

# Supplementary Materials for

## A Molecular Switch Framework for Reagentless Electrochemical Detection of Proteins

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## Supplementary Text

$$\theta = \frac{[AGNAB]}{[AGNAB] + [AGN^* AB]} = \frac{\frac{[AGN][AGN^* AB]}{[AGN^*]} K_{eq}}{\frac{[AGN][AGN^* AB]}{[AGN^*]} K_{eq} + [AGN^* AB]} = \frac{\frac{[AGN]}{[AGN^*]}}{\frac{[AGN]}{[AGN^*]} + \frac{1}{K_{eq}}}$$

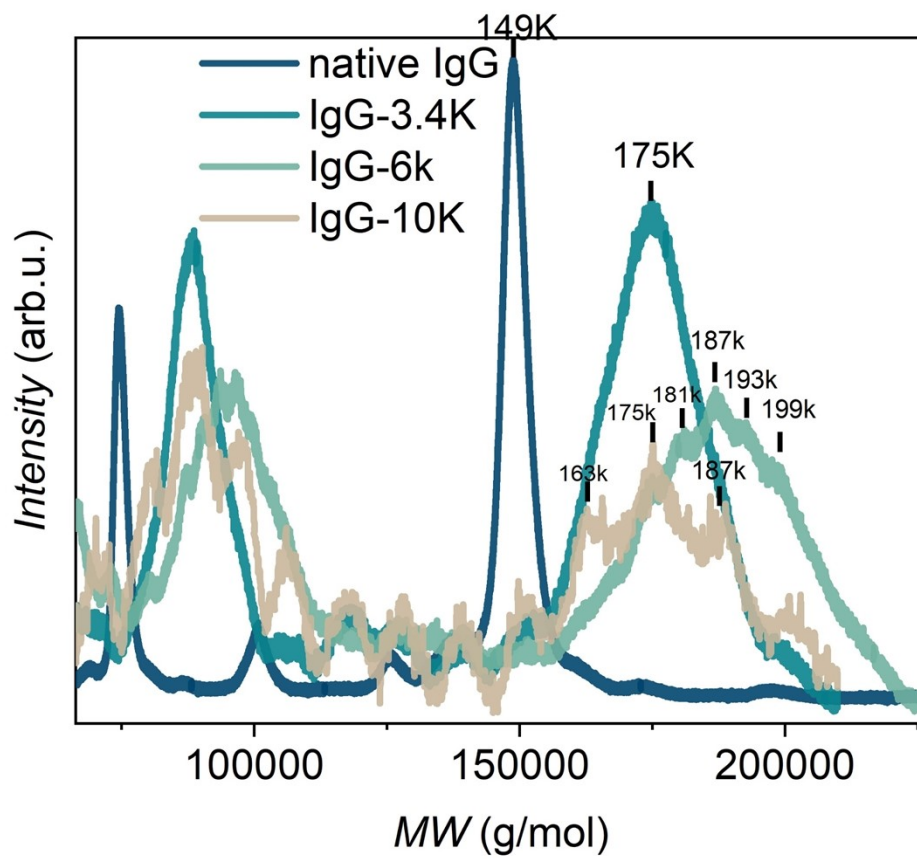
Using equation 4:

$$1/K_{eq} = EC_{50} / \frac{[AGN^*]_{50}}{[AGN]}$$

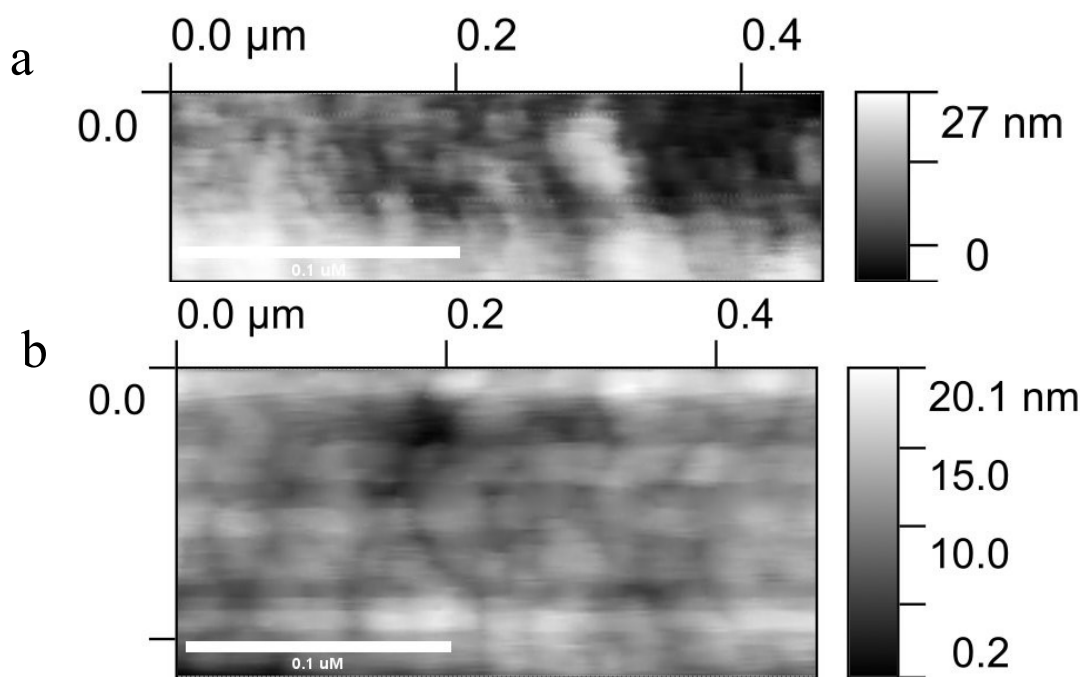
$$\theta = \frac{\frac{[AGN]}{[AGN^*]}}{\frac{[AGN]}{[AGN^*]} + \left(\frac{EC_{50}}{[AGN^*]_{50}}\right)} = \frac{1}{1 + \left(\frac{EC_{50}}{[AGN]} \times \frac{[AGN^*]}{[AGN^*]_{50}}\right)}$$

Thus:  $\frac{[AGN^*]}{[AGN^*]_{50}}$   
by approximating  $\theta \simeq 2[AGN^*]_{50}$

$$\therefore \theta = \frac{1}{1 + \left(\frac{EC_{50}}{[AGN]} \times 2\theta\right)} \Rightarrow \left(\frac{2 \times EC_{50}}{[AGN]} \theta\right) \theta^2 + \theta - 1 = 0 \Rightarrow \theta = \frac{[AGN] \left( \sqrt{\left(1 + \frac{8 \times EC_{50}}{[AGN]}\right)} - 1 \right)}{4 \times EC_{50}}$$

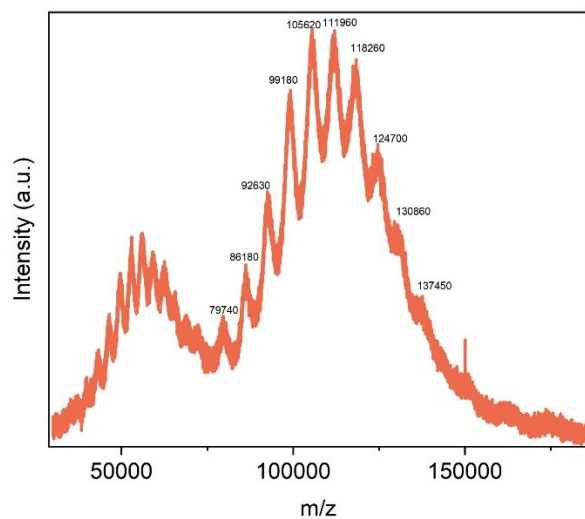


**Fig. S1.** MALDI spectra of Fc-tagged IgG with different arm sizes. The annotated numbers show the molecular weight corresponding to the peak. Based on these values, the number of arms attached is calculated.

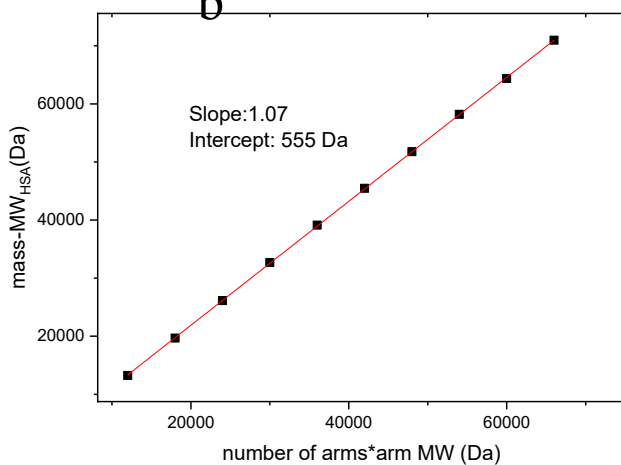


**Fig. S2.** The AFM image of (a) a bare working electrode and (b) with HSA-10K and antibody shows a rough surface. Scale bar = 100 nm.

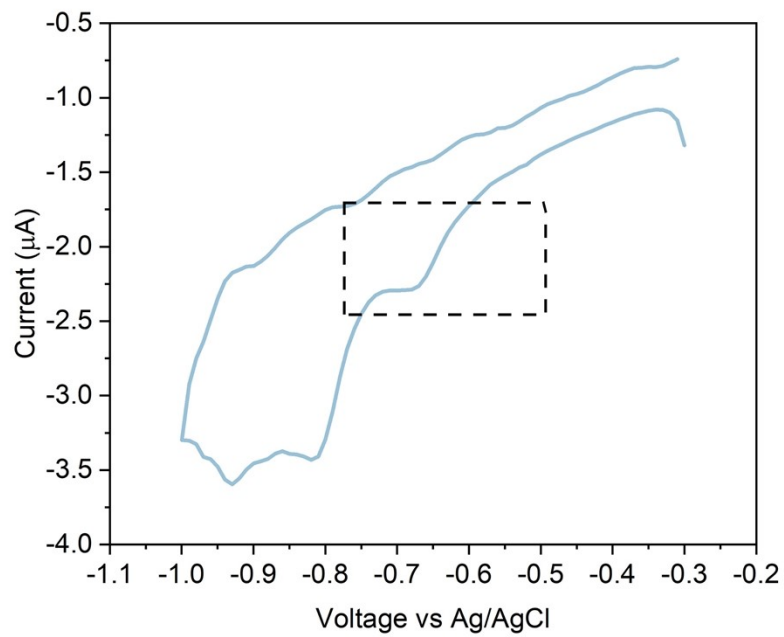
a



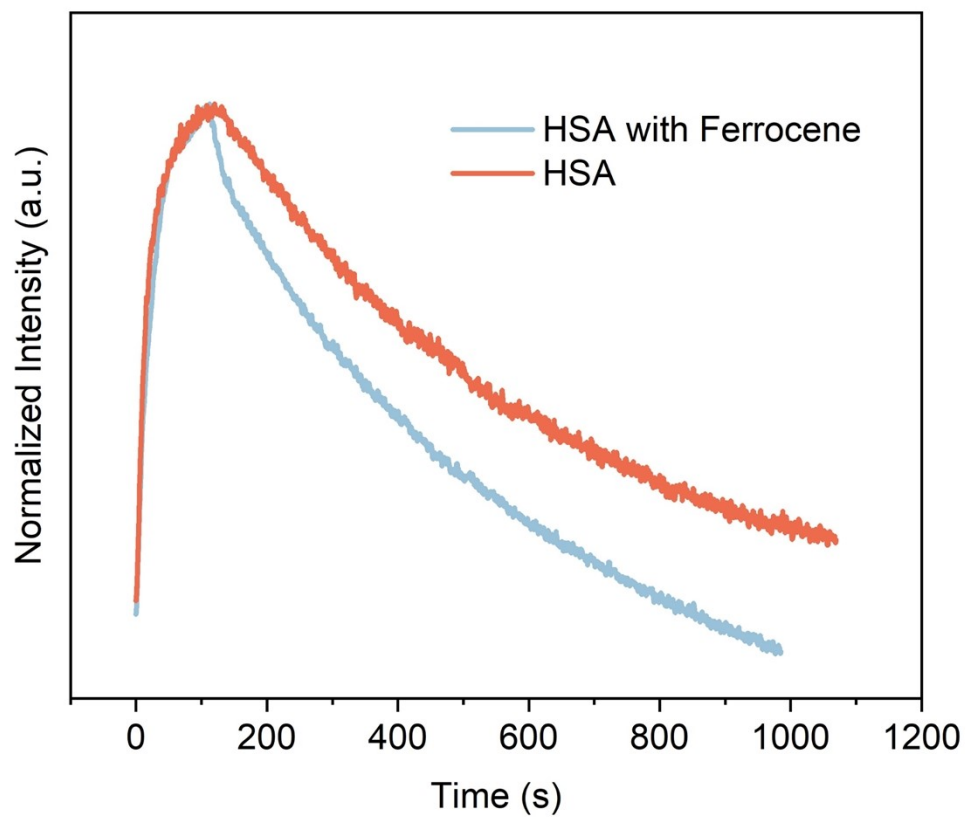
b



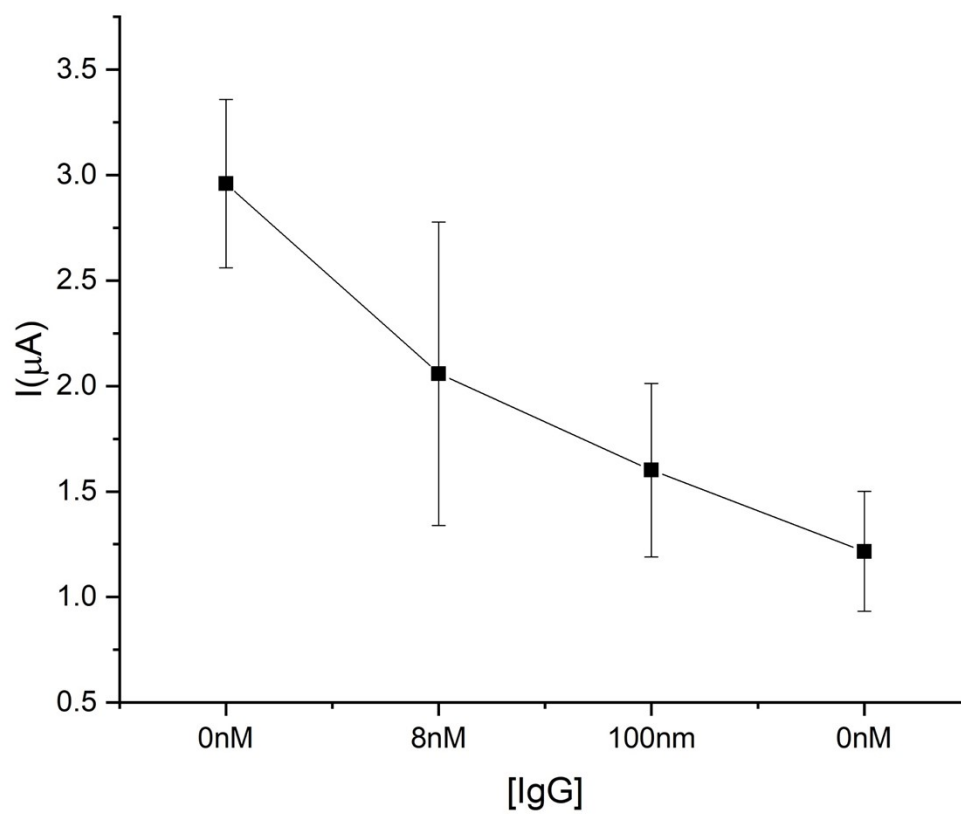
**Fig. S3.** a, MALDI spectra of HSA-6K were used to measure the number of Ferrocenes attached to the protein. Each number represents the corresponding mass associated with a protein with a certain number of arms ranging from 2 arms for 79740 to 11 for 137450. Please note that the average molecular weight of 6K PEG based on the fitting is 6400 Da. b, The relation between subtracted mass obtained from MALDI from HSA MW to integer multiplication of 6K PEG MW (6400 Da). The results show a linear relationship with the intercept of 555 indicating 2 Ferrocene attachment to each HSA.



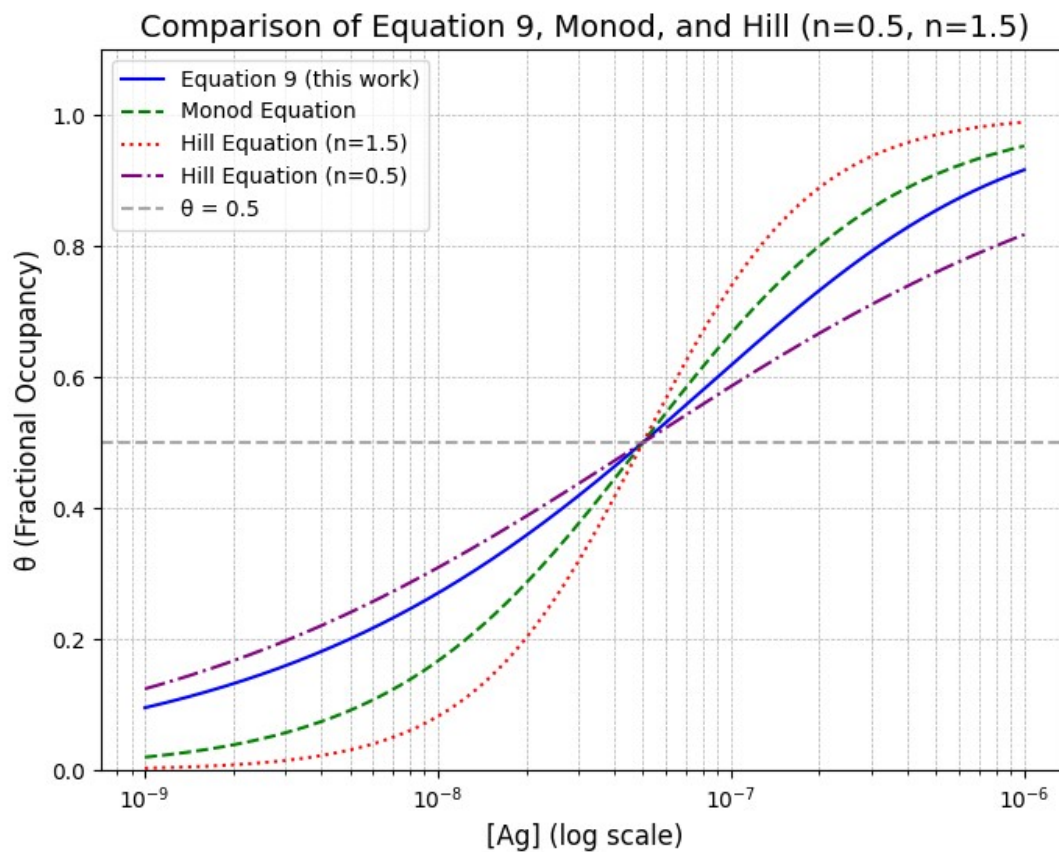
**Fig. S4.** Cyclic voltammogram for HSA-6K devices (with geometric surface area of 0.0314 cm<sup>2</sup>) in 10 mM NaOH. The peak area indicated by the rectangle is used to calculate total thiol coverage.



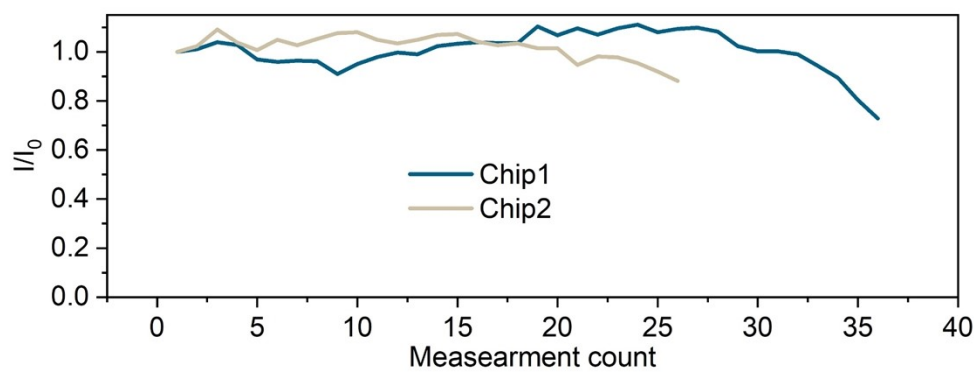
**Fig. S5.** SPR results show a faster dissociation of HSA with ferrocene compared with native HSA.



**Fig. S6.** SWV current using a Ferrocene-tagged IgG without any arm shows a decay in the signal strength from diffusing the ferrocene-tagged IgG proteins into the solvent.



**Fig. S7.** The comparison of the sigmoidal response from equation 9 to Monod's and Hill's equations.



**Fig. S8.** The stability test shows HSA ProSwitch devices withstanding several SWV measurements at 20-minute intervals in 1x PBS buffer (0 nM HSA).

**Table S1.** The Flory radius estimations. The Flory radii ( $R_f$ ) presented in supplementary Table 1 were calculated using the formula  $R_f = aN^{3/5}$ , where  $a$  represents the size of an individual ethylene glycol monomer ( $a=0.35\text{nm}$ ), and  $N$  is the degree of polymerization ( $N=77$ ,  $136$ , and  $227$  for PEG3.4k, PEG6k, and PEG10k, respectively).

PEG arm	Flory radius
3.4K	4.7nm
6K	6.7nm
10K	9.1nm

**Table S2.** The electron transfer ( $k_s$ ) results from fitting the  $I/f$  vs  $f$  at different concentrations measured for HSA-6K. The results were fitted with the following parabola function,  $(I/f)-(I/f)_{\max}=-ak_s^2(f^1-k_{\max}/k_s)^2$ . (1)  $k_{\max}$  was chosen as 0.9 for  $\alpha=0.5$ . (2)  $a= 3nFACD^{1/2}$ , where  $n$  here is the number of Ferrocene on each protein,  $F$  is the Faradaic number,  $C$  is the concentration of the Tagged antigen, and  $D$  is the diffusion rate of the tagged antigen.

Concentration (nM)	Fitted $k_s$ ( $s^{-1}$ )	$k_s$ standard error	$(I/f)_{\max}$	$a$
0	202.49736	3.85469	0.07163	0.0985
5	208.85341	4.74658	0.08409	0.08867
10	188.76568	2.27868	0.09236	0.10302
25	211.92957	4.51206	0.1116	0.11408
50	195.28691	1.48488	0.12621	0.16537
100	198.24986	3.54075	0.13562	0.14858

**Table S3.** The comparison of equation 9 from this work with Hill's and Monod's equations. n is cooperativity.

<b>Feature</b>	<b>Equation 9 (this work)</b>	<b>Monod Equation</b>	<b>Hill's Equation</b>
Low [AGN] behavior	$\theta \propto [\text{AGN}]^{1/2}$	$\theta \propto [\text{AGN}]$	$\theta \propto [\text{AGN}]^n$
High [AGN] behavior	Saturates at $\theta=1$	Saturates at $\theta=1$	Saturates at $\theta=1$
Transition steepness	Less steep than Hill's for $n>1$ and Monod, but steeper than Hill's for $n=0.5$	Smooth transition (linear binding curve).	Adjustable steepness (via n).

## References:

1. Š. Komorsky-Lovrić, M. Lovrić, Square-wave voltammetry of quasi-reversible surface redox reactions. *Journal of Electroanalytical Chemistry* **384**, 115-122 (1995).
2. Š. Komorsky-Lovrić, M. Lovrić, Measurements of redox kinetics of adsorbed azobenzene by “a quasireversible maximum” in square-wave voltammetry. *Electrochimica acta* **40**, 1781-1784 (1995).