Electronic Supplementary Information (ESI) for

An electrochemical approach for the detection of HIV-1 protease

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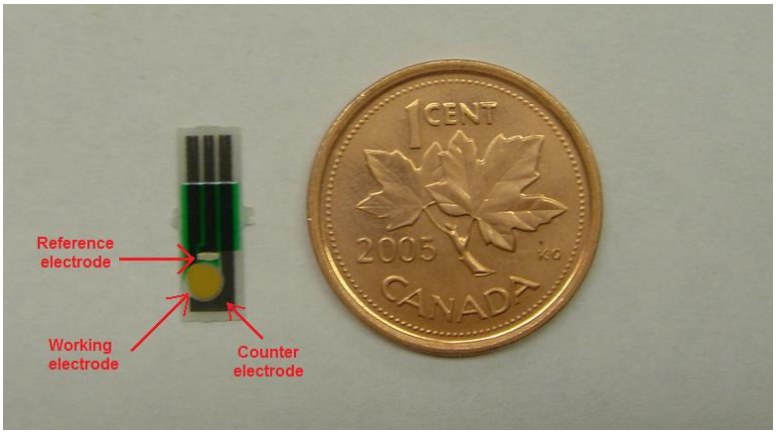
Reagents and apparatus:

HIV-1 protease (HIV-1 PR) recombinant expressed in E. coli (25 µg), pepsin (E. C. 3.4.23.1) from porcine stomach mucosa with 3460 U/mg were purchased from Sigma. DTT (± threo-2,3-dihydroxy-1,4-butanedithiol) was obtained from Fluka. The amino acid derivatives were purchased from Advanced ChemTech (Louisville, KY). Cytochalasin A (Calbiochem) and human serum albumin (HSA), human male serum AB (Sigma, H4522) were kindly donated by the Dept. of Biochemistry and Prof. David A. R. Sanders in the Dept. of Chemistry, University of Saskatchewan. The screen-printed gold electrodes were kindly donated by Prof. Eiichi Tamiya (Osaka University, Japan) and Biodevice Technology Ltd., (Ishikawa, Japan). Total length of the strip was 11 mm, and the geometric working area was 2.64 mm^2 (ESI Fig. 1). All the other chemicals were obtained from Merck & Co. Inc. and used as received.



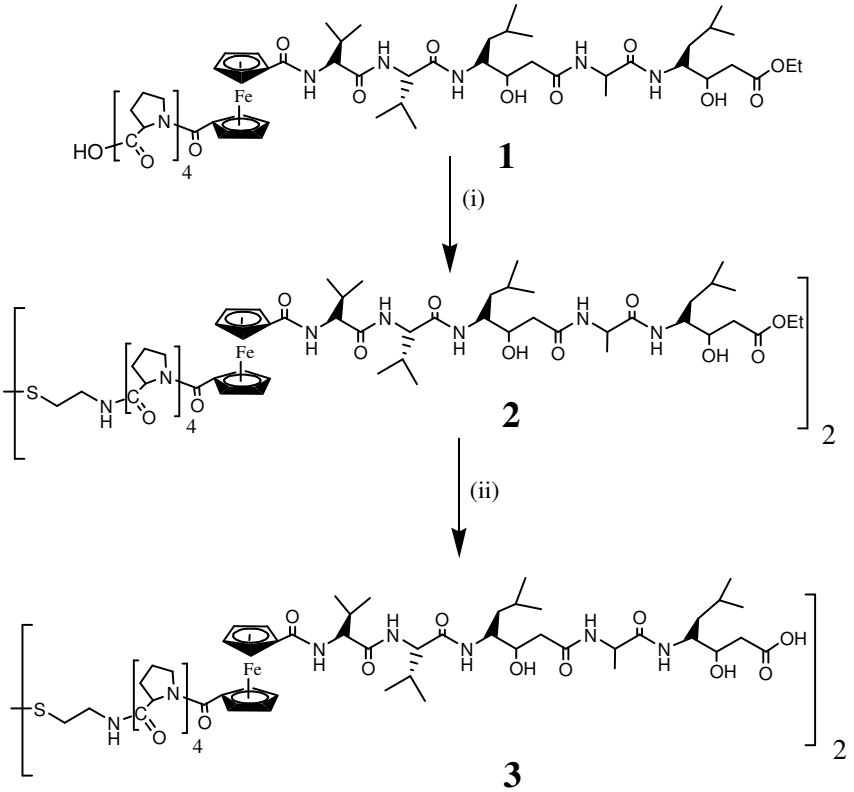
ESI Fig. 1. Screen-printed gold electrode (SPE) with a three-electrode system.

Synthesis of ferrocene (Fc)-conjugated pepstatin:

The synthesis of the Fc-conjugated pepstatin (Cys-(NH-Pro_4-C(O)-Fc-C(O)-Val_2-Sta-Ala-Sta-$OH)_2$) was achieved by employing the reported carbodiimide coupling methods in solution[1,2] and is summarized in Scheme 1.

General Procedure: All syntheses were carried out under dry nitrogen gas unless otherwise indicated. CH_2Cl_2 (BDH; ACS grade) used for synthesis was dried

(CaH_2) and distilled prior to use. $CDCl_3$ (Aldrich) was dried (CaH_2) , and stored over molecular sieves (8-12 mesh; 4Å effective pore size; Fisher) before use. 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDC.HCl), hydroxybenzotriazole (HOBt) and cystamine.2HCl (Nova), $MgSO_4$, and $NaHCO_3$ (VWR) were used as received. For column chromatography, a column with a width of 2.7 cm (ID) and a length of 45 cm was packed 18-22 cm high with 230-400 mesh silica gel (VWR). For TLC, aluminum plates coated with silica gel 60 F_{254} (EM Science) were used. NMR spectra were recorded on a Bruker Avance-500 spectrometer using a 5-mm broadband probe operating at 500.134 MHz (^{1}H) and 125.766 MHz (^{13}C{^{1}H}). Peak positions in ^{1}H spectra are reported in ppm relative to TMS. All otherwise it is described ^{1}H, ^{13}C{^{1}H} spectra are referenced to the CD_3OD signal at δ 3.31, 49.55, respectively. Mass spectrometry was carried out on a VG Analytical 70/20 VSE instrument. Infrared spectra were obtained with a Perkin-Elmer model 1605 FT-IR. H-Pro_4-C(O)-Fc-C(O)-Val_2-Sta-Ala-Sta-OEt (1) was synthesised according to our previously published procedures.[1]



ESI Fig. 2: Synthesis of the inhibitor 3, reagents and conditions. (i) Cystamine, HOBt, EDC, Et_3N, 0 °C, (ii) NaOH, dioxane, H_2O.

Synthesis of Cys-(NH-Pro$_4$-C(O)-Fc-C(O)-Val$_2$-Sta-Ala-Sta-OEt)$_2$ (2)

HOBt (0.25 mmol, 0.038 g), and EDAC·HCl (0.25 mmol, 0.043 g) in dry CH_2Cl_2 (30 mL, 0 °C) was added to a solution of HO-Pro_4-C(O)-Fc-C(O)-Val_2-Sta-Ala-Sta-$OEt)_2$ (1) in dry CH_2Cl_2 (0.25 mmol, 0.315 g), and the mixture was stirred for 30 min. Then, a solution of cystamine, obtained by treatment of Cystamine ·2HCl (0.12 mmol, 0.027 g) with Et_3N (0.5 mL) in dry CH_2Cl_2 (20 mL) was added and the stirring continued at room

temperature for 16 h. The reaction mixture was then treated consecutively with aqueous solutions of saturated $NaHCO_3$, citric acid (10%), again saturated $NaHCO_3$ and water, dried over Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, 90:10 CH_2Cl_2-MeOH) to give compound (2) as a yellow crystalline solid (yield: 0.184 g, 58%): mp 138-142 °C, ^{1}H-NMR CD_3OD, (δ in ppm): 0.7-0.9 (m, 36H), 0.9-1.2 (m, 6H), 1.3 (d, J = 8, 4H), 1.4-1.8 (m, 14H), 1.8-2.4 (m, 46H), 2.5-2.8 (m, 8H), 33-3.7 (m, 20H), 3.7-4.2 (m, 14H), 4.2-4.4 (m, 14H), 4.5-4.6 (d, J = 10, 8H), 4.6-4.9 (m, 12H), 5.0 (bs, 5H), 5.1 (bs, 4H); ^{13}C{^{1}H}-NMR CD_3OD, (δ in ppm): 14.3, 18.3, 18.6, 19.9, 22.2, 22.7, 23.0, 23.5, 24.9, 28.1, 28.9, 29.5, 39.5, 39.9, 40.8, 46.7, 47.0, 49.7, 51.1, 53.2, 59.0, 59.8, 60.6, 64.1, 64.1, 67.2, 67.2, 70.7, 72.0, 73.2, 136.7, 170.1, 171.3, 171.6, 171.8, 172.0, 172.6, 172.9, 173.4, 173.7; FT-IR (KBr, cm^{-1}) 3500, 3332, 1719, 1648, 1528, 1447, 1392; TOF-MS (m/z): calc for $C_{130}H_{198}Fe_2N_{20}O_{28}S_2$ $[M]^+$: 2663.2819; found: 2663.2883. Anal. calc. for $C_{130}H_{198}Fe_2N_{20}O_{28}S_2$: C 58.59; H 7.49; N 10.51. Found: C 58.62; H 7.52; N 10.49.

Synthesis of Cys-(NH-Pro$_4$-C(O)-Fc-C(O)-Val$_2$-Sta-Ala-Sta-OH)$_2$ (3)

To a solution of compound (2) (0.05 mmol, 0.133 g) in dioxane (2.0 mL), a solution of 1 N NaOH in water (2.6 mL) was added while stirring. The reaction was stored at room temperature for 6 h, then 1 N HCl (1.5 mL) was added. MeOH was removed in vacuo followed by cooling of the solution in a ice bath prior to the drop-wise addition of 2 N HCl (3.0 mL). The solution was then stored in the fridge for 3 h, subsequently, the precipitate was filtered off and washed three times with cold, distilled water (50 mL) and dried under reduced pressure overnight to give compound (3) as a yellow solid (0.090 g, 69%): mp 179-184 °C, ^{1}H-NMR CD_3OD, (δ in ppm): 0.7-0.9 (m, 30H), 0.9-1.2 (m, 6H), 1.3 (d, J = 8, 4H), 1.4-1.8 (m, 14H), 1.8-2.4 (m, 46H), 2.5-2.8 (m, 8H), 33-3.7 (m, 20H), 3.7-4.2 (m, 10H), 4.2-4.4 (m, 14H), 4.5-4.6 (d, J = 9.4, 8H), 4.6-4.9 (m, 17H), 5.0 (bs, 4H) ^{13}C{^{1}H}-NMR CD_3OD, (δ in ppm): 17.8, 18.2, 18.2, 19.3, 19.4, 22.2, 25.6, 28.1, 28.9, 29.5, 39.5, 39.9, 40.8, 46.7, 47.0, 49.7, 51.1, 53.2, 59.0, 59.8, 60.6, 64.1, 64.1, 67.2, 67.2, 70.7, 72.0, 73.2, 169.5, 171.3, 171.6, 171.8, 172.0, 172.6, 172.8, 173.3; FT-IR (KBr, cm^{-1}) 3433, 3299, 1639, 1545, 1387; TOF-MS (m/z): calc for $C_{126}H_{190}Fe_2N_{20}O_{28}S_2$ $[M]^+$: 2607.2193; found: 2607.2216. Anal. calc. for $C_{126}H_{190}Fe_2N_{20}O_{28}S_2$ C, 58.01; H, 7.34; N, 10.74. Found: C 58.04; H 7.36; N 10.70.

Preparation of bioconjugate-3 modified gold electrodes:

SPEs were incubated in 1 mM ethanolic solution of (3) for overnight (~15 h). The electrodes were rinsed with ethanol and then Millipore water (18.2 MΩ.cm). The electrodes were then incubated in 1 mM ethanolic hexanethiol solution for 10 min to produce the diluted film. Finally, the electrodes were rinsed with ethanol and then Millipore water.

Incubation with HIV-1 PR:

The stock solution of HIV-1 PR (200 nM) was prepared using the assay buffer containing 0.1 M sodium acetate (pH 4.7) with 2 M $NaClO_4$, 1 mM EDTA, 1 mM dithiothreitol (DTT), 10% dimethyl sulfoxide (DMSO). Several dilutions of the HIV-1 PR stock solution were prepared using the assay buffer. The electrodes were incubated with these HIV-1 PR samples for 1 h and then rinsed with Millipore water. Samples with

no HIV-1 PR and the electrodes with no peptide modification were also measured. For the control experiments, several dilutions of pepsin were prepared using buffer solutions at various pH conditions, i.e. 0.1 N HCl for pH 1, 0.1 M sodium acetate for pH 3 and 5, 50 mM phosphate buffer, and 20 mM Tris buffer for pH 7 and 9, respectively. For the studies with HSA and serum, various concentrations of HIV-1 PR were spiked into the assay buffer solutions containing HSA and serum as noted in the text. Human serum was diluted for 10 times using the assay buffer solution. For the inhibition studies, the inhibitor, Cytochalasin A, was spiked into the assay buffer solution at certain concentrations in the presence of 100 nM HIV-1 PR.

Electrochemical measurements:

Electrochemical experiments were carried out at room temperature using an electrochemical cell enclosed in a grounded Faraday cage. A Luggin capillary was used to make electrochemical connection to Ag/AgCl reference electrode. Pt wire was used as the counter electrode. Electrochemical measurements were performed in blank 0.1 M sodium acetate buffer solution, using 2 M $NaClO_4$ as the supporting electrolyte. Error bars in the plots indicate the standard deviation of the electrochemical responses from five repetitive measurements (n=5). Cyclic voltammetry experiments were performed at varying scan rates using a CHInstruments 660 potentiostat/galvanostat.

Reference:

1. Y. Xu and H.-B. Kraatz, *Tetrahedron Lett.* 2001, **42**, 2601-2603.