-1S - uncleaved
Normalized Absorbance
1
0.8
0.6
0.4
0.2
0
25
45
65
85
Temperature (°C)
Tm = 78.1 ± 1.6 °C


TA-2S - cleaved
Normalized Absorbance
1
0.8
0.6
0.4
0.2
0
5
25
45
65
85
Temperature (°C)
Tm = 34.0 ± 0.9 °C


TA-2S - uncleaved
Normalized Absorbance
1
0.8
0.6
0.4
0.2
0
25
45
65
85
Temperature (°C)
Tm = 69.2 ± 3.4 °C


TA-3S - cleaved
Normalized Absorbance
1
0.8
0.6
0.4
0.2
0
5
25
45
65
85
Temperature (°C)
Tm = 35.3 ± 1.0 °C

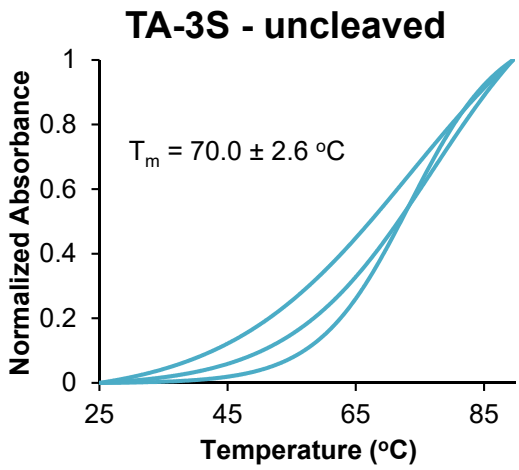
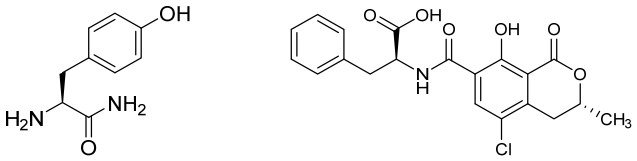

TA-3S - uncleaved
Normalized Absorbance
1
0.8
0.6
0.4
0.2
0
25
45
65
85
Temperature (°C)
Tm = 70.0 ± 2.6 °C

Figure S6. Chemical structures of L-Tym and OTA.



L-Tyrosinamide (L-Tym) Ochratoxin A (OTA)

Figure S7. Real-time monitoring of biosensor response upon un-caging in the presence of 6 mM L-Tym (red circles) overlaid with biosensor cleavage kinetics (blue line). [TA-1S] = 1 μM in 10 mM Tris-HCl, 100 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.5. All fluorescence data are normalized to reflect % displacement of the complementary strand from the aptamer.

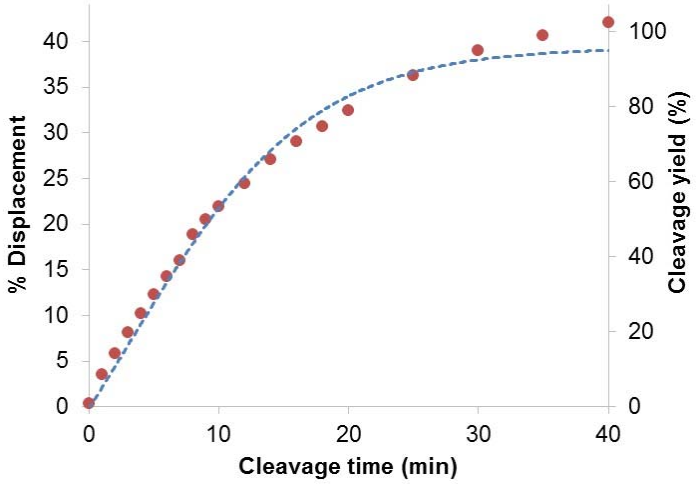


Table S4. Thermodynamic parameters for uncleaved and cleaved L-Tym biosensors. The normalized UV melting data in Figure S2 were used to obtain van 't Hoff plot (ln K_a vs. 1/T). The Gibbs free energy (ΔG) was calculated using equation 3, where T = 298 K:

$$\Delta G = \Delta H - T\Delta S \quad (3)$$

Errors represent the standard deviation of three independent trials.

Sequence	ΔH (kJ·mol^{-1})	TΔS (kJ·mol^{-1})	ΔG (kJ·mol^{-1})
TA-0S-uncleaved	-164.2 ± 27.0	-107.2 ± 22.7	-57.0 ± 4.3
TA-1S-uncleaved	-163.2 ± 13.5	-105.1 ± 11.6	-58.2 ± 2.0
TA-2S-uncleaved	-107.1 ± 5.0	-59.8 ± 4.3	-47.3 ± 0.7

TA-3S-uncleaved	-138.1 ± 29.3	-85.9 ± 25.0	-52.2 ± 4.4
TA-0S-cleaved	-123.8 ± 16.0	-83.40 ± 15.7	-40.4 ± 0.4
TA-1S-cleaved	-114.4 ± 15.0	-74.1 ± 14.7	-40.2 ± 0.4
TA-2S-cleaved	-112.8 ± 19.9	-72.9 ± 19.5	-39.8 ± 0.4
TA-3S-cleaved	-96.9 ± 18.2	-57.0 ± 18.24	-40.0 ± 0.2

Table S5. Tabular data for dose-dependent response of L-Tym biosensor in percent displacement. Errors represent the standard deviation of three independent trials.

	Cleavage time (min)					
[L-Tym] (μM)	0	1	2	5	10	20
0	0.00 ± 0.00	0.76 ± 0.54	1.14 ± 0.49	2.86 ± 0.33	2.77 ± 2.55	4.59 ± 0.58
1	0.16 ± 0.98	1.08 ± 0.82	1.86 ± 0.97	2.80 ± 1.25	3.65 ± 0.82	5.46 ± 0.35
3	-0.66 ± 1.18	0.72 ± 0.67	1.19 ± 1.11	2.90 ± 1.02	5.32 ± 2.40	7.16 ± 2.24
10	-0.77 ± 0.33	1.21 ± 1.03	1.65 ± 1.53	3.90 ± 1.92	7.04 ± 3.67	8.43 ± 1.78
30	0.07 ± 0.81	1.42 ± 0.64	1.50 ± 2.10	5.09 ± 2.20	8.61 ± 3.28	11.33 ± 2.31
100	0.05 ± 1.64	1.38 ± 0.38	4.79 ± 2.06	6.19 ± 2.79	9.33 ± 1.28	13.38 ± 2.44
300	0.46 ± 1.30	1.82 ± 0.26	5.10 ± 1.42	8.75 ± 2.62	12.91 ± 1.05	20.17 ± 4.72
1000	-0.13 ± 1.61	1.94 ± 0.31	4.52 ± 1.61	10.30 ± 2.02	18.37 ± 3.28	26.19 ± 3.80
3000	0.92 ± 0.54	2.25 ± 0.14	5.88 ± 1.57	11.06 ± 2.37	20.52 ± 2.51	29.89 ± 3.33
6000	0.34 ± 1.58	1.65 ± 0.67	5.61 ± 1.09	11.16 ± 1.37	19.86 ± 0.21	30.35 ± 1.80

% Displacement

Table S6. Tabular data for dose-dependent response of L-Tym biosensor in raw fluorescence values. Errors represent the standard deviation of three independent trials.

	Cleavage time (min)						
[L-Tym] (μM)	0	1	2	5	10	20	
0	12675 ± 1002	13166 ± 1024	13410 ± 925	14512 ± 1143	14446 ± 2440	15631 ± 767	Raw fluorescence data
1	12783 ± 374	13370 ± 1042	13873 ± 1038	14471 ± 1586	15017 ± 1470	16191 ± 1055	
3	12256 ± 970	13138 ± 1028	13447 ± 301	14534 ± 1537	16086 ± 2346	17271 ± 2286	
10	12178 ± 1223	13456 ± 1211	13746 ± 92	15173 ± 2078	17184 ± 3164	18089 ± 2022	
30	12727 ± 511	13591 ± 1018	13652 ± 414	15939 ± 2217	18200 ± 2938	19951 ± 2330	
100	12706 ± 1420	13561 ± 1137	15749 ± 1941	16647 ± 2576	18676 ± 1400	21274 ± 2347	
300	12974 ± 846	13845 ± 909	15952 ± 1502	18296 ± 2452	20981 ± 1211	25631 ± 3751	
1000	12589 ± 1426	13928 ± 825	15595 ± 217	19293 ± 2127	24476 ± 2843	29505 ± 3159	
3000	13271 ± 825	14121 ± 912	16461 ± 1090	19782 ± 2319	25868 ± 2367	31892 ± 2774	
6000	12898 ± 1106	13739 ± 616	16294 ± 322	19848 ± 1629	25456 ± 843	32200 ± 1803	

Table S7. K_D values of L-Tym biosensor as a function of cleavage time.

Cleavage time (min)	**K_D (μM)**
5	125
10	128
20	167

Table S8. Tabular data for real-time monitoring experiment using L-Tym biosensor in percent displacement. Errors represent the standard deviation of three independent trials.

	[L-Tym] (μM)				
Cleavage time (min)	0	10	100	1000	6000
0	0.00 ± 0.00	1.13 ± 1.20	1.28 ± 1.08	0.53 ± 1.11	0.37 ± 1.39
1	1.51 ± 0.12	2.81 ± 1.05	3.32 ± 0.98	3.32 ± 1.05	3.58 ± 1.28
2	1.67 ± 0.04	3.33 ± 1.02	4.56 ± 0.99	5.42 ± 1.04	5.80 ± 1.25
3	1.76 ± 0.06	3.69 ± 1.09	5.76 ± 0.87	7.41 ± 1.09	8.13 ± 1.25
4	1.98 ± 0.09	4.06 ± 1.23	6.78 ± 0.93	9.30 ± 1.14	10.26 ± 1.16
5	2.23 ± 0.28	4.60 ± 1.07	7.69 ± 0.83	10.94 ± 1.16	12.28 ± 1.09
6	2.40 ± 0.10	4.93 ± 1.09	8.59 ± 0.84	12.62 ± 1.22	14.27 ± 0.99
7	2.63 ± 0.28	5.49 ± 0.97	9.39 ± 0.84	14.12 ± 1.25	16.03 ± 0.84
8	4.35 ± 0.32	7.30 ± 0.80	11.79 ± 0.43	16.88 ± 1.27	18.88 ± 0.66
9	4.80 ± 0.37	8.05 ± 0.66	12.73 ± 0.40	18.42 ± 1.25	20.49 ± 0.78
10	4.89 ± 0.35	8.30 ± 0.70	13.51 ± 0.45	19.85 ± 1.18	21.96 ± 0.80
12	5.08 ± 0.37	8.76 ± 0.77	14.58 ± 0.20	22.03 ± 1.24	24.45 ± 0.86
14	5.33 ± 0.50	9.31 ± 0.50	15.75 ± 0.30	24.25 ± 1.52	27.02 ± 0.93
16	5.46 ± 0.42	9.63 ± 0.45	16.42 ± 0.17	25.70 ± 1.27	29.02 ± 1.02
18	5.57 ± 0.43	9.99 ± 0.26	17.12 ± 0.25	27.15 ± 1.11	30.64 ± 1.12
20	5.75 ± 0.75	10.41 ± 0.55	17.88 ± 0.29	28.81 ± 1.22	32.48 ± 1.22
25	5.95	11.04	19.21	31.78	36.25

% Displacement

	± 0.43	± 0.92	± 0.37	± 1.20	± 1.20
30	6.50 ± 0.53	11.32 ± 0.98	19.96 ± 0.43	33.72 ± 1.48	39.03 ± 1.41
35	6.47 ± 0.39	11.53 ± 1.01	20.57 ± 0.28	35.39 ± 1.53	40.68 ± 1.66
40	6.66 ± 0.40	11.87 ± 0.95	21.09 ± 0.32	36.20 ± 1.66	42.12 ± 1.70
45	6.77 ± 0.42	12.03 ± 0.91	21.55 ± 0.37	36.83 ± 1.73	42.90 ± 1.74
50	6.84 ± 0.53	12.04 ± 0.80	21.73 ± 0.32	37.52 ± 2.02	43.62 ± 1.80

Table S9. Tabular data for real-time monitoring experiment using L-Tym biosensor in raw fluorescence values. Errors represent the standard deviation of three independent trials.

	[L-Tym] (μM)					
Cleavage time (min)	0	10	100	1000	6000	
0	11418 ± 650	12109 ± 369	12201 ±71	11744 ± 42	11647 ± 228	Raw fluorescence data
1	12402 ± 713	13251 ± 249	13584 ± 37	13583 ± 553	13754 ± 208	
2	12485 ± 660	13558 ± 210	14342 ± 80	14895 ± 551	15140 ± 189	
3	12535 ± 617	13757 ± 140	15071 ± 155	16112 ± 641	16574 ± 198	
4	12662 ± 592	13972 ± 189	15675 ± 227	17251 ± 682	17855 ± 154	
5	12797 ± 647	14266 ± 49	16172 ± 312	18177 ± 761	19011 ± 105	
6	12894 ± 574	14454 ± 111	16709 ± 273	19186 ± 814	20202 ± 89	
7	13028 ± 648	14781 ± 39	17163 ± 450	20051 ± 782	21224 ± 30	
8	14337 ± 714	16324 ± 77	19331 ± 428	22752 ± 919	24096 ± 84	
9	14620	16791	19911	23702	25087	

	± 842	± 179	± 305	± 924	± 61
10	14653 ± 822	16918 ± 135	20364 ± 410	24555 ± 880	25958 ± 82
12	14767 ± 794	17196 ± 86	21032 ± 460	25949 ± 939	27546 ± 83
14	14898 ± 832	17508 ± 277	21712 ± 616	27266 ± 1191	29083 ± 147
16	14978 ± 813	17697 ± 310	22128 ± 602	28179 ± 991	30350 ± 210
18	15039 ± 795	17915 ± 447	22544 ± 633	29066 ± 874	31341 ± 311
20	15120 ± 892	18122 ± 384	22932 ± 629	29972 ± 949	32341 ± 367
25	15187 ± 834	18414 ± 586	23584 ± 668	31544 ± 955	34387 ± 380
30	15484 ± 917	18510 ± 755	23917 ± 730	32538 ± 1089	35870 ± 534
35	15460 ± 840	18624 ± 741	24271 ± 643	33527 ± 1114	36839 ± 682
40	15557 ± 836	18805 ± 679	24537 ± 626	33935 ± 1173	37626 ± 712
45	15599 ± 865	18857 ± 738	24734 ± 704	34179 ± 1217	37943 ± 741
50	15604 ± 926	18796 ± 650	24731 ± 633	34404 ± 1364	38145 ± 758

Table S10. Tabular data for dose-dependent response of OTA biosensor in percent displacement. Errors represent the standard deviation of three independent trials.

	Cleavage time (min)						
[OTA] (μM)	0	2	5	10	20	40	
0	0.00 ± 0.00	0.95 ± 0.36	1.24 ± 0.29	0.54 ± 0.25	0.96 ± 0.13	1.76 ± 0.25	% Displacement
0.001	-0.31 ± 0.17	0.69 ± 0.54	0.92 ± 0.30	0.72 ± 0.60	0.96 ± 0.25	1.87 ± 0.30	
0.01	-0.35 ± 0.14	0.75 ± 0.20	1.03 ± 0.27	1.03 ± 0.25	1.03 ± 0.18	1.60 ± 0.46	
0.1	-0.34 ± 0.07	0.97 ± 0.22	1.35 ± 0.22	1.17 ± 0.25	1.85 ± 0.10	3.34 ± 0.51	
1	-0.16 ± 0.13	1.67 ± 0.22	2.94 ± 0.12	2.94 ± 0.35	4.58 ± 0.43	8.22 ± 0.56	
10	-0.12 ± 0.08	1.71 ± 0.33	3.90 ± 0.33	4.86 ± 0.49	7.47 ± 0.35	14.19 ± 0.35	
100	-0.89 ± 0.20	0.64 ± 0.21	3.41 ± 0.39	5.22 ± 0.33	7.79 ± 0.20	15.50 ± 0.34	

Table S11. Tabular data for dose-dependent response of OTA biosensor in raw fluorescence values. Errors represent the standard deviation of three independent trials.

	Cleavage time (min)						
[OTA] (μM)	0	2	5	10	20	40	
0	12077 ± 109	12761 ± 328	13915 ± 362	12396 ± 346	11220 ± 111	13702 ± 121	Raw fluorescence data
0.001	11876 ± 32	12593 ± 453	13725 ± 354	12514 ± 388	11222 ± 199	13766 ± 145	
0.01	11850 ± 53	12630 ± 240	13795 ± 339	12714 ± 413	11261 ± 155	13604 ± 227	
0.1	11852 ± 67	12771 ± 249	13982 ± 338	12799 ± 450	11766 ± 104	14625 ± 260	
1	11971 ± 82	13229 ± 241	14929 ± 191	13946 ± 397	13438 ± 310	17488 ± 291	
10	12000 ± 64	13254 ± 296	15499 ± 389	15193 ± 490	15211 ± 257	20989 ± 174	
100	11496 ± 22	12561 ± 234	15207 ± 446	15423 ± 346	15412 ± 167	21753 ±188	

Table S12. K_D values of OTA biosensor as a function of cleavage time.

Cleavage time (min)	**K_D (μM)**
10	0.59
20	0.54
40	0.71

Figure S8. Real-time monitoring of OTA biosensor response upon un-caging in the presence of varying concentrations of OTA. [OA-1S] = 45 nM in 10 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 90 nM OA-CS, pH 8.5.

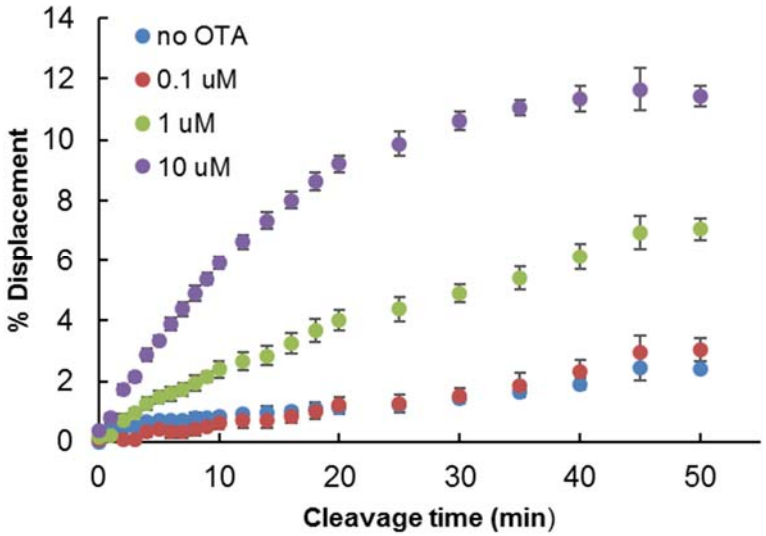


Table S13. Tabular data for real-time monitoring experiment using OTA biosensor in percent displacement. Errors represent the standard deviation of three independent trials.

	[OTA] (μM)			
Cleavage time (min)	0	0.1	1	10
0	0.00 ± 0.00	0.09 ± 0.23	0.15 ± 0.34	0.40 ± 0.22
1	0.43 ± 0.25	-0.03 ± 0.25	0.22 ± 0.39	0.78 0.30
2	0.46 ± 0.22	0.07 ± 0.25	0.73 ± 0.41	1.74 ± 0.30
3	0.50 ± 0.16	0.10 ± 0.08	0.98 ± 0.26	2.14 ± 0.31
4	0.67 ± 0.26	0.35 ± 0.34	1.26 ± 0.43	2.89 ± 0.43
5	0.73 ± 0.19	0.43 ± 0.22	1.48 ± 0.40	3.36 ± 0.31
6	0.72 ± 0.19	0.32 ± 0.39	1.59 ± 0.48	3.89 ± 0.45
7	0.73	0.33	1.73	4.38

% Displacement

	± 0.23	± 0.42	± 0.42	± 0.46
8	0.82 ± 0.31	0.41 ± 0.38	1.95 ± 0.50	4.93 ± 0.50
9	0.81 ± 0.14	0.52 ± 0.26	2.16 ± 0.37	5.39 ± 0.36
10	0.83 ± 0.31	0.61 ± 0.42	2.39 ± 0.58	5.92 ± 0.46
12	0.94 ± 0.25	0.70 ± 0.47	2.66 ± 0.59	6.63 ± 0.45
14	0.99 ± 0.36	0.72 ± 0.54	2.85 ± 0.65	7.33 ± 0.56
16	1.01 ± 0.30	0.86 ± 0.49	3.26 ± 0.68	8.01 ± 0.57
18	1.11 ± 0.40	1.00 ± 0.47	3.67 ± 0.78	8.64 ± 0.60
20	1.15 ± 0.25	1.21 ± 0.52	4.01 ± 0.69	9.22 ± 0.57
25	1.22 ± 0.26	1.28 ± 0.58	4.38 ± 0.79	9.87 ± 0.81
30	1.44 ± 0.19	1.53 ± 0.51	4.91 ± 0.58	10.62 ± 0.60
35	1.64 ± 0.28	1.87 ± 0.81	5.42 ± 0.79	11.05 ± 0.54
40	1.92 ± 0.37	2.33 ± 0.79	6.13 ± 0.83	11.34 ± 0.82
45	2.44 ± 0.83	2.96 ± 1.08	6.93 ± 1.09	11.66 ± 1.43
50	2.40 ± 0.14	3.06 ± 0.77	7.05 ± 0.73	11.44 ± 0.69

Table S14. Tabular data for real-time monitoring experiment using OTA biosensor in raw fluorescence values. Errors represent the standard deviation of three independent trials.

	[OTA] (μM)			
Cleavage time (min)	0	0.1	1	10
0	12965 ± 115	13019 ± 36	13055 ± 124	13208 ± 73
1	14247 ± 51	13951 ± 75	14116 ± 75	14476 ± 47
2	14064 ± 119	13821 ± 65	14237 ± 50	14870 ± 54
3	14013 ± 200	13766 ± 190	14302 ± 116	15007 ± 153
4	13964 ± 244	13778 ± 123	14313 ± 126	15264 ± 168
5	13950 ± 217	13776 ± 173	14390 ± 91	15490 ± 135
6	15345 ± 277	15096 ± 131	15887 ± 139	17321 ± 196
7	15342 ± 262	15095 ± 129	15951 ± 132	17579 ± 159
8	15376 ± 290	15127 ± 180	16054 ± 151	17848 ± 222
9	15336 ± 275	15170 ± 200	16137 ± 136	18054 ± 183
10	15336 ± 289	15211 ± 178	16247 ± 121	18303 ± 212
12	15387 ± 332	15245 ± 160	16379 ± 123	18666 ± 252
14	16870 ± 310	16705 ± 178	18022 ± 108	20793 ± 368
16	16844 ± 314	16757 ± 202	18224 ± 75	21132 ± 280
18	16907 ± 271	16843 ± 171	18445 ± 45	21422 ± 358
20	16933 ± 340	16966 ± 168	18623 ± 141	21700 ± 317
25	17011	17048	18854	22042

Raw fluorescence data

	± 343	± 194	± 147	± 336
30	17085 ± 307	17136 ± 113	19065 ± 107	22326 ± 411
35	17227 ± 321	17358 ± 131	19347 ± 45	22501 ± 431
40	18912 ± 349	19159 ± 136	21448 ± 199	24582 ± 463
45	19066 ± 406	19383 ± 115	21774 ± 154	24617 ± 520
50	19248 ± 388	19639 ± 168	21985 ± 101	24567 ± 480