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# A modular electrochemical peptide-based sensor for antibody detection†

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**We report a modular electrochemical peptide-based sensor targeting the anti-deamidated gliadin peptide (DGP) antibody. A recognition peptide, here DGP, is grafted onto a supporting peptide bearing a redox label. The fabricated peptide-based sensor supports the detection of the target antibody (anti-DGP antibody) in the nanomolar range.**

The need for point-of-care testing (POCT) as a cost-effective and easy-to-use platform for clinical diagnosis is pushing new routes in the research of new high-quality, miniaturized and portable devices which can be simple enough to be used at the primary care level and in remote settings with no laboratory infrastructure.[1] Recent years have seen an explosion in the number of well-characterized disease markers, especially antibodies that present in blood or at the surface of cells can be diagnostic of specific diseases such as HIV, C hepatitis, Alzheimer's disease,[2–4] or autoimmune diseases.[5] These antibodies often recognize a small epitope of antigenic proteins or polysaccharides. The epitopes are usually small aminoacidic sequence (peptides), which can be used as valuable recognition elements in the design of antibody sensing platforms. However, current methods to detect antibodies are either multistep or time-consuming procedures that require several reagents and laboratory skilled personnel. In this perspective, novel methods that allow detection of clinically relevant antibodies with a single measurement and in a reagentless fashion are urgently needed.

Recently, Plaxco, Lai and other groups have reported the development of electrochemical peptide-based (E-PB) sensors that use short antigenic peptides as recognition elements for the detection of diagnostically relevant antibodies.[2,6,7] In these platforms the recognition peptide is usually immobilized either by direct chemisorption of a thiol-end labelled peptide onto gold surfaces[8] or by attachment on alkylthiol self-assembled monolayers (SAMs) with terminal reactive groups *via* a "click-chemistry" approach;[9] the peptides are also labelled at one end with a redox reporter, most frequently ferrocene,[4,10–12] or methylene blue (MB).[2,6] Once fabricated, these E-PB sensors allow the monitoring of the target antibody in a reagentless fashion and thus appear suitable for the development of portable and easy-to-use sensors for antibody detection. However, E-PB sensors often display voltammetric baseline fluctuations and capacitance current drifts[6] presumably because the recognition peptide in the unbound state cannot be considered structure-free[13] and the secondary structure of the peptide chain cannot be controlled as in the case of, for example, short DNA oligomers.[14] Moreover, E-PB sensors are difficult to generalize because redox tag labelling on the recognition peptide might lead to changes in the specificity of the antigen–antibody binding and in the signalling of the sensor. Another possible drawback of these sensors is their short-term stability. In fact, while short helical peptides that are able to form compact layers[9,15,16] are relatively stable, recognition peptides are usually quite long and present disparate helix domains which are often unable to form highly ordered structures thus ultimately affecting stability.

In response to the above drawbacks, we report here a novel strategy for the development of a modular E-PB sensor. In this modular approach a short helical support peptide (SP) is first immobilized on the surface of a gold electrode and then labelled with an electrochemical tag (here Methylene Blue, MB) and with a recognition peptide (Fig. 1, left). As a model recognition peptide we have used here the alpha-2 deamidated gliadin peptide (DGP),[17,18] a 33-mer peptide containing the 56–88 residues of alpha gliadin from gluten. The binding of the target antibody (here the anti-DGP IgG monoclonal antibody) to the recognition peptide reduces the efficiency with which the support peptide can transfer electrons to the gold surface and thus leads to a signal decrease (signal off) (Fig. 1, right).

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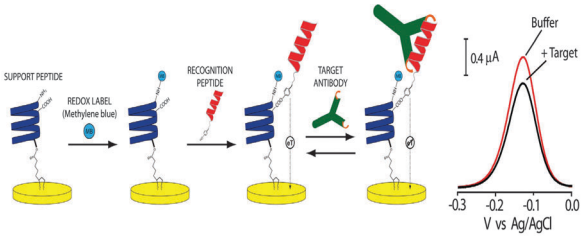


Fig. 1 Here we report a modular electrochemical peptide-based (E-PB) sensor employing a support peptide layer (in this work at a low density), labeled "on-the-fly" with a redox tag and further functionalized with a recognition peptide for the detection of a target antibody (left). The faradaic current arising from the flexible redox-labeled support peptide is significantly reduced in the presence of the target antibody (right) presumably because the formation of a large high-molecular weight complex reduces the efficiency with which the terminal redox tag collides with the electrode surface and transfers electrons.

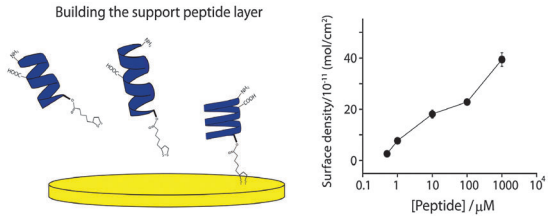


Fig. 2 Surface density of the support peptide is in direct relation with the concentration of the support peptide used during the immobilization step. A surface density ranging from (2.61 ± 0.18) to (39.4 ± 2.6) × $10^{-11}$ moles $cm^{-2}$ was found for the support peptide layer when used in a 0.5–1000 μM range. Here the surface density of the support peptide was achieved through linear sweep voltammetry and surface plasmon resonance measurements. Values reported are the average with standard deviation obtained with three different electrodes.

This modular approach should ensure high background currents in the absence of the target (even at low peptide surface densities) and, because of its modular nature, it could be amenable to generalization to other recognition elements.

We have first used a 9-mer support peptide that contains a lipoic acid moiety at one end to allow immobilization to a gold electrode surface through gold–thiol chemistry. The support peptide also contains a free amino group at the other end which can be used to functionalize it with a redox label (here MB). Finally, at the same extremity, the support peptide contains also a free carboxyl group able to covalently bind the hydroxyl group from the target. Of note, the sequence of such a short support peptide has been chosen so that it can fold in a regular α- or $3_{10}$-helical structure. This would allow the formation of an ordered layer on the electrode surface. Moreover, it was reported that with such helical structure the peptide dipole moments (DMs) could sum up to generate a macrodipole stabilized by head-to-tail interactions, parallel oriented with the helix axis. This secondary structure would thus provide an overall DM oriented from the electrode (acceptor) towards the redox label (donor)[19,20–24] that could significantly increase the electron transfer (ET) rate of the redox label ultimately providing a high electrochemical "blank" signal in the absence of the recognition peptide and target antibody.

We first immobilize the support peptide onto the surface of a gold electrode. To do this, the gold electrode was immersed in a support peptide solution (see Experimental section). To tune the peptide surface coverage on the electrode we adjusted the concentration of the support peptide during the immobilization procedure. The surface density of the support peptide was measured using both electrochemical (linear sweep voltammetry, Fig. SI1, ESI†) and surface plasmon resonance (SPR) methods[25,26] (Fig. SI2, ESI†). The results obtained for support peptide concentrations ranging from 10 to 100 μM using these two techniques were in good agreement and compared well with other previous reports that showed surface density for densely-packed helical peptide SAMs on gold between 10.9 and 18.8 × $10^{-11}$ moles $cm^{-2}$ [25] (Fig. 2).

The attachment of the electrochemical label (MB) to the support peptide was achieved through the reaction between MB-succinimidyl (MB-NHS) ester and the terminal free amino group of the support peptide at pH 8.5 (Fig. 3, left). Upon labelling, a clean and easily measurable SWV peak was observed with a peak potential consistent with that of MB (Fig. 3, center). To obtain information about the surface density of the electrochemically labelled support peptide and thus on the percentage degree of labelling we used a previously established relationship with ACV peak current.[27,28] The surface density of the unlabelled support peptide was compared with the surface density of the electroactive MB-labelled support peptide. This gave an estimation of the percentage labelling degree of our procedure. The highest labelling degree (around 15%) was obtained at low surface density (Fig. 3, right).

While decreasing the support peptide density provides an increased labelling degree, the stability of the support layer might be affected due to occurrence of defects in low-surface density peptidic films.[29,30] To overcome this possible problem we used different coadsorbants that were reported to increase the stability of peptidic layers on the gold electrode.[2,6] We observed increased insulating properties of the peptidic layer upon using a mixture of 6-mercaptohexanol (6-MH) and 6-hexandithiol (1,6-HDT) as coadsorbants (Fig. SI3, ESI†). These thiols are in fact reported to act as spacers and prevent the dioxygen adsorption thoroughly[28]

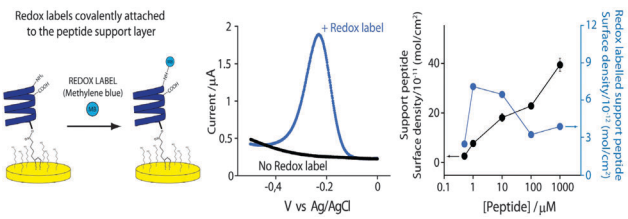


Fig. 3 The support peptide immobilized on the gold electrode surface can be labelled "on-the-fly" with a methylene blue redox tag *via* a simple click-chemistry reaction. As expected, after the labelling reaction, the sensor provides a well-defined current peak consistent with an immobilized methylene blue label (peak potential of −225 mV). The highest redox-labeled support peptide/support peptide ratio was achieved at relative low surface density (2.61 ± 0.18) × $10^{-11}$ moles $cm^{-2}$, while the lowest ratio was obtained for high surface density (39.4 ± 2.6) × $10^{-11}$ moles $cm^{-2}$ presumably due to steric hindrance generated by the compactness of the support layer (right).

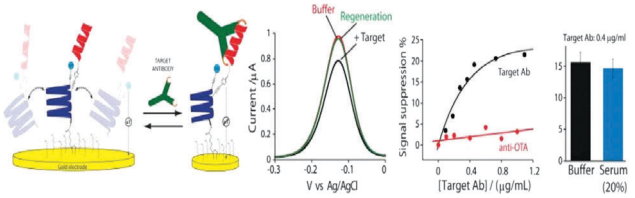


Fig. 4 Our modular E-PB sensor supports the detection of specific antibodies. Here we used as recognition peptide the DGP that is recognized by anti-gliadin antibodies that are diagnostic of the celiac disease. To attach the recognition peptide to the support peptide, the carboxyl groups of the support layer were first activated with 1-ethyl-3-(3-dimethylaminopropyl carbodiimide) and further reacted with 4-dimethylaminopyridine to bind the free phenolic groups of the recognition peptide under moderate acidic conditions. The electrochemical peptide sensor displays a stable methylene blue signal (buffer curve) that is suppressed upon binding of the target antibody to the recognition peptide (+target). Here a concentration of target antibody (anti-deamidated gliadin peptide monoclonal antibody) of 1 μg $mL^{-1}$ (6.7 nM) leads to a 22% signal suppression in 10 mM PBS, pH = 6. The signal suppression observed in the presence of the target antibody is specific and no significant signal change is observed in the presence of even saturated concentration of a random non-specific antibody (here anti-ochratoxin A antibody) used as a model interferent (see red curve). Moreover, a very similar affinity and signaling to those obtained in a pure buffer solution was observed testing our sensors in 1 : 5 diluted serum (right), further demonstrating the specificity and selectivity of our approach. Of note, after the electrode was washed with 100 mM HCl we observed an almost complete regeneration of the signal (center).

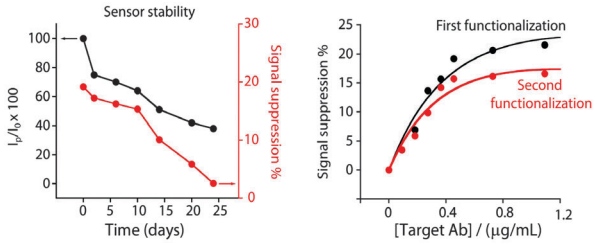


Fig. 5 The electrochemical peptide sensor is stable for several days (left). Here we show that the signal gain observed in the presence of the target antibody remains quite stable for as long as 10 days. However, this becomes indistinguishable from the buffer signal after 15 days. We also note that the sensor can be re-functionalized with the binding of a new recognition peptide layer. This allows us to observe sensitivities very similar to that observed with the original sensor. Sensors, when not in use, were stored in 10 mM HCl at 4 °C. Experiments were carried out in 10 mM PBS buffer at pH 6.

for similar E-DNA sensors.[6,13] This mixed layer was then chosen for the next experiments.

After the redox-labelling of the support peptide, we then focused on the attachment of the recognition module. We used as recognition peptide the alpha-2 deamidated gliadin peptide (DGP) which is specifically recognized by anti-DGP IgG monoclonal antibodies, diagnostic of celiac disease. The immobilization of this recognition peptide (*i.e.* DGP) on the support peptide was achieved *via* covalent binding of the phenolic groups of the tyrosine residues of DGP to the carboxyl groups of the support peptide using an adapted version of the Steglich esterification.[31] The successful immobilization of the recognition peptide was monitored through SWV measurements (Fig. SI4, ESI†). The binding of the target antibody (*i.e.* anti-DGP IgG) to the recognition peptide (Fig. 4, left) was monitored for concentrations of anti-DGP antibody ranging from 0.033 to 1.0 μg $mL^{-1}$ (0.22–6.7 nM). No interferences were observed when a random non-specific antibody was used as a model interferent in the same concentration range as the target antibody (Fig. 4, red curve).

As shown in Fig. 4 when the target antibody binds to the relatively flexible redox-labeled support peptide it significantly reduces its redox signal (Fig. 4). The electrochemical signal observed in the absence of the target antibody is likely due to both an electron transfer through the peptide backbone (as reported by Orlowski *et al.*[9]) and a collisional mechanism due to the relative flexibility of the support peptide that allows the MB to collide to and thus transfer electrons with the electrode surface with good efficiency. Under these conditions, antibody binding changes the efficiency with which the MB label collides with the surface and transfers electrons and this results in the signal suppression observed. Consistent with this mechanism, at the highest support peptide densities (dense package) we have tested, we obtained lower signal suppression probably because the electron transfer through the peptide backbone is dominant. Conversely, at the lowest support peptide densities (which give a sparse monolayer) we have observed the highest signal suppression (Fig. SI5, ESI†).

The stabilities of both the background signal in the absence of the target and the signal suppression at the target binding were monitored for ~3 weeks (Fig. 5). For this extended stability test the sensors were stored in 10 mM HCl solution, a condition under which the sensors demonstrated a slightly better stability than when stored in PBS at both pH 6 and 7. This unusual stability of the peptide sensor under acidic conditions might be due to the fact that the DGP receptor is well known to be stable under acidic conditions (unlike most peptides or proteins) since this epitope is resistant to the action of proteolytic enzymes in the acid medium from the gastrointestinal tract.[32,33] During this stability experiment the sensors retained a good response to the antibody target even after 10 days (Fig. 5, left). After this period, despite the sensor showing a measurable background signal, no significant signal suppression was observed upon the addition of the antibody (Fig. 5, left). We also demonstrated the possibility to reuse the modular sensor. To do this, the DGP block was cleaved by changing the pH to moderate basic values (pH = 8.5), and the surface was re-functionalized with a fresh recognition peptide solution. The newly regenerated sensor exhibited sensitivity towards antibody binding comparable to that of the original sensor (Fig. 5, right).

The modular approach of the E-PB sensor exploiting peptide–peptide temporary covalent binding might provide a promising tool for developing miniaturised biosensing platforms for high molecular weight targets. The first "module", the redox-labelled support peptide layer, ensures a high electrochemical signal. The recognition "module" can be selectively attached to the support peptide using simple esterification chemistry without prior chemical modifications. The quite simple and reversible "click-chemistry" immobilization procedure reported

 

here confers versatility to the modified surface making it available for implementing other recognition elements, peptides or small molecules containing free hydroxyl groups. Despite this, the procedure still needs to be optimized in order to reduce the preparation time and to improve the storage lifetime of the sensors. We also note that, despite that the sensitivity of our sensors appears to be not competitive with other label-free technologies, the electrochemical modular strategy proposed here could in principle be adaptable to portable low-cost instrumentation and to disposable, mass-producible sensors, thus ultimately making the approach suitable for point-of-care applications.

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## Notes and references

1 N. P. Pai, C. Vadnais, C. Denkinger, N. Engel and M. Pai, *PLoS Med.*, 2012, **9**, e1001306.
2 J. Y. Gerasimov and R. Y. Lai, *Chem. Commun.*, 2010, **46**, 395–397.
3 M. A. Sowole and H.-B. Kraatz, *Analyst*, 2012, **137**, 1120–1124.
4 H. Li, Y. Cao, X. Wu, Z. Ye and G. Li, *Talanta*, 2012, **93**, 358–363.
5 M. Ankelo, V. Kleimola, S. Simell, O. Simell, M. Knip, E. Jokisalo, M. Tarkia, A. Westerlund, Q. He, M. Viander, J. Ilonen and A. E. Hinkkanen, *Clin. Exp. Immunol.*, 2007, **150**, 285–293.
6 J. Y. Gerasimov and R. Y. Lai, *Chem. Commun.*, 2011, **47**, 8688–8690.
7 J. Adjémian, A. Anne, G. Cauet and C. Demaille, *Langmuir*, 2010, **26**, 10347–10356.
8 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004–2021.
9 G. A. Orlowski and H.-B. Kraatz, *Electrochim. Acta*, 2006, **51**, 2934–2937.
10 S. Yuan, C. Hu and S. Hu, *Electrochim. Acta*, 2006, **51**, 5274–5285.
11 S. Martic, M. Labib and H.-B. Kraatz, *Analyst*, 2011, **136**, 107–112.
12 S. Martić, M. Labib and H.-B. Kraatz, *Talanta*, 2011, **85**, 2430–2436.
13 F. Ricci, A. J. Bonham, A. C. Mason, N. O. Reich and K. W. Plaxco, *Anal. Chem.*, 2009, **81**, 1608–1614.
14 E. Gatto, L. Stella, F. Formaggio, C. Toniolo, L. Lorenzelli and M. Venanzi, *J. Pept. Sci.*, 2008, **14**, 184–191.
15 E. Gatto, M. Venanzi, A. Palleschi, L. Stella, B. Pispisa, L. Lorenzelli, C. Toniolo, F. Formaggio and G. Marletta, *Mater. Sci. Eng., C*, 2007, **27**, 1309–1312.
16 M. J. I. Andrews and A. B. Tabor, *Tetrahedron*, 1999, **55**, 11711–11743.
17 M. Barbato, G. Maiella, C. Di Camillo, S. Guida, F. Valitutti, G. Lastrucci, F. Mainiero and S. Cucchiara, *Dig. Liver Dis.*, 2011, **43**, 465–469.
18 M. M. P. S. Neves, M. B. Gonzalez-Garcia, H. P. A. Nouws and A. Costa-Garcia, *Analyst*, 2013, **138**, 1956–1958.
19 R. F. Carvalhal, R. Sanches Freire and L. T. Kubota, *Electroanalysis*, 2005, **17**, 1251–1259.
20 E. G. Petrov and V. May, *J. Phys. Chem. A*, 2001, **105**, 10176–10186.
21 K. Kitagawa, T. Morita and S. Kimura, *Thin Solid Films*, 2006, **509**, 18–26.
22 S. Okamoto, T. Morita and S. Kimura, *Langmuir*, 2009, **25**, 3297–3304.
23 H. S. Mandal and H.-B. Kraatz, *Chem. Phys.*, 2006, **326**, 246–251.
24 B. R. Chaudhry, J. D. E. T. Wilton-Ely, A. B. Tabor and D. J. Caruana, *Phys. Chem. Chem. Phys.*, 2010, **12**, 9996–9998.
25 E. Gatto, A. Porchetta, M. Scarselli, M. De Crescenzi, F. Formaggio, C. Toniolo and M. Venanzi, *Langmuir*, 2012, **28**, 2817–2826.
26 M. Puiu, O. Istrate, L. Rotariu and C. Bala, *Anal. Biochem.*, 2012, **421**, 587–594.
27 V. Biagiotti, A. Porchetta, S. Desiderati, K. Plaxco, G. Palleschi and F. Ricci, *Anal. Bioanal. Chem.*, 2012, **402**, 413–421.
28 F. Kuralay, S. Campuzano and J. Wang, *Talanta*, 2012, **99**, 155–160.
29 J. S. Albert and A. D. Hamilton, *Biochemistry*, 1995, **34**, 984–990.
30 M. Venanzi, G. Pace, A. Palleschi, L. Stella, P. Castrucci, M. Scarselli, M. De Crescenzi, F. Formaggio, C. Toniolo and G. Marletta, *Surf. Sci.*, 2006, **600**, 409–416.
31 B. Neises and W. Steglich, *Angew. Chem., Int. Ed. Engl.*, 1978, **17**, 522–524.
32 V. Kumar, A. K. Abbas, N. Fausto and R. Mitchell, *Robbins Basic Pathology*, Elsevier Health Sciences, 2012, vol. 14, pp. 577–582.
33 J. L. Piper, G. M. Gray and C. Khosla, *J. Pharmacol. Exp. Ther.*, 2004, **311**, 213–219.