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A novel and rapid assay for HIV-1 protease detection using magnetic bead mediation

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ABSTRACT

A simple sensing assay was established for label-free detection of HIV-1 protease. HIV-1 protease peptide substrate conjugated to magnetic beads via its N-terminus is directly fixed onto the sensor gold surface through the sulphur atom of cysteine. Surface plasmon resonance (SPR) was used to study the peptide substrate cleavage efficiency of the protease with magnetic beads of different sizes (1 μm and 30 nm). Cyclic voltammetry and faradic impedance spectroscopy were employed in order to characterize the functionalized gold electrode. It was found that the nano-sized beads are a more efficient sensing probe for the protease. Electrochemical biosensing showed a gradual decrease in charge transfer resistance after injection of the HIV-1 protease. The experimental data established a detection limit of 10 pg/ml, as well as demonstrated a drug screening assay. This HIV-1 protease biosensor represents a new detection approach which will lead to low-cost point-of-care devices for sensitive HIV-1 diagnosis, as well as high-throughput drug screening platforms.

1. Introduction

Infectious diseases are the main cause of the significant increase in human pathogenesis and death throughout the world surpassing even the cardiovascular and cancer sicknesses (Taubes, 2008). In developed countries, there has been a remarkable technological progress in sanitation to identify and control most infectious diseases (Martinez, 2008). However, many serious issues related to food contamination, hospital-acquired pathogens and sexually transmitted diseases are still not resolved (Batt, 2007; Salyers and Whitt, 2002). Acquired immunodeficiency syndrome (AIDS), first reported by the US Center for Diseases Control in 1981, represents the most problematic one in all over the world (Montagnier and Alizon, 1987). In fact, the World Health Organisation (WHO) estimates that among 40 million people living with human immunodeficiency virus (HIV), the causative agent in AIDS, only 1 of 10 people is aware of their HIV sero-status (UNAIDS, 2009). This official estimation questions the efficiency/efficacy of the current HIV screening methods and programs adopted worldwide.

HIV virus is detected using a variety of time-consuming methods applied on blood samples by specialised laboratories. These include enzyme immunoassay (EIA), enzyme-linked immunosorbent assay (ELISA) and western blot test to detect the presence of antibodies generated by the patient against the virus. Nevertheless, detection of HIV by this approach is only reliable after 3 weeks to 6 months because in most people, detectable level of anti-HIV antibodies is developed only after this "window period". Furthermore, in practical terms, these diagnostic techniques do not offer portability and user-friendliness as they require sophisticated instrumentation operated by highly-trained personnel. Alternatively, biosensors would circumvent these drawbacks by offering rapid, highly specific and real-time sensitive HIV detection systems soon after infection because detections are based on the direct molecular recognition of HIV and its components. Researchers start using surface plasmon resonance configuration for real-time assessment and life cycle biosensing of HIV-1 genes and proteins (Bianchi et al., 1997; Yoo et al., 1997; Rich and Myszka, 2003). SPR-based biosensors are capable of monitoring complex formations and molecular interactions, which led to the measurement of thermodynamic parameters (Glaser and Hausdorf, 1996; Roos et al., 1998), affinity constants (Ota and Ueda, 1998; Nair et al., 2000) and the identification of several drug candidates (Altermana et al., 2001; Markgren et al., 2001, 2002). Nevertheless, development of miniature and low-cost SPR biosensors are still far from achievement due to the complexity and the high cost of the SPR machines. Interestingly, electrochemical detection assays have the advantage of being inexpensive, robust and relatively simple to operate (Kerman et al., 2004; Hahn et al., 2005)

There are two main biorecognition mechanisms in biosensing: identification of genetic materials such as specific nucleic acid sequences (Tran et al., 2011; Darfeuille et al., 2002; Kim et al.,

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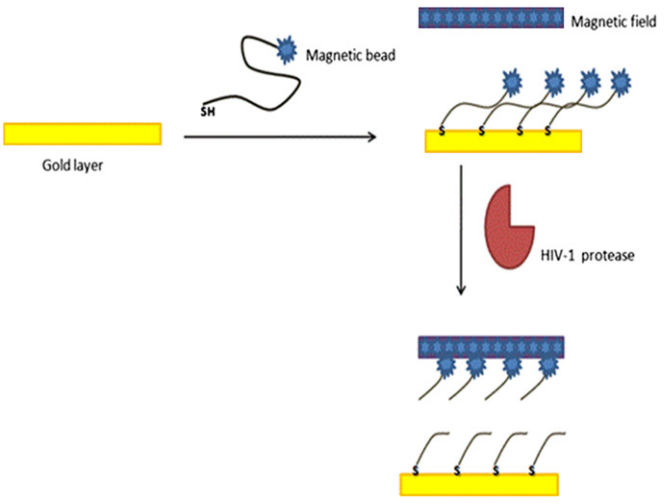
2002) and detection of biomolecules specific to the target of interest such as surface protein/antigens (Katz and Willner, 2003; Hoffman et al., 2000; Yang et al., 2001). In general, nucleic acid-based detection is more specific and sensitive than detection based on biomolecular interaction, while the latter is faster and more robust (Iqbal et al., 2000). While both methods directly identify HIV, they cannot determine whether the HIV is viable as they do not provide any information regarding the metabolic state of the virus. Therefore, the nucleic acid and biorecognition approaches in biosensing offer limited advantages when applied in drug screening and discovery. On the other hand, HIV detection based on the activity of HIV-1 protease, an enzyme essential to the assembly and maturation of HIV, might offer us an alternative to carry out HIV detection. In fact, with its integral role in the HIV life cycle, HIV-1 protease has been a prime target for drug discovery, which has led to drugs which inhibit key aspects of HIV replication. Biosensors that measure HIV-1 protease activity can lead to sensitive detection of HIV, as well as discovery of HIV-1 protease inhibitor drugs on a single sensing surface such as SPR chips and microelectrodes. Indeed, several biosensing platforms have been developed for the detection of HIV-1 proteases including SPR, impedance, quartz crystal microbalances. Mahmoud et al. (2008) were able to detect 0.8 pM of HIV-1 protease using impedance spectroscopy. However, their immobilization procedure of the recognition receptor is complex involving immobilization of ferrocene and thiol double-labelled carbon nanotubes onto gold surface (Mahmoud et al., 2008). Another method shown by Katz and Willner (2003) requires a secondary anti-HIV antibody peroxidase conjugate as a biomolecule reporter in order to detect 500 ng/ml of HIV-1 antibodies.

In this work, we have designed a very simple and inexpensive sensing surface for impedimetric HIV-1 protease detection. The probe consists of a specific HIV-1 protease substrate peptide carrying a magnetic bead via coupling to its N-terminus. This probe is attached onto the gold sensor surface at the C-terminus of the peptide, resulting in a layer of magnetic beads close to the sensor surface. Upon cleavage of the probe peptide by the HIV-1 protease, the physical link between the magnetic beads and the sensor surface is abolished. An externally applied magnetic field accelerates the dissociation of the magnetic beads from the sensor surface. In turn, a significant shift of the electrochemical signal is expected as a result of the protease-induced release of the magnetic beads from the sensor (Scheme 1). This label-free detection does not require washing or blocking step while offering high specificity and sensitivity. This very simple biosensing mechanism could be employed for lab-on-a-chip (LOC) biosensor elaboration, which will lead to efficient and reliable point-of-care HIV diagnostics.



Scheme 1. : Mechanism of HIV-1 protease detection on gold sensor surface.

2. Experimental

2.1. Materials and reagents

Carboxy-terminated magnetic beads of 1 μm of diameter were obtained from Bioclone Inc. (USA). Carboxy-terminated beads of 30 nm of diameter were provided by TurboBeads (Switzerland). Recombinant HIV-1 protease was purchased from EMD chemical (USA). The peptide sequences (short-linker: GSGSARVLAEAGSGSC; long-linker: GGGSGSGSARVLAEAGGGSGSGSC; control: GSGSAESG-ARSGSGSC) were synthesized at the Sheldon Biotechnology Center at McGill University (Canada). The HIV-1 protease inhibitor Saquinavir mesylate was obtained from Sigma-Aldrich (Canada).The coupling agent EDC [1-ethyl-3(3-dimethylaminopropyl) carbodiimide] was purchased from Sigma. The coupling buffer (10 mM potassium phosphate, 0.15 M NaCl, pH 5.5), wash/storage buffer (10 mM tris base, 0.15 M NaCl, 0.1% (w/v) BSA, 1 mM EDTA, 0.1% sodium azide, pH 7.5) and blocking buffer (1 M ethanolamine pH 8.0) were prepared from chemicals of analytical grade. The PBS buffer used for all electrochemical measurements was composed of 0.101 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 1.4 mM NaCl and 2.7 mM KCl.

2.2. Conjugation of the HIV-1 protease substrate peptide to magnetic bead

Magnetic beads suspension (20 mg/ml) was mixed with each peptide (0.5 mg/ml) and coupling agent EDC (0.57 mg/ml) with gentle shaking at room temperature for 24 h. The pH of the mixture was maintained between 4.5 and 6.0. The uncoupled peptides were removed by washing the beads 3 times using wash buffer. The reactive groups of the bead were then blocked by incubation with blocking buffer for 1 h. Finally, the beads are washed 3 times and stored at 4 °C in storage buffer.

2.3. Real-time monitoring of peptide immobilization and enzymatic reaction

The use of SPR to interrogate any bio-chemical interaction is based on total internal reflection of the incident light in a glass prism onto which a thin metal film is deposited. The delocalized free electrons of the metal generate a propagating wave along the surface which exerts an evanescent field in the far side of the metal layer (Iribe, 2007). In case of SPR imaging which is applied in our experiment, the intensity of the reflected light depends only on the refractive index of the ambient medium above the gold film.

SPR imaging device from GWC Technologies was employed to monitor in real time the peptide attachment to the gold electrode surface and HIV-1 protease detection by instantaneous calculation of reflected light. The gold coated chips were placed on top of the prism using oil matching liquids. The assembly was mounted above the flow cell and positioned in front of the lamp and CCD camera. The lamp is used to generate the incident light. The CCD camera detects and measures the reflected light intensity. The wash/storage buffer was used as blank baseline and washing step agent during all the SPR experiments. The flow rate applied was around 0.038 ml/min.

2.4. Electrode preparation and probe immobilization

The gold electrode surfaces were polished using 0.05 μm alumina for 20 min. The gold electrode was then incubated in ethanol ultrasonic bath for 5 min and immersed in piranha solution ($1/3H_2O_2$, $2/3H_2SO_4$) for 1 min. After washing with water and ethanol, cyclic voltammetry in 0.1 M H_2SO_4 was applied to the bare gold surface with potential ranging from 0 to 1.4 V using a scan rate of 100 mV/s for 20 rounds. This step permits a complete removal of micro-pollutants through successive oxidation and reduction of the gold. The cleaned gold electrode was immersed into the magnetic bead-peptide suspension for 10 h at room temperature. Then, the gold electrode was washed with ultrapure water and dried and mounted in the electrochemical cell for subsequent measurements.

2.5. Electrochemical characterizations

Autolab potentiostat/galvanostat was used to perform impedimetric measurements. The electrochemical impedance spectroscopy was applied at the potential of 0.25 V in the frequency range of 100 mHz and 50 kHz in order to characterize the magnetic bead-peptide deposition, to perform HIV-1 protease detection and to evaluate the inhibition effect of Saquinavir mesylate. All the experiments were carried out in a closed electrochemical cell inside a Faraday cage. The electrochemical cell is composed of a working electrode (gold electrode), a reference electrode (Ag/Ag^+) and a platinum electrode. The magnet is permanently attached at the bottom of the cell in order to attract free magnetic beads liberated during the enzymatic reaction. The number of cycles in each experiment was adjusted according to the stability of the electrochemical signal.

2.6. Biosensing of HIV-1 protease

Different concentrations of HIV-1 protease were incubated separately within the electrochemical cell. Each cleavage reaction was monitored through real-time measurement of the change in the couple resistance/capacitance of the Nyquist plot. Modelling and theoretical calculation, as well as Nyquist and cyclic votammetry plots were produced with the NOVA software. A calibration plot was established based on the values generated by the fitting calculations of the experimental curves. Detection of HIV-1 protease inhibition was carried out with a mixture of HIV-1 protease (10 ng/ml) and Saquinavir mesylate (0.12 nM) introduced to the magnetic bead-peptide modified gold electrode.

3. Results and discussion

3.1. Real-time monitoring of peptide immobilization and enzymatic reaction: elucidation of the double role of the magnetic beads

Before starting our main detection experiment using impedance spectroscopy, it was interesting for us to study the time dependant behaviour of our bio-chemical processes near gold surface. In fact, the surface plasmon resonance technique is considered in literature as a powerful tool for real time monitoring of any change in refractive index of the ambient medium on the far side of the gold film (Homola et al., 1999; Mitchell, 2010). Consequently, SPR biosensors can be used to study the interactions of any biological system from proteins, oligonucleotides, oligosaccharides and lipids to small molecules, phage, viral particles and cells.

Upon injection of the magnetic bead-peptide sample, a rapid and significant decrease in reflectivity from −1 to −3 PIU was observed followed by a slow and gradual drop of signal from −3 to −3.75 PIU before stabilization. The rapid SPR signal response indicates the presence of magnetic bead near gold surface which causes a significant change in refractive index of the buffer medium within the evanescent field. Subsequently, the stepwise attachment of the magnetic beads onto the gold surface through the peptide thiol group causes a gradual and slow decrease in SPR signal. On the other hand, during the washing step, a sharp and significant increase in reflection intensity, followed by stabilization of the signal around −1.75 PIU (Fig. 1a) was observed. Most importantly, the signal did not revert to the starting baseline. This partial reversal of the SPR signal towards the initial level suggests the dissociation and removal of weakly adsorbed magnetic beads from the evanescent field; whereas a significant portion of the peptide-beads are still stably attached onto the gold surface.

Upon addition of 100 ng/ml of HIV-1 protease, a slow and gradual decrease in reflectivity from −1.75 to −4.25 PIU was caused by the presence of protease at the vicinity of the gold layer. As the evanescent field generated by the oscillation of the Plasmon extends not more than 300 nm away from the gold surface (Homola, 2008; Mitchell et al., 2005), this shift of reflectivity signal indicates that the protease was able to migrate

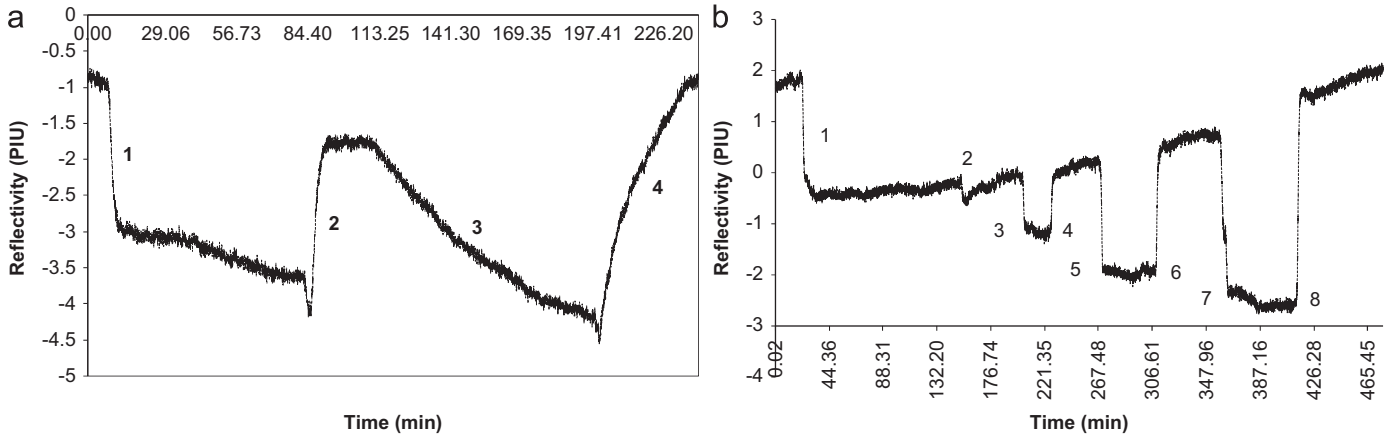


Fig. 1. (a) SPR signal indicating 1— the immobilization of 1 μm magnetic beads labelled with HIV-1 protease substrate peptide; 2— washing of the non-immobilized beads; 3— introduction of 100 ng/ml HIV-1 protease; 4— HIV-1 protease induced dissociation of the magnetic beads. (b) SPR signal indicating 1— the immobilization of 30 nm magnetic beads labelled with HIV-1 protease substrate peptide; 2— washing of the non-immobilized beads; 3— introduction of 100 pg/ml HIV-1 protease; 4— HIV-1 protease induced dissociation of the magnetic beads; 5— introduction of 1 ng/ml HIV-1 protease; 6— HIV-1 protease induced dissociation of the magnetic beads; 7— introduction of 10 ng/ml HIV-1 protease; and 8— HIV-1 protease induced dissociation of the magnetic beads.

across the micrometer-sized magnetic bead-peptide layer to reach the evanescent field area. Indeed, the large size of the magnetic beads (1 μm) provides sufficient free spaces for the protease to diffuse through to reach the gold surface. During this process, some of the protease could adsorb non-specifically onto the gold layer. Subsequent injection of the washing buffer, on the other hand, caused a slow and gradual reversal of the SPR signal back to the initial baseline level. This complete reversal of SPR signal not only indicates the added protease was being removed by the washing buffer, but also that the linkage between the gold surface and magnetic bead was abolished, which resulted in the dissociation of all the beads from the surface. Since the abolishment of this linkage was caused by the cleavage of the peptide sequence by the HIV-1 protease, the reversal of the SPR signal represents a direct indication of enzymatically active protease.

3.2. Effect of magnetic bead size on sensitivity

We explored the effect of bead size in order to improve sensitivity and response time of the SPR-based detection mechanism. We hypothesized that a smaller bead size might lead to a higher loading efficiency, better surface coverage and enhancement of the accessibility of the peptide cleavage sequence to the protease. An enhanced accessibility of the peptide cleavage sequence to the protease is expected to give higher signal amplitude and shorter response time, which will result in a more rapid detection with a lower detection limit.

Using 30 nm-sized magnetic beads, we noticed that the shift in reflectivity was three times more in comparison with the signal caused by 1 μm beads (Fig. 1b), suggesting a significant increase in the amount of immobilized magnetic bead-peptide on the gold surface. This result matches well with our initial hypothesis that a lower bead size might result in a higher surface coverage and in turn, more peptide attachment. With an enhanced bead loading capacity, the subsequent biosensing experiments revealed a marked improvement in the detection limit and response time. In fact, the sensing platform was able to detect 100 pg/ml of HIV-1 protease during 60 min (Fig. 1b). In contrast, the detection limit does not exceed 100 ng/ml using large sized magnetic bead (Fig. 1a) with total response time of 130 min. Fig. 1b shows the increasing rate of SPR signal reversal with addition of HIV-1 protease at increasing concentrations. After injection of 10 ng/ml of the enzyme, the SPR signal returned back to the initial SPR signal level of bare gold surface, indicating the complete removal of magnetic beads from the surface by HIV-1 protease cleavage of the substrate peptide.

3.3. HIV-1 protease biosensing

The injection of HIV-1 protease at the concentration of 1 ng/ml immediately induces an increase in the diameter of the Nyquist plot corresponding to charge transfer resistance (Fig. 2a). Subsequently, a gradual decrease in diameter of the semi-circle is observed with time (Fig. 2a). After 90 min, a significant shift of the Nyquist plot was observed with respect to the initial signal. The diameter of the semi-circle continues to decrease over time. On the other hand, the Warburg diffusion is changing positively. The same electrochemical signals were also observed using nano-sized magnetic beads. Interestingly, the response time and

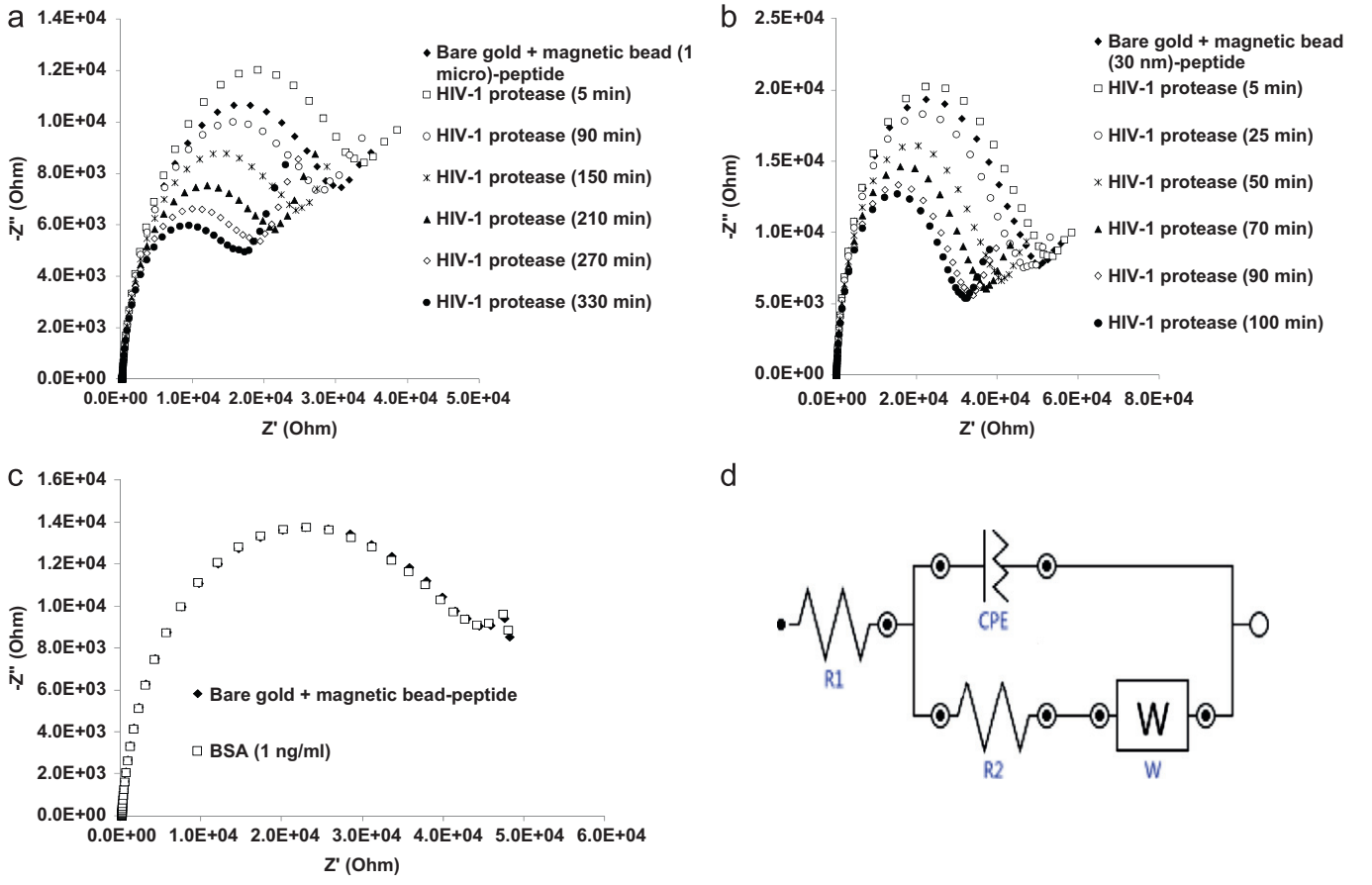


Fig. 2. Electrochemical biosensing of HIV-1 protease. Nyquist plots corresponding to the real-time detection of (a) 1 ng/ml of HIV-1 protease using 1 μm-sized magnetic bead-peptide layer, (b) 10 pg/ml of HIV-1 protease using 30 nm-sized magnetic bead-peptide layer, (c) 1 ng/ml of BSA using 1 μm sized magnetic bead-peptide layer and (d) equivalent circuit of the modified gold electrode.

sensitivity were markedly improved with a lower detection limit (10 pg/ml) with nano-sized beads. Significant variation of the charge transfer resistance (Fig. 2b) was observed after only 5 min. Injection of a negative control containing 1 ng/ml BSA onto the magnetic bead-peptide layer had no effect on the electrochemical resistance (Fig. 2c).

The temporary increase in charge transfer resistance observed upon the addition of the protease can be explained by the presence at the proximity of the gold surface. As the proteolytic cleavage progresses, magnetic beads continue to dissociate from the surface. The dissociation of the magnetic from the gold surface is further enhanced by the external magnet installed opposite to the electrode. More electrode surface is then exposed, causing a decrease in the charge transfer resistance and an enhancement in ion diffusion (Fig. 2a). The experimental curves were fitted with an equivalent circuit (Fig. 2d) to obtain values of each component parameter corresponding to the real time plots (Table 1). The values show clearly the decrease in resistance caused by the HIV-1 protease induced dissociation of the magnetic beads from the gold surface. Higher capacitance and diffusion values (Table 1) also indicate the increase of the number of pores and free gold surface inside the magnetic bead-peptide layer.

For each HIV-1 protease concentration and within the defined duration, it is possible to establish a new parameter named charge transfer resistance variation (ΔR) which corresponds to the subtraction of magnetic bead-peptide layer resistance after enzyme addition from the magnetic bead-peptide layer resistance before enzyme addition. The calibration plot ($\Delta R = f$ [HIV-1 protease]) shows an apparent linear shape with a slight saturation behaviour towards 10 ng/ml of HIV-1 protease. Indeed, using a higher concentration of enzyme induces an acceleration of the proteolytic cleavage allowing a big reduction of the available peptides close to the gold surface. The signal therefore shows an inhibition effect due to the lack of substrate. The detection limit attained 10 pg/ml which corresponds to 1 pM considering the molecular weight of the HIV-1 protease (Fig. 3). The advantage of the developed method stems from its simplicity, ease of single probe immobilization and the elimination of reporter or secondary biomolecules when compared to other methods (Katz and Willner, 2003; Mahmoud et al., 2008). Furthermore, the sensing system could also be used in drug screening through evaluation of HIV-1 protease inhibition.

3.4. Effect of spacer length on detection sensitivity

A similar sensor construct with a non-cleavable control peptide did not cause a significant response at different concentrations of HIV-1 protease ranging from 10 pg/ml to 10 ng/ml, which demonstrates the specificity of the detection mechanism (Fig. 3). We have also observed a significant improvement in sensitivity (Fig. 3) after an increase in the peptide Gly-Ser spacer length on each termini of the substrate sequence from 4 to 8 residues. The spacer enhanced the access of the protease to the substrate sequence near the sensor surface and magnetic bead layer by providing additional degree of freedom to the target molecules.

3.5. Drugs inhibition assay

The injection of HIV-1 protease (10 ng/ml) pre-incubated with 0.12 nM of Saquinavir mesylate induces less variations of the charge transfer resistance of the magnetic bead-peptide layer (Fig. 4a and b). This decrease in enzymatic activity is explained by a partial inhibition of the enzyme after incubation with Saquinavir mesylate. The calculation of the ratio of that electrochemical resistance drop by the total $\Delta R_{\text{without inhibitor}}$ value allows the deduction of the inhibition percentage (I) extremely important for the drug evaluation: ΔR_a (without inhibitor) = 10,355 Ω (Fig. 4a); ΔR_b (with inhibitor) = 4294 Ω (Fig.4b); I = 58.53%.

Since that the inhibition constant (K_i) of Saquinavir mesylate is around 0.12 nM, the enzymatic activity of HIV-1 protease, in our experiment, should be reduced by 50% which is not far from our founded experimental value (58.53%). This slight increase in

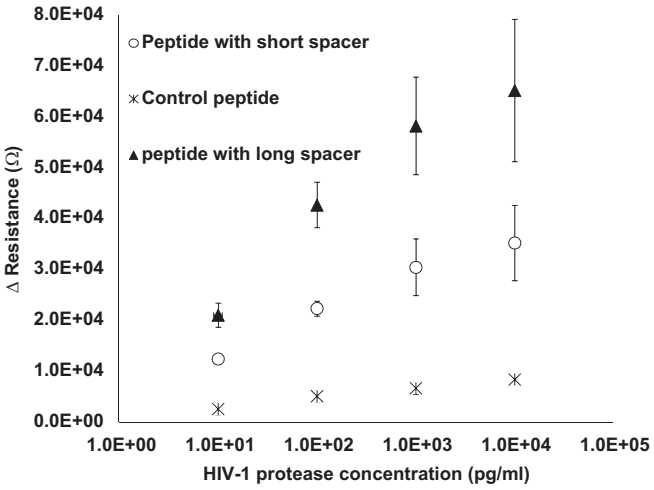


Fig. 3. Calibration plot: ΔR corresponds to the subtraction of magnetic bead-peptide layer resistance after enzyme addition from the magnetic bead-peptide layer resistance before enzyme addition.

Table 1
Values of the electrical component parameters of the equivalent circuit.

parameter	Bare gold	30 nm sized magnetic bead-peptide	1 ng/ml HIV-1 protease (5 min)	1 ng/ml HIV-1 protease (25 min)	1 ng/ml HIV-1 protease (50 min)	1 ng/ml HIV-1 protease (70 min)	1 ng/ml HIV-1 protease (90 min)	1 ng/ml HIV-1 protease (100 min)
Solution resistance (*R1*) (Ω)	196.53 (0.546%)	211.99 (0.682%)	213.6 (0.711%)	212.73 (0.695%)	212.69 (0.636%)	212.64 (0.607%)	12.77 (0.584%)	212.89 (0.575%)
Capacitance (CPE) *Y0* (μS) *N*	–	*Y0* = 0.526 (0.543%) *N* = 0.9216 (0.08%)	*Y0* = 0.534 (1.810%) *N* = 0.9217 (0.267%)	*Y0* = 0.545 (1.816%) *N* = 0.9225 (0.267%)	*Y0* = 0.5590 (1.722%) *N* = 0.9237 (0.252%)	*Y0* = 0.5702 (1.689%) *N* = 0.9244 (0.246%)	*Y0* = 0.5817 (1.667%) *N* = 0.9250 (0.242%)	*Y0* = 0.5876 (1.666%) *N* = 0.92531 (0.242%)
Charge transfer resistance (*R2*) (Ω)	102.14 (2.324%)	44,088 (0,665%)	46,101 (0.911%)	41,442 (0.884%)	36,290 (0.806%)	32,812 (0.766%)	29,722 (0.734%)	28,277 (0.724%)
Capacitance (*C*) (nF)	558.32 (3.622%)	–	–	–	–	–	–	–
Warburg diffusion (*W*) (μS)	243.77 (0.593%)	84.087 (4.24%)	79.374 (5.417%)	85.063 (5.123%)	90.071 (4.397%)	94.585 (4.017%)	98.5 (3.676%)	100.27 (3.532%)

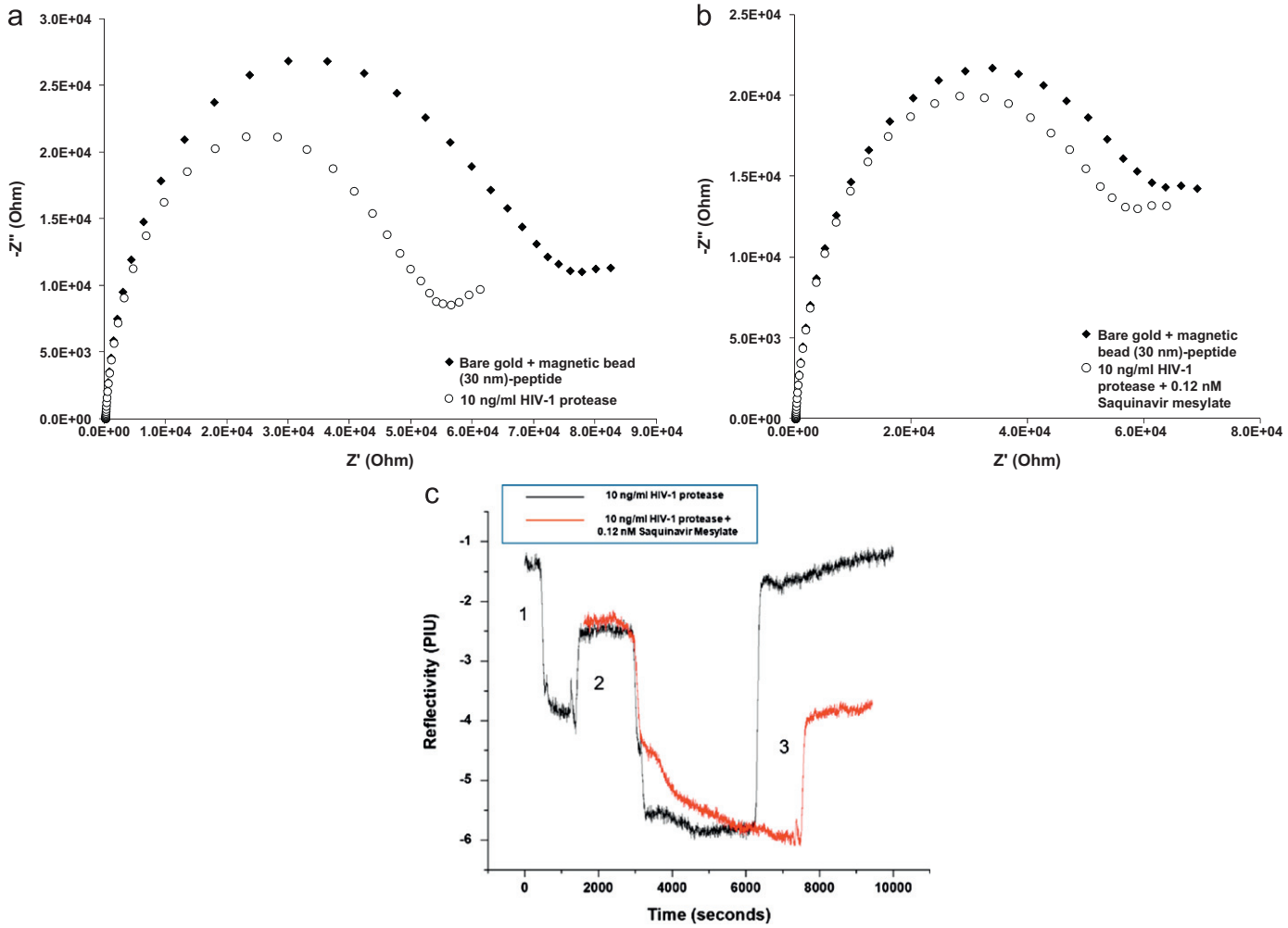


Fig. 4. Measurement of the inhibitory activities of anti-HIV drug by electrochemical impedance and SPR. (a) Electrochemical detection of 10 ng/ml of HIV-1 protease. (b) Electrochemical detection of 10 ng/ml of HIV-1 protease pre-incubated with 0.12 nM of Saquinavir mesylate. (c) SPR detection of HIV-1 protease inhibition: 1: injection of magnetic bead 30 nm-peptide; 2: washing non-immobilized magnetic bead-peptide complexes; and 3: HIV-1 protease induced dissociation of the magnetic beads.

inhibition effect comparing to the theoretical value could be explained by the negative effect of the immobilization process on the total availability of the substrate. In fact, those theoretical values of inhibition percentage are calculated when the substrate and the inhibited enzyme are mixed together in free solution. Therefore, our simple biosensing interface has also the potential to be applied also in drug screening and discovery.

Surface plasmon resonance evaluation of HIV-1 protease activity in the presence of 0.12 nM of Saquinavir mesylate reveals roughly the same percentage of inhibition founded in the electrochemical impedance assessment (Fig. 4c). In fact, the calculation of the ratio of the drop in reflectivity in the presence of the inhibitor by the initial total optical signal corresponding to the total HIV-1 protease activity gives a value close to 50% (2.5/4.5 = 55%).

4. Conclusions

Electrochemical detection of HIV-1 protease was performed using a sensitive monolayer of magnetic bead-peptide. The proteolytic cleavage of the substrate peptide induces a significant variation of charge transfer resistance due to the dissociation of the magnetic beads from the gold surface. The sensor was able to detect HIV-1 protease at concentration as low as 10 pg/ml (1 pM) within 25 min. Furthermore, the same sensor was capable of detecting the inhibitory activities of the anti-HIV drug Saquinavir mesylate with acceptable accuracy. This sensor offers superior simplicity in the probe immobilization procedure and the elimination of reporter biomolecules facilitates the development of point-of-care HIV screening. Furthermore, the observed diffusion phenomena at the magnetic bead layer would lead to simple approaches to overcome the difficulties caused by interferrants in complex sample media by obstructing the contaminants from reaching gold surface. This sensing mechanism can also be implemented in other detection platform coupled to novel transducers such as localized surface plasmon resonance (LSPR), which would lead to further enhancement of detection sensitivity and specificity. The sensing mechanism also represents an elegant approach amenable to high-throughput drug screening.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.08.049.

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