Supplementary Material (ESI) for Analyst

Supporting Information for

Towards an early diagnosis of HIV infection: An electrochemical approach for detection of HIV-1 reverse transcriptase enzyme

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Supplemental information

Synthesis of the Fc-labeled cystamine derivative

Synthesis of $[CH_3OOC\text{-}Fc\text{-}CONHCH_2CH_2S]_2$
1'-methoxycarbonylferrocene-1-carboxylic acid (1 equiv.), HBTU (1.2 equiv.), Et_3N (2 equiv.) were stirred in 100 mL DCM for 2 hours. After which cystamine (0.75 equiv.) was added and the reaction mixture was stirred for 72 hours. 150 mL DCM was added and the reaction mixture was washed with two 150 mL portions of H_2O and the organic layer collected and dried over Na_2SO_4. The crude mixture was filtered and the solvent removed *in vacuo* to leave an orange oil. The oil was purified by flash chromatography on silica using diethyl ether as the eluent. The product was in the third fraction and was concentrated *in vacuo*. The product was isolated as feathery crystals upon addition of hexane and the yield was 62%. The NMR spectra and chemical shifts are provided in Fig. S1 (ESI†).

Synthesis of $[HOOC\text{-}Fc\text{-}CONHCH_2CH_2S]_2$
$[CH_3OOC\text{-}Fc\text{-}CONHCH_2CH_2S]_2$ (1 equiv.), LiOH (5 equiv.), H_2O (1 mL) and THF (15 mL) were stirred at room temperature for 48 hours. The THF was removed *in vacuo* and 10 mL of 0.1 M NaOH was added. The aqueous solution was poured into 1 M HCl to precipitate the product which was collected in a sintered glass crucible and the yield was 21%. The NMR spectra and chemical shifts are provided in Fig. S2 (ESI†).

Synthesis of $[C_6H_4N_3OOC\text{-}Fc\text{-}CONHCH_2CH_2S]_2$
$[HOOC\text{-}Fc\text{-}CONHCH_2CH_2S]_2$ (1 equiv.), HOBt (1.5 equiv.), and EDC (1.5 equiv.) were stirred for 2 hours in 15 mL of DCM at room temperature. The reaction mixture was

diluted with DCM and washed with saturated sodium bicarbonate solution, 10% citric acid solution, and H_2O. The organic phase was dried over Na_2SO_4, filtered and the solvent was removed *in vacuo*. The crude product was purified using flash chromatography on silica using ethyl acetate as the eluent. The product was isolated as an orange solid and the yield was 20%. The NMR spectra and chemical shifts are provided in Fig. S3 (ESI†).

Preparation of the peptide-modified gold electrodes

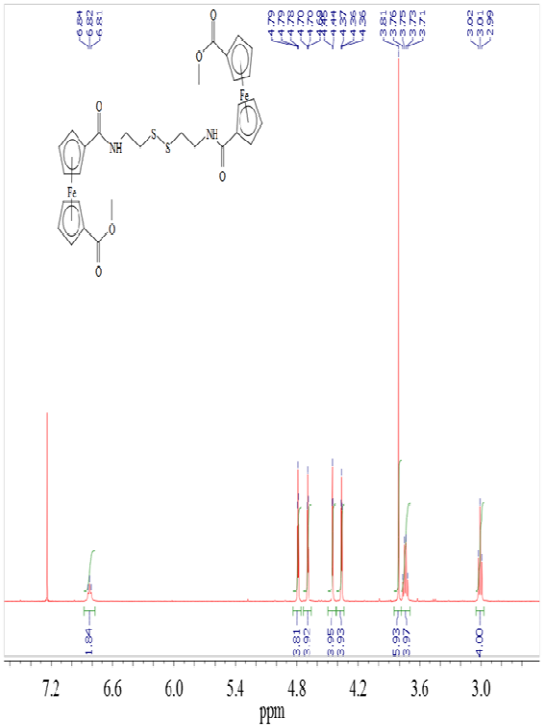
Prior to the experiments, the gold electrodes were polished with 0.3 and 0.05 μm alumina slurry, cleaned in 0.5 M KOH, and then washed thoroughly with Millipore water. Next, the electrodes were cleaned by electrochemical sweeping in 0.5 M H_2SO_4 within the potential range of 0 to 1.5 V until a stable gold oxidation peak at 1.1 V *vs.* Ag/AgCl was obtained. Consequently, the gold surface was scanned cyclically between –3 and –0.2 V until a stable baseline at 0 V was formed, indicating the reduction of any gold oxide. Finally, the gold electrodes were soaked in an ultrasonic bath with ethanol for 5 min, and then dried with N_2. The self-assembly of either the lipoic acid NHS ester or the Fc-labeled cystamine derivative on the gold surface was performed by dipping the electrode into an ethanolic solution containing 2 mM of either the active ester or the Fc-labeled cystamine derivative for 24 h, at room temperature. Afterwards, the electrode was incubated with 1 mM of the RT-specific peptide (VEAIIRILQQLLFIH) in 10 mM sodium phosphate buffer, pH 7, overnight at 4 °C. The remaining active esters were quenched by incubating the peptide-modified electrode in ethanolic 100 mM ethanolamine solution for 1 h at room temperature. Subsequently, the peptide-modified electrode was incubated with an ethanolic solution of 1 mM hexanethiol for 10 min to back-fill the empty spots of the electrode surface, thus reducing the non-specific hydrophobic protein adsorption on the surface. Finally, the electrode was rinsed with ethanol and Millipore water to give the peptide-modified sensor surface.

X-ray photoelectron spectroscopy (XPS) and Time-of-Flight secondary ion mass spectrometry (TOF-SIMS) analyses

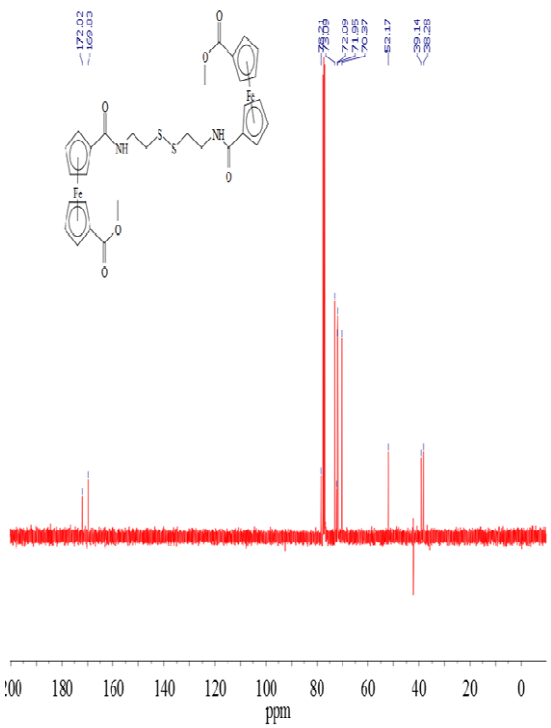
The XPS spectra were acquired with a Kratos Axis Ultra spectrometer (Kratos Analytical, UK) using a monochromatic Al-Kα X-ray source (15 mA, 14 kV). The takeoff angle between the film surface and the photoelectron energy analyzer was 90°. A typical operating pressure was around 5×10^{-10} Torr in the analysis chamber. Survey spectra (0–1100 eV) were taken at constant analyzer pass energy of 160 eV and were applied on an analysis area of 300 × 700 μm. High-resolution analyses were carried out at a pass energy of 20 eV on the same surface area. The binding energies were referenced to Au $4f_{7/2}$ at 83.96 eV and the spectrometer dispersion was adjusted to give a binding energy of 932.62 eV for the Cu $2p_{3/2}$ line of metallic copper. Acquired spectra were charge-corrected to the main line of the carbon 1s spectrum (adventitious carbon) set at 284.8 eV and analyzed using CasaXPS software (version 2.3.14).

TOF-SIMS experiments were performed using TOF-SIMS IV (ION-TOF GmbH, Münster, Germany) which was equipped with a Bi liquid metal ion source. For all

measurements, a 25 keV Bi_3^+ cluster primary ion beam with a pulse width of 12 ns (target current of ~1 pA) was utilized. The cycle time for the bombardment processes and detection was 100 μs (or 10 kHz). A pulsed, low energy electron flood was used to neutralize sample charging. For each sample, spectra were collected from 128 × 128 pixels over an area of 500 μm × 500 μm for 60 s. Positive and negative ion spectra were internally calibrated by using H^+, H_2^+, CH_3^+, H^-, C^-, and CH^- signals, respectively. Two spots per sample were analyzed by using a random approach.

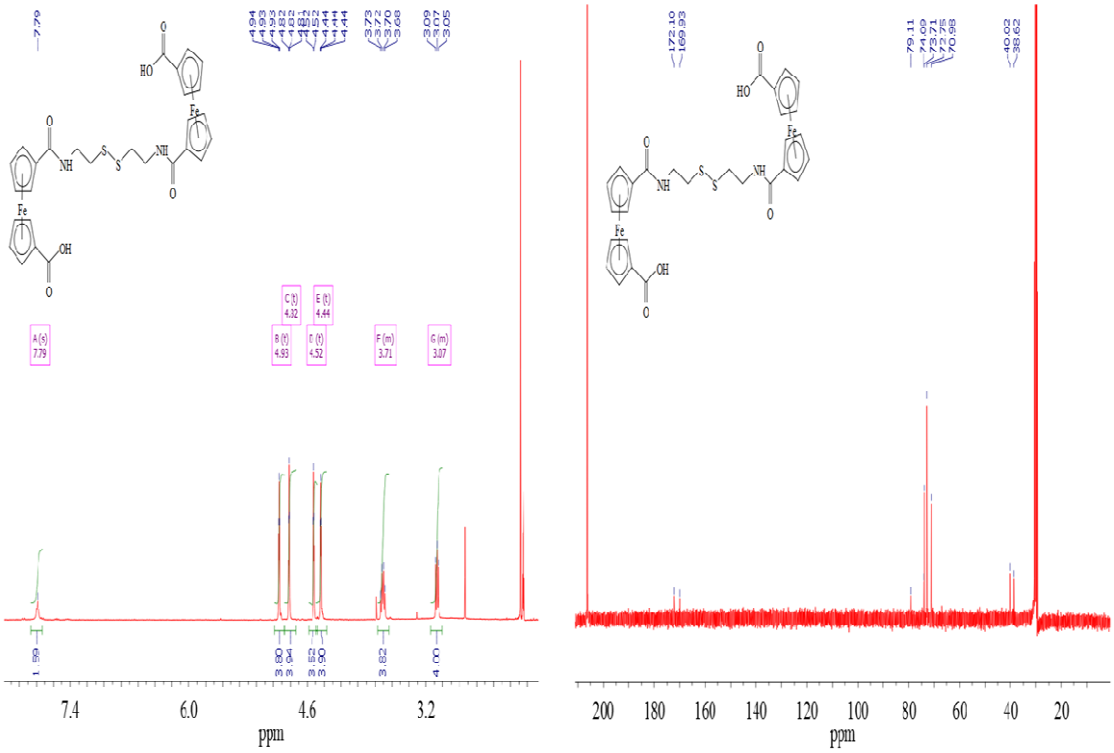


^{1}H NMR (400 MHz, $CDCl_3$) δ 6.88 (s, 2H), 4.83 – 4.75 (m, 4H), 4.75 – 4.66 (m, 4H), 4.51 – 4.42 (m, 4H), 4.40 – 4.33 (m, 4H), 3.80 (s, 6H), 3.73 (q, *J* = 6.4, 4H), 3.00 (t, *J* = 6.5, 4H).



^{13}C NMR (101 MHz, cdcl$_3$) δ 172.0, 169.8, 78.2, 73.1, 72.4, 72.0, 71.9, 70.4, 52.2, 39.1, 38.3. *m/z* = 715.0312 (Na^+) : 692.0401 (calc.)

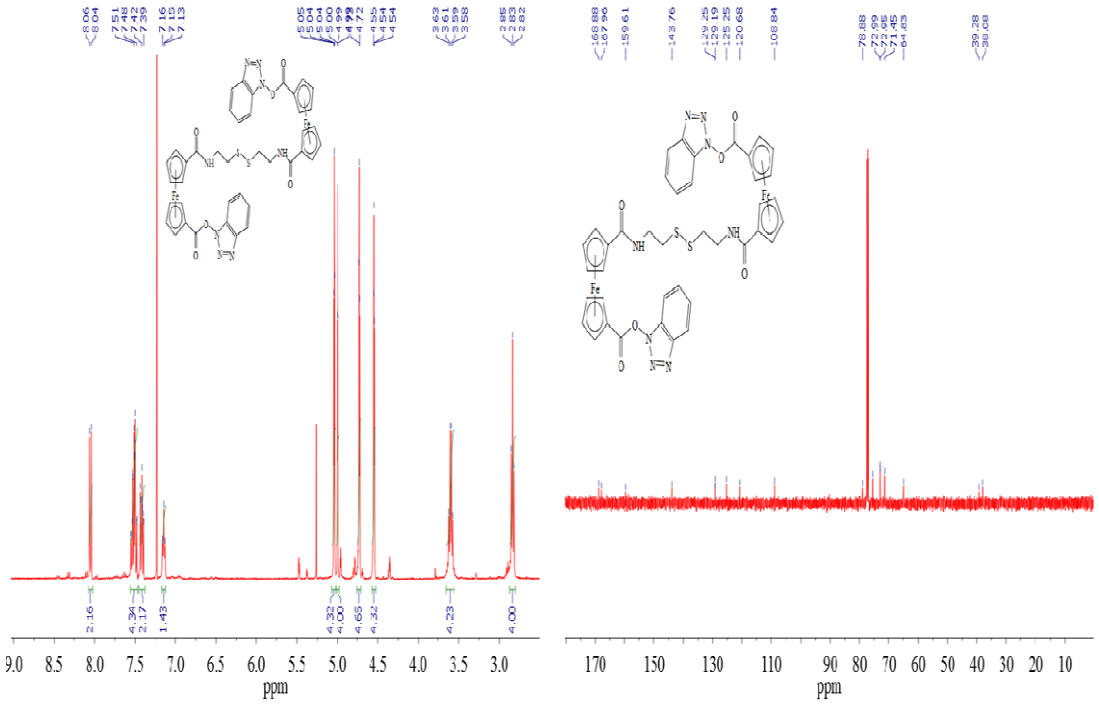
Fig. S1 NMR spectra and chemical shifts of $[CH_3OOC\text{-}Fc\text{-}CONHCH_2CH_2S]_2$.



^{1}H NMR (400 MHz, acetone) δ 7.79 (s, 2H), 4.93 (t, *J* = 1.9, 4H), 4.82 (t, *J* = 1.9, 4H), 4.52 (t, *J* = 1.9, 4H), 4.44 (t, *J* = 1.9, 4H), 3.77 – 3.64 (m, 4H), 3.14 – 3.00 (m, 4H).

^{13}C NMR (101 MHz, acetone) δ 172.1, 169.9, 79.1, 74.1, 73.7, 72.8, 71.0, 40.0, 38.6. *m/z* = 686.9988 (Na^+); 664.0088(calc.).

Fig. S2 NMR spectra and chemical shifts of [HOOC-Fc-CONHCH$_2$CH$_2$S]$_2$.



^{1}H NMR (400 MHz, cdcl$_3$) δ 8.05 (d, *J* = 8.4, 2H), 7.56 – 7.47 (m, 4H), 7.41 (ddd, *J* = 8.3, 6.5, 1.5, 2H), 7.15 (t, *J* = 5.8, 1H), 5.07 – 5.02 (m, 4H), 5.02 – 4.97 (m, 4H), 4.76 – 4.71 (m, 5H), 4.57 – 4.52 (m, 4H), 3.66 – 3.56 (m, 4H), 2.83 (t, *J* = 6.6, 4H).

^{13}C NMR (101 MHz, cdcl$_3$) δ 168.9, 168.0, 159.6, 143.8, 129.3, 129.2 125.3, 120.7, 108.8, 78.9, 75.4, 73.0, 72.9, 71.45, 64.8, 39.3, 38.1. *m/z* = 921.0619 (Na^+); 898.0742 (calc.)

Fig. S3 NMR spectra and chemical shifts of $[C_6H_4N_3OOC\text{-}Fc\text{-}CONHCH_2CH_2S]_2$.

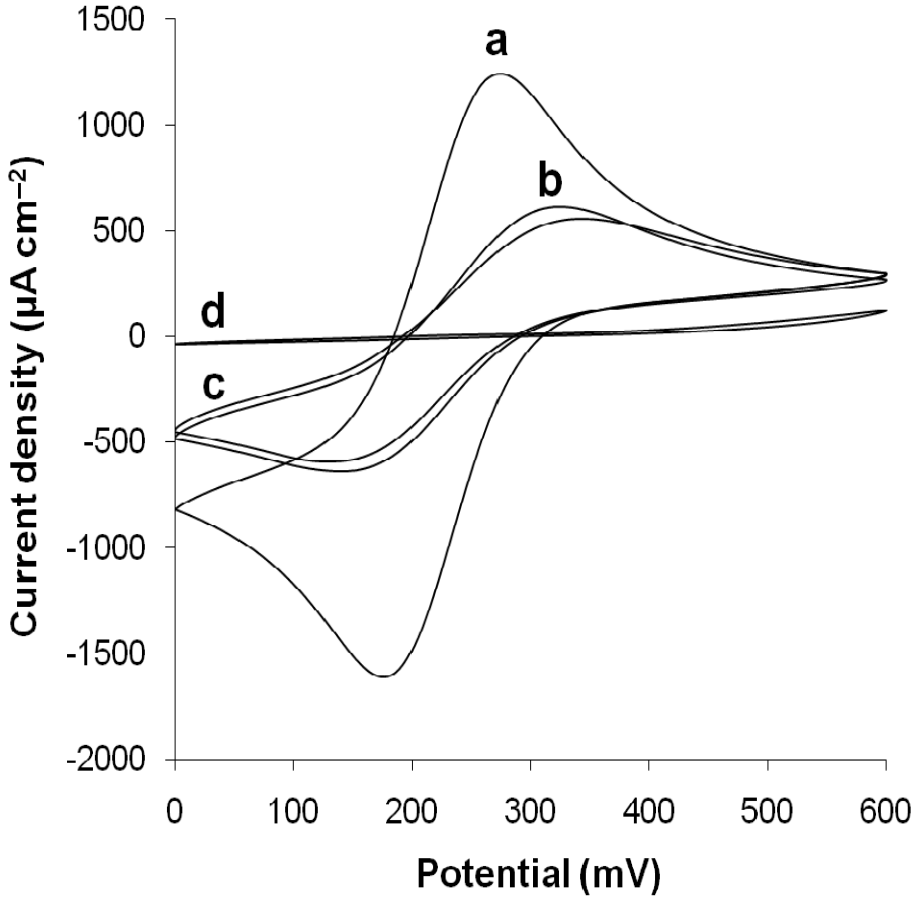


Fig. S4 Cyclic voltammograms of the RT biosensor after each immobilization or binding step in 10 mM sodium phosphate buffer (pH 7), containing 5 mM $NaClO_4$ and 5 mM $K_4Fe(CN)_6$. The cyclic voltammograms were recorded at a scan rate of 100 mV $s,^{-1}$ where (*a*) bare gold; (*b*) after coating with lipoic acid NHS ester; (*c)* after covalent binding of the RT-specific peptide; (*d*) after back-filling with 1 mM hexanethiol.

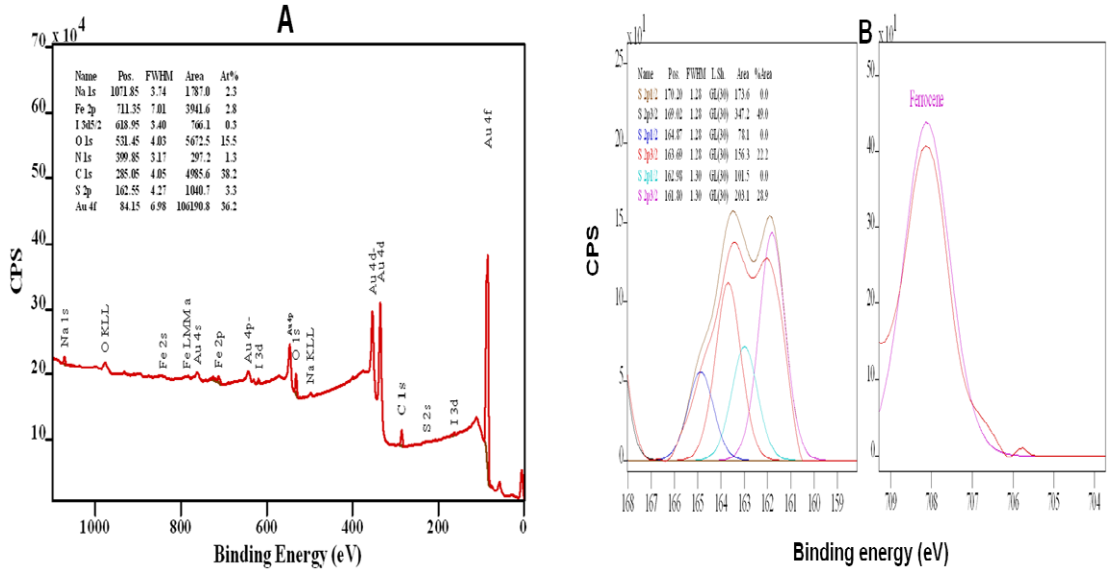


Fig. S5 X-ray photoelectron spectroscopy (XPS) of the Fc-labeled cystamine derivative-modified gold surface. **(A)** Survey scan of the film covering the electrode surface. **(B)** High-resolution XPS spectrum showing two S 2p doublets, the first corresponds to S $2p_{3/2}$, with binding energy values of 169, whereas the second represents the S $2p_{1/2}$ with a binding energy of 164.9 eV. It also showed Fe $2p_{3/2}$ peak with a binding energy value of 708.1 eV, which is characteristic for the Fc label.

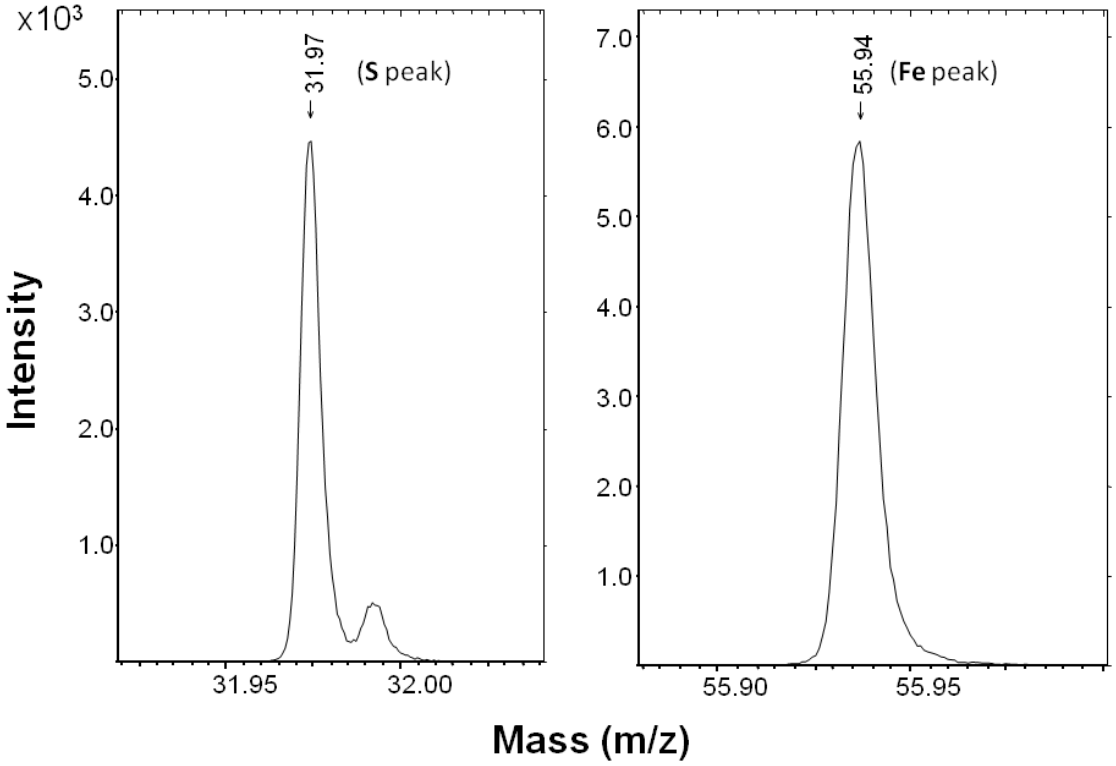


Fig. S6 Time-of-flight secondary ion mass spectrometry (TOF-SIMS) of the Fc-labeled cystamine derivative-modified gold surface. The TOF-SIMS spectra showed a high Fe^{+} fragment ion intensity at *m/z* = 55.9 in addition to a high S^{-} fragment ion intensity at *m/z* = 31.9 at the positive and negative modes, respectively.

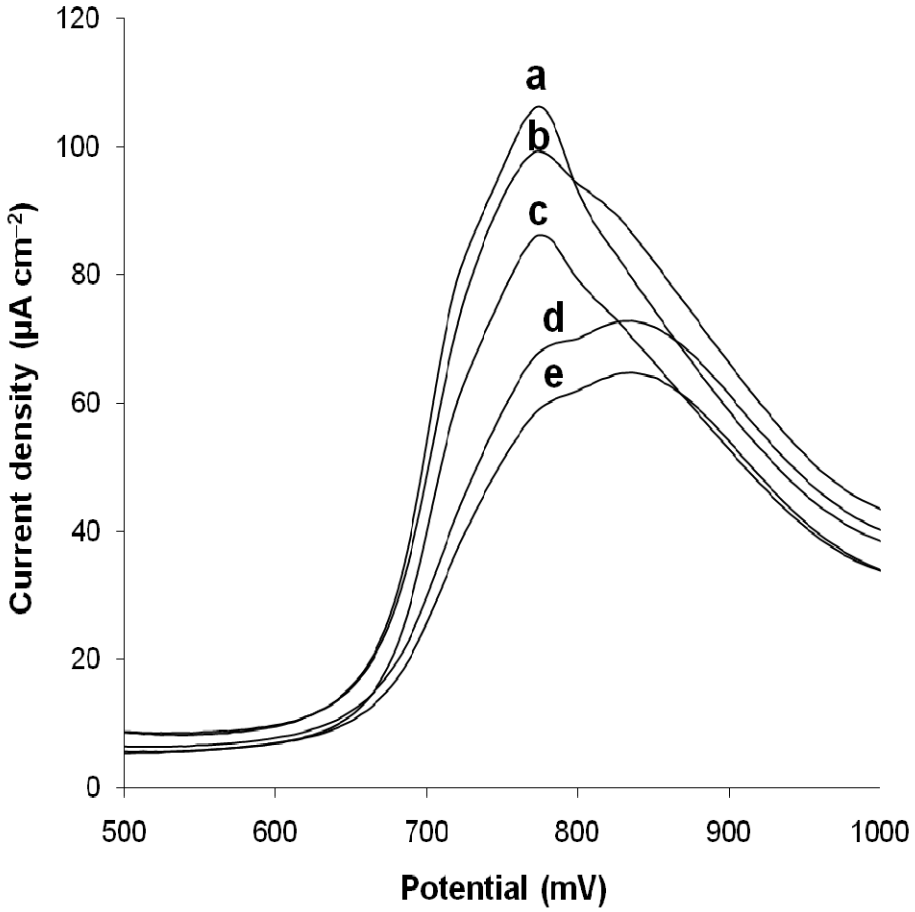


Fig. S7 Differential pulse voltammograms of the Fc-labeled cystamine derivative-modified gold electrode, covalently coupled to RT-specific peptide, in the presence of increasing RT concentrations: (*a*) 75; (*b*) 100; (*c*) 250; (*d*) 500; (*e*) 750 pg $mL,^{-1}$ in 10 mM sodium phosphate buffer, pH 7. Scans were performed in the range from 500 to 1000 mV with a step potential of 4 mV and amplitude of 25 mV.

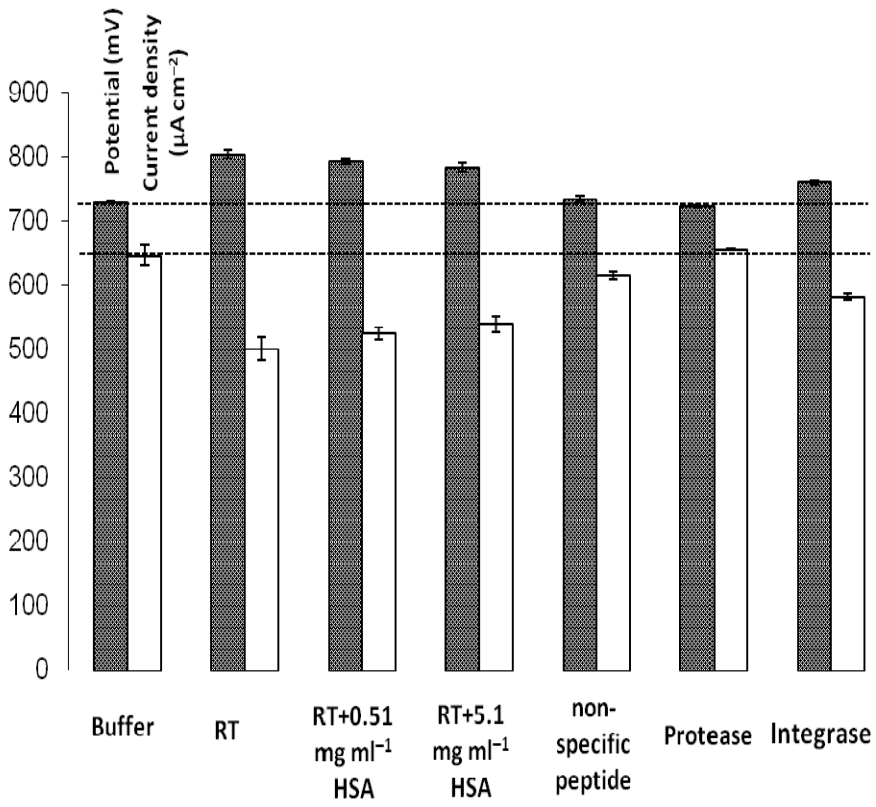


Fig. S8 A column chart showing the change in formal potential (*E*) and current density after incubation of the developed biosensor with a fixed concentration of RT (500 pg mL^{-1}) either alone or with two different concentrations of HSA, 5.1 and 0.51 mg $mL,^{-1}$ each at a time to test the specificity. Selectivity experiments were performed using: the assay buffer alone, 500 pg mL^{-1} protease, 500 pg mL^{-1} integrase, and 500 pg mL^{-1} RT using a non-specific peptide. Error bars indicate standard deviation of triplicate measurements (n = 3).

Table S1

Equivalent circuit element values for the impedance spectroscopy employed for surface characterization of developed non-labeled biosensor

Surface coverage	R_S (Ω cm^2)	CPE (μF cm^2)	n	R_{CT} (Ω cm^2)	W (μF$^{0.5}$ cm^2)
Bare gold	5.2(0.05)	808(68.5)	0.7(0.02)	31(2.7)	8514(729.8)
After SAM formation	2.5(0.02)	57(2.2)	0.9(0.03)	818(31.2)	3502(133.5)
After peptide binding	2.5(0.03)	54(3.6)	0.9(0.02)	948(63.5)	2982(199.7)
After back-filling with hexanethiol	2.3(0.01)	38(0.8)	0.9(0.01)	2040(70.4)	2104(72.7)

[a] The values in parentheses represent the standard deviations from at least three electrode measurements.

Table S2

Equivalent circuit element values for the lipoic acid NHS ester-modified gold electrode, covalently coupled to RT-specific peptide, in the presence of increasing concentrations of RT

RT conc. (Pg mL^{-1})	R_S (Ω cm^2)	*CPE* (μF cm^2)	*n*	R_{CT} (Ω cm^2)	*CPE* (μF cm^2)	*n*	R_X (Ω cm^2)
0	2.3(0.01)	38.2(0.8)	0.9(0.01)	2040(70.4)			
75	2.3(0.01)	38.4(0.8)	0.9(0.01)	2168(64.7)	87.6(4.3)	0.4(0.01)	1118(27.7)
100	2.2(0.01)	37.5(0.2)	0.9(0.01)	2251(24.2)	74.8(9.4)	0.4(0.01)	1164(145.5)
250	2.3((0.02)	32.2(2.9)	0.9(0.01)	2635(257.5)	66.7(3.9)	0.4(0.01)	1356(79.7)
500	2.3(0.01)	26.3(0.8)	0.9(0.01)	3248(71.6)	61.2(2.4)	0.4(0.01)	1637(63.5)
750	2.3(0.01)	23.6(1.6)	0.9(0.01)	3486(144.3)	59.4(4.4)	0.4(0.01)	1812(132.8)

[a] The values in parentheses represent the standard deviations from at least three electrode measurements.

Table S3

Equivalent circuit element values for the Fc-labeled cystamine derivative-modified gold electrode, covalently coupled to RT-specific peptide, in the presence of increasing concentrations of RT

RT conc. (Pg mL^{-1})	R_S (Ω cm^2)	CPE (μF cm^2)	n	R_{CT} (Ω cm^2)	W ($\mu F^{0.5}$ cm^2)
75	4.7(0.01)	118.9(4.4)	0.9(0.01)	16240(600.4)	52.46(1.9)
100	4.7(0.01)	107.4(8.3)	0.9(0.01)	34270(2690.5)	41.26(1.8)
250	4.7(0.05)	94.6(9.2)	0.9(0.02)	105800(10276.8)	11.9(0.7)
500	4.8(0.01)	91.7(6.4)	0.9(0.01)	238600(16627.7)	2.6(0.2)
750	4.8(0.03)	88.5(7.3)	0.9(0.02)	372800(30715)	1.8(0.5)

[a] The values in parentheses represent the standard deviations from at least three electrode measurements.