Supporting Information

An electrochemical peptide-based biosensing platform for HIV detection†

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MATERIALS AND METHODS

All buffers, salts, acids, bases, creatinine, 9-mercapto-1-nonanol, 6-mercapto-1-hexanol, and IgG antibodies were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA) and used as received. HIV-1 p24 mouse antibodies were purchased from ProSpec, Inc. (Rehovot, Israel) and used without further purification. The dual labeled peptide probe with the sequence EAAEWDRVHK was custom-synthesized by ChinaTech Peptide (Suzhou City, China). 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 geometric area of 0.0314 cm² 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). 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. The cleaned electrodes were then incubated in a solution containing 2.5 µM of the peptide probe in 10 mM phosphate buffer saline, 0.1 M NaCl, pH 7.4 for 5.5 hours, followed by an 18-hour incubation in 2 mM 9-mercapto-1-nonanol or 6-mercapto-1-hexanol in a Phys2 buffer (20 mM Tris, 140 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, pH 7.4). The sensor electrode was treated with a 30-sec DI water rinse prior to introduction to the electrochemical cell for target interrogation.

The sensor performance was analyzed by cyclic voltammetry and AC voltammetry. The sensor interrogation solutions used were either a pure Phys2 buffer or a solution...
containing equal parts of Phys2 buffer and synthetic urine (containing deionized water, urea, magnesium sulfate, calcium chloride, sodium chloride, pH ~ 7.9) (Ricca Chemical Company, Baitsville, IN, USA) supplemented with 11 mM creatinine. AC voltammograms were recorded from 0 V to -0.5 V vs. Ag/AgCl with a 1 Hz, 25 mV AC potential. Cyclic voltammograms were recorded from 0.3 V to -0.6 V at a scan rate of 1 V/s. Prior to target interrogation, sensors were allowed to equilibrate until they exhibited less than 1% change in the peak current in the course of 10 minutes. The sensors were then interrogated at 10-min intervals in the target solution (IgG or anti-p24 antibodies) for a total of 60 min. 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.
S.I. 1 Structure of the dual-labeled peptide used in this study.
S.I. 2 The E-PB sensor responses to its target in minutes. At a target antibody concentration of 43 nM, complete signal saturation time is ~100 min, but the majority of the response (>75 %) is achieved within 60 min.
S.I. 3 E-PB sensors fabricated with C6-OH as the diluent show similar selectivity as the C9-OH passivated sensors; however, sensor sensitivity appears to be adversely affected since less signal suppression is observed under the same experimental condition. Shown here are the sensors’ responses in AC voltammetry when interrogated with (A) 43 nM IgG and (B) 43 nM anti-p24 antibodies in a Phys2 buffer. Conditions: AC frequency: 10 Hz; AC amplitude: 25 mV; incubation time: ~60 min.
S.I. 4 The linear relationship between peak currents and scan rates confirms that the redox species (MB) is confined to the electrode surface.