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Optimization of gold nanoparticle-based real-time colorimetric assay of dipeptidyl peptidase IV activity

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Abstract

Dipeptidyl peptidase IV (DPP-IV also referred to as CD-26) is a serine protease enzyme with remarkable diagnostic and prognostic value in a variety of health and disease conditions. Herein, we describe a simple and real-time colorimetric assay for DPP-IV/CD-26 activity based on the aggregation of gold nanoparticles (AuNPs) functionalized with the peptide substrates: Gly-Pro-Asp-Cys (GPDC) or Val-Pro-ethylene diamine-Asp-Cys (VP-ED-DC). Cleavage of the substrates by DPP-IV resulted in aggregation of the AuNPs with accompanying colour change in the solution from red to blue that was monitored using either a UV-visible spectrophotometer or by the naked eye. Factors, such as pH, ionic strength and the structure of the substrate that influence the cleavage reaction in solution were investigated. The effects of potential interference from serum proteins (lysozyme, thrombin and trypsin) on the analytical response were negligible. The detection limits when GPDC or VP-EN-DC functionalized AuNPs were used for DPP-IV assay were 1.2 U/L and 1.5 U/L, respectively. The VP-EN-DC method was preferred for the quantitative determination of DPP-IV activity in serum because of its wide linear range 0 - 30 U/L compared to 0-12 U/L for the GPDC assay. Recoveries from serum samples spiked with DPP-IV activity, between 5-25U/L, and using the VP-EN-DC modified AuNPs method ranged between 83.6-114.9%. The two colorimetric biosensors described here are superior to other conventional methods because of their simplicity, stability, selectivity and reliability.

Keywords

gold nanoparticles, colorimetric assay, peptide substrates and DPP-IV enzyme activity.

1. Introduction

Dipeptidyl peptidase IV/CD26 (DPP4/ DPP-IV) is a type II transmembrane serine protease glycoprotein belonging to the S9 prolyl oligopeptidase family [1] and capable of splitting N-terminal dipeptides from polypeptide substrates with proline in the penultimate position, thereby acting on a variety of compounds such as chemokines, neuropeptides, peptide hormones and incretins. The enzyme is implicated in immune regulation, oncological, neurodegenerative and inflammatory processes, and also in nutritional control and nociception [2,3]. The focus of ongoing research is in the development of drugs which target DPP-IV production for the therapeutic management of type II diabetes and its use as a biomarker for the management of heart failure [4]. It has been suggested that the measurement of DPP-IV activity can be used as a diagnostic and prognostic marker for various inflammatory disorders, tumours and haematological malignancies [5–9]. Therefore, there is demand for simple, selective and sensitive assays that can be used to measure the activity of DPP-IV in health and disease.

To date, the most commonly used methods for the determination of DPP-IV enzymatic activity are based on the deployment of chromogenic, fluorogenic, bioluminescent and electrochemical [10–13] sensors (Table 1). Although sensitive, most fluorogenic probes suffer from poor aqueous solubility and therefore, require the use of organic co-solvents which have been reported to reduce enzyme activity and thus limit their application to cell-free assays [14]. A further drawback with the use of the current chromogenic substrates is that they contain over reactive leaving groups, which render them easily susceptible to non-enzymatic cleavage by interfering proteins in biological samples [15].

Nanoplasmonic colorimetric assays are highly sensitive because of the high extinction coefficients of gold nanoparticles (AuNPs), 10^2-10^3 times higher than those of conventional colored dyes [16].Using thiol chemistry, recognition substrates, e.g. peptides with cysteine residues, alkane thiolate or tiopronin [17–19] can be coupled to the AuNPs surface to form a

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colloidal solution whose stability is affected in the presence of the enzyme resulting in the aggregation of the AuNPs with accompanying colour change. Label-free colorimetric assays in which the substrate reporter molecule is not covalently attached to the AuNPs but through electrostatic and hydrophobic interactions have also been reported for the detection of thrombin [20] and alkaline phosphatase [21]. Detection of enzymatic activity by using metal (typically gold) nanoparticle based colorimetric assays is an attractive alternative to traditional enzymatic assays because color changes are visible to the naked eye. This color change caused by direct or indirect induced AuNP aggregation forms the basis for the colorimetric detection. A variety of enzymes (lipase, protease, kinase and DNAse) have been detected using this approach [22–25].

Of critical importance to this type of application is to ensure that the colloidal solution is stable and the resultant aggregation is triggered by the analyte and not due to any non-specific changes in the solution. In order to improve the stability of the colloidal solution, Lévy and co-workers, (2004) designed a pentapeptide sequence (CALNN) using a combinatorial approach in order to produce an extremely stable, water soluble peptide ligand, which has been used as an additional stabilizer in colorimetric platforms for the detection of a variety of analytes [26,27]. Takeshi *et al.*, 2010, in an attempt to produce a stable colloid, explored a different approach in which the reduction of the gold salt and functionalization with phosphorylated dipeptide pTyr-Arg substrate occurred simultaneously in the presence of 0.1M HEPES buffer (pH 7.2) to detect alkaline phosphatase activity [28]. However, with this approach, the colloidal solution may not be stable enough to withstand changes caused by matrix constituents in complex biological samples.

Most of the AuNP-based assays for the measurement of enzyme activity are largely based on the AuNPs aggregation initiated by the enzyme reaction. The length of the substrate is one of the essential parameters that are of crucial importance to the performance of the assay based

on functionalized AuNPs. A well-designed substrate confers steric stabilization on the AuNP colloidal solution, and provides optimum distances between the P-AuNPs to allow the enzyme easy access to the substrate.

Recently, Xia *et al.* used unmodified Au NPs as a colorimetric probe to measure DPP-IV activity [29]. Their method was based on the design of peptide sequence (Arg-Pro-Arg) with 2 binding tags to crosslink unmodified Au NPs. In their method, the Au NPs were not functionalized with the peptide substrate making their method prone to possible interferences from the sample matrix. More importantly, their two-stage method is not amenable to real-time monitoring of enzyme activity.

Herein we investigate and compare the performances of Au NPs functionalized separately with two designed peptide probes [(Gly-Pro-Asp-Cys (GPDC) and Val-Pro-NH-$(CH_2)_2$-NH-Asp-Cys (VP-EN-DC)] in the development of a rapid and simple colorimetric assay for the measurement of DPP-IV activity. This method is a novel non-crosslinking AuNP-based colorimetric method for detection of DPP-IV activity.

2. Materials and methods

2.1 Reagents and Materials

The peptide Gly-Pro-Asp-Cys (~ 90%) pure was purchased from Thermo Fisher Scientific Co. Ltd (Germany), Val-Pro-NH-$(CH_2)_2$-NH-Asp-Cys (> 95%) pure was purchased from Cambridge Research Biochemicals (UK). DPP-IV enzyme from porcine kidney was purchased from Merck Chemicals (Germany). Human lysozyme, trypsin from porcine pancreas, hydrogen tetrachloroaurate(III) ($HAuCl_4.3H_2O$), 99.99% pure, and sodium citrate dihydrate ($Na_3C_6H_5O_7.2H_2O$), 99% pure, were purchased from Sigma-Aldrich Co. Ltd (UK) and used without further purification. Thrombin from bovine plasma was purchased from GE HealthCare (UK). Normal human serum control was purchased from Thermo Scientific.

The DPP-IV solutions were prepared in 50mM Tris buffer solution of pH 8.3. A 10 mM citrate buffer solution at pH 6 was used to dissolve the GPDC peptide, VP-EN-DC was dissolved in 50 mM Glycine buffer solution (pH 10).All solutions were prepared using deionized water with a resistivity of 18.2 MΩ cm^{-1} prepared with a Milli-Q Academic Purification equipment from Millipore (UK).

2.2 Preparation of citrate-capped AuNPs

Citrate AuNPs (∼15 nm) diameter was prepared by citrate reduction of HAuCl$_4$ [30]. Briefly, 10 ml of 38.8 mM sodium citrate dihydrate at 50-60°C was added to 100 ml of 1 mM boiling HAuCl$_4$ under vigorous stirring to form a ruby red colored solution instantaneously. After cooling, the solution was filtered through a 0.22 μm Millipore syringe filter to remove any precipitate, the pH was adjusted to 7.4 using 0.5 M NaOH solution, and the filtrate was stored at 4°C.

2.3 Preparation of peptide-capped AuNPs bioconjugate (P-AuNPs)

The Gly-Pro-Asp-Cys conjugated AuNPs were prepared by addition of an aqueous solution of 2 mM peptide in citrate buffer pH 6, to the solution of 8 nM citrate AuNPs (based on an extinction coefficient of ~1.61×10^8 M^{-1} cm^{-1} at 520 nm for AuNPs) in a volume ratio of 1 to 10. After overnight reaction at room temperature, excess peptides were removed by centrifugation (13000 rpm, 30 min at 4°C) using an Eppendorf centrifuge (Eppendorf, Germany), the pelleted AuNPs were resuspended in deionized water and stored at 4°C. Val-Pro-NH-(CH$_2$)$_2$-NH-Asp-Cys modified AuNPs were prepared by the same procedure with slight modification to the rate and time of centrifugation used for separating the modified AuNPs (14500 rpm, 20 min at 4 °C). The pellets were resuspended in Tris buffer pH 8.3 and stored at 4°C.

2.4 Instrumentation for gold nanoparticle characterization

The ultraviolet-visible (UV-vis) absorption spectra were recorded on a microplate reader (Tecan 2000, San Francisco, USA) using transparent 96 well microplates (Nunclon,

Thermo Scientific, UK). ESI-Mass spectra of the peptides were obtained on an API-150EX MS single quadrupole LC/MS system (Applied Biosystems). Dynamic light scattering (DLS), (Malvern Zetasizer Nano-ZS) for sizing measurements were carried out using a 10mm disposable sizing cuvette and the particle size measurements were performed in triplicate.

The XPS spectra were obtained on a VG Escalab 210 Photoelectron Spectrometer. The X-ray source was a non-monochromated Al Kα source (1486.6eV), operated with an X-ray emission current of 20 mA and an anode high tension (acceleration voltage) of 12 kV. The freeze-dried sample was placed on a standard sample stud employing double-sided adhesive tape and the take-off angle was fixed at 90º relative to the sample plane.

Transmission electron microscope (TEM) images were taken on a CM200 Tecnai TF20. Field emission gun TEM (FEGTEM) operated at an accelerating voltage of 200 kV and equipped with a Gatan Orius SC600A CCD camera to capture images of the AuNPs. Samples were prepared by placing a drop of the functionalized-AuNPs solution on a copper grid. The films on the TEM grids were allowed to dry for 5 minutes under a heat bulb.

2.5 Colorimetric assay of DPP-IV activity

To perform the colorimetric DPP-IV assays, 100 µL aliquots of the modified AuNPs solutions were mixed in the wells of a transparent 96-well microtiter plate with the enzyme dissolved in 50mM Tris buffer pH 8.3. The contents were gently mixed using a plate shaker at 200 rpm for 60 seconds, and the 96 well plates were incubated at 37 °C for 15 minutes. The UV-vis absorption spectrum of each solution was recorded in absorbance scan mode from 400-900nm. To study the reaction dynamics, the P-AuNPs were exposed to a series of activities of DPP-IV ranging from 2 to 40 U/L and the aggregation of modified AuNPs was monitored by recording the changes in the absorption spectra.

3. Results and Discussion

The first of the two peptide substrates, GPDC was designed to mimic the structure of the chromogenic substrate Gly-Pro-*p*-nitroanilide, which is one of the most frequently used for DPP-IV detection. The rationale for the choice of this sequence include the presence of the following: (i) the enzyme cleavage site in the peptide (NH_2-GP) for DPP-IV, as the enzyme functions selectively on a dipeptide sequence that contain a protonated amino group (Lambier et al., 2003), (ii) cysteine (C) as anchor to facilitate immobilization of the peptide to the AuNP surface, (iii) aspartic acid (D) in order to impart a negative charge on the peptide thus enhance the stability of the colloidal solution. In the second peptide, VP-EN-DC ethylene diamine was incorporated into the structure in order to act as a spacer, so that possible steric hindrance caused by the curvature of the AuNPs is reduced. A schematic diagram of the enzymatic reaction followed by AuNP aggregation is shown in Fig.1.

The UV-Vis spectra of the as-prepared AuNPs stabilized with citrate ions, and functionalized with either peptide substrates is shown in Fig. 2a. Confirmation of the peptide substrate binding was obtained from X-ray photoelectron spectroscopy (XPS) measurements. The S 2p peak at approximately 161.8 eV is consistent with the formation of a thiolate linkage, in comparison with the S in the free thiol which appears at 164.8 eV as shown in Fig. 2b.

DLS measurements showed that the peptide modified AuNPs have an average hydrodynamic diameter of 36.5 nm when functionalized with GPDC peptide and 45.1 nm for VP-EN-DC in comparison to 21.9 nm for the citrate stabilized nanoparticles. This difference in diameter is indicative of the surface functionalization of the AuNPs with each peptide. The extent of the AuNPs surface modification was assessed by zeta potential measurements, which changed upon the addition of each of the substrate. The zeta potential shifted from -19±1.5 mV for citrate stabilized AuNPs to -28±4mV for VP-EN-DC capped and -23.5 ± 2.5 mV for GPDC-capped AuNPs, respectively. Solutions of the functionalized AuNPs were stable for several months when stored at 4°C (Supplementary information Fig. S1).

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The addition of DPP-IV/CD 26 to each substrate functionalized AuNPs solution resulted in a colour change from red to pale blue. The absorbance of the surface plasmon resonance band (SPR) at 525nm decreased with accompanying band broadening between 600-800 nm as shown in Figures 3a and 3b for both VP-EN-DC and GPDC substrates, respectively indicative of the formation of aggregates. The color intensity increased with higher DPP-IV activity as more of the peptide substrates were cleaved by DPP-IV causing further aggregation. Furthermore, increasing DPP-IV activity was associated with the gradual shift of absorbance band to a higher wavelength with resultant colour change and band broadening for both peptide substrates. Results from transmission electron microscopy (TEM) measurements show that the dispersed substrate functionalized AuNPs aggregate in the presence of DPP-IV with an increase in the average hydrodynamic diameter (Figure 4a-d). Dynamic light scattering (DLS) data are consistent with the TEM results, which showed that with the addition of DPP-IV, their sizes after aggregation changed from 36.5 and 45.1nm for both the substrates GPDC and VP-EN-DC functionalized Au NPs to 342 nm and 413 nm, respectively. The broad band obtained for the VP-EN-DC functionalized Au NPs is indicative of the formation of large Au NPS aggregates.

In order to establish the optimum time for enzymatic hydrolysis of the functionalized AuNPs, changes in UV-visible spectra were measured at different time points. The time-dependent absorbance changes at Abs_{642}/Abs_{522} for GPDC functionalized AuNPs with different enzyme activities are shown in Supporting Information Fig. S2a. DLS measurements were used to follow the changes in the size of AuNPs over time following enzyme addition. The DLS data show that the hydrodynamic size of the AuNPs gradually increased after the addition of the enzyme and plateaus after about 10 minutes (Supporting Information Fig. S2b).

To further assess the selectivity of the method, experiments were performed with potential interfering proteins and enzymes such as trypsin, thrombin, lysozyme human serum albumin (HSA) and denatured DPP-IV, to examine whether this treatment with any of these compounds could trigger AuNPs aggregation. As shown in Fig. 5a, the proteases thrombin and trypsin and the non-protease HSA and lysozyme did produce signals that were commensurate with the background, revealing that our assay is selective and enzyme activity-dependent. The same experiments were performed with the VP-EN-DC functionalized AuNP, and comparable results were observed (Figure 5`b). In order to test that the same response is not obtained without modification of the AuNPs, different concentrations of DPP-IV (0, 2.5, 5, 10, 15, 20, and 25 U/L) were added to the unmodified citrate Au-NP suspensions and no color change was observed thus confirming that DPP-IV alone does not induce aggregation of the AuNP (Supporting Information Fig. S3).

For quantitative DPP-IV/ CD26 activity measurement, the activities of DPP-IV (2.5, 5, 10, 15, 20, 25, 30, 35 and 40) in Tris buffer of were used to calibrate the assay. Linear regression analysis showed very good linearity for both AuNPs assays ($r^2 = 0.9924$ for GPDC AuNPs, $r^2 = 0.9761$ for VP-EN-DC AuNPs) as shown in Figs 5c,d. The GPDC functionalized AuNPs displayed a narrow linear range 2–12 U/L with the signal levelling out at higher activities. The detection limit (LOD) of 1.2 U/L DPP-IV, based on $3\sigma b$/slope, where σb was the standard deviation of the blank samples. A wider calibration range was obtained with VP-EN-DC modified AuNPs, and the calibration plots displayed a good linear relationship between the red shifts of the LSPR peak ($\Delta\lambda$ max) and DPP-IV activities in the range of 0 - 40 U/L, with a LOD of 1.5 U/L DPP-IV.

Normal human DPP-IV activity in serum range between 17 - 52.6 U /L [31]. The developed colorimetric VP-EN-DC AuNPs assay is suitable for the qualitative and quantitative determination of DPP-IV activity in human serum samples. For this purpose, we

spiked human serum samples with varying DPP-IV activities (5, 10, 15, 20 and 25 U/L) to investigate the analytical properties of the colorimetric assay (see Figure S4 in the Supporting Information). Table 2 shows that the recoveries of the practical samples are in the range 83.6% to 114.9%. The very good recoveries and precision values are an indication of the reliability of the proposed method for detection of DPP-IV in biological samples.

The stability of the colloidal system is mainly governed by steric hindrance and electrostatic repulsion forces between the charged peptide capping ligands. The DPP-IV protease recognizes the sequence of Xaa-Pro-, and cleaves at the C-terminus of the underlined proline residue, shortens the length of the peptide arm and thus the distance between particles decreases which in turn affects the plasmon resonance of the functionalized nanoparticles due to the removal of steric barriers between the particles, thus resulting in irreversible AuNPs aggregation. Overall, both substrates proved to be useful candidates for the detection and measurement of DPP-IV activity with high sensitivity and selectivity. However, the additional advantage in using VP-EN-DC for quantitative determination is that it offers a wider linear range that covers the levels of DPP IV activity in serum. It is probable that the incorporation of the spacer arm in its design provides extra space for the hydrolytic enzyme cleavage resulting in the formation of larger AuNPs aggregates as can be seen from the results of the DLS measurement of the hydrodynamic radius.

4. Conclusions

We have designed and developed a novel, simple real-time and one-step colorimetric detection method for the measurement of DPP-IV enzymatic activity based on the aggregation of functionalized AuNPs in the presence of the enzyme. The relevance of this nanoparticle-based assay resides in the fact that visual detection or simple instrumentation can be used for this analysis. In principle, aggregation of GPDC or VP-EN-DC functionalized

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AuNPs can be used to measure the activity of DPP-IV after hydrolysis by the enzyme. The aggregation of AuNPs induced a gradual colour change that can be observed by the naked eye. The detection limits for GPDC and VP-EN-DC were 1.2 and 1.5 U/L, respectively making them suitable for the detection of DPP-IV activity, but the VP-EN-DC based assay is preferred because of the wider linear calibration range. More importantly, the developed VP-EN-DC method has been successfully applied to the detection of DPP-IV activity in spiked serum samples. The developed method offers a new approach for developing simple, cheap, reliable and sensitive sensors for DPP-IV detection.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval:

This article does not contain any studies with human participants or animals performed by any of the authors.

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Figures and Captions:

Fig.1 Schematic representation of the working principle for measurement of DPP-IV/ CD26 activity. DPP-IV hydrolyzes the amide bond between Xaa-Pro and aspartic acid or ethylene diamine. **(In color)**

Fig.2 (a) UV-Vis absorption spectra of citrate stabilized AuNPs (blue), P- modified AuNPs (red), (b) Detection of covalent thiolate - gold bond (S-Au) by high resolution XPS spectra. A Weak signal of a free thiol (R-SH) appeared at (164.8 eV) while the sulphur atom that bound to Au revealed prominent signal at a binding energy of (161.8 eV). Error bar represents the standard deviation (n = 3), c) Average Hydrodynamic radius of AuNPs before and after modification with GPDC and VP-EN-DC peptides separately d) zeta potential measurements of bare AuNPs (black), GPDC-capped AuNPs (blue) and VP-EN-DC capped AuNPs verified
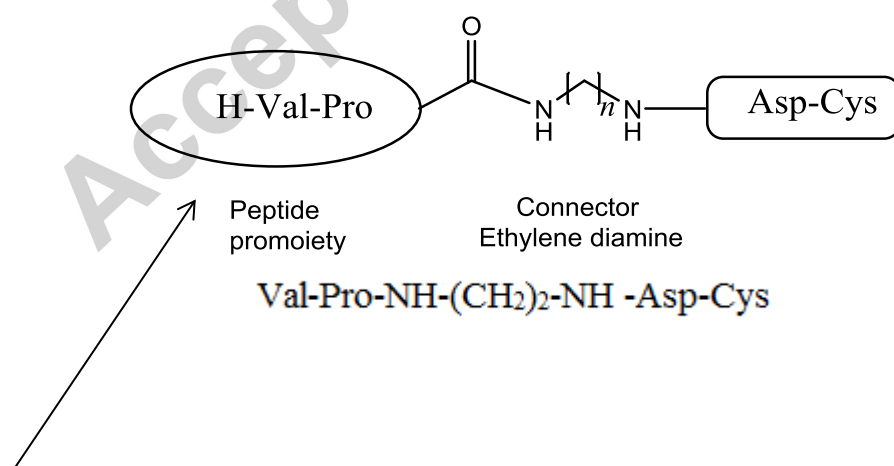
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the surface functionalization of the particles. Error bar represents the standard deviation (n = 3). **(In color)**

Fig.3 (a) UV−vis Absorption spectra of VP-EN-DC capped AuNPs & b) GPDC capped AuNPs after incubation with different activities of DPP-IV. The DPP-IV/CD-26 activity ranged from 0 to 40 U/L. (Inset: Solution color changes after the addition of DPP-IV (right to left) 2.5, 5, 7.5, 10, 15, 20, 25, 30 and 40 U/L to the VP-EN-DC AuNPs assay. **(In color)**

Figure 4 TEM images of (a,b) GPDC-AuNPs and (c, d) VP-EN-DC-AuNPs without DPP-IV (panels (a) and (c)) and (b, d)with DPP-IV (20 U/L) . All samples were suspended in Tris (pH 8.3, 50 mM) at 37 °C. Scale bar = 100 nm. **(In color)**

Figure 5 Response of the modified AuNP solution incubated with 20 U/L of DPP-IV compared to effect produced by other enzymes, proteins such as lysozyme (1μg/mL), thrombin (20 U/mL), trypsin (20 μg/L) and Denatured DPP-IV (heating at 80 °C 15 minutes) under the same conditions. Error bar represents the standard deviation (n = 3) when mixed with a) GPDC functionalized AuNPs, b) VP-EN-DC functionalized AuNPs
c) Calibration curve of Abs_{642}/Abs_{522} versus the different activities of DPP-IV incubated with (GPDC functionalized AuNPs). (Inset: Expanded linear region for DPPIV activity in the range of 2-12 U/L). Error bar represents the standard deviation (n = 3). d) Calibration curve of Abs_{700}/Abs_{525} versus the different activities DPP-IV incubated with (VP-EN-DC functionalized AuNPs).
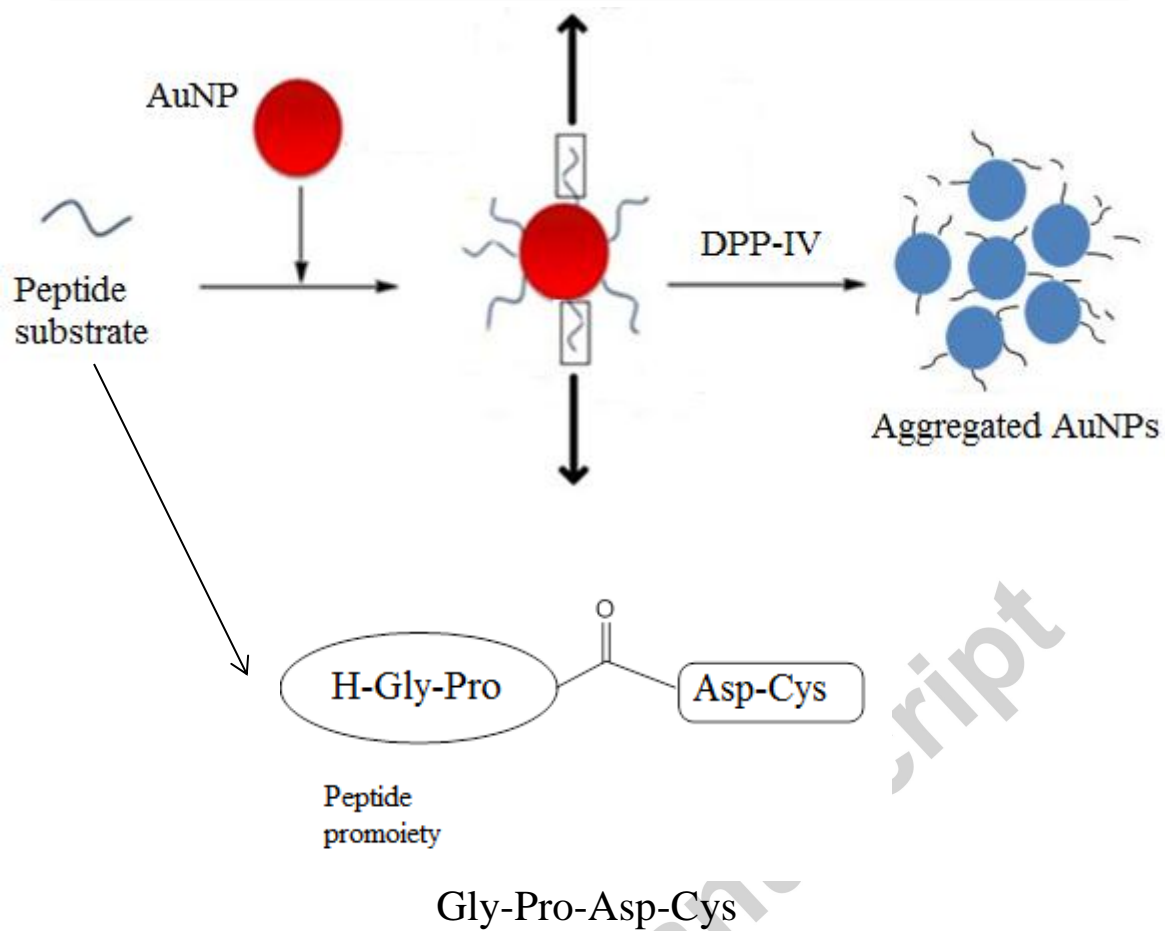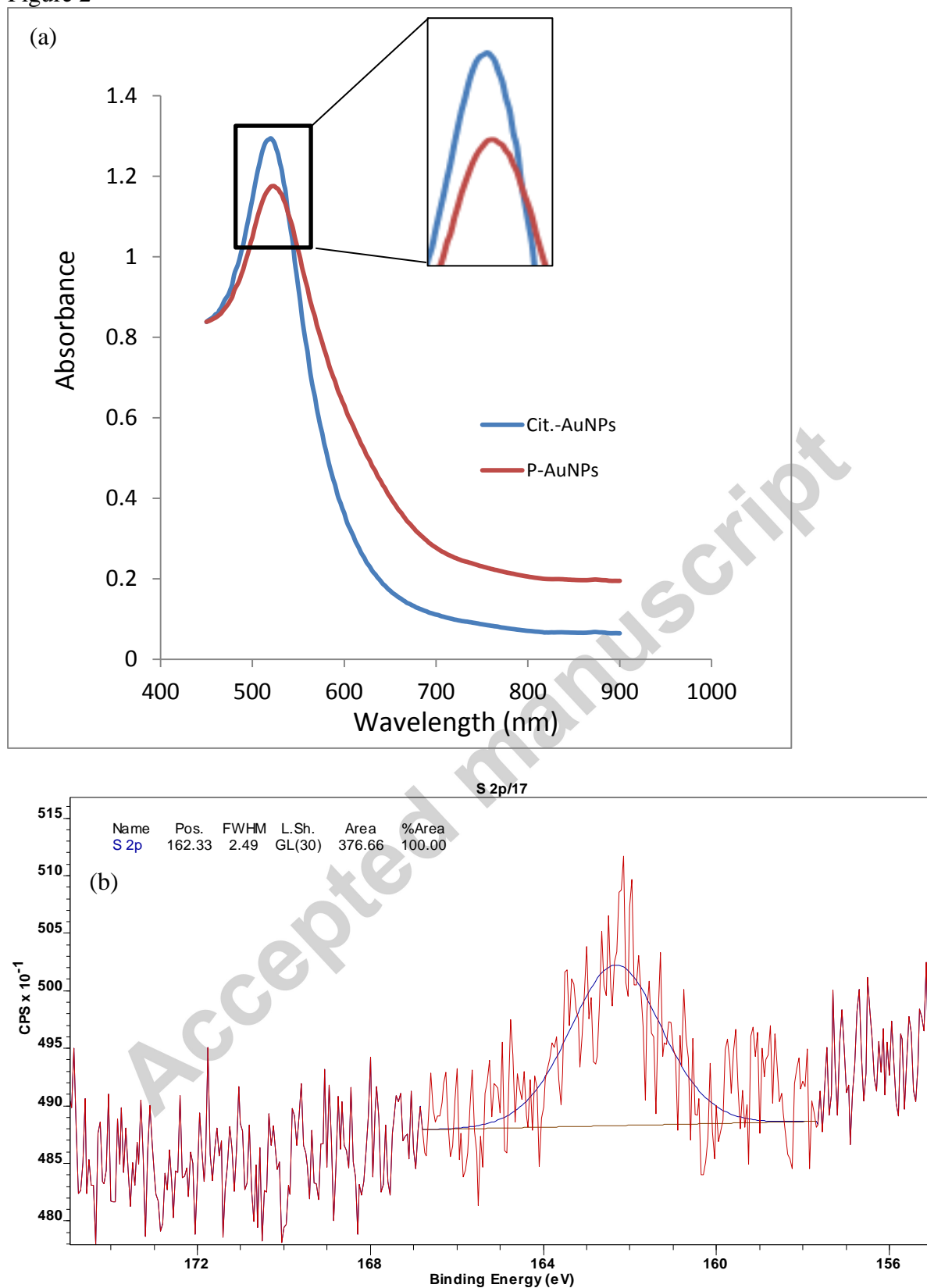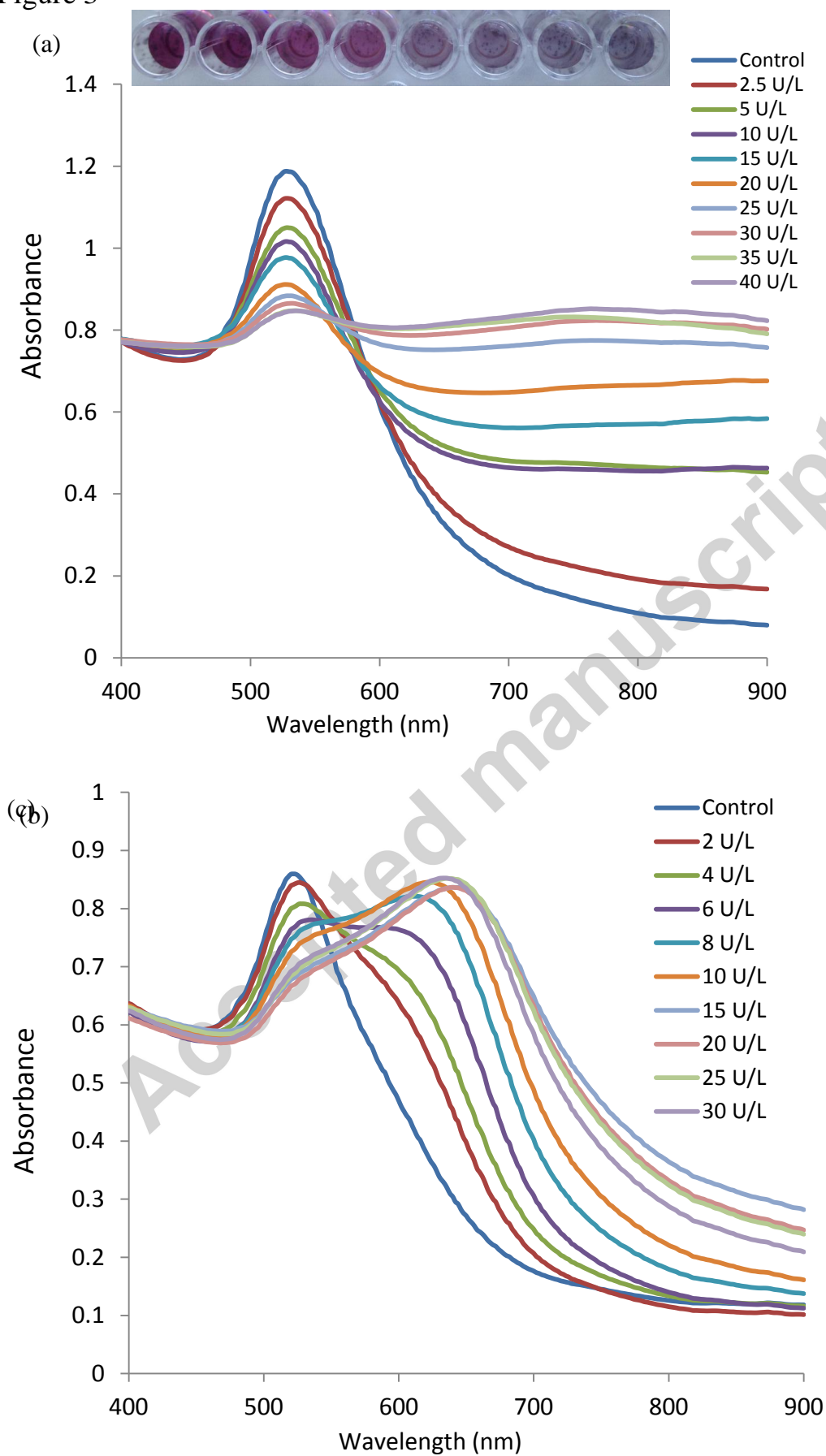
Figure 1

Val-Pro-NH-(CH₂)₂-NH -Asp-Cys

Gly-Pro-Asp-Cys

Figure 2

(a)

(b)

S 2p/17

Name	Pos.	FWHM	L.Sh.	Area	%Area
S 2p	162.33	2.49	GL(30)	376.66	100.00

CasaXPS (This string can be edited in CasaXPS.DEF/PrintFootNote.txt)
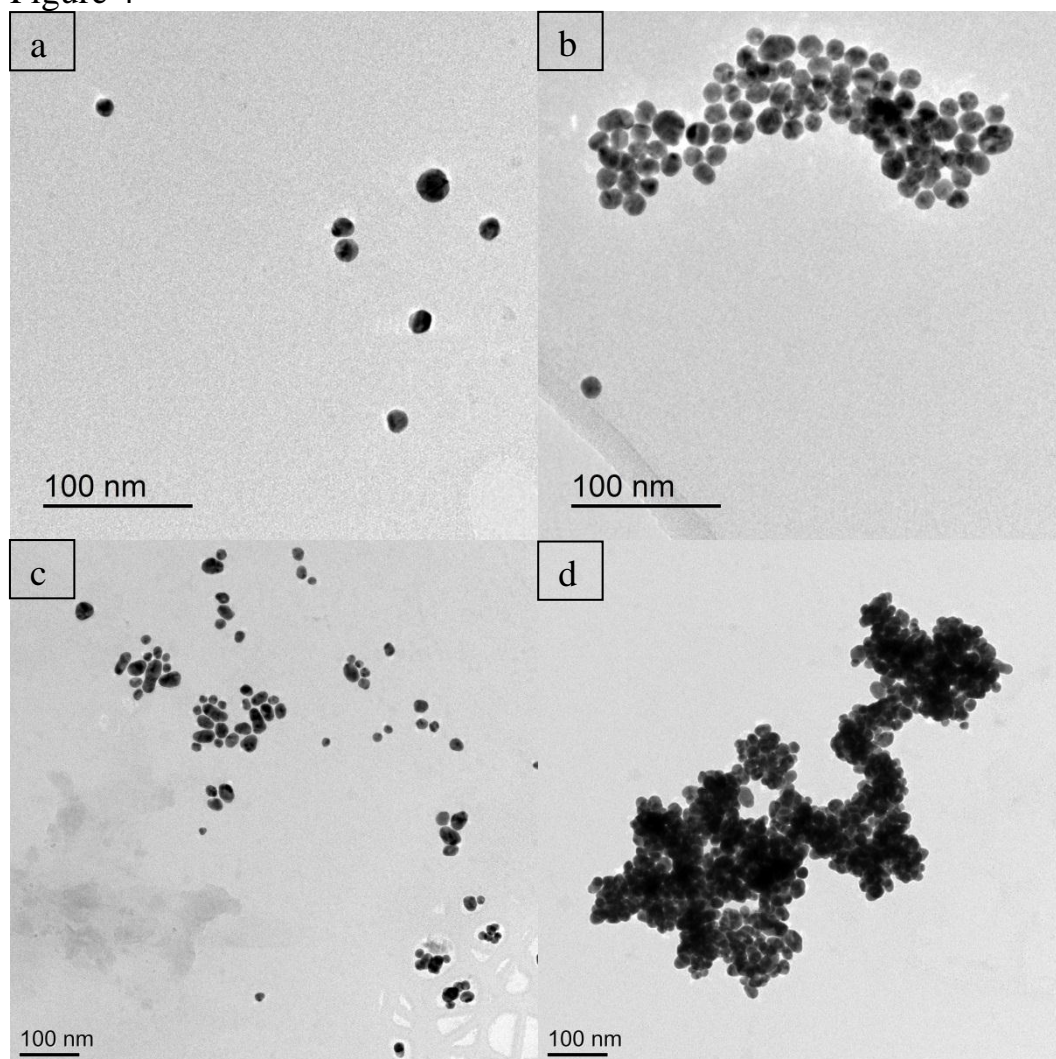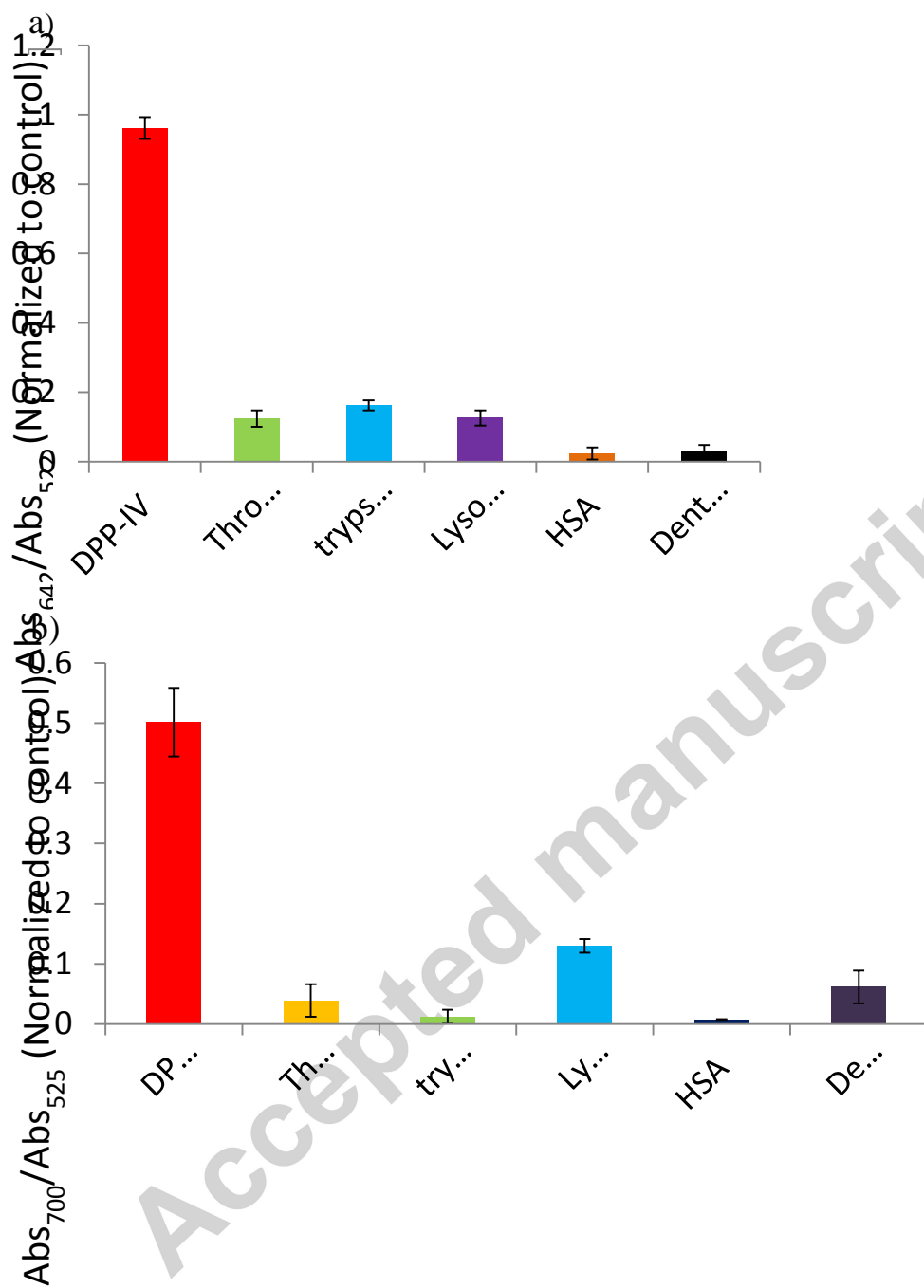
17

Figure 3
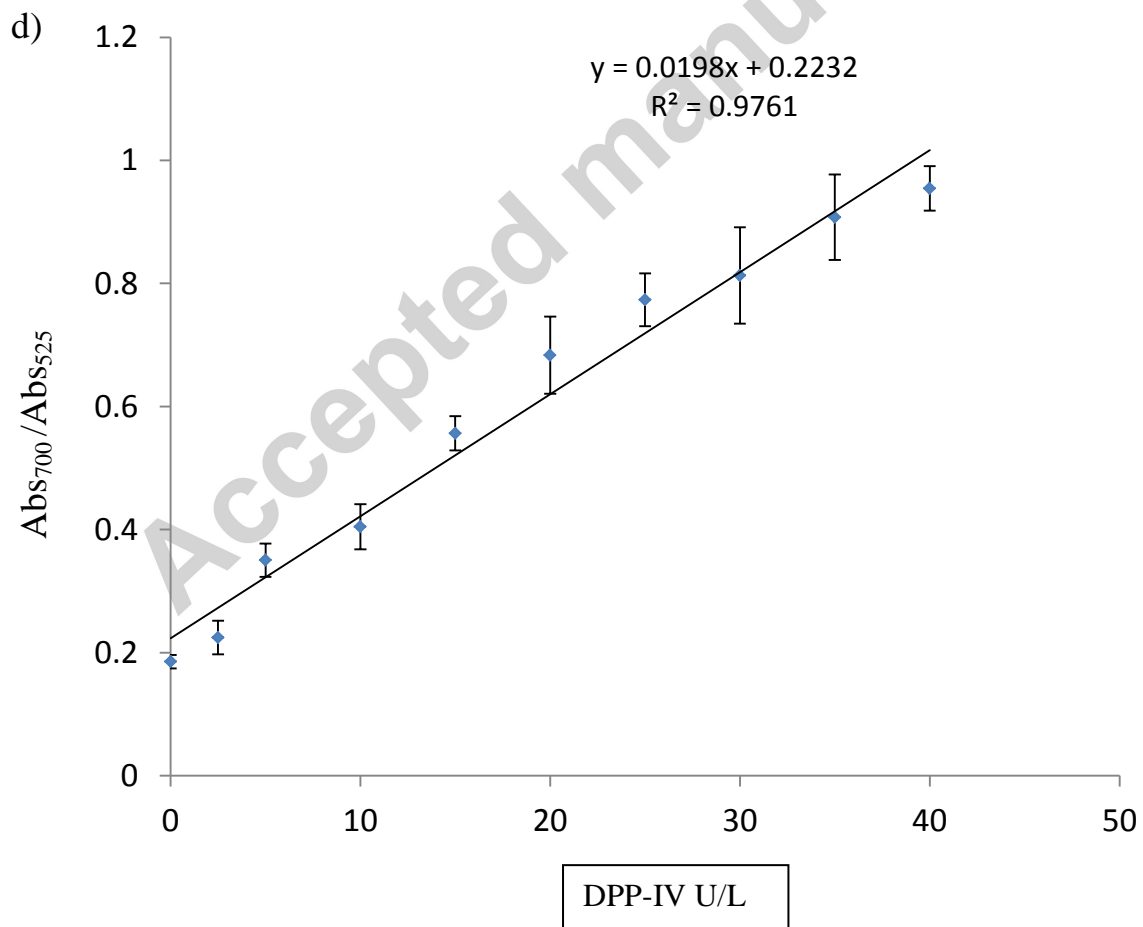
(a)
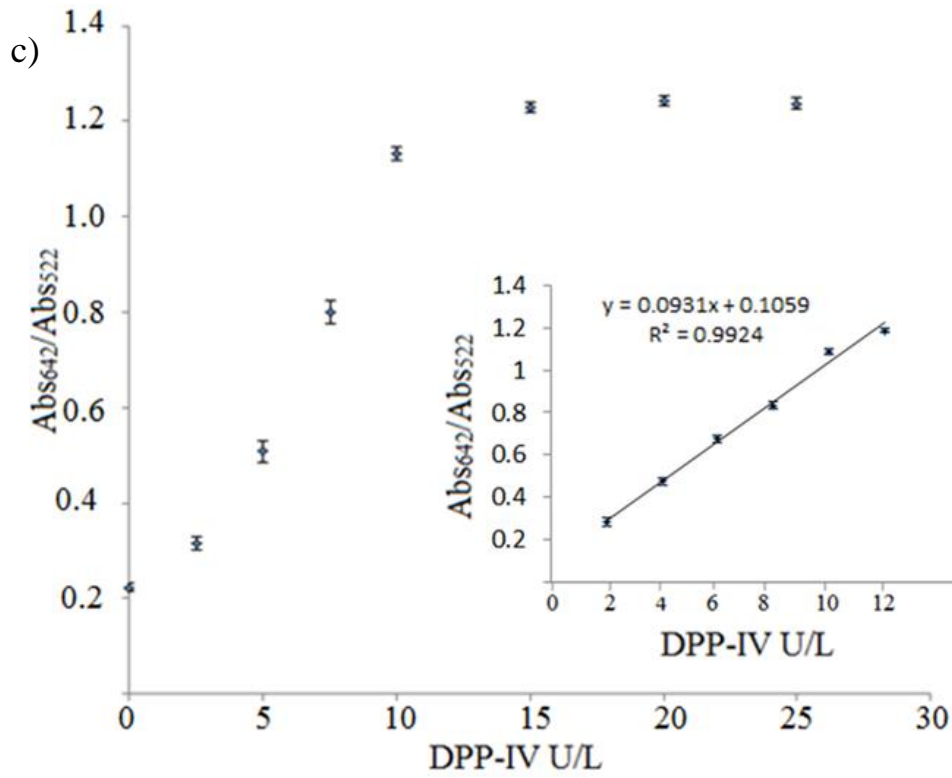

(c) (b)

Figure 4

Figure 5

a)

c)

d)

Table 1 Comparison of the assay sensitivity as defined by the limit of detection (LOD) and

limit of quantification (LOQ) of colorimetric, fluorometric and luminescent DPP-IV assays.

 (GP: glycyl proline; Me: methoxy; NA: naphthylamide; AMC: amino methyl coumarin; Fc:

Ferrocene)

Substrate	LOD (U/L)	LOQ (U/L)	Type of Assay
Gly-Pro-p-nitroanilide	1.56	2.92	Colorimetric [32]
Gly-Pro-4-Me-β-NA)	0.10	0.26	Fluorometric [32]
Gly-Pro-AMC	0.18	0.27	Fluorometric [32]
DPPIV-Glo™ Protease*	0.035	0.040	Luminescent [12]
Fc-Tyr-Pro-Phe-Phe-NH$_2$	$3.9 * 10^{-5}$	$0.5 * 10^{-3}$	Electrochemical [33]
Arg-Pro-Arg Au NP	0.07	0.1	Colorimetric [29]

Table 2 Analytical results for DPP-IV in spiked serum samples

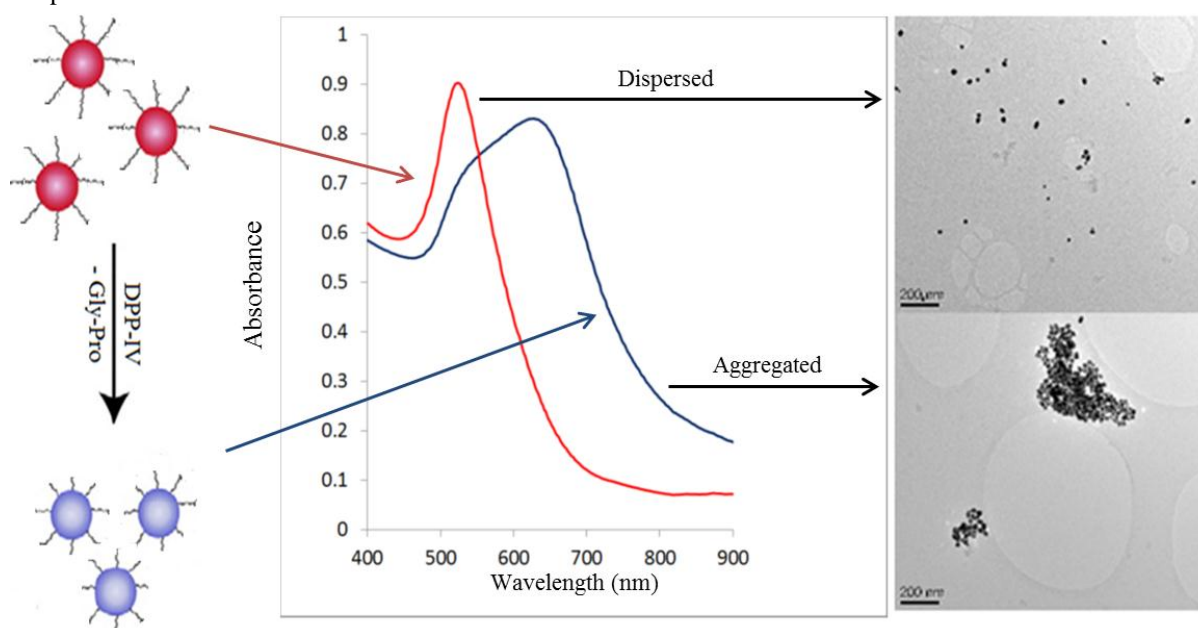| Add (U/L)	Found (U/L)*	Recovery (%)	RSD (%)
5	5.04± 0.19	100.8	3.23
10	8.36± 0.23	83.6	2.37
15	15.89± 0.33	105.9	2.1
20	22.99± 0.91	114.9	4
25	26.77± 0.34	107	1.31

* Average of three determinations ± standard deviation.

Highlights

- **Two colorimetric assays based on enzyme peptide substrates functionalized gold nanoparticles for the measurement of dipeptidyl peptidase IV/CD26 (DPP-IV) activity have been developed.**
- **Aggregation of the gold nanoparticles in the presence of DPP IV with resultant color change followed by UV/Vis spectrometry was used to measure the activity of the enzyme.**
- **The analytical performance of the two designed enzyme peptide substrates, Gly-Pro-Asp-Cys (GPDC) and Val-Pro-ethylene diamine-Asp-Cys (VP-ED-DC), respectively were investigated.**

- **A spacer moiety (ethylene diamine) was introduced in one of the sequences to enhance the distance of hydrolysable moiety from gold nanoparticle surface and to investigate its effect on aggregation of the gold nanoparticles.**

- **Quantitative determination of activity can be achieved by plotting absorbance ratio at two fixed wavelengths with DPP-IV activity.**

- **Excellent recoveries were obtained in spiked serum samples.**

- **The developed biosensors were simple, fast and reliable and thus suitable for point of care and real-time DPP-IV activity measurements.**

Graphical abstract