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Recognition-induced covalent capturing and labeling as a general strategy for protein detection

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ABSTRACT

In this work we have developed a peptide-based method for protein detection, termed as "Recognition-induced Covalent Capturing and Labeling" (RCCL). In this method, upon binding of the peptide with the target protein, electrochemically controlled and metal catalyzed oxidative cross-linking can be induced between the peptide and the target protein. Specifically, the peptide and the target protein are cross-linked by the formation of dityrosine between tyrosine moieties of the two molecules. Meanwhile, the dityrosine formed in this manner also has fluorescent signal readout. Therefore, the proposed method needs only one probe for the target protein, and the initial non-covalent molecular recognition can be finalized by cross-linking between the peptide and the target, while the dityrosine formed between peptide and protein can also act as a signal reporter, thereby greatly simplifying the design. Moreover, the robust covalent capturing via RCCL also enables detection in complex biological and clinical samples. These results point to the prospect of using RCCL as a promising method in protein detection in the future.

1. Introduction

Protein bioanalysis is an indispensible tool for biomedical research, and its clinical application is also essential for disease screening and diagnosis (He et al., 2015; Wu and Qu, 2015). Over the last few decades, the technology of protein biosensing has evolved dramatically (Howes et al., 2014; Muenzer et al., 2013; Ren et al., 2014). Many aspects of protein biosensing have benefited from this progress (Peng et al., 2015), while the essential mechanism follows the classic design: two targeting ligands (usually antibodies) bind with the target protein, thereby coupling bio-recognition with subsequent signal generation and signal amplification. For the classic design we have the following considerations: First, antibodies need various other biosensing elements of signal amplification to achieve satisfactory analytical properties (Zhu et al., 2015), and high performance is often accompanied by relatively complicated design. Second, recent development of biosensing progresses introduce and promote the synthetic targeting agents (Feng et al., 2014; Toh et al., 2015; Wu et al., 2015), on the basis of their readiness of chemical modification; but their application using the classic design may be hampered with several factors: (1) the classical design requires at least two different probes for the same target protein, but the proper synthetic candidates are not always available; (2) the classical design employs the non-covalent but strong binding between antibody and protein to work properly. Synthetic probes are somewhat inferior in terms of the strength of binding. This may greatly restrict the application in bio-sample analysis, which requires violent rinsing to reduce background interference. Such rinsing may dissolve the biosensing complex formed between the synthetic probe and the target protein. In light of these technological restrictions, we propose a design more suitable for the recently introduced synthetic protein-targeting probes. In this design, only one probe is required for each target protein, and probe/protein binding can induce cross-linking between the peptide probe and the target protein, so the performance of synthetic probes can be augmented by covalent bonds. Moreover, the product of cross-linking can have direct fluorescent signal readout, so the complexity of the design is greatly reduced.

This method, termed "Recognition-induced Covalent Capturing and Labeling" (RCCL), is first made possible by the fact that a lot of protein-targeting peptide probes have been screened out or

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designed during the search of protein-targeted therapeutic and biochemical reagents (Gray and Brown, 2014; Hosseinkhani et al., 2013; Linh and Tap, 2015; Milroy et al., 2014; Qin, 2015). Second, function groups of many proteins and peptides have chemo-selective reactivity under certain conditions (Chen et al., 2011; Schoneich, 2000). These interesting features of protein, peptide and their interaction have been noticed and taken advantage of in this work. Here tyrosine is selected to exemplify the proposed strategy. Tyrosine can be catalytically oxidized to form 3,4-Dihydroxyphenylalanine (DOPA) or dityrosine product, depending on the absence/presence of a second tyrosine in the vicinity (Dalsgaard et al., 2011). This enables the peptide probe to covalently capture target after the initial peptide/protein binding, via a dityrosine ligation driven by proximity. At the same while, surplus probes can mask themselves by the formation of DOPA. This points to another favorable fact that the original tyrosine, the dityrosine indicating the presence of target protein, and DOPA indicating its absence, all can give out fluorescent peak responses of distinct quantum yields at distinct wave lengths (Andreev et al., 2002; Kehr et al., 1972). To guarantee a quantitative conversion of protein abundance to signal readout, the RCCL mechanism is further secured by electrochemical control. In biosensing, electrochemistry is mostly employed as a form of signal readout. But electrochemistry is also apt for controlling the reductive/oxidative status, therefore the state of activity of bio/chemo-catalysts. So Cu (I)/(II) ion as a catalyst is incorporated into the sequence of the peptide probe. In this manner, through electrochemically controlling the availability of the catalyst, the ligation step can be controlled spatiotemporally to take place only at the correct stage of protein sensing. The above aspects constitute the proposed RCCL method, which has the following merits as a novel strategy of protein detection: (1) only one probe is required for each target protein; (2) the dityrosine formed between peptide and protein can also act as a signal reporter, so the design is greatly simplified; (3) non-covalent probe/target binding is finalized by covalent capturing immediately. Violent rinsing then becomes feasible, enabling biosample analysis. Using this method, several different disease-associated biomarker proteins have been quantitatively determined and their detection in complicated biological and clinical samples has been preliminarily realized.

2. Material and methods

2.1. Chemicals and biological samples

Peptide probe NH_2-GHK-G_6-TSFAEYWNLLSP-K(11-mercaptoundecanoic acid, MUA)-COOH, NH_2-GHK-G_6-DESDPEELMYWWEFLSED-PSS-K(MUA)-COOH, NH_2-GHK-G_6-RGTFEGKF-K(MUA)-COOH for murine double minute 2 (MDM2), G Protein alpha (G-pro) and amyloid beta 1–42 (A beta), respectively, were manufactured by Shanghai Science Peptide as lyophilized powder, purity $>95\%$. Powder of the peptide probes was dissolved with 10 mM phosphate buffer solution (PBS) (pH 7.4) to the desired concentrations. MDM2 was purchased from ProSpec-Tany TechnoGene Ltd.; G-pro was from Novus Biologicals; A beta was from Sigma Aldrich. Analytical-grade was attained for all of the other reagents. The serum-spiked sample of MDM2 was prepared by dissolving the protein powder with 10 mM PBS (pH 7.4) and then diluting it to desired concentrations with the fetal bovine serum. The original solution (50 mM Tris–HCl, pH 8.0) of G-pro was first diluted with 10 mM PBS (pH 7.4) and subsequently diluted with fetal bovine serum to desired concentrations. A beta lyophilized powder was firstly dissolved in 1,1,1,3,3,3-hexafluoro-2-propa-nol (HFIP) at a concentration of 1 mg/mL to eliminate pre-existing aggregates. HFIP was subsequently evaporated under a gentle stream of high purity nitrogen, leaving behind a film that was later reconstituted with DMSO to 2.2 mM. This resuspension was further monomerized via sonication for 1 min, followed by being diluted to 440 μM with 10 mM PBS, pH 7.4, the above prepared solution of A beta was transparent without evident aggregation. This stock solution was kept at 4 °C for store and could be diluted to the desired concentrations with fetal bovine serum. Redistilled water for preparation of all the solutions was prepared from distilled water with a Milli-Q purification system, the resistance of 18 MΩ cm was achieved to guarantee the purity. Cancerous tissue samples of patients with non-small cell lung cancer were obtained from the Clinical Laboratory of the Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School, after elected consent by the local ethical committee. Immediately after resection, the tissue samples were sliced, lysed and fractioned using a Nuclear Extract Kit (Active Motif, CA), the nuclear fraction was retained and 100 × diluted with 10 mM PBS (pH 7.4) for MDM2 quantification.

2.2. Electrode treatment

Transparent Au slides (Aldrich, layer thickness 100 Å) were cut to fit the size of the cuvette used for fluorescence measurement. These slides were cleaned by sonicating for 20 min in ethanolamine (20 wt%) at around 50 °C, followed by immersion for 10 s in piranha solution (*Caution: piranha solution is dangerous and should be handled with care*). After dried under mild stream of nitrogen, the slides were immerged in the assembly solution (2.5 μM peptide probes and 5 mM Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) in 10mM PBS, pH 7.4) at 4 °C for 16 h, TCEP was adopted to prevent disulphide formation between peptide probes. The slides were then immerged in 9-mercaptononanol (MN) solution (1 mM MN in 10 mM PBS, pH 7.4) at room temperature for 3 h.

2.3. Protein detection

The prepared slides were immerged in serum spiked samples or fractioned tissue samples containing the target enzyme and kept at 37 °C for proper time for the target/probe recognition and binding to proceed. Then, using the slides as the working electrode, the whole mixture was brought under 10 min of cyclic voltammetric scans with proper scanning parameters to induce and regulate the Recognition-induced Covalent Capturing and Labeling (RCCL) process. Then, after violent rinsing with SDS, the slides were ready for measurement.

2.4. Experimental measurements

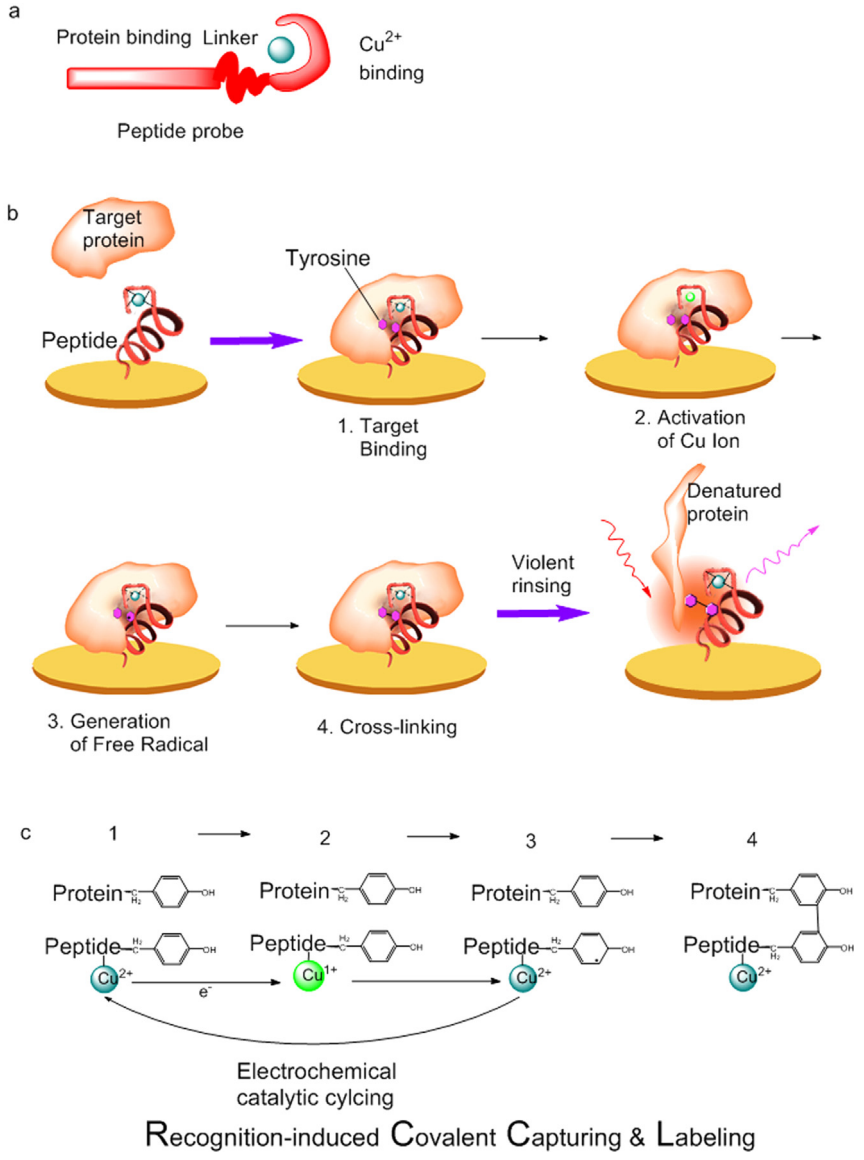
Isothermal titration calorimetry (ITC) measurements were conducted using a MicroCal ITC200 System (GE healthcare life sciences). The titration was conducted at 25 °C. The titration schedule consisted of 38 consecutive injections of 1 μL with at least a 120 s interval between injections. Heats of dilution, measured by titrating beyond saturation, were subtracted from each data set. All solutions were degassed prior to titration. The data were analyzed using Origin 7.0 software. Fluorescence emission spectra of surfaces were measured using a QM-4/2005 fluorescence spectrometer (Photon Technology International, Inc., Birmingham, NJ) equipped with a xenon lamp. This light source and the detector were in the same plane at right angle to each other. The slides were kept in a water-filled cuvette at 60° from the base of the cuvette for fluorescence measurements. The surface with gold film was kept away from the light source and towards the detector. Activating peak wavelength for dityrosine is 325 nm, while that for 3,4-Dihydroxyphenylalanine (DOPA) is around 360 nm. Electrochemical measurements were carried out on a CHI660D Potentiostat (CH Instruments) with a conventional three-

electrode system: the modified electrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the counter electrode. The experimental parameters for EIS: bias potential, 0.224 V vs. SCE; amplitude, 5 mV; frequency range, 0.1 Hz–10 kHz. Electrolyte solution: 5 mM $Fe(CN)_6^{3-/4-}$ with 1 M KCl. The data are obtained from at least three times of repetition of independent experiment, error bars are shown in the figures.

3. Results and discussion

3.1. Design principle

The above proposed RCCL method of protein detection has been illustrated in Scheme 1. As shown in Scheme 1a, the peptide probe possesses two functional motifs, Gly-His-Lys (GHK) trimeric Cu(II)-binding motif, and the protein-targeting motif, the sequence of which is variable according to the target protein. These two motifs are connected together by a flexible Gly_6 linker. In the detection procedure (Scheme 1b), the target proteins in the sample are first recognized by the surface-tethered probes. This brings tyrosine moieties on the target protein to the vicinity of the tyrosine moieties on the probe, as well as to the vicinity of the Cu(II) catalyst. The GHK-coordinated Cu(II) catalyst can be activated by the electrode (Scheme 1c) and reduced to Cu(I), which, due to its low stability, is rapidly oxidized back to Cu(II), giving rise to Tyr-derived radical (Smith et al., 2007). This radical, either on the probe or the target, is then received by the tyrosine moieties on its counterpart to form the dityrosine product. On the other hand, for the surplus probe that has not been occupied by a target, the radical that is formed is received by the solvent (H_2O) to form a DOPA product. The dityrosine formed between the peptide and the target protein, has a fluorescent peak emission around 400–420 nm, with large quantum yield (QY), while DOPA formed in the absence of the target protein only has an emission of low QY, around 500 nm. In this way, the amount of target protein can be converted quantitatively to dityrosine fluorescence peak response by the RCCL mechanism proposed.



Scheme 1. (a) Design of the peptide probe, (b) the procedure of protein detection with RCCL (step 1–4 as shown in the scheme). (c) The scheme of chemical reaction of RCCL. Not drawn to scale.

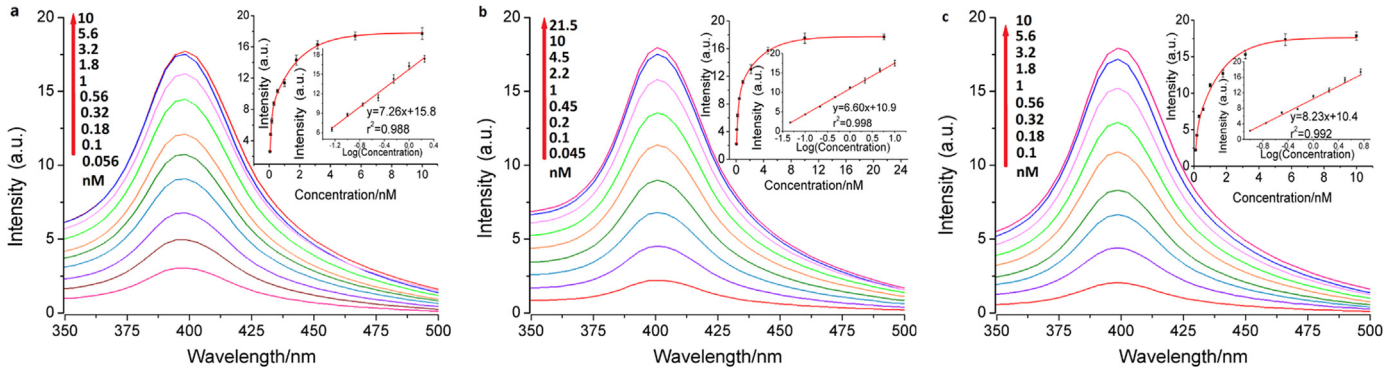


Fig. 1. Quantitative analysis of MDM2 (a), G pro (b) and A beta (c) using RCCL-based method, recorded as the fluorescence peak responses of dityrosine (Excitation wavelength: 325 nm). Insets shows the peak responses in each panel plotted as a function of protein concentration. The linear range and the corresponding formula obtained via regression analysis are shown beside the plot. The error bars represent standard deviation from average ($n=3$).

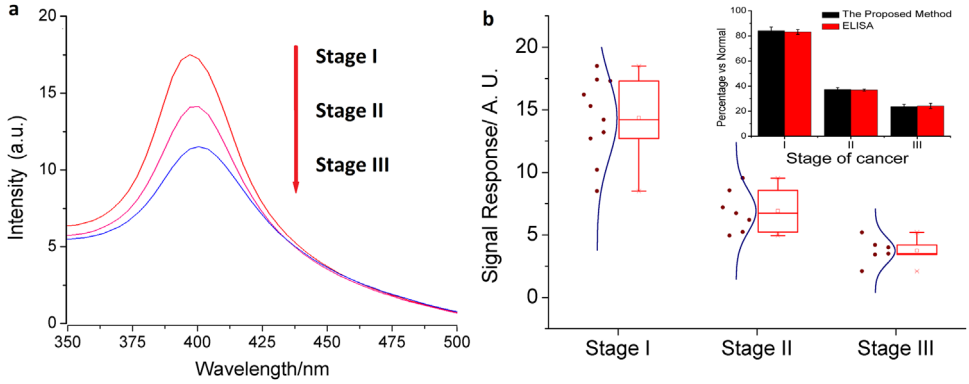


Fig. 2. (a) Representative fluorescence peak responses of dityrosine obtained from the clinical samples of non small cell lung cancer (excitation wavelength: 325 nm). (b) The box chart showing the distribution of signal responses in detecting these clinical samples. Each box includes the maximum, minimum, mean, 1st and 99th percentile marked on the graph, in addition to the 25th, median, and 75th percentiles. The raw data is included as a column scatter plot to the left of each box. A curve corresponding to normal distribution is also displayed on top of the scatter plot. Inset is the comparison of the results in detecting the above samples in (b) using the proposed method and ELISA.

3.2. *Validation of design principle*

To realize the above depicted method, the electrochemically controlled catalytic forming of dityrosine linkage should be investigated first. Cu ion catalyzed oxidation of tyrosine, as well as the electro-activity of peptide-complexed Cu ion have already been characterized in a protein modification-associated context, but the specific application in protein detection requires more study on this metal-catalyzed oxidative coupling. Recorded and shown in Fig. S1a is the cyclic voltammetric response of surface-complexed Cu ion in catalyzing the oxidative coupling between free tyrosine molecules and tyrosine-containing surface tethered peptide probes. Successive decrease of oxidative peak response is observed at the same time with corresponding gradual increase of reduction peak response. This can indicate that more and more electrochemically generated Cu(I) ion is oxidized by the bio-molecules to form the tyrosine-derived radical; while more and more biochemically generated Cu(II) ion awaits to be electrochemically reduced back to Cu(I) to continue the subsequent cycles of catalysis. So the observed response of Cu ion is coherent with the mechanism described above. Besides, this mechanism also involves the formation of a by-product, H_2O_2, accompanying the dityrosine ligation (Smith et al., 2007). An evolution of H_2O_2 can indeed be recorded in the above process of catalyzed oxidation (Fig. S1b). The product of the Cu ion catalyzed coupling reaction is then examined. By varying the amount of free tyrosine molecule employed, the response of dityrosine and DOPA varies accordingly (Fig. S1c and d), suggesting the feasibility of copper-catalyzed oxidative coupling, and the success of electrochemical control for this reaction to proceed in a quantitative manner.

This RCCL mechanism is then extended to protein detection. Murine double minute 2 (MDM2) is first employed as a model target to validate the design. The MDM2-targeting probe is derived from p53, and it contains one tyrosine moiety in direct contact with tyrosine 100 of MDM2, upon their binding. This molecular recognition is first verified by isothermal titration calorimetry (ITC) method (Fig. S2a), showing a strong binding consistent with previous reports (Pazgiera et al., 2009). This essential step may bring the probe and target together, to be conjugated via the copper-catalyzed coupling as validated above. The success of covalent coupling is confirmed in Fig. S2b (curve a). As a control, sequence-scrambled probe, HSA and denatured target protein have all been examined. None can produce response much more evident than the background (curve b–d), confirming the indispensible role the molecular recognition played in RCCL. To gain a deeper insight on this point, the time courses are recorded for ligations without molecular recognition as the pre-step, namely, the ligations involving the above control targets. For both the scrambled probe and HSA, if maintained under the electrochemical catalytic environment for a duration much longer than that for the detection with the normal probe, dityrosine response (Fig. S3a and b) as well as EIS response (Fig. S3c and d) increase gradually with time, although in the short interval for target detection, no significant response can be achieved. These prolonged time courses point to the fact that the ligation itself has no specificity, and the long electrochemical treatment may have denatured the proteins to expose tyrosine moieties that cannot be accessed by molecular recognition under natural condition. Together, the results of Figs. S2b and S3 signify the essential point of RCCL: molecular recognition can be exploited to accelerate the kinetics of covalent

coupling by binding-induced proximity of reactant; at the same time, molecular recognition and the resulted proximity also render the simple coupling target-specific.

The ligation enables thorough and even denaturing rinsing, so the interference in complicated biological and clinical samples can be greatly reduced. As shown in Fig. S4, for protein detection in a complicated biological environment, only the proposed method can produce a neat response indicating high performance, while the control experiment without ligation shows lowered peak response, since its molecular recognition is compromised to some extent by the rinsing (Fig. S4b). For the control without thorough rinsing, the lowered peak response, as well as the increased half-width, can indicate fouling and disruption of surface packing by interfering species adsorbed on the surface (Fig. S4c).

The procedure of protein detection by RCCL is followed by EIS (Fig. S2c). The bear surface appears as a straight line indicating no impedance to interface electron transfer (curve 1). Peptide modification results in a moderate increase of impedance, represented by a semi-circular part of the curve 2. Subsequent incubation with complicated bio-sample containing the target protein gives rise to evident increase of impedance (curve 3), suggesting protein binding and adsorption to the surface. The following step of RCCL leads to slight decrease of impedance (curve 4), while a subsequent thorough rinsing further reduce the impedance (curve 5), due to the removal of absorbed interfering species.

3.3. Optimization of experimental conditions

Major procedures of detection are studied and optimized. Before the final signal readout, the RCCL-based method involves only two pre-steps: protein binding and RCCL, the latter is the more sophisticated of the two and is optimized first. Since the above results in Fig. S3 suggest non-specific coupling as a result of much too prolonged or excessively processed ligation, the intensity and duration of RCCL have both been studied. The time course of protein detection in biological environment has been recorded by both the response of unreacted tyrosine (Fig. S5a) and the final response of dityrosine (Fig. S5b). For both, two-phase decrease and increase are evident. The much more retarded second phase may be associated with the non-specific ligation unassisted by target-specific recognition. So the inflexion point bordering the two phases can be selected as the optimal time for electro-ligation. Similarly, the range of potential scanning for RCCL has also been optimized (Fig. S5c and d). While the smaller range creates insufficient overpotential to drive the reaction, scans over large potential range tends to cause a second phase of non-specific coupling, so the optimal range is moderate. Then the conditions for incubation with target proteins are optimized. First, pH value influencing the conformation of probe and target, coordination of Cu(II), as well as kinetics of electro-ligation, is optimized, in the detection under biological environment (Fig. S6). Only the mild pH range can give rise to satisfactory response. Based on these optimized conditions, the incubation time for the 3 target proteins have been optimized (Fig. S7).

3.4. Analytical performance

The above optimized RCCL method is applied to the detection of serum-spiked murine double minute 2 (MDM2), G Protein alpha (G-pro) and amyloid beta 1–42 (A beta). For all three target proteins, the signal response can increase proportionally with the logarithm of the concentration of target (Fig. 1a–c). Linear ranges of measurement can be established for all three proteins (Fig. 1a–c, inset), and the limit of detection (LOD) defined at signal-to-noise ratio of 3:1 is 0.023 nM for MDM2, 0.019 nM for G-pro, and 0.052 nM for amyloid beta. For MDM2 and G-pro, the LOD of the proposed method is comparable with the ELISA methods, while the LOD for amyloid beta is comparable with the previous report (Li et al., 2012). The standard deviations of repetitions are all below 5%. The specificity of the method is then examined using various control species, as shown in Fig. S8, all controls result in only the responses of background level, so the specificity is acceptable. The efficiency of the proposed method in detecting clinical samples has been evaluated by quantifying MDM2 in cancerous tissue samples from non-small cell lung cancer (NSCLC) patients. As is shown in Fig. 2, advancing of NSCLC inversely correlates with MDM2 detected with the proposed RCCL method, consistent with previous reports using antibody-based methods (Wang et al., 2005) and a control using ELISA kit (inset of Fig. 2b). In detecting these samples, the performance of the proposed method is comparable to commercially available methods (Table S1). However, the proposed method also has some limitations. The cross-linking might be limited to some extent by the right configuration of molecular recognition, while the limit of detection is not comparable with the latest reported immuno-sandwich methods.

4. Conclusion

In summary, a new method for protein detection has been developed and named as “Recognition-induced Covalent Capturing and Labeling” (RCCL). The molecular recognition between target protein and peptide probe are exploited to bring tyrosine moieties on the two molecules to close proximity. So that these tyrosine moieties can be covalently bound together by electrochemically controlled Cu(II)/(I) ion-catalytic cycling of oxidative ligation. In this manner, not only the initial non-covalent molecular recognition can be finalized by the formation of covalent ligation between the probe and the target, but the product of ligation can simultaneously act as a signal reporter, therefore greatly simplifying the design of the biosensing method. The RCCL method needs only one probe for one target, so the “two-for-one” requirement in the classical method can be dispensed with. Moreover, the robust covalent capturing via RCCL also enables detection in complex biological and clinical samples. These results point to the prospect of using RCCL as a promising alternative of the traditional immunomethod in protein detection in the future.

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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.2016.02.012.

References

Andreev, O.A., Reshetnyak, Y.K., Goldfarb, R.H., 2002. Photochem. Photobiol. Sci. 1, 834–836.
Chen, H.-H., Chen, C.-Y., Chow, L.-P., Chen, C.-H., Lee, Y.-T., Smith, C.V., Yang, C.-Y., 2011. Biol. Chem. 392, 859–867.
Dalsgaard, T.K., Nielsen, J.H., Brown, B.E., Stadler, N., Davies, M.J., 2011. J. Agric. Food Chem. 59, 7939–7947.
Feng, C., Dai, S., Wang, L., 2014. Biosens. Bioelectron. 59, 64–74.
Gray, B.P., Brown, K.C., 2014. Chem. Rev. 114, 1020–1081.

He, X.-P., Zang, Y., James, T.D., Li, J., Chen, G.-R., 2015. Chem. Soc. Rev. 44, 4239–4248.
Hosseinkhani, H., Hong, P.-D., Yu, D.-S., 2013. Chem. Rev. 113, 4837–4861.
Howes, P.D., Chandrawati, R., Stevens, M.M., 2014. Science 346, 53.
Kehr, W., Carlsson, A., Lindqvis., M., 1972. N.-S. Arch. Pharmacol. 274, 273.
Li, H., Cao, Y., Wu, X., Ye, Z., Li, G., 2012. Talanta 93, 358–363.
Linh, T., Tap, H.-D., 2015. Peptides 69, 86–91.
Milroy, L.-G., Grossmann, T.N., Hennig, S., Brunsveld, L., Ottmann, C., 2014. Chem. Rev. 114, 4695–4748.
Muenzer, A.M., Michael, Z.R., Star, A., 2013. ACS Nano 7, 7448–7453.
Pazgiera, M., Liu, M., Zou, G., Yuan, W., Li, C., Li, C., Li, J., Monbo, J., Zella, D., Tarasov, S.G., Lu, W., 2009. Proc. Natl. Acad. Sci. USA 106, 4665–4670.
Peng, Y., Xiong, B., Peng, L., Li, H., He, Y., Yeung, E.S., 2015. Anal. Chem. 87, 200–215.
Qin, Z., 2015. Peptides 67, 64–73.
Ren, C., Zhang, J., Chen, M., Yang, Z., 2014. Chem. Soc. Rev. 43, 7257–7266.
Schoneich, C., 2000. J. Pharm. Biomed. Anal. 21, 1093–1097.
Smith, D.G., Cappai, R., Barnham, K.J., 2007. BBA Biomembr. 1768, 1976–1990.
Toh, S.Y., Citartan, M., Gopinath, S.C.B., Tang, T.-H., 2015. Biosens. Bioelectron. 64, 392–403.
Wang, Y.C., Lin, R.K., Tan, Y.H., Chen, J.T., Chen, C.Y., 2005. J. Clin. Oncol. 23, 154–164.
Wu, L., Qu, X., 2015. Chem. Soc. Rev. 44, 2963–2997.
Wu, X., Chen, J., Wu, M., Zhao, J.X., 2015. Theranostics 5, 322–344.
Zhu, C., Yang, G., Li, H., Du, D., Lin, Y., 2015. Anal. Chem. 87, 230–249.