CSIRO PUBLISHING

Aust. J. Chem. **2013**, *66*, 613–618
http://dx.doi.org/10.1071/CH13043

Full Paper

Demonstrating the Use of Bisphenol A-functionalised Gold Nanoparticles in Immunoassays

Joshua R. Peterson,[A] *Yang Lu,*[B] *Erwann Luais,*[A,C] *Nanju Alice Lee,*[B,E] *and J. Justin Gooding*[A,D,E]

[A]School of Chemistry, University of New South Wales, Sydney, NSW 2052, Australia.
[B]School of Chemical Engineering, University of New South Wales, Sydney, NSW 2052, Australia.
[C]Current address: GREMAN, University François Rabelais, Parc de Grandmont, 37200 Tours, France.
[D]Australian Centre for NanoMedicine, University of New South Wales, Sydney, NSW 2052, Australia.
[E]Corresponding authors. Email: justin.gooding@unsw.edu.au, alice.lee@unsw.edu.au

Spherical gold nanoparticles (5-nm diameter) were modified with a small-molecule thiolated bisphenol A (BPA) ligand to achieve an estimated coverage of $\sim 3.3 \times 10^{-10}$ mol cm^{-2}, or 180 ligands per particle. The modified particles were tested in an enzyme-linked immunosorbent assay (ELISA) format to measure functionality and were shown to bind specifically to anti-BPA antibody while resisting the non-specific adsorption of an antibody with no affinity for BPA. It was found that the use of 10% ethanol as a co-solvent was required in the ELISA as aqueous buffers alone resulted in poor binding between anti-BPA antibody and the functionalised nanoparticles. This is likely due to the hydrophobic nature of the BPA ligand limiting its solubility, and therefore its availability for antibody interactions, in purely aqueous environments. To our knowledge, this is the first example of a nanoparticle modified with a small organic molecule being used in an ELISA assay.

Manuscript received: 25 January 2013.
Manuscript accepted: 5 March 2013.
Published online: 8 April 2013.

Introduction

A dizzying array of uses for gold nanoparticles has been reported in recent years and spans an impressive variety of research areas from energy[1] to healthcare,[2] food,[3] and chemical and biological analysis.[4] The use of nanoparticles in sensing applications is of particular interest as they have many advantageous properties that make them suitable for incorporation into sensing devices: they are easily synthesised across a range of sizes, they have a high surface area-to-volume ratio, it is relatively straightforward to functionalise their surfaces with biocompatible ligands, and they possess unique optical and electronic properties that can be exploited in several different analytical formats. As a result, there has been a large output of research aimed at using nanoparticles for the detection of analytes ranging from malignant cells to metal ions and small organic compounds.[4] In several studies, nanoparticles have demonstrated the potential for improved analyte capture, detection, and sensitivity, making nanoparticles an attractive option when considering analytes that are present in low levels or with limited water solubility, for example small organic molecule contaminants.

Bisphenol A (BPA) is one such contaminant that has garnered a great deal of attention over the past decade owing to its activity as an endocrine disrupter, capable of interfering with male and female reproductive development *in utero*, and also its association with increased risk of certain cancers.[5] A monomer used in the production of polycarbonate plastics, and produced on the billions of kg per year scale, BPA is ubiquitous in the environment.[6] Although human exposure is predominantly the result of its use in food packaging, both the US Environmental Protection Agency and Food and Drug Administration have taken steps to reduce human exposure to BPA, as have the relevant agencies in many other countries around the world.[7]

As a result, a great deal of research has been directed at the detection of BPA in a variety of media including human serum,[8] food products,[9] and environmental samples.[10] In addition, a variety of techniques have been developed for the extraction and detection of BPA such as HPLC-mass spectroscopy (MS), gas chromatography (GC)-MS, fluorescence, electrochemical detection, and immunochemistry methods.[11]

Recent advancements in the detection of many analytes, including BPA, have combined immunochemistry with nanoparticles, resulting in assays that have the specificity of antibodies but increased sensitivity compared with traditional enzyme-linked immunosorbent assay (ELISA). Modification of nanoparticles with antibodies has been shown to increase sensitivity in ELISA for cancer biomarkers.[12] Magnetic nanoparticles have been shown in the ELISA format to preconcentrate analyte, lowering the limit of detection by an order of magnitude,[13] and to reduce ELISA assay time from hours to a matter of minutes.[14] Graham and coworkers have used modified gold nanoparticles in an ELISA with surface-enhanced

resonance Raman scattering (SERRS) to increase sensitivity.[15] Most recently, nanoparticles modified with anti-BPA aptamers have been used for the visual detection of BPA down to 0.1 μg L^{-1}.[16]

Although the use of modified gold nanoparticles in detection systems, including ELISA assays, has been reported, the nanoparticles have been almost exclusively modified with antibodies or large proteins. In the few studies that have reported the use of small-molecule-functionalised nanoparticles, great potential has been shown in both medical[17] and biosensor applications.[18] To our knowledge, there have been no studies in which gold nanoparticles modified with a small organic molecule have been tested in an ELISA. The combination of ELISA and nanoparticles is potentially useful in developing detection methods for many analytes, including BPA. There are, however, broader implications for this research than using them in ELISA. Demonstrating the functionality of a nanoparticle sensing element could be invaluable in generating immunogenic species as a substitute to hapten–protein conjugates and also in designing new electrochemical immunosensors that incorporate nanoparticles.[19] For these reasons, we decided to test BPA-functionalised nanoparticles in a sandwich ELISA assay to assess their functionality and their suitability for future incorporation into immunosensors. In this paper, we discuss the use of nanoparticles modified with the small organic molecule bisphenol A in an ELISA assay (Fig. 1), the need for an organic co-solvent, and the specific binding exhibited by the modified nanoparticles.

Results and Discussion

Preparation and Characterisation of Functionalised Gold Nanoparticles

Synthesis of the thiol-modified BPA ligand followed a common amide coupling procedure to first activate the acid group of BPA-valeric acid with *N*,*N*′-dicyclohexylcarbodiimide/*N*-hydroxysuccinimide (DCC/NHS), followed by reaction with cysteamine (Fig. 2). Although the yield of purified **cysBPAv** ligand was low, it was sufficient to allow functionalisation of a large number of nanoparticles because so little is used. For example, 1 mg of **cysBPAv** ligand is theoretically sufficient to prepare 145 mL of functionalised nanoparticles, enough to perform more than 14000 ELISA assays.

The **cysBPAv** ligand was added to the nanoparticle solution, which, after overnight incubation, was centrifuged and decanted to remove any **cysBPAv** not bound to the nanoparticle surface. The addition of **cysBPAv** to the nanoparticle surface significantly increases the hydrophobicity of the nanoparticles themselves even though BPA has been reported as a 'moderately soluble' compound, with a water solubility in the range of 120 to 300 mg L^{-1}.[20] In this case, the **cysBPAv** ligand is not sufficiently water soluble to allow nanoparticle functionalisation and redispersion in pure water (which would be the most desirable conditions considering that immunochemistry reactions in the ELISA are optimised for aqueous conditions). However, the resulting modified nanoparticles were highly dispersible in absolute ethanol,

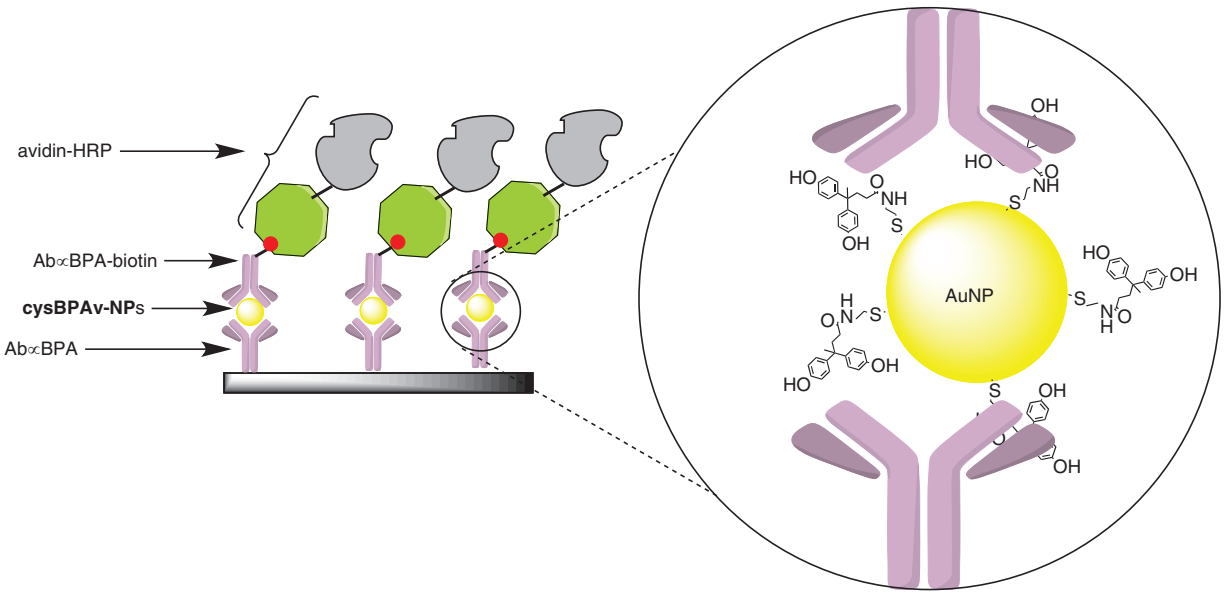

Fig. 1. Schematic of BPA nanoparticle sandwich ELISA (NP, nanoparticle).

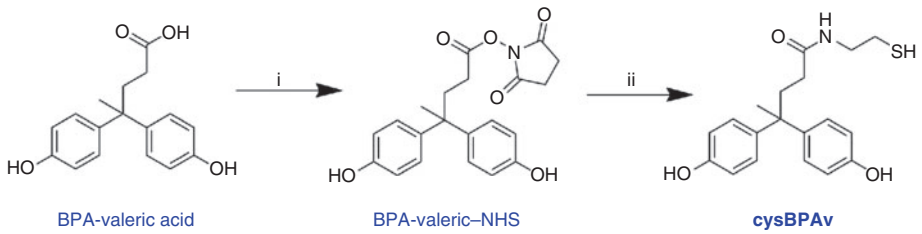

Fig. 2. Synthesis of **cysBPAv** from BPA-valeric acid via NHS activation and amide coupling with cysteamine: (i) DCC, NHS, dry THF, 22 h, rt, 36 % yield; (ii) cysteamine, DIPEA, dry THF, 3 h, rt, 11 % yield.

and the precipitated particles could be resuspended in a small volume of ethanol before dilution with water to the final mixture of 10 % ethanol in water. In this ethanol/water mixture, the **cysBPAv-NP** suspension was stable for several months.

The modified particles used for these studies were prepared by the addition of 20 μL of a 1 mM solution of **cysBPAv** to 1 mL of nanoparticle solution. Assuming spherical bare nanoparticles of 5.3-nm diameter (s.d. = 1.6 nm, $n = 107$, based on transmission electron microscopy (TEM) measurements), and a concentration of 3.2×10^{13} nanoparticles mL^{-1} calculated using the methods of Haiss and coworkers,[21] the ligand added is sufficient to achieve a theoretical surface coverage of 7.1×10^{-10} mol thiol ligand cm^{-2} (equivalent to ~380 **cysBPAv** ligands per particle). This is slightly less than the maximum coverage of close-packed alkanethiols on gold that was reported by Porter and coworkers[22] as 9.3×10^{-10} mol cm^{-2} (equivalent to 500 **cysBPAv** ligands per particle). In fact, the bulkiness of the **cysBPAv** ligand would not allow close packing on the surface as one would expect for straight-chain alkanethiols, so the surface density of **cysBPAv** ligand would likely be even less than 7.1×10^{-10} mol cm^{-2}. Basic modelling shows the footprint of the **cysBPAv** ligand to be ~50 $Å^2$. Using this figure, a surface coverage of 3.3×10^{-10} mol cm^{-2} can be calculated, which equates to ~180 ligands per nanoparticle.

The modified nanoparticles (**cysBPAv-NP**s) were characterised by TEM, X-ray photoelectron spectroscopy (XPS), and UV-vis. TEM confirmed that the centrifuged and resuspended particles were on average 5.4 nm in diameter (s.d. = 1.4 nm, $n = 228$), essentially unchanged compared with the unmodified particles (Fig. 3a). UV-vis showed a shift in the surface plasmon resonance (SPR) peak from 517 nm to 529 nm (Fig. 3b), which is consistent with shifts predicted for particles coated with an organic compound.[21]

The functionalisation of gold nanoparticles was demonstrated through XPS characterisation of gold nanoparticles before and after modification with the **cysBPAv** ligand. Comparing the survey spectra (Fig. 4a), new XPS signals appear for sulfur and for nitrogen. These XPS signals are from the thiolated **cysBPAv** ligand, which contains an amide bond in addition to the sulfur. Evidence that the **cysBPAv** ligand is bound to the gold nanoparticles surface is provided by the S 2p XPS signal for functionalised nanoparticles (Fig. 4b), fitted with two components corresponding to the XPS S 2p splitting signal for S 2p3/2 and S 2p1/2 at respective binding energies of 162.1 and 163.4 eV. These binding energy values match with bound thiol on gold surfaces whereas free or uncomplexed thiol typically exhibits values at higher energies.[23] XPS C 1s scans are displayed in Fig. 4c. For gold nanoparticles synthesised with citrate present, as was done in the present study, the carbon material on the gold nanoparticle surface is the citrate coating, and the C 1s signal can be fitted in three components. The first component, at a binding energy value of 285.6 eV, is attributed to aliphatic carbon whereas at higher binding energies, two other components are present resulting from carbon bound to more electronegative elements, in this case oxygen. The second component, at a binding energy of 286.7 eV, corresponds to the carbon–hydroxyl functional group whereas the third component at 288.9 eV is for carbon atoms in a carboxylic environment. After the functionalisation with the **cysBPAv** ligand, the C 1s signal is very different and is fitted with four components. The first component, at 284.6 eV, corresponds to carbon atoms in an sp^2 hybridisation state from the two phenyl rings in the ligand structure. At a binding energy value of 285.3 eV, the second component is for aliphatic carbon atoms. For carbon in a more oxidised state, a component at 286.3 eV is attributed to carbon atoms bound to sulfur, nitrogen, and oxygen (phenol group). The last component, at a binding energy of 288.1 eV, corresponds to carbon atoms engaged in the amide bond.[24] The presence of amide is also confirmed through the XPS N 1s signal (Fig. 4d) where the N 1s signal is fitted with a single component at a binding energy of 400.9 eV.[24] Evidence that the citrate capping is removed by the self-assembled monolayer ligand on the gold nanoparticle surface is also supported by the decrease in atomic percentage of Na 1s, which drops from 4.05 atom-% in the unmodified particles to 0.83 atom-% in the **cysBPAv-NP**s.

Use of Functionalised Gold Nanoparticles in the Sandwich ELISA Assay

The **cysBPAv-NP**s were tested in an ELISA assay to ascertain the binding characteristics to anti-BPA antibodies. Polyclonal antibodies to BPA-valerate (Ab∝BPA) were raised as described previously.[9b] The assay was run in a sandwich ELISA format (Fig. 1) where the surface of the ELISA plate was first coated with Ab∝BPA, followed by subsequent incubations in **cysBPAv-NP**s, Ab∝BPA–biotin and then avidin–horseradish peroxidase (HRP). The resulting complex could then be measured using standard ELISA colorimetric methods.

Initial ELISA results showed poor binding between the **cysBPAv-NP**s and Ab∝BPA in phosphate buffered solution (PBS) buffer (Fig. 5). This is likely due to the hydrophobic nature of the **cysBPAv** ligand, which could strongly associate with the nanoparticle surface and adjacent ligands rather than extending out into the solution phase where it would be available as an antibody binding site. This is even more likely in an

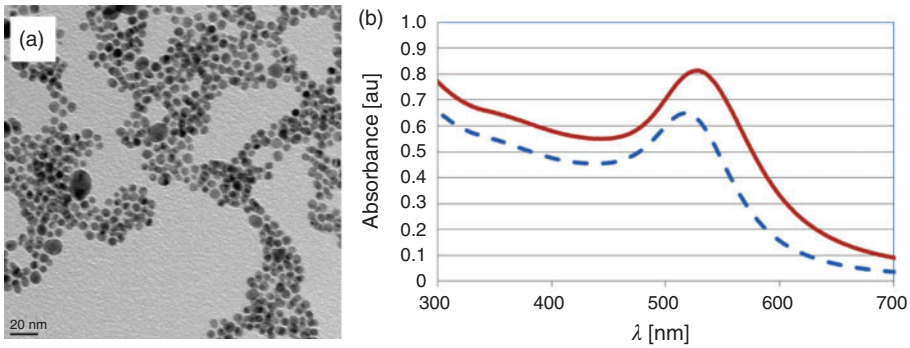

Fig. 3. (a) TEM of **cysBPAv-NP**s. Particle diameter = 5.4 nm (s.d. 1.4 nm, $n = 228$); and (b) UV-vis spectra showing shift in SPR peak from 517 nm (dotted line, unmodified particles) to 529 nm (solid line, **cysBPAv-NP**s) when particles are modified with **cysBPAv** ligand.

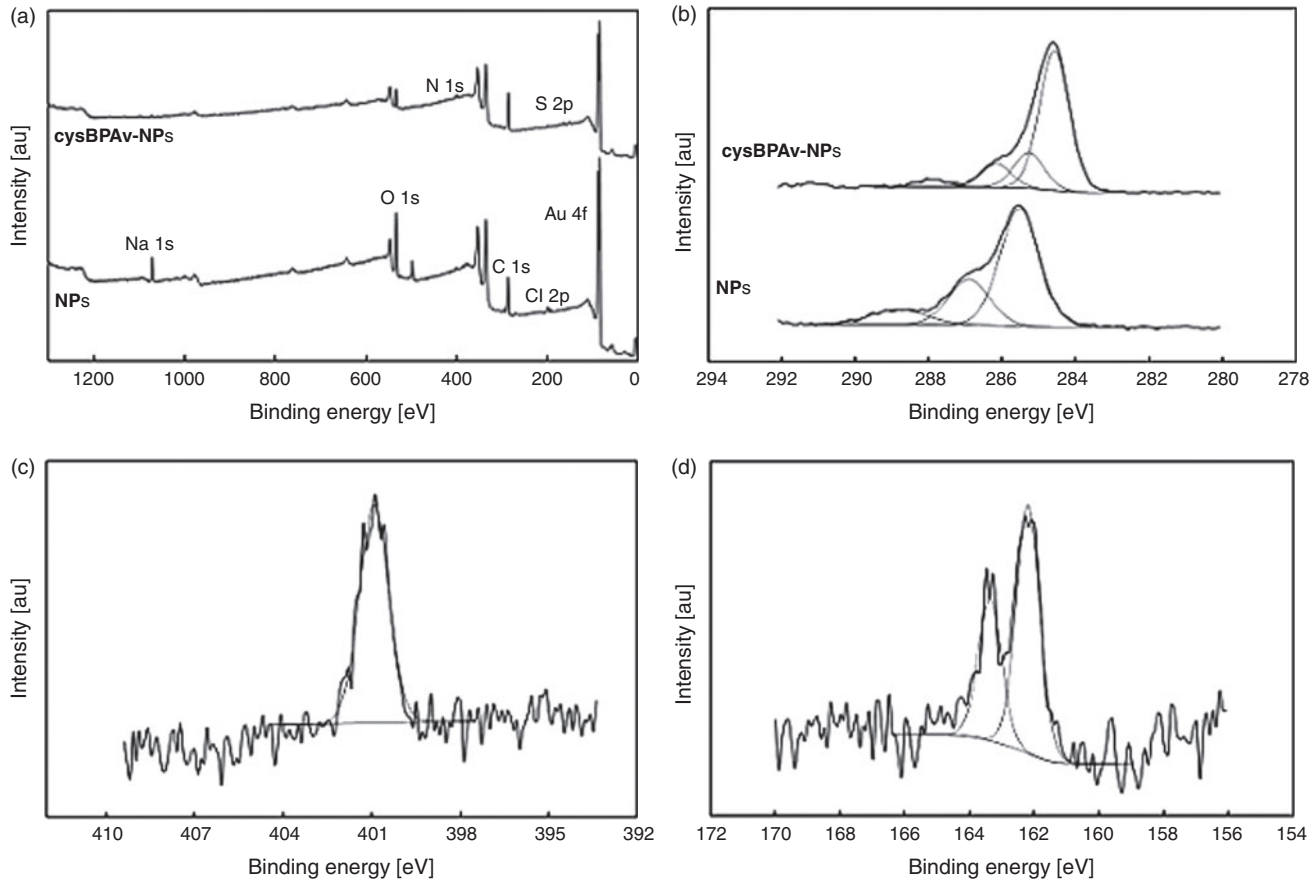

Fig. 4. (a) XPS survey of unmodified gold nanoparticles and of **cysBPAv**-modified gold nanoparticles; (b) XPS C 1s scans of unmodified particles and **cysBPAv-NPs**; (c) XPS N 1s spectra of **cysBPAv-NPs**; and (d) XPS S 2p spectra of **cysBPAv-NPs**.

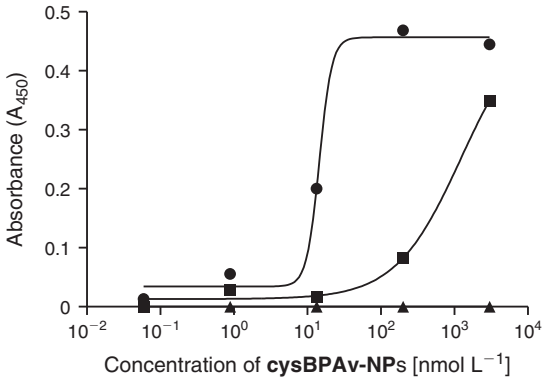

Fig. 5. Dose–response between **cysBPAv-NPs** and Ab∝BPA with and without ethanol present: ● = **cysBPAv-NPs** measured in PBS/10 % EtOH; ■ = **cysBPAv-NPs** measured in PBS alone. The results demonstrate the need for 10 % EtOH as a co-solvent to allow efficient binding of the antibody to the **cysBPAv** ligand on the modified nanoparticles. The data for unmodified nanoparticles measured in PBS/10 % EtOH (▲) show that no binding occurs when the **cysBPAv** ligand is absent.

aqueous environment with high salt content such as PBS, so in the standard ELISA method, there is probably very little surface-bound BPA ligand available in solution for the antibody to bind. To make the bulk solution more favourable for the **cysBPAv** ligand, an organic co-solvent was added to the PBS. It was found that the addition of 10 % (v/v) ethanol to the PBS resulted in much better binding between the Ab∝BPA and **cysBPAv-NPs**, and the data were on par with what would be considered a typical ELISA binding curve. As shown in Fig. 5, significant binding between Ab∝BPA and **cysBPAv-NPs** in 10 % ethanol can be measured at nanoparticle concentrations as low as 10 nM **cysBPAv-NP** with the colorimetric response signal being saturated at 100 nM **cysBPAv-NP**. In contrast, without ethanol the binding cannot be reliably measured below a concentration of 100 nM **cysBPAv-NP** and the colorimetric response is not saturated even at a nanoparticle concentration of 5000 nM. In other words, it takes a great deal fewer nanoparticles to achieve a colorimetric response in the ELISA when the ELISA is carried out in 10 % ethanol. As noted above, this is likely due to the small amount of organic co-solvent being sufficient to allow some of the surface-bound **cysBPAv** ligands to be available to the bulk solution where they can interact with the Ab∝BPA antibodies.

To ensure that the observed binding was due to specific interactions between **cysBPAv-NP** and Ab∝BPA and not simply hydrophobic interactions, the ELISA assay was run comparing the response of Ab∝BPA with an antibody with no affinity for BPA (Ab∝HareI#3, which was raised against 17α-ethinylestradiol). In the ELISA assay, the **cysBPAv-NPs** showed specific binding with an anti-BPA antibody (Ab∝BPA) with the maximum absorbance value difference of 0.58 in the range of 3×10^{-1} to 1×10^{-10} M **cysBPAv-NPs** (Fig. 6). The antibody with no affinity for BPA (Ab∝HareI#3) showed no binding to the modified nanoparticles across a wide range of concentrations. It is clear from the data that incorporation of 10 % ethanol is sufficient to alter the environment at the surface of the modified particles to an extent that allows specific interactions with the

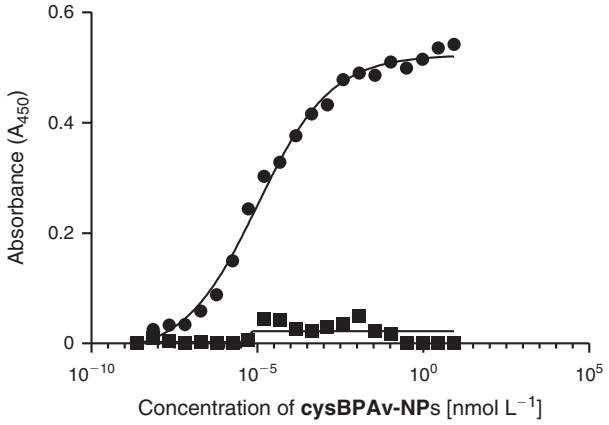

Fig. 6. Dose–response between **cysBPAv-NP**s and specific and non-specific antibodies in PBS with 10 % (v/v) EtOH: ● = **cysBPAv-NP**s/Ab∝BPA; ■ = **cysBPAv-NP**s/Ab∝HareI#3. Ab∝BPA is an anti-BPA antibody, whereas Ab∝HareI#3 is an antibody with no affinity for BPA. The results demonstrate a selective interaction between BPA antibody and the **cysBPAv**-modified gold nanoparticles.

anti-BPA antibodies. This demonstration of selective interaction between the anti-BPA antibody and the **cysBPAv-NP**s is promising for their use in an electrochemical immunosensor.

Conclusions

Gold nanoparticles ~5 nm in diameter were synthesised and modified with a thiol-bisphenol A ligand. The modified particles were characterised by TEM, XPS, and UV-vis and were estimated to have a surface coverage of 3.3×10^{-10} mol cm^{-2}, or an average of 180 ligands per particle. The modified particles were characterised by ELISA assay and demonstrated functionality with specific binding to anti-BPA antibody while resisting the non-specific adsorption of an antibody with no affinity for BPA. A key feature of the ELISA assay was the need to use 10 % ethanol as a co-solvent, presumably owing to the hydrophobic nature of the BPA ligand, which may limit its availability for antibody interactions at the nanoparticle surface in purely aqueous environments. Future work will focus on additional characterisation of interactions of modified nanoparticles with specific and non-specific antibodies and incorporation of these nanoparticles as sensing elements in electrochemical immunosensors.

Experimental

Chemicals and Instruments

4,4-Bis(4-hydroxyphenyl)valeric acid (BPA-valeric acid), DCC, NHS, *N*,*N*-diisopropylethylamine (DIPEA), hydrogen tetrachloroaurate(III) hydrate, sodium borohydride, thimerosal and 3,3′,5,5′-tetramethylbenzidine (TMB) were purchased from Aldrich. Trisodium citrate, potassium phosphate (dibasic), sulfuric acid, hydrochloric acid, nitric acid, and hydrogen peroxide were purchased from Ajax Finechem. Avidin-HRP was purchased from Sigma. The preparation of anti-BPA antibody (Ab∝BPA) has been described previously.[9b]

Methanol (MeOH, HPLC grade), and absolute ethanol (EtOH, Ajax Finechem) were used as received. Dichloromethane (DCM), ethyl acetate (EtOAc), and hexane (drum grade, Ajax Finechem) were distilled before use and tetrahydrofuran (HPLC grade, preservative-free, Fisher Scientific) was dried by a solvent purification system (Innovative Technologies, Inc.). *N*,*N*-dimethylformamide (DMF, Ajax Finechem) was dried with alumina (Sigma–Aldrich, activated, neutral, Brockmann I) and stored over 4-Å molecular sieves (Ajax Finechem) before use. Deionised water was prepared with a Millipore Milli-Q Academic system (18.2 MΩ cm). Silica chromatography medium was purchased from Grace Davison (Davisil LC60A, 40–63 μm). Maxisorp polystyrene 96-well plates were obtained from Nunc (ThermoFisher Scientific, Hvidovre Denmark).

NMR measurements were performed on a 300 MHz Bruker Avance III. UV-vis measurements were performed on a Varian Cary 50 Bio UV-visible spectrophotometer. TEM measurements were performed on a Phillips CM200 fitted with an SIS CCD camera and image analysis carried out using *ImageJ* software (version 1.42q, National Institutes of Health, USA). Centrifugation was performed using a Sigma 1–14 Laboratory Table-top Microcentrifuge (14800 rpm = 16163*g*). An ELISA plate reader (SpectraMax M2) was obtained from Molecular Devices (Sunnyvale, CA, USA).

The chemical environments of gold nanoparticles before and after functionalisation with the **cysBPAv** ligand were analysed by X-ray photoelectron spectroscopy using an EscaLab 250-IXL spectrometer with a monochromated Al Kα source (1486.6 eV). Take-off angle was normal (90°) to the surface. Survey and narrow scans were acquired with a pass energy of 100 and 20 eV respectively. The sample preparation consisted of drop-casting a solution of gold nanoparticles onto a silicon chip and letting it dry until the solvent evaporated.

Synthesis of BPA-valeric–NHS

A mixture of BPA-valeric acid (1.51 g, 5.26 mmol), DCC (1.30 g, 6.30 mmol), and NHS (0.732 g, 6.36 mmol) in dry THF (40 mL) was stirred for 22 h at room temperature. The mixture was filtered, the solvent removed under vacuum, the residue dissolved in dry DCM, dried over Na_2SO_4 and filtered, and the solvent removed under vacuum. The product was purified by column chromatography (silica, 8 : 2 DCM/MeOH, $R_f = 0.8$) to give the product as a white solid (0.719 g, 36 %). δ_H (300 MHz, $CDCl_3$) 7.06–7.02 (m, 4H), 6.78–6.73 (m, 4H), 4.90 (br s, 2H), 2.83 (s, 4H), 2.51–2.34 (m, 4H), 1.58 (s, 3H). *m/z* (HRMS-ESI) 406.1255 ($[M + Na]^+$ requires 406.1261).

Synthesis of *CysBPAv*

To a stirred solution of BPA-valeric–NHS (0.439 g, 1.14 mmol) in dry THF (30 mL) under a nitrogen atmosphere was added a suspension of cysteamine (90.3 mg, 1.17 mmol) and DIPEA (0.2 mL, 1.2 mmol) in dry THF (5 mL). The mixture was stirred at room temperature for 3 h, concentrated under vacuum, then ethyl acetate added and the precipitate removed by filtration. The solvent was removed under vacuum and the crude product purified twice by column chromatography (silica, first column EtOAc ($R_f = 0.5$); second column 2 : 1 EtOAc/hexane ($R_f = 0.25$)) to afford the product as a white solid (42 mg, 11 %). δ_H (300 MHz, CD_3OD) 7.03–6.98 (m, 4H), 6.72–6.65 (m, 4H), 3.28–3.23 (t, 2H), 2.56–2.52 (t, 2H), 2.36–2.31 (m, 2H), 1.99–1.96 (m, 2H), 1.54 (s, 3H). *m/z* (HRMS-ESI) 346.1467 ($[M + H]^+$ requires 346.1471), 368.1285 ($[M + Na]^+$ requires 368.1291).

Preparation of Ab∝BPA-biotin Conjugate

Biotin (0.2 g, 0.08 mmol), DCC (0.025 g, 0.12 mmol), and NHS (0.014 g, 0.12 mmol) were dissolved in anhydrous THF (10 mL). Subsequently, the mixture was stirred overnight at room

temperature, filtered to remove the precipitate, and then concentrated under reduced pressure to yield the biotin active ester.

The active ester dissolved in DMF was added slowly in droplets into Ab∝BPA solution with a biotin-antibody molar ratio of 75 to form Ab∝BPA–biotin. The reaction was allowed to proceed for 2 h at room temperature followed by 16 to 24 h at 4°C. Finally, the crude conjugate was dialysed with 50 mM K_2HPO_4 for 1.5 days with three buffer changes. After the addition of thimerosal, the antibody–biotin conjugate was stored at 4°C until use.

Preparation of Functionalised Gold Nanoparticles

All glassware was cleaned with piranha (H_2SO_4/H_2O_2 3 : 1 v/v) followed by aqua regia (HCl/HNO_3 1 : 1 v/v), then rinsed with copious amounts of distilled water before nanoparticle synthesis. A solution of 0.25 mM $HAuCl_4$ and 0.25 mM trisodium citrate in 100 mL deionised water was stirred vigorously during the addition of 1 mL of an ice-cold solution of 100 mM $NaBH_4$. The resulting mixture was stirred vigorously for an additional 10 min, resulting in a suspension of 5-nm diameter spherical gold nanoparticles.

To 1 mL of the above gold nanoparticle solution, 20 µL of 1 mM **cysBPAv** solution (in absolute ethanol) was added and the mixture left overnight at room temperature. After centrifugation at 14800 rpm (16163*g*) for 15 min, the solution was decanted and the precipitated particles were redispersed in 100 µL of absolute ethanol followed by the addition of 900 µL of distilled water.

Nanoparticle ELISA

Wells were modified with anti-BPA antibodies (Ab∝BPA) by adding 110 µL of a 10 µg mL^{-1} antibody solution and incubating overnight. After washing three times with washing solution, 1 % soy bean protein in PBS (SBP-PBS) was incubated in the wells for 1 h. Following the blocking step, the plate was washed three times with the washing solution (0.05 % Tween-20/reverse osmosis (RO) water). **CysBPAv-NP**s diluted in 10 % EtOH/PBS were added to each well and incubated for 30 min. Thereafter, the plate was washed another three times, and then 1.17 µg mL^{-1} Ab∝BPA-biotin in 10 % EtOH/PBS was added over 1 h. After three washes, avidin-HRP conjugate was added to the plate and incubated for 30 min. The plate was washed five times before the TMB substrate solution was incubated (150 µL per well) in all the microwells for 20 min to develop the colour. Stop solution (1.25 M sulfuric acid) was added (50 µL per well) to the wells to stop further colour reaction and measurement of the absorbance was conducted using a microplate reader with a dual wavelength mode at 450 and 650 nm.

Acknowledgements

The authors would like to thank the CSIRO Flagship Collaboration Fund research cluster program for financial support. Yang Lu is grateful for a CSIRO Flagship Scholarship.

References

[1] S. E. Lohse, C. J. Murphy, *J. Am. Chem. Soc.* **2012**, *134*, 15607. doi:10.1021/JA307589N

[2] E. C. Dreaden, A. M. Alkilany, X. Huang, C. J. Murphy, M. A. El-Sayed, *Chem. Soc. Rev.* **2012**, *41*, 2740. doi:10.1039/C1CS15237H

[3] M. Cao, Z. Li, J. Wang, W. Ge, T. Yue, R. Li, V. L. Colvin, W. W. Yu, *Trends Food Sci. Technol.* **2012**, *27*, 47. doi:10.1016/J.TIFS.2012.04.003

[4] K. Saha, S. S. Agasti, C. Kim, X. Li, V. M. Rotello, *Chem. Rev.* **2012**, *112*, 2739. doi:10.1021/CR2001178

[5] C. A. Richter, L. S. Birnbaum, F. Farabollini, R. R. Newbold, B. S. Rubin, C. E. Talsness, J. G. Vandenbergh, D. R. Walser-Kuntz, F. S. vom Saal, *Reprod. Toxicol.* **2007**, *24*, 199. doi:10.1016/J.REPROTOX.2007.06.004

[6] P. Fu, K. Kawamura, *Environ. Pollut.* **2010**, *158*, 3138. doi:10.1016/J.ENVPOL.2010.06.040

[7] (a) US EPA *Bisphenol A Action Plan* **2010** Available at: http://www.epa.gov/oppt/existingchemicals/pubs/actionplans/bpa_action_plan.pdf (Verified 16 October 2012)
(b) US FDA, *Bisphenol A (BPA): Use in Food Contact Application* 2010. Available at: http://www.fda.gov/newsevents/publichealthfocus/ucm064437.htm – current (Verified 16 October 2012)

[8] H. Ohkuma, K. Abe, M. Ito, A. Kokado, A. Kambegawa, M. Maeda, *Analyst* **2002**, *127*, 93. doi:10.1039/B103515K

[9] (a) Z. Brenn-Struckhofova, M. Cichna-Markl, *Food Add. Contam.* **2006**, *23*, 1227. doi:10.1080/02652030600654382
(b) Y. Lu, J. Peterson, J. Gooding, N. Lee, *Anal. Bioanal. Chem.* **2012**, *403*, 1607. doi:10.1007/S00216-012-5969-8
(c) D. Podlipna, M. Cichna-Markl, *European Food Research and Technology Zeitschrift Lebensmittel A.* **2007**, *224*, 629. doi:10.1007/S00217-006-0350-9

[10] (a) H. Fromme, T. Küchler, T. Otto, K. Pilz, J. Müller, A. Wenzel, *Water Res.* **2002**, *36*, 1429. doi:10.1016/S0043-1354(01)00367-0
(b) G. Gatidou, N. S. Thomaidis, A. S. Stasinakis, T. D. Lekkas, *J. Chromatog. A.* **2007**, *1138*, 32. doi:10.1016/J.CHROMA.2006.10.037
(c) L. Patrolecco, S. Capri, S. De Angelis, S. Polesello, S. Valsecchi, *J. Chromatog. A.* **2004**, *1022*, 1. doi:10.1016/J.CHROMA.2003.09.050
(d) Y. Watabe, T. Kondo, M. Morita, N. Tanaka, J. Haginaka, K. Hosoya, *J. Chromatogr. A* **2004**, *1032*, 45. doi:10.1016/J.CHROMA.2003.11.079

[11] A. Ballesteros-Gómez, S. Rubio, D. Pérez-Bendito, *J. Chromatogr. A* **2009**, *1216*, 449. doi:10.1016/J.CHROMA.2008.06.037

[12] (a) A. Ambrosi, F. Airò, A. Merkoçi, *Anal. Chem.* **2010**, *82*, 1151. doi:10.1021/AC902492C
(b) C.-P. Jia, X.-Q. Zhong, B. Hua, M.-Y. Liu, F.-X. Jing, X.-H. Lou, S.-H. Yao, J.-Q. Xiang, Q.-H. Jin, J.-L. Zhao, *Biosens. Bioelectron.* **2009**, *24*, 2836. doi:10.1016/J.BIOS.2009.02.024

[13] C.-L. Mao, K. D. Zientek, P. T. Colahan, M.-Y. Kuo, C.-H. Liu, K.-M. Lee, C.-C. Chou, *J. Pharm. Biomed. Anal.* **2006**, *41*, 1332. doi:10.1016/J.JPBA.2006.03.009

[14] (a) A. Radoi, M. Targa, B. Prieto-Simon, J. L. Marty, *Talanta* **2008**, *77*, 138. doi:10.1016/J.TALANTA.2008.05.048
(b) K. Chuah, L. M. H. Lai, I. Y. Goon, S. G. Parker, R. Amal, J. J. Gooding, *Chem. Commun.* **2012**, 3503. doi:10.1039/C2CC30512G

[15] F. M. Campbell, A. Ingram, P. Monaghan, J. Cooper, N. Sattar, P. D. Eckersall, D. Graham, *Analyst* **2008**, *133*, 1355. doi:10.1039/B808087A

[16] Z. Mei, H. Chu, W. Chen, F. Xue, J. Liu, H. Xu, R. Zhang, L. Zheng, *Biosens. Bioelectron.* **2013**, *39*, 26. doi:10.1016/J.BIOS.2012.06.027

[17] R. Weissleder, K. Kelly, E. Y. Sun, T. Shtatland, L. Josephson, *Nat. Biotechnol.* **2005**, *23*, 1418. doi:10.1038/NBT1159

[18] L. M. H. Lai, I. Y. Goon, K. Chuah, M. Lim, F. Braet, R. Amal, J. J. Gooding, *Angew. Chem. Int. Ed.* **2012**, *51*, 6456. doi:10.1002/ANIE.201202350

[19] (a) G. Z. Liu, S. G. Iyengar, J. J. Gooding, *Electroanalysis.* **2012**, *24*, 1509. doi:10.1002/ELAN.201200233
(b) G. Z. Liu, S. G. Iyengar, J. J. Gooding, *Electroanalysis* **2012**, in press.

[20] C. A. Staples, P. B. Dome, G. M. Klecka, S. T. Oblock, L. R. Harris, *Chemosphere* **1998**, *36*, 2149. doi:10.1016/S0045-6535(97)10133-3

[21] W. Haiss, N. T. K. Thanh, J. Aveyard, D. G. Fernig, *Anal. Chem.* **2007**, *79*, 4215. doi:10.1021/AC0702084

[22] C. A. Widrig, C. Chung, M. D. Porter, *J. Electroanal. Chem.* **1991**, *310*, 335. doi:10.1016/0022-0728(91)85271-P

[23] D. G. Castner, K. Hinds, D. W. Grainger, *Langmuir* **1996**, *12*, 5083. doi:10.1021/LA960465W

[24] E. Luais, C. Thobie-Gautier, A. Tailleur, M. A. Djouadi, A. Granier, P. Y. Tessier, D. Debarnot, F. Poncin-Epaillard, M. Boujtita, *Electrochim. Acta* **2010**, *55*, 7916. doi:10.1016/J.ELECTACTA.2010.02.070