ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2015, **51**, 402

Received 22nd September 2014,
Accepted 10th November 2014

DOI: 10.1039/c4cc07474b

www.rsc.org/chemcomm

Signal enhancement of electrochemical biosensors *via* direct electrochemical oxidation of silver nanoparticle labels coated with zwitterionic polymers†

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A new electrochemical label has been developed, which is made up of silver nanoparticles (AgNPs) coated with a mixture of zwitterionic and biotinylated zwitterionic polymers. These polymers improve colloidal stability in physiological medium and ensure biorecognition while direct electrochemical oxidation of silver nanoparticles strongly enhances the detection signal. The resulting hybrid nanomaterials are used as labels in the electrochemical sensing of avidin using sandwich assays elaborated using the biotin–avidin biorecognition system.

In recent years, major progress has been made in the development of biosensors.[1–4] New research in this field faces new challenges such as the ability to enhance the sensitivity for the detection of traces of disease markers and infectious agents and the possibility of directly working in biological media.

Nanomaterials are at the core of solutions commonly employed to improve sensitivity.[5,6] They have been used as well as in optical[7] and electrochemical[8–10] biosensors either due to their large specific surface area (to increase the transducer area or to immobilize more labels) or due to their physical properties. For instance, in optical biosensors, slight and long life fluorescence of quantum dots, surface plasmon resonance (SPR), surface enhanced Raman spectroscopy (SERS) and metal enhanced fluorescence (MEF) of metallic nanoparticles have been particularly exploited. For electrochemical devices, metallic nanoparticles, carbon nanotubes and graphene have been used to catalyze electrochemical reactions and to improve electron transfer. Nanoparticles have also been used as labels capable of generating intense signals in comparison with electroactive molecules or organometallic complexes like ferrocene. Indeed, due to their high number of atoms, a huge number of electrons can be exchanged through oxidation or reduction. In the initial studies reporting the latter use, the analysis procedure was tedious, as a chemical dissolution step was realized before performing titration by anodic stripping.[11–15] These supplementary steps are time consuming, can be the origin of mistakes and constitute a limit to the development of such labels in electrochemical biosensors that otherwise present many advantages like speed, selectivity, low cost and ease of production and use. Hence, recent research in this field focuses on direct oxidation of labels especially silver nanoparticle tags.[16–18] Oxidation of silver deposits is also often encountered in biosensor devices to reveal a biorecognition event and increase the electrochemical signal.[19–23] As mentioned previously, the colloidal stability of labels in a biological environment is an additional crucial requirement for many applications especially in the field of clinical diagnostics. Zwitterionic polymers due to their remarkable anti-fouling properties (resistance to cell, bacteria and protein adsorption) are becoming choice materials in biomedical fields where high stability is required.[24–27] In the present work, we report on a novel label for electrochemical biosensors based on hybrid nanoparticles with a silver core and a zwitterionic polymer shell that will be able to satisfy all requirements (Fig. 1). Indeed, the electrochemical oxidation of silver will contribute to signal enhancement while the zwitterionic polymer chains grafted onto the silver surface will provide excellent stability. Besides these advantages, this elaborated label is very versatile due to the presence of a carboxylic acid as its terminal function, allowing further biofunctionalization with a lot of bioreceptors. This therefore opens up the electrochemical biosensor devices to be directed towards various targets. The sensor structure developed to test these hybrid labels is shown in Scheme 1. The biotinylated labels were used in sandwich assays for the detection of avidin as a model protein.

Zwitterionic polymers were synthesized by reversible addition–fragmentation chain transfer (RAFT) polymerization of *N*,*N*′-dimethyl-(methacrylamido propyl)ammonium propanesulfonate (SPP)

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† Electronic supplementary information (ESI) available: Experimental details for the synthesis, biofunctionalization and characterization of zwitterionic polymers and hybrid nanoparticles. Biosensor elaboration and voltammograms. See DOI: 10.1039/c4cc07474b

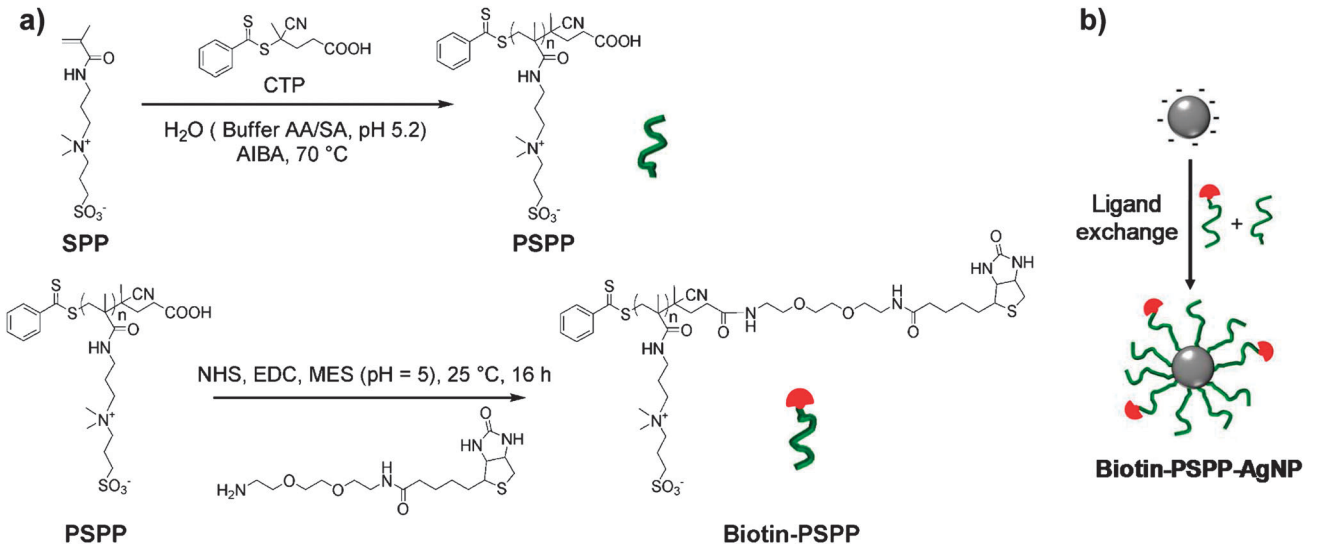
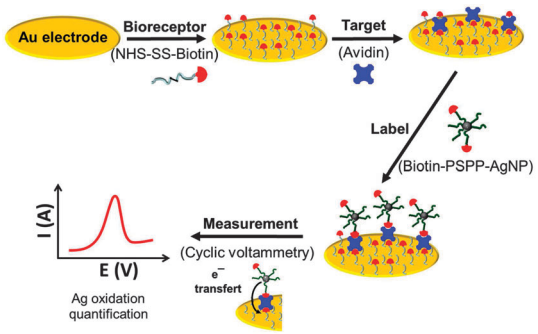


Fig. 1 Elaboration of the electrochemical label. (a) Synthesis of zwitterionic polymers, PSPPs, by RAFT polymerization and biotin–PSPPs by coupling of PSPPs with amino-PEG_2–biotin. (b) Ligand exchange procedure using a mixture of PSPPs and biotin–PSPPs on 13 nm diameter silver nanoparticles.



Scheme 1 Principle of the electrochemical biosensor.

monomers using 4-cyanopentanoic acid dithiobenzoate (CTP) as the RAFT agent. The RAFT agent used in this work is essential to allow the synthesis of zwitterionic polymers (PSPPs) with well-controlled average molar mass, dispersity and end-functionality. The as-synthesized PSPPs were biofunctionalized with amino-PEG_2–biotin (biotin–PSPPs) *via* peptidic coupling (Fig. 1a). The characterization of the synthesized and modified polymers using size exclusion chromatography and NMR spectroscopy is shown in Fig. S1 and S2 and Table S1 in the ESI.† Then, the electrochemical label is obtained by ligand exchange procedure using a mixture of PSPPs and biotin–PSPPs on 13 nm-diameter silver nanoparticles (Fig. 1b). The prepared biotin–PSPPs–AgNPs exhibit excellent colloidal stability in high ionic strength solution as confirmed by the UV-vis spectra, thanks to the properties of zwitterionic polymer chains (Fig. S4, ESI†).[10]

The electrochemical biosensor was elaborated step by step as follows (Scheme 1): gold disk electrodes of 500 μm diameter were biotinylated using sulfo-NHS-SS-biotin and then used to immobilize avidin protein, which was employed as a model target for biosensing tests. Finally, the electrochemical labels, biotin–PSPPs–AgNPs, were added by a second biorecognition between avidin and biotin. The electrode modification was followed step by step by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) measurements employing $Fe(CN)_6^{3-/4-}$ in PBS as a redox probe (Fig. 2). The successive modification steps of the electrode with biotin and avidin induce a decrease of the current and an increase of the potential difference

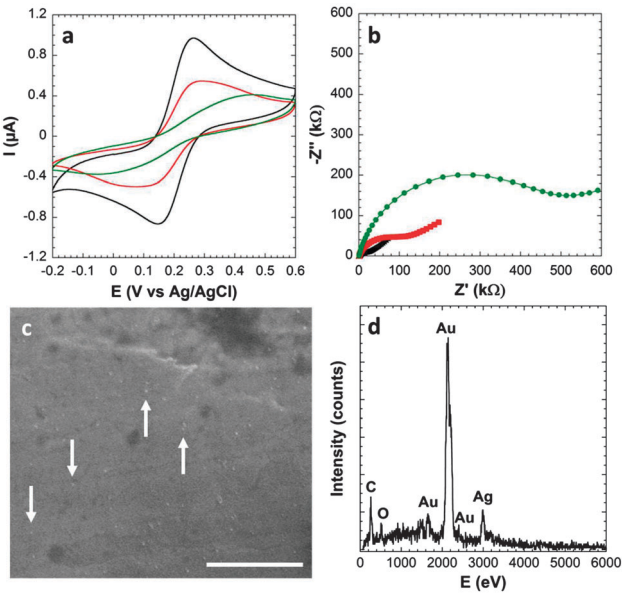


Fig. 2 (a) Cyclic voltammograms of various modified electrodes in 0.1 M PBS (pH 7.0) solution containing 1 mM $Fe(CN)_6^{3-/4-}$ at a scan rate of 50 mV s^{-1}: gold electrode (black), Au/biotin (red), Au/biotin/avidin (green). (b) Nyquist plots corresponding to various modified electrodes in 0.1 M PBS (pH 7.0) solution containing 1 mM $Fe(CN)_6^{3-/4-}$ at around 0.24 V: gold electrode (black), Au/biotin (red), Au/biotin/avidin (green). (c) SEM images of biosensor's gold electrodes functionalized with biotin–PSPPs–AgNPs, [avidin] = 15 nM. The arrows represent silver nanoparticles deposited on gold electrodes. The scale bar is 1 μm. (d) EDX spectrum of the biosensor's electrode.

between oxidation and reduction peaks, both indicating a slower electron transfer due to hindrance and characteristics of modification of the electrode. The electrode modification was also validated by EIS measurements (Fig. 2b). Here, the small semicircle observed for a bare gold electrode gradually increases after each modification indicating the progressively more difficult mass transfer of $Fe(CN)_6^{3-/4-}$ and consequently the effective biotinylation followed by avidin immobilization.

The presence of biotin–PSPPs–AgNPs on gold electrodes after the last immobilization step was confirmed by SEM images (Fig. 2c). The EDX spectrum shows a peak related to silver at 2964 eV, which proves the presence of immobilized biotin–PSPPs–AgNPs on the modified electrode (Fig. 2d). The estimated density is 10 nanoparticles per μm^2. This absence of a full monolayer of nanoparticles is coherent with previous results reporting that dipping an electrode modified with a self-assembled monolayer (SAM) made from NHS–SS–biotin in low concentration solutions (<10 nM) of avidin did not lead to a full avidin monolayer.[28] In addition, it is necessary to take into account the desorption of avidin when biotinylated nanoparticles are introduced due to competitive binding of avidin between biotin immobilized on the electrode and biotin from labels. Such a phenomenon was also described and the authors reported that 1 mM biotin solution is able to remove almost a monolayer of avidin.[29] In our study, the NP concentration is estimated to be at the nanomolar level, which explains that we do not remove all the avidin. Sensing tests of the elaborated biosensor using biotin–PSPPs–AgNPs as electrochemical labels were carried out by performing cyclic voltammetry in phosphate buffer solution (Fig. 3a). The cyclic voltammogram presents an oxidation peak with a maximum at 210 mV and a reduction peak at -5 mV. These peaks are characteristics respectively of oxidation of silver coated with PSPPs in PBS medium in a silver precipitate and of the reverse reaction. This result shows that silver from the hybrid label developed in this study can be electrochemically oxidized. This signal is only related to the specific adsorption of biotin–PSPPs–AgNPs *via* biotin–avidin complexation. Indeed, sensing tests carried out using Au and Au/biotin electrodes after being dipped in a biotin–PSPPs–AgNPs suspension do not induce any oxidation or reduction peak (Fig. S7 and S8, ESI†).

In addition, the oxidation charge of assemblies realized with various amounts of avidin was investigated. The charge variation with the avidin concentration is plotted in Fig. 3b. It is noteworthy that a straight-line is obtained and it constitutes a calibration curve for determination of avidin. It should be noticed that the calibration curve has not been extended to higher avidin concentrations to characterize the linear calibration range as we focused on the electrochemical oxidation of silver nanoparticles. The limit of quantification obtained here is 1.5 nM, corresponding to 90 ng mL^{-1} or 500 pM. Moreover, the surface density of the immobilized nanoparticles was estimated to be 10 nanoparticles per μm^2 and considering the electrode geometric area of 196 300 μm^2 and an average of 100 000 Ag atoms per nanoparticle, the oxidation charge expected from this sensor is 32 nC $\pm$ 5. This value is slightly lower than the experimental value determined by cyclic voltammetry but gives evidence to the high rate of silver nanoparticle oxidation. The latter conclusion confirms that the intense signal can be obtained using silver nanoparticles as expected.

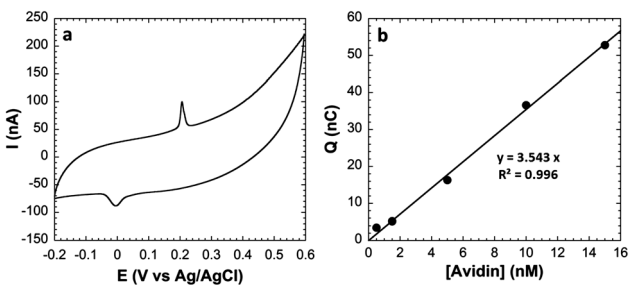


Fig. 3 (a) Cyclic voltammogram of Au/biotin/avidin/biotin–PSSPs–AgNPs in 0.1 M PBS (pH 7.0) solution at a scan rate of 50 mV s^{-1}, [avidin] = 5 nM. (b) Calibration curve of the relationship between the oxidation charge of AgNPs and avidin concentration used in sandwich elaboration.

In summary, we developed a new versatile label for electrochemical biosensors based on zwitterionic polymer-coated silver nanoparticles. The zwitterionic polymer of the coating, synthesized by RAFT polymerization, provides both versatility, as it can be easily biofunctionalized with various bioreceptors directed towards new target molecules, and high colloidal stability. On the other hand, the silver core contributes to signal enhancement and its electrochemical oxidation allows easy sensing processes. The present work paves the way for the detection of biological marker traces like proteins directly in biological media.

This project was financially supported by Région Ile de France (C'nano IdF). The SEM-FEG instrument was facilitated by the IMPC (Institut des Matériaux de Paris Centre FR2482) financially supported by the C'Nano projects of the Region Ile-de-France. The authors thank X. Xu and D. Montero for technical assistance with electron microscopy and EDX analysis and M. Hanafi for SEC analysis.

Notes and references

1 A. P. F. Turner, *Chem. Soc. Rev.*, 2013, **42**, 3184–3196.
2 B. V. Chikkaveeraiah, A. A. Bhirde, N. Y. Morgan, H. S. Eden and X. Chen, *ACS Nano*, 2012, **6**, 6546–6561.
3 E. Katz and I. Willner, *Angew. Chem., Int. Ed.*, 2004, **43**, 6042–6108.
4 Y. Li, H. Schluesener and S. Xu, *Gold Bull.*, 2010, **43**, 29–41.
5 A. N. Shipway, E. Katz and I. Willner, *ChemPhysChem*, 2000, **1**, 18–52.
6 M. Pumera, A. Ambrosi, A. Bonanni, E. L. K. Chng and H. L. Poh, *TrAC, Trends Anal. Chem.*, 2010, **29**, 954–965.
7 G. Wang, Y. Wang, L. Chen and J. Choo, *Biosens. Bioelectron.*, 2009, **25**, 1859–1868.
8 X. Luo, A. Morrin, A. J. Killard and M. R. Smyth, *Electroanalysis*, 2006, **18**, 319–326.
9 J. Wang, *Analyst*, 2005, **130**, 421–426.
10 S. Alwarappan, A. Erdem, C. Liu and C. Z. Li, *J. Phys. Chem. C*, 2009, **113**, 8853–8857.
11 M. Dequaire, C. Degrand and B. Limoges, *Anal. Chem.*, 2000, **72**, 5521–5528.
12 J. Wang, R. Polsky and D. Xu, *Langmuir*, 2001, **17**, 5739–5741.
13 J. A. Hansen, J. Wang, A.-N. Kawde, Y. Xiang, K. V. Gothelf and G. Collins, *J. Am. Chem. Soc.*, 2006, **128**, 2228–2229.
14 J. Wang, G. Liu, M. R. Jan and Q. Zhu, *Electrochem. Commun.*, 2003, **5**, 1000–1004.
15 M. Wang, C. Sun, L. Wang, X. Ji, Y. Bai, T. Li and J. Li, *J. Pharm. Biomed. Anal.*, 2003, **33**, 1117–1125.
16 H. Karadeniz, A. Erdem, A. Caliskan, C. M. Pereira, E. M. Pereira and J. A. Ribeiro, *Electrochem. Commun.*, 2007, **9**, 2167–2173.

17 L. Kashefi-Kheyrabadi and M. A. Mehrgardi, *Biosens. Bioelectron.*, 2012, **37**, 94–98.
18 B. P. Ting, J. Zhang, Z. Gao and J. Y. Ying, *Biosens. Bioelectron.*, 2009, **25**, 282–287.
19 J. Ghilane, F.-R. F. Fan, A. J. Bard and N. Dunwoody, *Nano Lett.*, 2007, **7**, 1406–1412.
20 T. H. Degefa, S. Hwang, D. Kwon, J. H. Park and J. Kwak, *Electrochim. Acta*, 2009, **54**, 6788–6791.
21 F. Gao, Z. Zhu, J. Lei, Y. Geng and H. Ju, *Biosens. Bioelectron.*, 2013, **39**, 199–203.
22 M. Javanbakht, F. Divsar, A. Badiei, F. Fatollahi, Y. Khaniani, M. R. Ganjali, P. Norouzi, M. Chaloosi and G. M. Ziarani, *Electrochim. Acta*, 2009, **54**, 5381–5386.
23 G. Lai, L. Wang, J. Wu, H. Ju and F. Yan, *Anal. Chim. Acta*, 2012, **721**, 1–6.
24 E. Muro, T. Pons, N. Lequeux, A. Fragola, N. Sanson, Z. Lenkei and B. Dubertret, *J. Am. Chem. Soc.*, 2010, **132**, 4556–4557.
25 Z. G. Estephan, P. S. Schlenoff and J. B. Schlenoff, *Langmuir*, 2011, **27**, 6794–6800.
26 L. Mi and S. Y. Jiang, *Angew. Chem., Int. Ed.*, 2014, **53**, 1746–1754.
27 K. P. Garcia, K. Zarschler, L. Barbaro, J. A. Barreto, W. O'Maley, L. Spiccia, H. Stephan and B. Graham, *Small*, 2014, **10**, 2516–2529.
28 H. Kuramitz, K. Sugawara and S. Tanaka, *Electroanalysis*, 2000, **12**, 1299–1303.
29 V. H. Perez-Luna, M. J. O'Brien, K. A. Opperman, P. D. Hampton, G. P. Lopez, L. A. Klumb and P. S. Stayton, *J. Am. Chem. Soc.*, 1999, **121**, 6469–6478.