Supporting information for

Investigation of Protein Detection Parameters using Nano-Functionalized Organic-Field Effect Transistors

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Quantification of AuNP Spacing, Diameter, and DNA Loading

Atomic force microscopy (AFM) has been known to distort the lateral dimension (diameter) of AuNPs due to their convolution with the AFM tip.[72] Therefore, we have used scanning electron microscopy (SEM) to measure the average diameter of the AuNPs (D_{AuNP}) (Figure S1), though the values obtained from both AFM and SEM measurements are provided for comparison (Table S1). As determined from SEM, D_{AuNP} = 4.8 ± 0.6 nm, 10.3 ± 0.9 nm, and 10.1 ± 3.6 nm for AuNP cast from the 34, 105, and 1376 kDa PS-P2VP, respectively. While the standard deviation in D_{AuNP} for the AuNPs cast from the 34 and 105 kDa polymers is quite small, indicating near monodispersity, those produced from the 1376 kDa polymer are polydisperse. However, it should be noted that the smallest D_{AuNP} value observed for the AuNPs cast from the 1376 kDa polymer was 5.4 nm, while the largest was 18.7 nm, indicating less variation than would be suggested from the AFM images alone.

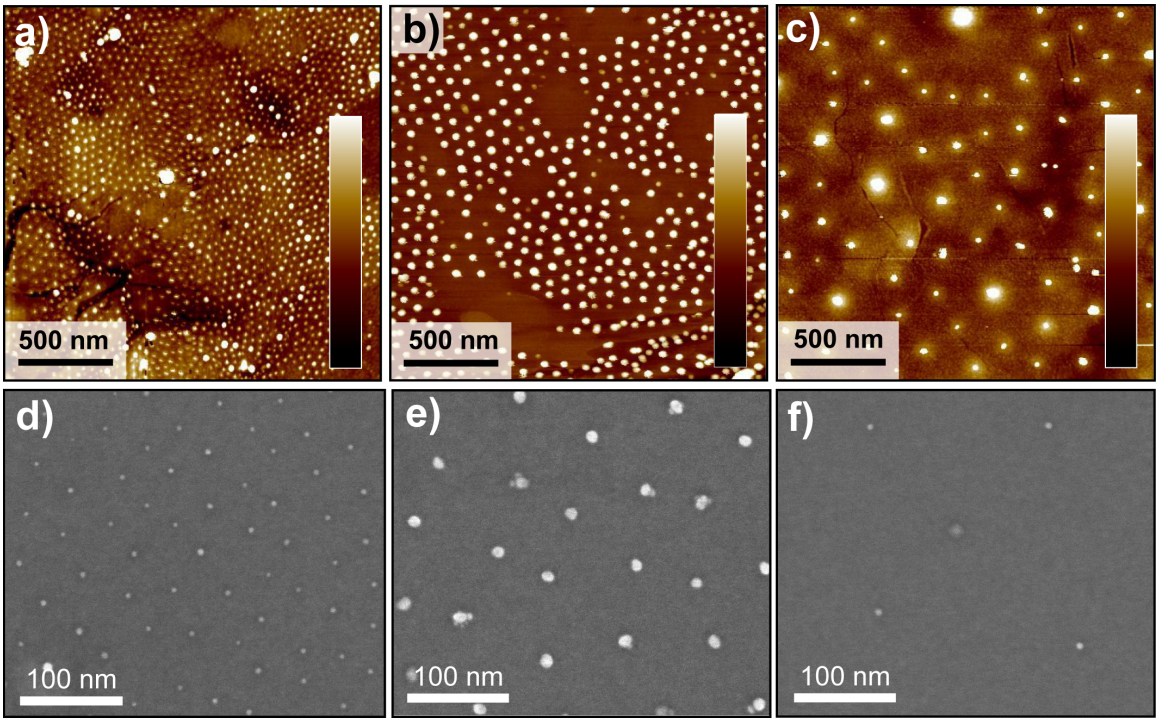


Figure S1. Characterization of AuNPs cast from various M_n PS-P2VP. Large area AFM measurement of AuNPs cast from a) 34, b) 105, and c) 1376 kDa PS-P2VP. The diameters of the AuNPs (D_{AuNP}) are distorted due to convolution between the AuNP and the AFM tip.[72] AFM height bars = a) 20 nm, b) 25 nm, and c) 25 nm. Zoomed in SEM images of AuNPs cast from d) 34, e) 105, and f) 1376 kDa PS-P2VP, from which accurate D_{AuNP} measurements can be made.

Table S1. Average AuNP diameter (D_{AuNP}) as measured by AFM and SEM.

	34 kDa		105 kDa		1376 kDa	
	SEM	AFM	SEM	AFM	SEM	AFM
Avg.	4.8	7.7	10.3	27.3	10.1	26.4
StDev.	0.6	4.1	0.9	11.1	3.6	15.8

The average center-to-center spacing (d_{avg}) determined from AFM and SEM measurements are reasonably in agreement with one another (Table S2). As the d_{avg} reported from the AFM measurements were calculated using a MATLAB script that calculated d_{avg} by identifying the centers of the AuNPs, they are not affected by the tip convolution that misrepresents D_{AuNP}. As significantly more AuNP were imaged with AFM, the d_{avg} values reported from the AFM data are likely more accurate; however, d_{avg} from both SEM and AFM measurements are provided for comparison. As determined by AFM, d_{avg} = 39.4 ± 0.2 nm 70.4 ± 0.6 nm, and 158.7 ± 8.0 nm for AuNPs cast from the 34, 105, and 1376 kDa PS-P2VP, respectively. For simplicity throughout the text, we have referred to these values as d_{avg} ~40, ~70 and ~160 nm, respectively. The increased value in the standard deviation for the 1376 kDa polymer demonstrates the more polydisperse nature of the AuNPs cast from this polymer.

Table S2. Average AuNP center-to-center spacing (d_{avg}) as measured by AFM and SEM.

	34 kDa		105 kDa		1376 kDa	
	SEM	AFM	SEM	AFM	SEM	AFM
Avg.	47.1	39.4	80.8	70.4	186.2	158.7
StDev.	8.3	0.2	8.7	0.6	74.4	8.0

The DNA loading per AuNP has also been quantified (Table S3). AuNPs were spun cast onto 1.5 cm x 1.5 cm substrates as previously described. 100 μL (enough to fully cover the surface) of a 100 μM DNA solution in 0.0001x TBB, pH 5.5 was introduced to the AuNP-containing substrates, followed by incubation at room temperature for 60 min in a desiccator under slight

vacuum. Afterward, the substrates were then thoroughly rinsed in a 1x PBS solution to remove nonspecifically bound DNA from the surface. To dissolve the AuNPs and release the bound DNA into solution,[73] 200 μL of a 100 μM NaCN solution was subsequently added to the AuNPs for 30 min (Figure S2). The DNA-containing solution was collected and concentrated using a vacufuge (Eppendorf). This process was repeated a total of 2 times for the 34 kDa PS-P2VP, 4 times for the 105 kDa PS-P2VP, and 6 times for the 1376 kDa PS-P2VP in order collect enough DNA for measurement. The DNA was resuspended in 3 μL of 1x TBB, pH 5.5, and quantified using a NanoDrop 1000 spectrophotometer. The DNA loading per AuNP was calculated, taking into account the appropriate concentration ratio (determined from the number of surfaces used to collect the DNA) and AuNP surface density (ρ), and assuming that the DNA could only access half the surface area of the AuNP due to the geometry on the surface. The DNA loading on the AuNPs cast from the 34 kDa polymer was determined to be 2.9 ± 0.5 DNA strands/ AuNP, while that on the AuNPs cast from the 105 kDa polymer was 9.2 ± 1.8 DNA strands/ AuNP. The high salt concentration of the sample collected from the AuNP cast from the 1376 kDa polymer prevented accurate quantification of DNA loading, though it is likely within the range of the DNA loading for the AuNPs cast from the other M_n PS-P2VP due to the similarities in AuNP size. These values are similar to those extrapolated from the literature;[73,74] however, it should be noted that parameters such as D_{AuNP}, AuNP radius of curvature, DNA sequence composition, salt concentration, and the use of sonication have been identified as important factors in altering the DNA loading density on these AuNP.[73,74] Since our DNA loading studies were performed in low salt (0.0001x TBB) on small AuNP without sonication, and with only half of the surface area available for binding, low DNA loading values are expected.

Table S3. DNA loading on AuNPs.

	34 kDa		105 kDa		1376 kDa	
	ρ (AuNP/μm^2)	Loading (DNA/ AuNP)	ρ (AuNP/μm^2)	Loading (DNA/ AuNP)	ρ (AuNP/μm^2)	Loading (DNA/ AuNP)
Avg.	598.3	2.9	128.3	9.2	22.4	N/A
StDev.	1.4	0.5	5.0	1.8	4.3	N/A

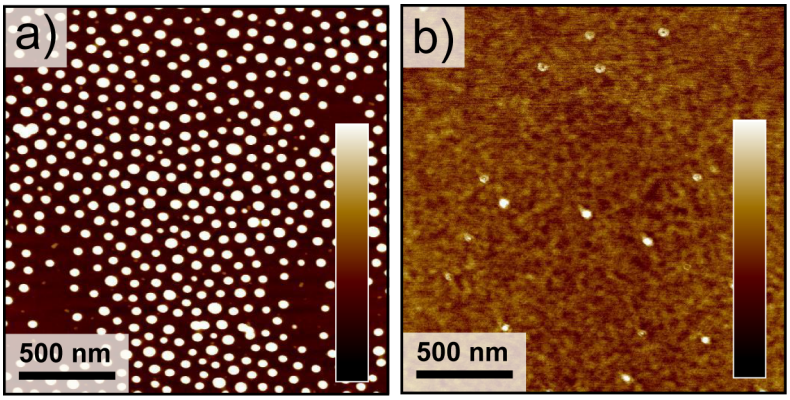


Figure S2. AFM characterization of AuNPs cast from 105 kDa PS-P2VP. Images of AuNPs a) before and b) after treatment with 100 mM NaCN. AFM height bars are a) 25 nm, b) 2 nm.

Optimization of Surface Blocking

Concentration of bovine serum albumin (BSA) and time of functionalization process were varied in an attempt to achieve near-monolayer surface coverage to block the surface of the OFET device. The BSA concentration was varied between 0.1 and 1% v/v in a 1x phosphate buffered saline (PBS) solution and deposited for 0-120 min (Figure S3). The contact angle of the surface was measured in order to determine the progress of the BSA functionalization reaction, and the optimized conditions (0.1% BSA in 1x PBS, deposited for 60 min) were taken to be at the minimum contact angle achieved in the shortest amount of time. The contact angle for these BSA-blocked devices was found to be similar to that reported in the literature.[38]

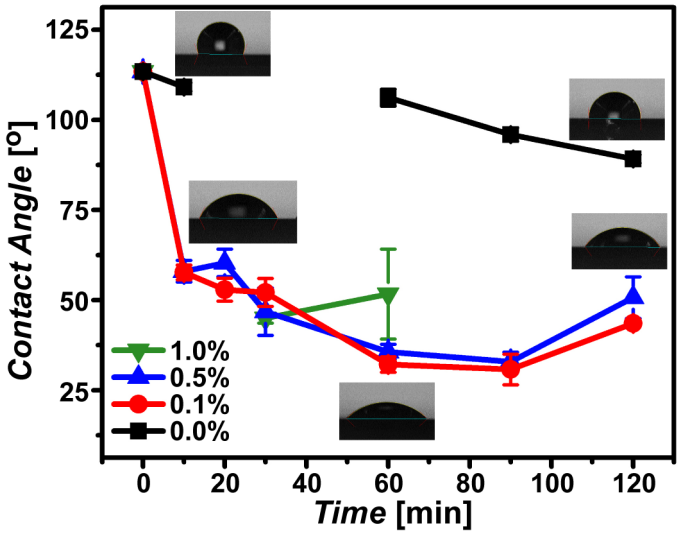


Figure S3. Contact angle measurements of optimization of BSA surface blocking treatment. Surface contact angle resulting from deposition of solutions of varying concentration of BSA in 1x PBS (0.0% = black squares, 0.1% = red circles, 0.5% = blue triangles, 0.1% = green triangles) for various durations. Lines presented are a guide to the eye.

In Situ Device Responses for AuNP-Decorated OFETs

Transfer (drain-source current (I_{DS}) *vs*. gate-source voltage (V_{GS}), Figure S4) and output (I_{DS} *vs*. drain-source voltage (V_{DS}), Figure S5) plots for devices functionalized with the thrombin binding aptamer were recorded before (Figure S4, blue lines and Figures S5a, S5c, and S5e) and after (Figure S4, black lines and Figures S5b, S5d, and S5f) exposure to thrombin *in situ*. It was found that a positively charged target protein (at pH 5.5) shifted the threshold voltage (V_T) to the left (Figure S4a), as expected given the measured decrease in current after addition of thrombin to the device (Figure 6a, Figure S5a *vs*. S5b). This response is consistent with previously reported results for positively charged proteins interacting with a p-type device.[54] Neutrally charged thrombin (pH 7.5) was not found to shift V_T (Figure S4b) or change I_{DS} in any appreciable manner (Figure 6b, Figure S5c *vs*. S5d). The effect of the negatively the negatively charged protein on V_T is not clear (Figure S4c), as a result of the high contact resistance of the device. However, there is an increase in I_{DS} after introduction of thrombin (Figure 6c, Figure S5e *vs*. S5f), as expected for interaction of a negatively charged group with the surface of a p-type transistor.[17]

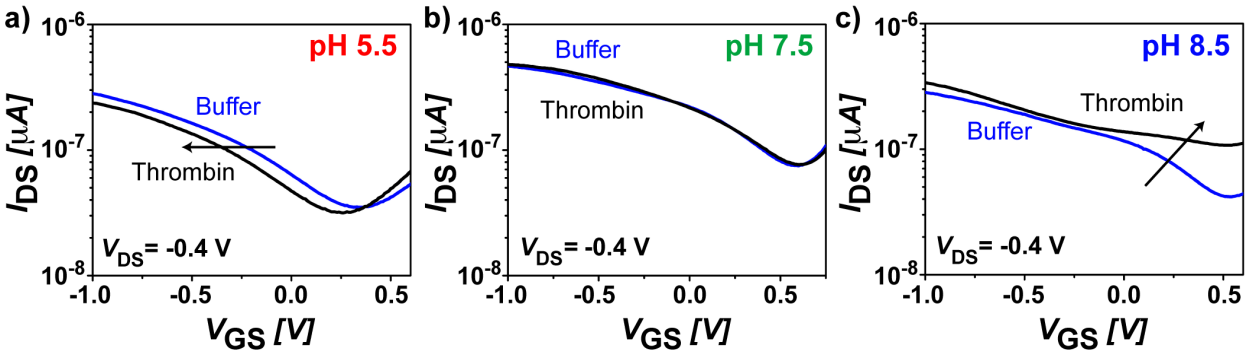


Figure S4. *In situ* transfer plots at various pH. Drain-source current (I_{DS}) *vs*. gate-source voltage (V_{GS}) plots for DNA-decorated OFET devices before (blue lines) and after (black lines) exposure to thrombin at pH a) 5.5, b) 7.5, and c) 8.5.

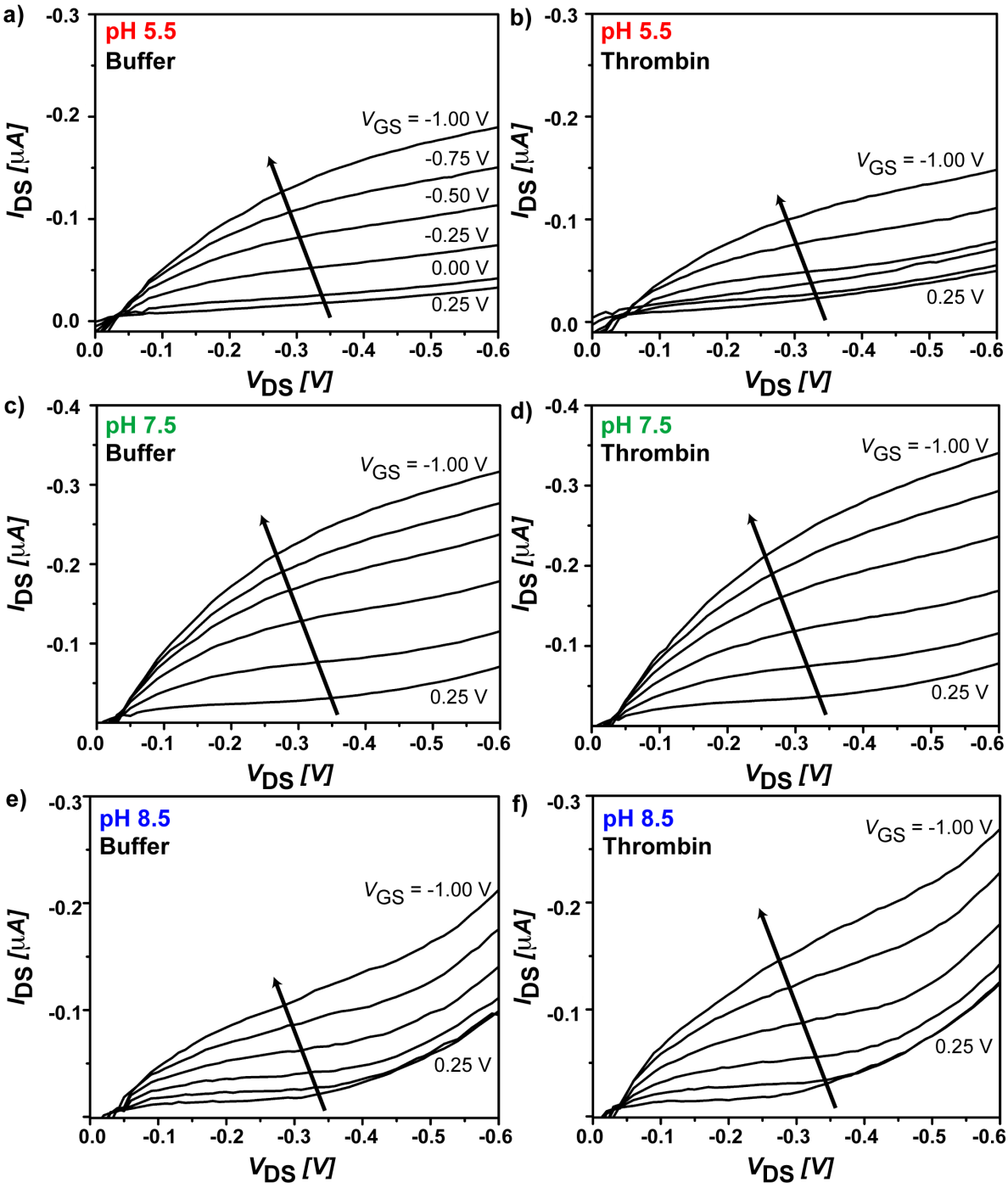


Figure S5. *In situ* output plots at various pH. Drain-source current (I_{DS}) *vs*. drain-source voltage (V_{DS}) plots for DNA-decorated OFET devices before thrombin exposure at pH a) 5.5, c) 7.5 and e) 8.5. I_{DS} *vs*. V_{DS} for DNA-decorated OFET devices after thrombin exposure at pH b) 5.5, d) 7.5 and f) 8.5.

Determination of the Equilibrium Dissociation Constant

The equilibrium dissociation constant of the thrombin aptamer-thrombin complex (k_D) can be determined using the Langmuir binding isotherm, using a method described in literature.[58] Briefly, I_{DS} is normalized by the transconductance (g_m) of the device (taken from the linear region of the conductance (I_{DS}/V_{DS}) *vs*. V_{GS}) plot. Using the method presented by Duan *et al*.,[58] equations for the association (k_1) and dissociation (k_{-1}) constants can be determined from a plot of the normalized I_{DS}/g_m *vs*. time (Figure 2d) as follows:

$$\frac{I_{DS}}{g_m} = V_{eq}(1 - e^{-(k_1 c_{thrombin} + k_{-1})t}) \quad \text{(S1)}$$

$$\frac{I_{DS}}{g_m} = V_{eq} e^{k_{-1}t} + V_T \quad \text{(S2)}$$

The k_D for thrombin can then be calculated using Equation S3.

$$k_D = \frac{k_{-1}}{k_1} \quad \text{(S3)}$$

Fitting these parameters to the data collected resulted in a k_D ~140 nM for this system, which is highly comparable for those values previously reported for this thrombin aptamer.[35,37]

Non-Linear Relationship Between Sensitivity and Analyte Concentration

Sensitivity was plotted at various thrombin concentrations ($c_{thrombin}$) and while the relationship between the two was non-linear, a log-log relationship between the two variables was discovered. This relationship was found to hold across various pH and ionic strengths of the buffer, as well as for different AuNP spacing (d_{avg}) (Figure S6).

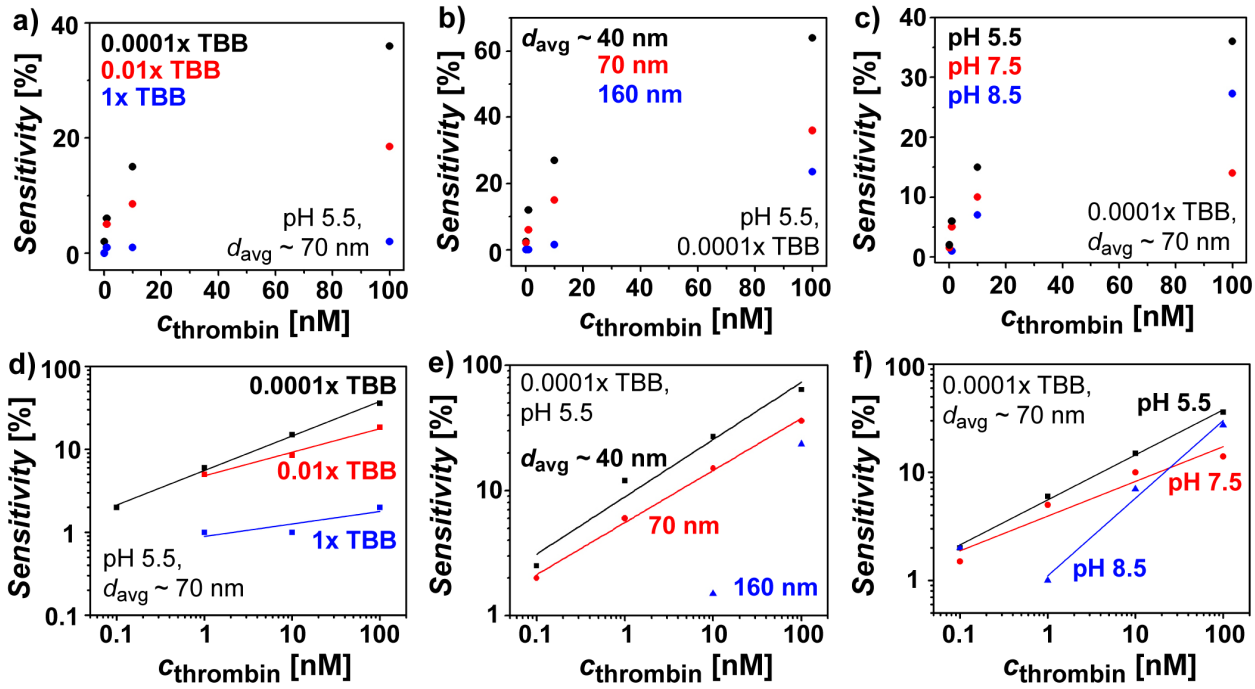


Figure S6. Relationship between sensitivity and thrombin concentration ($c_{thrombin}$) a, d) at various ionic strengths (pH 5.5, AuNP spacing (d_{avg}) ~ 70 nm), b, e) at various d_{avg} (pH 5.5, 0.0001x TBB), and c, f) at various pH (0.0001x TBB, d_{avg} ~70 nm). Plots b, e, and f demonstrate the log-log relationship between sensitivity and $c_{thrombin}$, and lines are fit of data.

Determination of Net Charge of Thrombin at Various pH

The magnitude and charge of a protein can be varied by adjusting the pH of the buffer in which it is suspended. Proteins are composed of individual amino acids, whose side chains may be neutral, acidic, or basic. By varying the pH of the buffer solution, these side chain moieties can be dissociated into their ionic forms, which exist in equilibrium with their non-dissociated forms in solution. The acid dissociation constant for this reaction (K_a) is defined as follows:

$$COOH \overset{K_a}{\Leftrightarrow} COO^- + H^+$$

$$NH_3^+ \overset{K_a}{\Leftrightarrow} NH_2 + H^+$$

$$K_a = \frac{[COO^-][H^+]}{[COOH]} = \frac{[NH_2][H^+]}{[NH_3^+]} \quad \text{(S4)}$$

The negative log of this K_a value is defined as the pK_a value. When the pH of the solution is equal to the pK_a, there is an equal number of charged and uncharged species in solution. Using the definition of pK_a, one can determine the fraction of charged species for acids and bases using Equations S5 and S6, respectively:

$$\frac{[COO^-]}{[COOH]} = 10^{(pH - pK_a)} \quad \text{(S5)}$$

$$\frac{[NH_3^+]}{[NH_2]} = 10^{(pK_a - pH)} \quad \text{(S6)}$$

Upon condensation of individual amino acid residues to form a polypeptide, the carboxylic acid and amine base terminals of the individual amino acids are converted into amide linkages, and are no longer capable of undergoing this equilibrium reaction. Thus, only the side chains (R groups) of the amino acid residues are capable of contributing to the overall charge of the protein, and only a particular subset of amino acids (aspartic/ glutamic acid (D/E), cysteine (C), tyrosine (Y), lysine (K), histidine (H), and arginine (R) (Figure S5)) contain R groups that can be

charged. The ratios in Equations S5 and S6 can thus be written for each of amino acid residues containing a side chain that can be charged. The net charge of a protein can then be determined as:

$$\text{Net charge} = \#\,\text{groups} * \text{charge} * \text{fraction groups charged} \tag{S7}$$

To determine the net charge of thrombin, its sequence (courtesy of the Protein Data Bank) was examined and classified according to charged residues, and then the calculations for fraction of charged residues were performed at each of the three pHs investigated, namely 5.5, 7.5, and 8.5. The resulting net charge was determined to be +7.90 (+8), + 1.33 (+1), and -2.91 (-3) at pH 5.5, 7.5, and 8.5, respectively (Table S4).

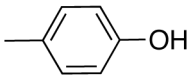
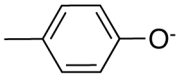
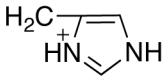
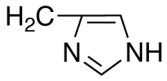
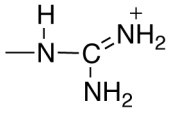
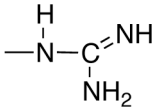
Group		Acid		Base + H^+		pK_a Value
Aspartic/ Glutamic Acid	(D/E)	COOH	⇌	COO^- + H^+		4.4
Cysteine	(C)	—SH	⇌	S^- + H^+		8.5
Tyrosine	(Y)		⇌	 + H^+		10.0
Lysine	(K)	$—NH_3^+$	⇌	$—NH_2$ + H^+		10.0
Histidine	(H)		⇌	 + H^+		6.0
Arginine	(R)		⇌	 + H^+		12.0

Figure S7. Organization of charged amino acids by equilibrium reaction and pKa value.

Table S4. Net charge of thrombin at various pH.

Residue	# Groups	Charge	Fraction Groups Charged			Net Charge		
			pH 5.5	pH 7.5	pH 8.5	pH 5.5	pH 7.5	pH 8.5
E/D	39	-1	0.93	1.00	1.00	-36.13	-38.97	-39.00
C	8	-1	0.00	0.09	0.50	-0.01	-0.73	-4.00
Y	10	-1	0.00	0.00	0.03	0.00	-0.03	-0.31
H	4	1	0.76	0.03	0.00	3.04	0.12	0.01
K	20	1	1.00	1.00	0.97	20.00	19.94	19.39
R	21	1	1.00	1.00	1.00	21.00	21.00	20.99
							Total	
						pH 5.5	pH 7.5	pH 8.5
						+7.90 (+8)	+1.33 (+1)	-2.91 (-3)