Supporting Information

Design and characterization of an electrochemical peptide-based sensor fabricated via "click" chemistry †

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MATERIALS AND METHODS

All buffers, salts, acids, bases, 9-mercapto-1-nonanol, trifluoroethanol, and IgG antibodies were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA) and used as received. Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl] amine (TBTA) was purchased from Soltec Ventures (Beverly, MA, USA). 11-azidoundecane-1-thiol was purchased from Prochimia (Gdansk, Poland). HIV-1 p24 mouse antibodies were purchased from ProSpec, Inc. (Rehovot, Israel) and used without further purification. The alkyne and methylene blue (MB)-modified peptide probe with the sequence TINEEAAEWDRVHPK was custom-synthesized by Xaia Custom Peptides (Göteborg, Sweden). The unmodified peptide probe with the sequence TINEEAAEWDRVHP was custom-synthesized by GenScript USA Inc. (Piscataway, NJ, USA). Electrochemical measurements were performed at room temperature (22±1°C) using a CHI 1040A Electrochemical Workstation (CH Instruments, Austin, TX). Polycrystalline gold disk electrodes (CH Instruments, Austin, TX) with a radius of 1 mm were used as working electrodes. The counter electrode used was a platinum wire electrode and a Ag/AgCl (3M KCl) electrode served as the reference electrode, both from CH Instruments (Austin, TX). All CD spectra were recorded on a Jasco-810 spectropolarimeter (Jasco, Easton, MD, USA) using a quartz cell with a 1 mm optical path length.

Sensor Fabrication and Interrogation

Prior to sensor fabrication, gold working electrodes were electrochemically cleaned by cycling between -0.4 and 1.6 V vs. Ag/AgCl in 0.5 M sulfuric acid until the gold oxide formation region of the voltammograms displayed three distinct peaks and successive scans showed minimal to no change. To form the mixed azide self-assembled monolayer

(SAM), electrochemically cleaned electrodes were incubated in an ethanolic solution containing 8 μM 11-azidoundecane-1-thiol and 142 μM 9-mercapto-1-nonanol for 10 minutes, after which they were thoroughly rinsed and transferred to a 2 mM solution of 9-mercapto-1-nonanol for 19 hours. Conjugation of the peptide probe to the surface was achieved by subsequent incubation in the "click" mixture (50% DMSO/ 50% water, 65 mM NaCl, 1.4 mM TBTA, 0.44 mM $CuSO_4$, 0.83 mM sodium-L-ascorbate, 4.3 μM alkyne and MB-modified peptide probe).

All the E-PB sensors were analyzed using alternating current voltammetry (ACV) with an amplitude of 25 mV and at various AC frequencies. The density of peptide probes on the electrode surface, Γ, was determined by integration of charges under the reduction peaks of cyclic voltammetry at low scan rates (eq. 1).

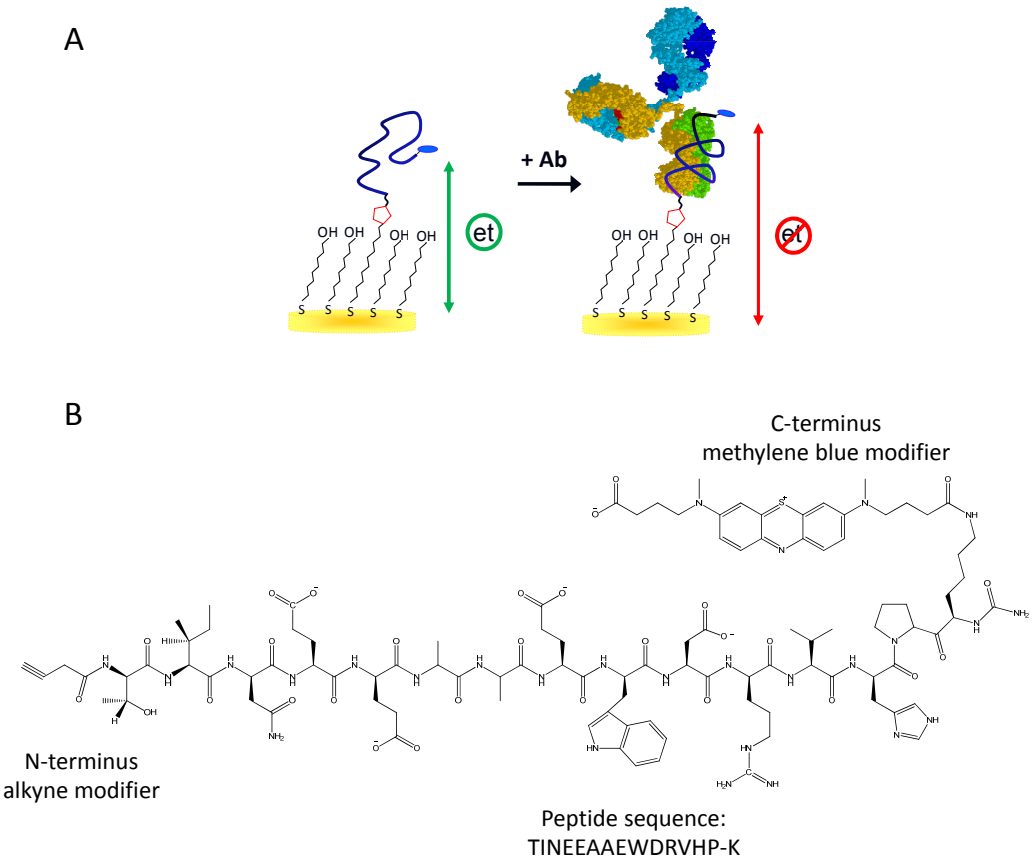
$$\Gamma = Q/nFA \quad (1)$$

where Q is the integration of charges under the reduction peaks of CVs, n is the number of electrons transferred per redox event (n = 2, MB), F is the Faraday current, and the A is the gold electrode area. Γ is given by the average values obtained at different potential scan rates (20, 50 and 100mV/s).

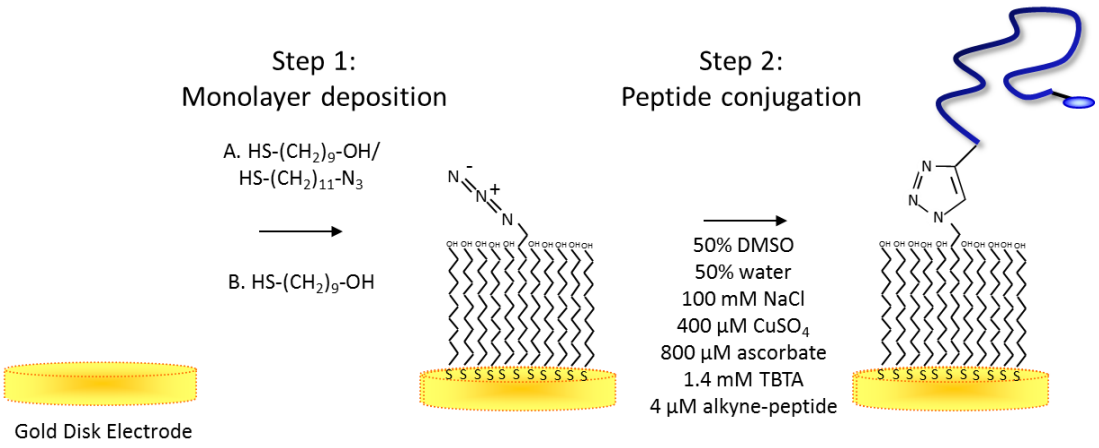
Prior to sensor interrogation, the sensors were equilibrated in a phosphate buffer (8 mM H_2NaPO_4, 2 mM HNa_2PO_4, 100 mM NaCl, pH 7.4) until they exhibited less than 5% change in the peak current in the course of 90 min. The sensors were then interrogated at 90-min intervals in the target solution (IgG or anti-p24 antibodies). The ratio between the peak current in the target solution and the peak current in the target-free solution was used to calculate the signal suppression caused by the target.

Circular Dichroism Spectroscopy (CD)

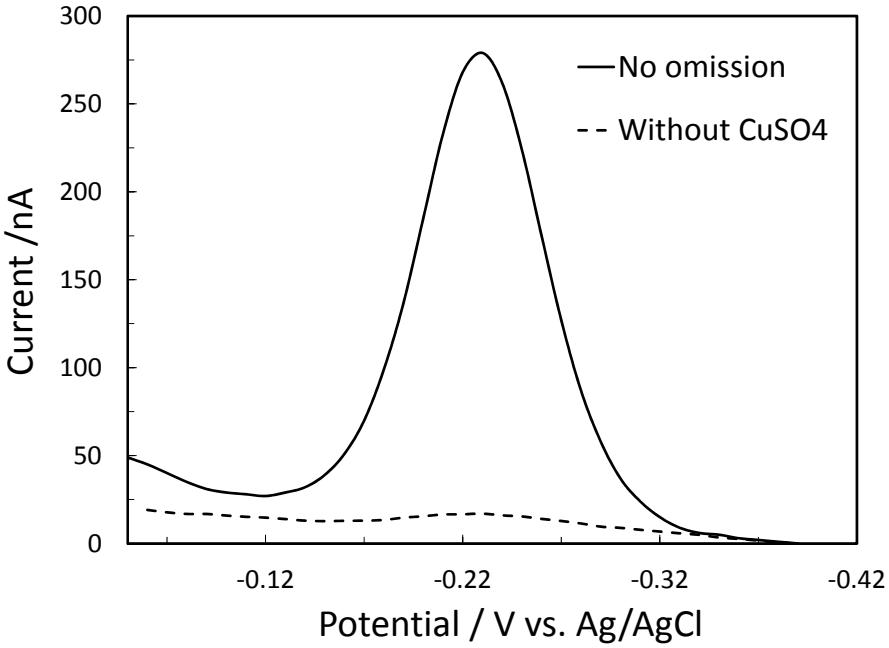
All CD experiments were performed in either pure deionized (DI) water, 1:9 DI water:TFE. CD spectra of the unmodified peptide probe (200 μM) were obtained at a scan rate of 50 nm/min and a data integration time of 2 seconds at room temperature (20^oC). To determine the effect of temperature on the structure of the peptide probe solvated in 1:9 DI water:TFE, the temperature was ramped from 20°C to 90°C at a rate of 2^0C/min. CD spectra were collected both before and after the temperature ramping.



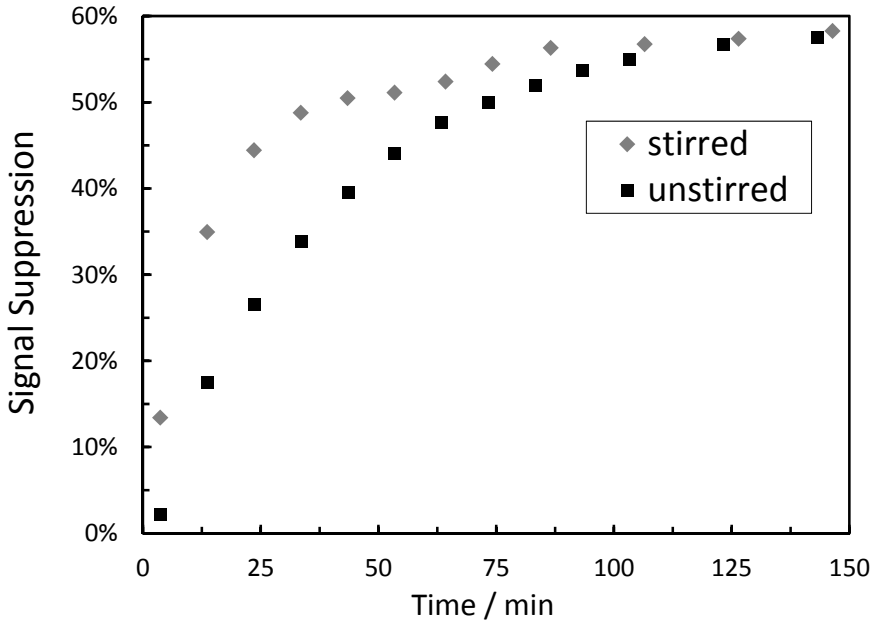
S.I. 1 (A) The signaling mechanism of the E-PB sensor should be similar to that of the LP E-DNA sensor. The signaling relies mostly on the change in the dynamics of the peptide probe upon binding to the target. In absence of the target (HIV-1 anti-p24 antibody), the electron transfer between the electrode and the MB label on the probe is efficient, giving rise to a large MB current when interrogated using AC voltammetry. Upon binding to the large antibody target (~150 kDa), the flexibility of the probe is significantly reduced, thereby impeding efficient collisional electron transfer, which is reflected in the reduction of the MB peak current. Additionally, the MB label could be physically trapped inside or adjacent to the binding domain of the antibody, a location where electron transport might not be efficient or at all facile. (B) The structure of the alkyne and methylene blue-modified peptide probe used in this study.



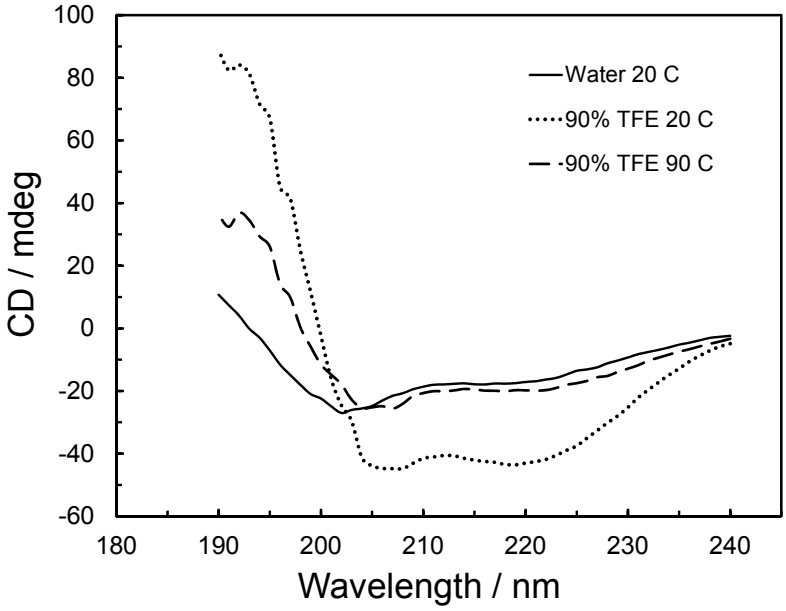
S.I. 2 Fabrication of a "click"-based E-PB sensor involves two steps. Post electrochemical cleaning, the gold electrode was first incubated in a mixture of azide- and hydroxy-terminated alkanethiols at a relatively low concentration for 10 min. The partially formed monolayer was then "back-filled" with hydroxy-terminated alkanethiols for 19-22 hours. The monolayer-modified electrode was then subjected to the "click" reaction, where alkyne-modified peptides were conjugated to the azide functional groups on the monolayer surface.



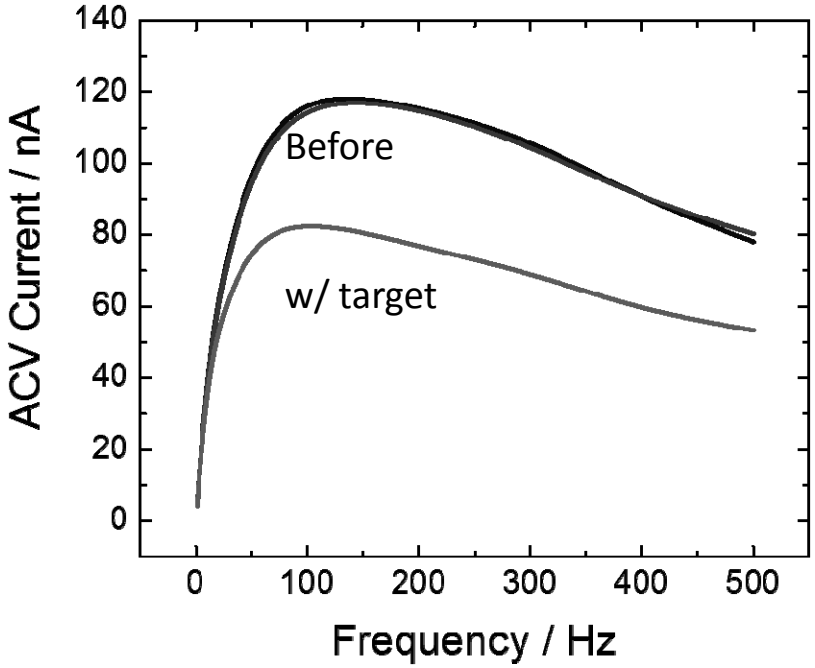
S.I. 3 Representative AC voltammograms of "click" chemistry-based E-PB sensors fabricated in absence and presence of copper sulfate. The probe coverage for the sensor fabricated in absence of copper sulfate is ~7 x 10^9 molecules/cm^2, which is ~31 times lower than that obtained with the sensor fabricated without the omission (~2.16 x 10^{11} molecules/cm^2).



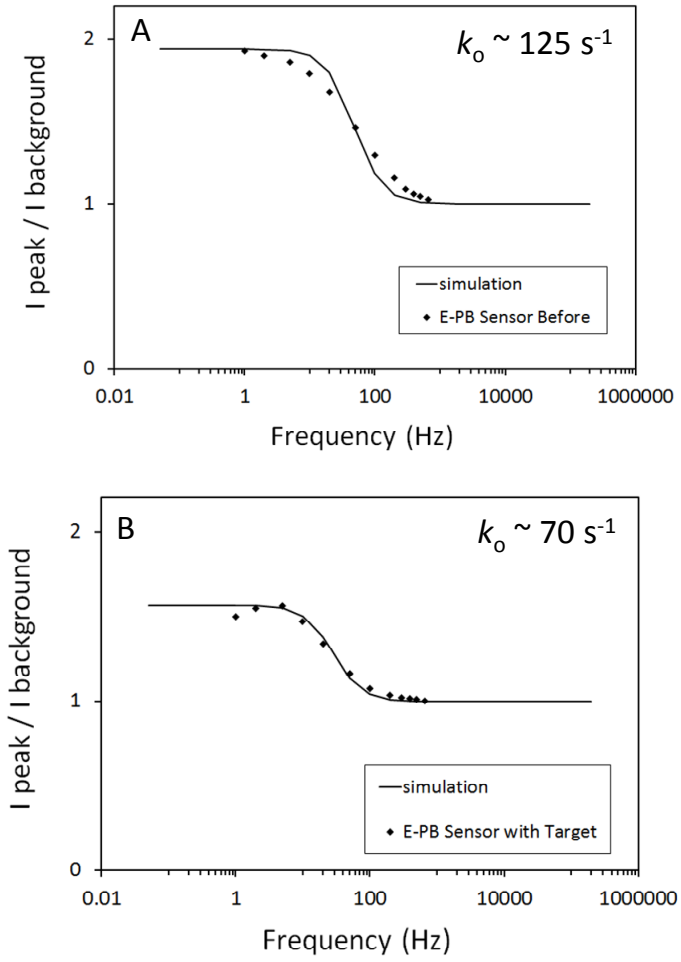
S.I. 4 Shown are the binding kinetic curves of two E-PB sensors interrogated in a stirred and unstirred solution, both containing 240 nM anti-p24 antibody. As observed, the sensor response time appears to be highly dependent on the diffusion of the target to the electrode surface. However, while magnetic stirring allows the acquisition of a response within 30 min rather than 90 min, the disturbance caused by the stirring also contributes to voltammetric baseline fluctuations which can greatly affect sensor to sensor response reproducibility.



S.I.5 Circular dichroism spectra of the unmodified peptide probe in water at 20°C (solid line), 90% TFE at 20°C (dotted line), and 90% TFE at 90°C (dashed line).



S.I.6 Shown are b-spline traces showing the AC frequency-dependence of the MB peak current in the E-PB sensor in absence and presence of the target antibody. Due to the limited data points collected within the frequency range, the resolution is not adequate to show a distinct shift in the frequency at which the AC peak current reaches a maximum; the b-spline traces, however, show that the maximum frequency does indeed shift to a lower value upon target binding (40 nM anti-p24 antibodies). This suggests that the electron transfer rate of MB is likely to be slower when compared to that obtained in absence of the target.



S.I.7 Shown are $I_{peak}/I_{background}$ vs. log (frequency) plots for the E-PB sensor before (A) and after (B) incubation in 40 nM anti-p24 antibody for 90 min. While the fit of the data is not optimal (precluding the determination of precise electron transfer rates), the discrepancy in the inflection points of the two curves indicates that electron transfer is slower upon target binding. The k_o of MB is estimated to be ~125 s^{-1} in absence of the target and ~ 70 s^{-1} in presence of the target.

Of note, to obtain electron transfer rates of MB on the peptide probe in absence and presence of the target, the following fitting parameters were utilized: C_{dl} = 2.0 X 10^{-6} F cm^{-2}; solution resistivity = 5 Ω cm; electrode area = 0.0314 cm^2. Each k_o measurement was reproduced at least on three different sensors of similar probe coverage. The electron transfer rates reported are averages of the values obtained.