Supplemental Material

Modular protein-oligonucleotide signal exchange

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Supplementary Data S1: Kinetic modeling of the exchange process

A set of chemical reactions that describe the operation of the exchange process are as follows:

$$W + P \xrightarrow[k_{r,1}]{k_{r,1}} WP; \quad K_d = \frac{[W][P]}{[WP]},$$
 (S1)

$$W + XY \xrightarrow[k_{r,2}]{k_{r,2}} WY + X, \tag{S2}$$

$$X + OZ \xrightarrow{\mathbf{k}_{\mathrm{f},3}} XZ + O.$$
(S3)

Here, aptamer W binds to protein P with a dissociation constant K_d . k_f and k_r are the forward and reverse rate constants respectively and the associated subscripts with them represent the reaction number. To relate the output concentration with the input concentration accurately, we used mass-action kinetics of Reactions S1-S3 to model the input-output relation at the steady-state:

$$\frac{d[W]}{dt} = k_{r,1}[WP] - k_{f,1}[W][P] + k_{r,2}[X][WY] - k_{f,2}[W][XY],$$
(S4)

$$\frac{d[P]}{dt} = k_{r,1}[WP] - k_{f,1}[W][P],$$
(S5)

$$\frac{d[WP]}{dt} = -k_{r,1}[WP] + k_{f,1}[W][P],$$
(S6)

$$\frac{d[X]}{dt} = k_{f,2}[W][XY] - k_{r,2}[X][WY] + k_{r,3}[O][XZ] - k_{f,3}[X][OZ],$$
(S7)

$$\frac{d[XY]}{dt} = -k_{f,2}[W][XY] + k_{r,2}[X][WY],$$
(S8)
$$\frac{d[WY]}{dWY} = k_{r,2}[W][YY] - k_{r,2}[Y][WY]$$
(S9)

$$\frac{t[WY]}{dt} = k_{f,2}[W][XY] - k_{r,2}[X][WY],$$
(S9)
$$\frac{d[O]}{dt}$$

$$\frac{d[O]}{dt} = -k_{r,3}[O][XZ] + k_{f,3}[X][OZ],$$
(S10)

$$\frac{d[OZ]}{dt} = k_{r,3}[O][XZ] - k_{f,3}[X][OZ],$$
(S11)

$$\frac{d[XZ]}{dt} = -k_{r,3}[O][XZ] + k_{f,3}[X][OZ].$$
(S12)

Here and elsewhere W, P, WP, XY, WY, X, OZ, XZ and O species are referred in general for any exchange process, and [] represents the molar concentration of the species. We referred equations S4-S12 as the ODE model for the exchange process and solved it numerically using MATLAB. In this model, K_d and $k_{f,1}$ values of a specific aptamer-protein interaction for an exchange process were taken from the literature (see Table S2) and the rate constants of strand-displacement reactions ($k_{f,2}$, $k_{r,2}$, $k_{f,3}$, $k_{r,3}$) were calculated using the rate constant model of toehold-mediated strand-displacement reaction.¹ The rate constant model requires an accurate calculation of the toehold binding energy for invading and incumbent species involved in the strand-displacement reaction. For example, in Reaction 2 (see Figure 2 in the main paper), tb toehold of W strand is invading XY complex by displacing the incumbent strand X from XY complex. Therefore, to determine the forward rate constant ($k_{f,2}$) of this reaction, we need to calculate the binding energy (ΔG) of the invading strand (W) to incumbent complex. To calculate the reverse rate constants, for Reactions 2 and 3, ($k_{r,2}$ and $k_{r,3}$) X and O will be the invading strands, and WY and XZ complexes will be the incumbent complexes respectively. To calculate the ΔG for each complex, we used NUPACK simulator² (shown in Table S1) and then equations S13- S16 were used to calculate the rates of Reactions 2 and 3 (see Table S2):¹

$$\text{Rate Constant} = \frac{k_c k_b k_{\text{incumbent offrate}}}{k_c k_{\text{incumbent offrate}} + k_b k_{\text{invading offrate}} + k_{\text{incumbent offrate}} k_{\text{invading offrate}}},$$
(S13)

where

$$k_b = \frac{400}{l^2},\tag{S14}$$

$$k_{\text{invading offrate}} = k_c \frac{2}{l} e^{\frac{\Delta G_{\text{invading energy}}}{RT}},$$
(S15)

$$k_{\text{incumbent offrate}} = k_c \frac{2}{l} e^{\frac{\Delta G_{\text{incumbent energy}}}{RT}}.$$
(S16)

Here, for Reaction 2, l is the total number of base-pair of d and c domains and for Reaction 3, it is the total number of base-pair of d domain. $k_c = 3.5 \times 10^6 \text{ M}^{-1} \text{s}^{-1}$, $T = 297 \text{ }^{o}\text{K}$ and R is the gas constant (8.31 J mol⁻¹ K⁻¹).

Supplementary Table S1: Calculated binding energies of the complexes involved in each exchange process. These values were determined using NUPACK simulator.² A correction factor of 2.38 kcal/mol was added in energies listed here to account the conversion from moles to molar when they were used to calculate the rates.¹

Complex	ΔG (kcal/mol)	ΔG (kcal/mol)	ΔG (kcal/mol)	ΔG (kcal/mol)
	Thrombin Sensor W1-O1	Thrombin Sensor W2-O1	VEGF Sensor W3-O1	Thrombin Sensor W4-O2
XY	-25 (X1Y1)	-33.42 (X2Y2)	-35 (X3Y3)	-23.95 (X4Y4)
WY	-25.85 (W1Y1)	-34.78 (W2Y2)	-35.65 (<i>W3Y3</i>)	-24.66 (W4Y4)
OZ	-20.2 (O1Z1)	-20.2 (O1Z1)	-20.2 (<i>O1Z1</i>)	-19.23 (<i>O</i> 2 <i>Z</i> 2)
XZ	-21.7 (X1Z1)	-21.64 (X2Z1)	-21.32 (X3Z1)	-22.14 (X4Z2)

Supplementary Table S2: Parameters values for the reactions involved in each exchange process. Here, $k_{f,2}$, $k_{f,3}$, and $k_{r,3}$ were calculated using equations S13- S16.¹ The aptamer-protein interaction was modeled using K_d and $k_{f,1}$ values that were taken from the literature ($k_{r,1} = K_d k_{f,1}$). The modified values of $k_{f,2}$ (shown inside the brackets) was determined through the least-squares fitting of the measured protein-free response of each exchange process. Here, n can be 1, 2, 3 and 4 representing, respectively, *Thrombin Sensor W1-O1*, *Thrombin Sensor W2-O1*, *VEGF Sensor W3-O1* and *Thrombin Sensor W4-O2*.

	$K_{d,n}$	$k_{f,1,n}$	$k_{f,2,n}$ (modified values)	$k_{r,2,n}$	$k_{f,3,n}$	$k_{r,3,n}$
Sensor	(nM)	$(10^4 \text{ M}^{-1} \text{s}^{-1})$	$(10^6 \text{ M}^{-1} \text{s}^{-1})$	$(10^6 \mathrm{M}^{-1} \mathrm{s}^{-1})$	$(10^6 \mathrm{M}^{-1}\mathrm{s}^{-1})$	$(10^6 \text{ M}^{-1} \text{s}^{-1})$
Thrombin Sensor W1-O1	5.2^{3}	7.45 ⁴	2.82 (0.065)	0.67	3.24	0.26
Thrombin Sensor W2-O1	0.5^{5}	2^{6}	3.18 (0.005)	0.32	3.22	0.28
VEGF Sensor W3-O1	0.37	1.927	2.62 (0.0004)	0.88	3.04	0.46
Thrombin Sensor W4-O2	5.2^{3}	7.45 ⁴	2.69 (0.08)	0.81	3.47	0.03



Supplementary Figure S1: NUPACK simulator predicted no interaction between the domain *d* (highlighted in yellow), which was composed of solely of A's and T's, and its respective original aptamer for each modified aptamer. In the simulation, the salt conditions were the same as in the experimental work (Na⁺ = 140 mM and Mg²⁺ = 1 mM).

Supplementary Data S2: Designing Thrombin Sensor W1-O1

Reliable operation of the exchange process requires that there should be no crosstalk between species that are not programmed to interact. In particular, when the aptamer forms WIYI complex by displacing XI from XIYI in Reaction 2 (see Figure 2 in the main paper), the aptamer should no longer be able to bind to the input protein. Specifically, this means that the WIYI complex should not interact with the protein through the *b* domain of the aptamer. Such an interaction could lower the concentration of the output strand. Likewise, the XI strand that is released from this reaction contains *c* domain which is presented in single-stranded form. It is also critical that this XI strand not be able to interact with the input protein. These interactions may be avoided by choosing short *b* and *c* domains.

Further, as we require that any change in concentration of the WI strand should quickly be reflected in the output, the length of toehold domains should be four base-pair or longer in order to allow Reactions 2 and 3 to reach the steady-state quickly. For the reversible toehold-mediated strand-displacement reactions, the rate constants can typically vary from $1 \text{ M}^{-1}\text{s}^{-1}$ (zero toehold) to $6 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ (7 toehold or longer).¹ Finally, *tc* domain should not share any complementary regions with *c* domain of the aptamer in order to prevent the reversibility of Reaction 3. Based on these and other design considerations (see 'Design' section in the main paper), we designed *Thrombin Sensor W1-O1* to exchange thrombin concentration into output strand *O1* using a modified TBA15 DNA aptamer.^{3,4}

Supplementary Table S3: Sequences of the DNA strands used in *Thrombin Sensor W1-O1* that uses modified TBA15 DNA aptamer to sense input protein thrombin. Here, domains c, tb and b represent the sequence of the original aptamer which folds into a two stacked G-quadruplexes and binds to thrombin with a dissociation constant (K_d) of 5.2 nM.^{3,4}

Name of the DNA Strand	Domains	Sequence
W1	d c tb b	TAATTTATAATTATT GG TTGGT GTGGTTGG
X1	td d C	ATTATG TAATTTATAATTATT GG
Y1	tb* <mark>c</mark> * d* td*	ACCAA CC AATAATTATAAATTA CATAAT
Z1	F tc* d* td*	/56-FAM/ CTCTT AATAATTATAAATTA CATAAT
01	d tc q	TAATTTATAATTATT AAGAG /3IABkFQ/



Supplementary Figure S2: Electrophoresis mobility shift assay for characterizing the interaction of thrombin with the modified TBA15 DNA aptamer. Lane 1 is a 10 base-pair ladder (ThermoFisher Scientific) while lane 2-5 and 6-9, respectively, indicating a fixed concentration of pre-annealed 15-mer TBA15 and modified TBA15 (W1) aptamers that were incubated with different amounts of thrombin (see Table S4) at 25 °C for 30 min before loading the samples into the gel (see 'Materials and Methods' section in the main paper). (A) Detection of the DNA with SYBR Gold staining. (B) Detection of thrombin with EZBlue staining. An increase in thrombin concentration increases its migration in the presence of 15-mer TBA15 or modified TBA15 aptamer by almost the same amount, suggesting the affinity of the modified aptamer towards thrombin may not be significantly altered compared to the original aptamer. Detailed stoichiometry of the gel matrix is shown in Table S4.

Lane	Original TBA15 aptamer (μ M)	Thrombin protein (μ M)	Modified TBA15 aptamer (μ M)
2	5	0	-
3	5	1	-
4	5	2.5	-
5	5	5	-
6	-	0	5
7	-	1	5
8	-	2.5	5
9	_	5	5

Supplementary Table S4: Stoichiometry of the gel matrix shown in Figure S2.



Supplementary Figure S3: Electrophoresis mobility shift assay to detect the interaction between b or c domain with thrombin. Lane 1 is a 10 base-pair ladder (ThermoFisher Scientific), lane 2-4 indicating different concentrations of thrombin and *W1Y1* complex, which has a single stranded b domain (see Figure 2 in the main paper), in the ratio of 1:0, 0:1 and 1:1 while lane 5-7 indicating thrombin and *X1* strand, which has c domain, in the ratio of 1:0, 0:1 and 1:1 respectively (see Table S5). (A) Detection of the DNA with SYBR Gold staining. (B) Detection of thrombin with EZBlue staining. In the gel matrix, no bands of thrombin were observed in the presence of *W1Y1* complex or *X1* strand, suggesting that b or c domain alone has no affinity towards thrombin. Detailed stoichiometry of the gel matrix is shown in Table S5.

Lane	Thrombin (μ M)	$W1Y1 \ (\mu M)$	$Xl~(\mu M)$
2	5	0	-
3	0	5	-
4	5	5	-
5	5	-	0
6	0	-	5
7	5	-	5

Supplementary Table S5: Stoichiometry of the gel matrix shown in Figure S3.



Supplementary Figure S4: Electrophoresis mobility shift assay for characterizing the interaction of thrombin with aptamer in the presence of non-aptamer DNA. Lane 1 is a 10 base-pair ladder (ThermoFisher Scientific) while lane 2-5 and 6-9, respectively, indicating a fixed concentration of pre-annealed 15-mer TBA15 and modified TBA15 (W1) aptamers (see 'Materials and Methods' section in the main paper). Lane 11-12 and lane 13-14 have different concentrations of thrombin in the presence of either W1Y1 complex or X1 strand respectively while lane 10 has thrombin only. Detailed stoichiometry of the gel matrix is shown in Table S6. A similar interaction between aptamer and thrombin was observed as was shown in Figure S2 in the presence of non-aptamer DNA X1 strand and thrombin (lane 14).

Supplementary	Table S6:	Stoichometry	of the ge	l matrix showr	in Supr	olemental	Figure S	S4.

Lane	TBA15 aptamer (µM)	Thrombin (μM)	Modified TBA15 (µM)	W1Y1 (μ M)	$X1 (\mu M)$
2	5	0	-	-	-
3	5	1	-	-	-
4	5	2.5	-	-	-
5	5	5	-	-	-
6	-	0	5	-	-
7	-	1	5	-	-
8	-	2.5	5	-	-
9	-	5	5	-	-
10	-	5	-	0	-
11	-	0	-	5	-
12	-	5	-	5	0
13	-	0	-	-	5
14	-	5	-	-	5

Supplementary Data S3: Calibration fluorophore dyes to determine the concentration of the output strand from the measured responses

In this study, while measuring the responses of each exchange process without or with protein, we recorded the changes in the fluorescence intensity before and after adding the input molecule, which was aptamer or protein. We then normalized the measured fluorescence signal with respect to the background, which was measured in the absence of the input molecule. The normalized value corresponds to a specific concentration of XZ complex present in the reaction volume at the steady-state. In order to compare the experimental data with the model data accurately, we calibrated the fluorophore molecule so that we can relate the measured fluorescence signal with the exact concentration of XZ complex.

To determine the relation between the fluorescence signal and the [XZ], we need to know the exact amount of XZ present in a reaction mixture at the steady-state. Because in the current design of transduction stage, Reaction 3 is reversible, it is not straightforward to relate the [XZ] complex with input [X] at the steady-state without knowing the exact values of the rates for this reaction. Therefore, to reduce the number of unknown parameters, we used a DNA strand $(Z1^*)$ that can form a duplex with Z1 by displacing O1 from O1Z1 complex (Figure S5). Because $Z1^*$ is completely complementary to Z1, this reaction is irreversible as no toehold will be available for O1 to interact with $Z1^*Z1$ duplex, once $Z1^*$ strand hybridized with Z1. At the steady-state, the concentration of $Z1^*Z1$ complex must be equal to the input concentration of $Z1^*$. Therefore, by diving the measured normalized fluorescence signal with the input concentration of $Z1^*$, we can calculate the average calibration factor that relates the concentration of $Z1^*Z1$ with the measured fluorescence signal.

To determine the calibration factor, a fixed concentration of pre-annealed O1Z1 complex (500 nM) was mixed with different concentrations $Z1^*$ (50, 100, 150, 200 and 250 nM) and the changes in the fluorescence intensity were recorded before and after adding $Z1^*$ (Figure S6A) and average calibration factor was calculated. Similar experiments were performed to calibrate the FAM and HEX fluorophore labeled complex of *Thrombin Sensor W4-*O2 (Figure S6B and S7 respectively) to determine the calibration factor. Using these values, we converted the concentration of the input $Z1^*$ strand in fluorescence unit and a close agreement between the measured and the calculated responses was observed (Figure S6 and S7).



Supplementary Figure S5: Irreversible strand-displacement reaction to characterize the measured fluorescence intensity and the concentration of the presented fluorophore labeled complex.

Due to the fact that any change in the [XZ] would also represent the same change in the [O] strand, in this work, we reported the measured response as a change in the concentration of the output strand. Further, for each experiment, we ran similar calibration measurements as mentioned above so that the measured fluorescence signal can be converted into concentration accurately.



Supplementary Figure S6: Fluorescence calibration curves of FAM fluorophore. Variations in the fluorescence intensity were recorded before and after adding different amounts of respective Z^* strand in the mixtures where the respective [OZ] = 500 nM (ZI^* and O1ZI for (A), and $Z2^*$ and O2Z2 for (B)). (A) For *Thrombin Sensor W1-O1*, *Thrombin Sensor W2-O1* and *VEGF Sensor W3-O1*, a fluorescence unit is equal to 14.26 pM of Z^* strand while (B) for *Thrombin Sensor W4-O2*, it is 9.47 pM. It should be noted that *Thrombin Sensor W1-O1*, *Thrombin Sensor W2-O1* and *VEGF Sensor W3-O1* use the same O1Z1 complex compared to *Thrombin Sensor W4-O2* that uses O2Z2 complex that is completely different from O1Z1.



Supplementary Figure S7: Fluorescence calibration curve of HEX fluorophore used in Thrombin Sensor W4-O2. Variations in the fluorescence intensity were recorded before and after adding different amounts of $Z2^*$ strand in the mixtures having [O2Z2] = 500 nM. For HEX fluorophore, a fluorescence unit is equal to 13.54 pM of $Z2^*$ strand.

Supplementary Data S4: Measuring the forward rate constant of Reaction 2 for Thrombin Sensor W1-O1

For the calculated value of $k_{f,2}$, we found that the ODE model of the exchange process is not able to predict the measured response of *Thrombin Sensor W1-O1* accurately in the absence of thrombin (see Figure S8). This may be due to the G-quadruplex folding of the TBA15 aptamer that may result in reducing its interaction with the *X1Y1* complex through *tb* toehold. To test this hypothesis, we used the modified *X1Y1* complex of *Sensor W1-O1* by labeling the *X1* and *Y1* strands with a fluorophore and a quencher molecule respectively (see Figure 9A; 'Materials and Methods' section in the main paper) and then measured the changes in the fluorescence intensity with different amounts of *W1* strand (see Figure 9B). We found the best fit between the measured response and model data for a $k_{f,2}$ value of $6.49 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$, which is much smaller than the calculated value ($2.82 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$).



Supplementary Figure S8: Comparison of measured protein-free response with the model data when the calculated value of $k_{f,2}$ (2.82 × 10⁶ M⁻¹s⁻¹) was used (see Table S2).



Supplementary Figure S9: **Determining the forward rate constant of Reaction 2 for Thrombin Sensor W1-O1.** (A) A schematic representation of Reaction 2 alone where *X1Y1* complex was labeled with a fluorophore dye and a quencher (see 'Materials and Methods' section in the main paper) so that any change in the concentration of *X1* strand can be directly detected when *W1* strand displaces *X1* strand from *X1Y1* complex. (B) We added different concentrations of *W1* stand in mixtures that have 250 nM of *X1Y1* and changes in the fluorescence intensity were recorded before and after adding the *W1* strand. The best fit between the measured and the model data was found when $k_{f,2} = 6.49 \times 10^4 \text{ M}^{-1} \text{s}^{-1}$. (C) The measured fluorescence intensities were converted into concentration through directly calibrating the fluorescence to [*X1*] relation. A linear regression (shown in dashed line) with a zero intercept was used to fit the measured response (shown in dashed-dot line) and the corresponding R-square value is 0.98.



Supplementary Figure S10: Predictable response of Thrombin Sensor W1-O1 at different ranges of input protein concentrations. Measured response of *Thrombin Sensor W1-O1* where thrombin was added in different incremental amounts in the presence of (A) [W1] = [X1Y1] = [O1Z1] = 50 nM, and (B) [W1] = [X1Y1] = [O1Z1] = 500 nM.



Supplementary Figure S11: **Thrombin Sensor W1-O1 operates reliably**. (A) Measured response of *Thrombin Sensor W1-O1* in the presence and absence of *W1* strand (500 nM) and [X1Y1] = [O1Z1] = 500 nM. In the absence of *W1* strand, the concentration of the output strand does not change significantly in the presence of different amounts of thrombin, suggesting that *X1Y1* or *O1Z1* do not interact with the thrombin. (B) When 50% H₂O/glycerol mixture in which thrombin was dissolved was added instead of thrombin in the presence of [W1] = [X1Y1] = [O1Z1] = 500 nM, no significant change in the output signal was observed with respect to the value when no 50% H2O/glycerol was added. This suggests that H₂O/glycerol base mixture does not affect the operation of the exchange process adversely.

Supplementary Data S5: Designing Thrombin Sensor W2-O1 and VEGF Sensor W3-O1

For *Thrombin Sensor W2-O1* and *VEGF Sensor W3-O1*, we added the same 15-mer sequence (d domain) at the 5'-ends of TBA29^{5,6} and 3R02⁷ aptamers as used for *Thrombin Sensor W1-O1*. This enabled us to produce the same *O1* strand for *Thrombin Sensor W2-O1* and *VEGF Sensor W3-O1* as in *Thrombin Sensor W1-O1*, even though, they use totally different aptamers and/or sense different proteins. We then grouped the core sequence of each aptamer into c, tb and b domains while minimizing the possibility of *WY* or *X* species binding with the input protein through b or c domain respectively for each exchange process. This is achieved by allowing limited base-pairs for overhang b domain and a short c domain. We used the same sequence of the toehold td for each Y strand for *Thrombin Sensor W2-O1* and *VEGF Sensor W3-O1* as used for *Thrombin Sensor W1-O1*, so that the same *O1Z1* complex could be used for *Thrombin Sensor W1-O1*, *Thrombin Sensor W2-O1* and *VEGF Sensor W3-O1*.

Supplementary Table S7: Sequences of the DNA strands used in *Thrombin Sensor W2-O1* that uses modified TBA29 DNA aptamer to target thrombin. Here, domains c, tb and b represent the sequence of the original aptamer that binds to thrombin with a dissociation constant (K_d) of 0.5 nM.^{5,6}

Name of the DNA Strand	Domains	Sequence
W2	d c tb b	TAATTTATAATTATT AGTCCGTG GTAGG GCAGGTTGGGGTGACT
X2	td d c	ATTATG TAATTTATAATTATT AGTCCGTG
Y2	tb* c* d* td*	CCTAC CACGGACT AATAATTATAAATTA CATAAT
Z1	Ftc* d* td*	/56-FAM/ CTCTT AATAATTATAAATTA CATAAT
01	d tc a	TAATTTATAATTATT AAGAG /3IABkFQ/

Supplementary Table S8: Sequences of the DNA strands used in *VEGF Sensor W3-O1* that uses modified 3R02 DNA aptamer to target VEGF. Here, domains c, tb and b represent the sequence of the original aptamer that binds to VEGF with a dissociation constant (K_d) of 0.3 nM.⁷

Name of the DNA Strand	Domains	Sequence
W3	<u>d</u> c tb b	TAATTTATAATTATT TGTGGGGG TGGA CTGGGTGGGTACC
ХЗ	td d c	ATTATG TAATTTATAATTATT TGTGGGGG
Y3	tb* c* d* td*	ТССА СССССАСА ААТААТТАТАААТТА САТААТ
Z1	Ftc* d* td*	/56-FAM/ CTCTT AATAATTATAAATTA CATAAT
01	d tc q	TAATTTATAATTATT AAGAG /3IABkFQ/



Supplementary Figure S12: Thrombin Sensor W2-O1 and VEGF Sensor W3-O1 can relate the free aptamer concentration with the output signal. *Thrombin Sensor W2-O1* uses the modified TBA29 aptamer (*W2*) as a thrombin binding aptamer and thrombin as an input protein while *VEGF Sensor W3-O1* uses modified 3R02 aptamer (*W3*) to detect VEGF as an input protein. Protein free response of (A, C) *Sensor W2-O1* where [*X2Y2*] = [O1Z1] = 100 nM and (B, D) *Sensor W3-O1* when [*X3Y3*] = [O1Z1] = 100 nM. In each case, ODE model of the exchange process was used to predict the response while using the $k_{f,2}$ that was either (A, B) calculated or (C, D) found through the least-squares fitting (see Data S1 for details).



Supplementary Figure S13: A reliable predictable response of Thrombin Sensor W2-O1 at different ranges of input protein concentrations. Measured response of *Thrombin Sensor W2-O1* where thrombin was added in different incremental amounts in the presence of (A) [W2] = [X2Y2] = [O1Z1] = 50 nM, and (B) [W2] = [X2Y2] = [O1Z1] = 500 nM. For each variation in thrombin concentration, model data can track the output.



Supplementary Figure S14: A reliable predictable response of VEGF Sensor W3-O1 at different ranges of input protein concentrations. Measured response of *VEGF Sensor W3-O1* where thrombin were added in different incremental amounts in the presence of (A) [W3] = [X3Y3] = [O1Z1] = 50 nM, and (B) [W3] = [X3Y3] = [O1Z1] = 500 nM. For each variation in VEGF concentration, model data can track the output.

Supplementary Data S6: Designing Thrombin Sensor W4-O2

Thrombin Sensor W4-O2 takes thrombin as in input protein and passes this information to an output strand O2 that has no sequence in common with the output strand O1 of other exchange processes we designed so far. This is achieved by changing the sequence of d and tc domains compared to Thrombin Sensor W1-O1. They both use the same sequence of the original TBA15 aptamer (see Table S3).

Supplementary Table S9: Sequences of the DNA strands that are used in *Thrombin Sensor W4-O2*. This sensor uses TBA15 DNA aptamer to target thrombin but produces a different output strand (O2) compared to the output strand (O1) of the other three sensors.

Name of the DNA Strand Domains		Sequence
W4	d c tb b	TAATATATAATAATA GG TTGGT GTGGTTGG
X4	td d C	TTAATG TAATATATAATAATA GG
Y4	tb*c* d* td*	ACCAA CC TATTATTATATATATA CATTAA
Z2	<mark>F tc* d* td</mark> *	/56-FAM/ TTTTC TATTATATATATATA CATTAA
02	d tc q	TAATATAAATAATA GAAAA /3IABkFQ/



Supplementary Figure S15: A reliable, predictable response of Thrombin Sensor W4-O2 at different ranges of input protein concentrations. Measured response of *Thrombin Sensor W4-O2* where thrombin were added in different incremental amounts in the presence of (A) [W4] = [X4Y4] = [O2Z2] = 50 nM, and (B) [W2] = [X4Y4] = [O2Z2] = 500 nM. For each variation in thrombin concentration, model data can track the output.

Supplementary Data S7: Modeling the combined response of Thrombin Sensor W2-O1 and VEGF Sensor W3-O1

To model the combined response of *Thrombin Sensor W2-O1* and *VEGF Sensor W3-O1*, we used the following set of reactions:

W2 + P2
$$\frac{\mathbf{k}_{f,1,2}}{\mathbf{k}_{r,1,2}}$$
 W2 P2; $K_{d,2} = \frac{[W2][P2]}{[W2P2]}$, (S17)

W2 + X2 Y2
$$\frac{k_{f,2,2}}{k_{r,2,2}}$$
 W2 Y2 + X2, (S18)

$$X2 + O1Z1 \xrightarrow[k_{f,3,2}]{k_{f,3,2}} X2Z1 + O1,$$
 (S19)

W3 + P3
$$\frac{k_{f,1,3}}{k_{r,13,}}$$
 W3 P3; $K_{d,3} = \frac{[W3][P3]}{[W3P3]}$, (S20)

W3 + X3 Y3
$$\frac{k_{f,2,3}}{k_{r,2,3}}$$
 W3 Y3 + X3, (S21)

$$X3 + O1Z1 \frac{k_{f,3,3}}{k_{r,3,3}} X3Z1 + O1.$$
(S22)

Here, $k_{f1,2}, k_{r,1,2}, k_{f2,2}, k_{r,2,2}, k_{f3,2}, k_{r,3,2}$ and $k_{f1,3}, k_{r,1,3}, k_{f2,3}, k_{r,2,3}, k_{f3,3}, k_{r,3,3}$ are the rates for *Thrombin* Sensor W2-O1 and VEGF Sensor W3-O1 respectively. Using mass-action kinetics, we derived the coupled differential equations model of Reactions S17-S22 and solved them numerically using the rates shown in Table S2.



Supplementary Figure S16: Modified exchange process to produce a DNA output strand of completely independent sequence from the original aptamer's sequence. By adding an additional strand-displacement reaction (green) into the existing transduction stage (cyan), a new DNA output strand (VI) can have any sequence as the sequence of the added the *d* domain, which is added in the original aptamers, will not be a part of the VI strand. VI would still be able to reflect the concentration of the input protein.



Supplementary Figure S17: Varying K_d values up to five-times does not significantly alter the model data. Model response for (A-C) *Thrombin Sensor W1-O1*, (D-F) *Thrombin Sensor W2-O1*, (G-I) *VEGF Sensor W3-O1* and (J-L) *Thrombin Sensor W4-O2* are compared with the measured responses while considering different K_d values from the nominal values mentioned in Table S2.



Supplementary Figure S18: An alternative scheme of protein-oligonucleotide signal exchange processes where different protein inputs can control the concentration of the different DNA strand outputs using two exchange processes with different inputs and outputs. This operation can be realized using *VEGF Sensor W3-O1* and any of *Thrombin Sensor*.

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