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Short communication

Development of a colorimetric, superparamagnetic biosensor for the capture and detection of biomolecules

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ABSTRACT

Polydiacetylenes are ideal sensing materials due to their ability to change color in response to external stimuli. Here we report a new colorimetric, magnetically separable biosensor using a diacetylene monomer modified with a hydrophilic lysine derivative and superparamagnetic iron oxide nanoparticles. The particles demonstrated a distinct blue to red color transition when added to ethanol and could be quickly separated from solution with a common magnet. Surface functionalization of the particles with bovine serum albumin (BSA) showed the ability to capture and detect anti-BSA antibody from antiserum with a colorimetric response, demonstrating our biosensors can serve as a platform for diagnostic applications including point-of-care testing.

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1. Introduction

Recent years have seen an increasing demand for point-of-care testing (POCT) devices to address the urgent need for fast and accurate diagnostic tools, especially in clinical medicine. These devices are typically operated by detecting biomarkers such as pleural and serum proteins, at specific concentrations relevant to the disease being diagnosed. Ideal devices are simple of manufacture, portable, are operated with relative ease, and provide easily interpretable diagnostic results (Yager et al., 2008). Consequently, the practicality of such devices extends beyond medicine to other applications ranging from personal care to food safety to hazardous material detection. In the clinical setting, these devices are critically needed for diseases with rapidly developed adverse effects such as sepsis, and especially in areas of poor healthcare infrastructure. In this communication, we report for the first time a surface-functionalizable core–shell particle biosensor composed of a superparamagnetic iron oxide nanoparticle (SPION) core and an amphiphilic polydiacetylene (PDA) shell, capable of measurable colorimetric sensing and simple, rapid magnetic separation for the capture and detection of biomarkers (Scheme 1).

Polydiacetylenes are a class of conjugated polymers that possess unique optical properties, making them ideal platforms for biosensing applications (Lee et al., 2010). The alternating ene-yne polymer backbone structure of PDAs allow them to absorb multiple photons (Abe et al., 1992). Thus, PDA-based structures such as thin films and vesicles are capable of displaying colorimetric transitions in response to external stimuli, expressing a range of colors from dark blue to purple to bright red (Schott, 2006; Thongmalai et al., 2011). Current consensus of the underlying mechanisms indicate that conformational changes in the ene-yne backbone are responsible (Schott, 2006). Intense research efforts to exploit these properties have resulted in PDA-based sensing systems of varying capacities, from protein sensing (Kolusheva et al., 2006) to optical detection of foodborne pathogens (dos Santos Pires et al., 2011; Scindia et al., 2007). Previous studies of PDA films and vesicles have demonstrated that the PDA surfaces can be functionalized with various biomolecules to act as sensing systems (Ahn and Kim, 2008), where binding of ligands to receptors at the PDA surfaces causes stress on polymer side chains and results in the reported color changes (Rubner, 1986).

Most PDA-based sensors typically have been studied using simplified, manipulated solutions. However, these strategies become impractical for sensing applications with complex solutions, requiring the ability to capture the target analytes and then discard the solution to eliminate its interference effects. Solution-based platforms are beneficial over solid-state platforms since they increase the probability of interactions between sensor and

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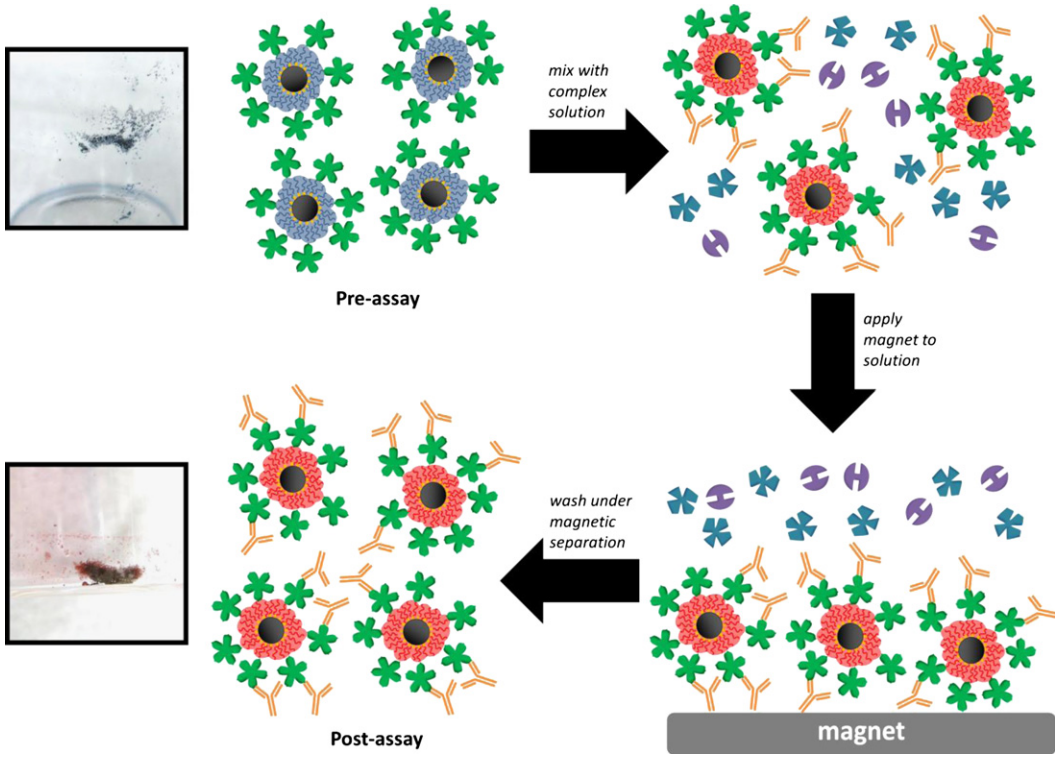

Scheme 1. Schematic representation of colorimetric, magnetically separable functionalized Lys-PCDA/SPION particles, describing the capturing and sensing of a target biomolecule from a complex solution. On the left are photographs of actual magnetically separated Lys-PCDA/SPIONs before and after the detection of ethanol.

analyte and allow for biomolecule-based analytes preserve their native form and function when in solution (Lee et al., 2010). To achieve rapid, simple particle separation, we utilized SPIONs because of their ability to act similarly to ferromagnetic materials when an external magnetic field is applied (Lu et al., 2007; Neuberger et al., 2005). To demonstrate the sensing performance of our particles in this study, we functionalize the particle surface with bovine serum albumin (BSA) to detect anti-BSA antibodies from antiserum, chosen because of their availability and relatively low costs.

2. Materials and methods

2.1. Synthesis of hydrophobic superparamagnetic iron oxide nanoparticles

Oleic acid-coated SPIONs (OA-SPIONs) were synthesized according to a previously described coprecipitation method, with minor modifications (Cui et al., 2007). Briefly, excess oleic acid was used to promote a uniform coating on the SPIONs, while undecylenic acid was not used. The OA-SPIONs were characterized using a Philips CM10 transmission electron microscope. The hydrophobic coating was verified by dispersing the OA-SPIONs in a chloroform/water mixture (see Supplementary Information).

2.2. Amphiphilic modification of diacetylene monomer

The diacetylene monomer 10,12-pentacosadiynoic acid (PCDA) (Alfa Aesar) was modified with a hydrophilic lysine derivative, N_{α},N_{α}-bis(carboxymethyl)-L-lysine hydrate (BCML) (Sigma Aldrich) via two-step coupling chemistry using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) (see Supplementary Information). The NHS-activated PCDA was reacted with a 1.2 M excess of BCML, precipitated and washed with 1 M hydrochloric acid, extracted with chloroform/ethanol (1:1), then filtered and dried to obtain a purplish white paste. To confirm the synthesis of the lysine-modified PCDA (Lys-PCDA), the product was characterized using ^{1}H nuclear magnetic resonance spectroscopy (Bruker Avance 300 MHz).

2.3. Fabrication of Lys-PCDA/SPION core–shell particle

To prepare the Lys-PCDA/SPION particles, the OA-SPIONs and Lys-PCDA were mixed together in tetrahydrofuran at various weight ratios. Self-assembly of Lys-PCDA onto OA-SPIONs was achieved through dropwise addition of the mixture into Milli-Q water under constant stirring. The particles were washed several times with water, then irradiated at 254 nm with a UV crosslinker (UVP CL-1000), causing polymerization of the Lys-PCDA surface coating. This was marked by a visible color change of the particles from brown to a black to dark blue color, depending on the percent concentration of Lys-PCDA added.

2.4. Detection of anti-bovine serum albumin antibodies

Particles used for the detection of anti-BSA antibodies were synthesized as before, excluding UV irradiation. To functionalize the particle surface with BSA, they were activated with EDC and N-hydroxysulfosuccinimide (sulfo-NHS) in a 0.1 M MES buffer, pH 4.7, and washed, repeated twice, then mixed end-over-end with 20 mg/ml BSA in 0.1 M PBS buffer, pH 7.2, overnight at 4 °C. The particles were washed to remove unconjugated BSA and mixed with 40 mM ethanolamine in water, pH 8.3, to block unreacted sulfo-NHS sites. The particles were washed several times again, then the Lys-PCDA coating was crosslinked at 254 nm in 10 bursts of 1 mJ/cm^2. All washes were performed with PBS buffer and magnetic separation.

The assay was performed by incubating approximately 1.25 mg/ml of BSA/Lys-PCDA/SPION particles in 1 ml aliquots of

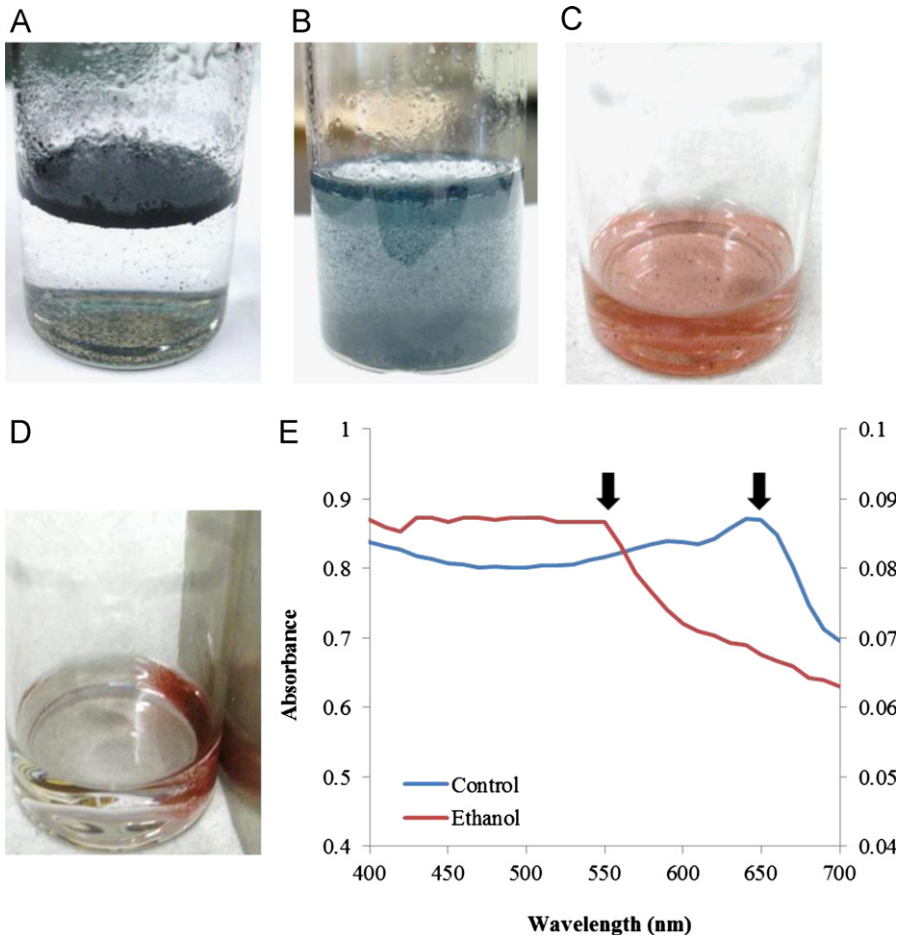

Fig. 1. Demonstration of the colorimetric and magnetic properties of Lys-PCDA/SPIONs. (A) PCDA/SPIONs in water, (B) Lys-PCDA/SPIONs in water, (C) in ethanol, (D) while applying a magnet, and (E) visible absorbance spectra before and after addition of ethanol. The increase at 550 nm and decrease at 650 nm marked with black arrows correspond to the color transition.

various concentrations of rabbit anti-BSA antiserum, of which anti-BSA polyclonal antibodies formed a ~10% fraction of the antiserum proteins according to manufacturer's specifications (Rockland Immunochemicals Inc.). The particles were continuously mixed with a low speed vortex mixer overnight, then washed several times with PBS buffer using magnetic separation. The particle color transition was measured with a BioTek Epoch UV–visible spectrophotometer in the visible absorbance spectrum.

The color transition was quantified as a colorimetric response (*CR %*), using the following (Kolusheva et al., 2001):

$$CR\,\% = \frac{(PB_0 - PB_1)}{PB_0} \times 100\%$$

where $PB = A_{blue}/(A_{blue}+A_{red})$, A was the UV–visible absorbance value of the particle's "blue" state at 650 nm or the "red" state at 550 nm, and PB_0 and PB_1 were the respective colorimetric ratios of the particles before and after the assay.

3. Results and discussion

3.1. Characterization of Lys-PCDA/SPION-based biosensors

Our objective in this study was to develop a magnetically separable sensor capable of displaying a distinct colorimetric response to receptor–ligand interactions, and thus suitable for POCT sensing applications. By incorporating a hydrophilic lysine derivative, the Lys-PCDA/SPIONs display sustained suspension in aqueous solutions compared to particles formed with unmodified PCDA (Fig. 1(A–B)), essential for maximizing particle interaction with aqueous soluble analytes such as serum proteins. To confirm the color functionality of the sensor, the particles were added to ethanol, resulting in a sharp color transition from a blue to a red state, similar to previously described PCDA vesicles (Charoenthai et al., 2011; Kolusheva et al., 2006; Scindia et al., 2007). This also resulted in an absorbance peak shift from 650 nm to 550 nm (Fig. 1(C–E)). Ethanol has been previously reported to cause PDA vesicles to change from blue to red, due to swelling of the PDA layers as ethanol interacts with the PDA carboxyl group through hydrogen bonding (Charoenthai et al., 2011). Applying a magnetic field to the particles in solution resulted in quick particle separation without causing changes in color. In addition, the particles remained magnetically separable after undergoing the color transition. This was marked by the solution becoming clear and colorless and was easily observed with the naked eye due to the particle size (Fig. 1(D)), illustrating the successful capture of particles after a sensing event. In rapid diagnostic applications involving complex or non-manipulated fluids, the ability to quickly and easily separate analytes for analysis without adversely affecting the sensor is a necessary functionality, which our particles demonstrated through this initial performance characterization.

3.2. Colorimetric performance of Lys-PCDA/SPION sensors

Biosensors developed for POCT applications must meet several criteria (Yager et al., 2008), including: yielding easily interpretable

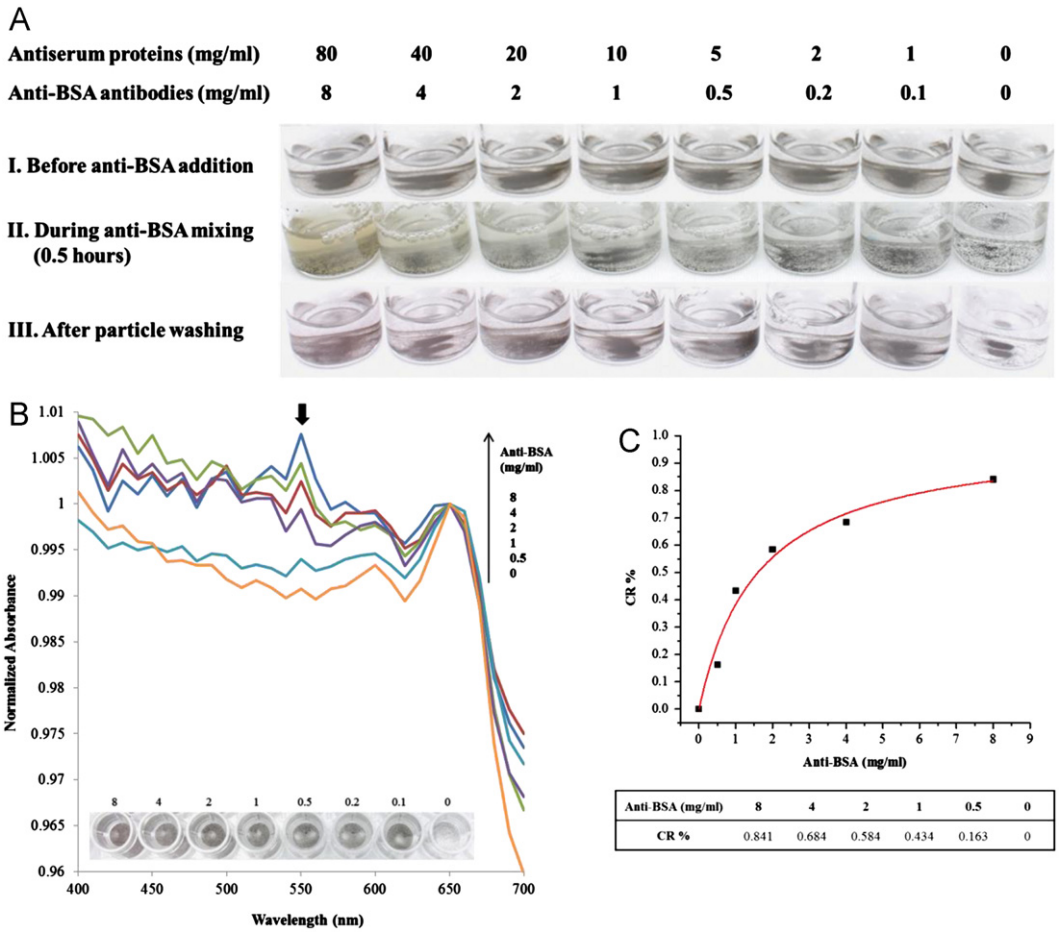

Fig. 2. Biosensor performance of BSA-functionalized Lys-PCDA/SPIONs. (A) Particles before, during, and after incubation in anti-BSA antiserum. (B) Comparison of colorimetric response after detection of anti-BSA. Responses are measured at the "red" state peak at 550 nm, indicated by the bolded black arrow. Overlaid is a photograph of a subsection of the 96-well microplate used, labeled with the anti-BSA concentrations. (C) Regression analysis of the colorimetric response values. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

results, requiring little to no sample manipulation, and requiring no specialized training or equipment to operate. Many serum proteins of interest are commonly present at low concentrations, requiring signal amplification of the detection event, such as a colorimetric response. To generate easily interpretable, equipment-free results, it is critically important that the detection of specific concentrations or concentration ranges correspond to distinguishable colors or color gradients. In this study, we sought to demonstrate the suitability of the Lys-PCDA/SPIONs for POCT applications by demonstrating the detection of receptor–ligand interactions, using BSA-functionalized sensors to detect anti-BSA antibodies. Previous studies of PDA-based sensors have demonstrated Similarly, our functionalized Lys-PCDA/SPIONs sensors exhibit a visible, increasing colorimetric response as the concentration of anti-BSA are increased, ranging from a blackish blue color to a gradient of increasingly lighter purples (Fig. 2(A)).

The use of anti-BSA in antiserum format highlights the importance of magnetic separability for analyte capture and detection, especially for POCT applications. As shown in Fig. 2(A-II), the purified antiserum appears as a clear reddish yellow solution, which hindered our ability to accurately judge the degree of color response with the naked eye at the end of the assay. Based on our current results and previous studies of PDA-based sensors (dos Santos Pires et al., 2011), PDAs are known to exhibit a color response ranging from dark purple to bright red in response to stimuli. It can be safely inferred that PDA-based sensors used with non-manipulated blood or other dark colored samples would mask any color transitions and make accurate interpretations of the results with the naked eye very difficult. As a result of the SPION cores, the particles demonstrated quick separation from solution with a common magnet, avoiding the need for specialized equipment to wash and observe the particles, and allowing us to accurately observe the increased expression of purple among the different samples (Fig. 2(A-III)). Based on the visual observations alone, Lys-PCDA/SPION particles can serve as a platform for the colorimetric detection and capture of analytes from solution, both ideal properties for POCT applications.

To verify the accuracy of the visual observations and illustrate the analytical performance of Lys-PCDA/SPION sensors, the visible absorbance spectra of the samples were measured and normalized against the "blue" state peak at 650 nm. The absorbance at 550 nm (the "red" state), which is used to calculate the degree of color change for PDA-based sensors, showed an increasing trend as the concentration of anti-BSA was increased (Fig. 2(B)). Similar trends of increasing "red" state absorbance values in response to increasing analyte concentrations have previously been reported, including in the detection of *S. typhimurium* cultures (Scindia et al., 2007) and amino acids (Kolusheva et al., 2006). Quantitative analysis, expressed as CR %, described an increasing colorimetric response of 0.163, 0.434, 0.584, 0.684, and 0.841% for samples with 0.5, 1, 2, 4, and 8 mg/ml anti-BSA, respectively, compared to particles from the 0 mg/ml anti-BSA sample (Fig. 2(C)). In comparison, when the particles were added to ethanol, a maximum CR % of only 1.796% was achieved, indicating that approximately half of the possible color response range was achieved with our particles.

We expect that greater color distinctions between differing amounts of analyte and expression of the entire color response range can be achieved by optimization of the ratios of: 1) receptor molecules to a given sensor surface area, and 2) Lys-PCDA to SPIONs. This is suggested by the different color response achieved with anti-BSA compared to the response to ethanol. Previous studies of PDA-based sensors, such as with ethanol (Charoenthai et al., 2011) and bacteria (dos Santos Pires et al., 2011), have demonstrated that chemical and electrostatic interactions are responsible for the color transitions, respectively. Such interactions involve the entire PDA system interacting, whereas receptor–ligand binding at the PDA surface achieves localized stress-induced responses, thus requiring optimization of the ratio of receptors to sensor surface area to achieve maximum color response. Secondly, the results indicated that optimization of the ratio of Lys-PCDA to SPION is needed. It has been previously demonstrated that lower ratios of PDA to non-PDA material results in lower color responses and higher ratios should result in larger responses (Kang et al., 2012). As shown in Fig. 1B, the particles tested with ethanol contained a higher ratio of Lys-PCDA to SPION, which formed particles with a more intense initial blue color. At a lower ratio, the sensors appeared less intensely blue since less PDA material was present (Fig. 2A), which may have also caused the difference in color response to anti-BSA compared to the response to ethanol. Nonetheless, the colorimetric response values of the functionalized Lys-PCDA/SPION sensors showed a hyperbolic regression with a coefficient of determination of 0.9806 (see Supplementary Information), also indicating that if larger amounts of anti-BSA had been tested, the full color response range would have likely been realized. Ultimately, these results demonstrate that our particles can be used as analytical biosensors in addition to semi-quantitative POCT applications.

4. Conclusions

In this study, we have successfully illustrated the simple synthesis and application of novel Lys-PCDA/SPION particles as a platform for the capture and detection of serum proteins. Although only a proof-of-concept investigation was performed, our biosensor showed a colorimetric response that corresponded to an increasing concentration of anti-BSA antibodies, with a lower detection limit of 0.5 mg/ml. Thus, in its current form, our biosensor would be useful for diagnostic applications where high biomarker concentrations are indicative of disease, as well as non-clinical applications where analyte detection is needed in the mg/ml range. Additionally, the colorimetric response was both easily visualized with the naked eye and could be measured with a basic UV–vis spectrophotometer. The response could be accurately measured because the particles could be quickly separated from a mixed solution with a common magnet, eliminating the need for advanced equipment such as high-speed centrifuges or laborious wash techniques. We are currently studying ways to improve the sensing performance of the particles and achieving a greater range of colorimetric response, including increasing the Lys-PCDA to SPION ratio and varying the amount of receptor molecules used, and believe we have clearly demonstrated that our particles can be quickly developed for clinical applications to address a pressing need for POCT platforms.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.10.008.

References

Abe, S., Schreiber, M., Su, W.P., Yu, J., 1992. Physical Review B 45 (16), 9432–9435.
Ahn, D.J., Kim, J.-M., 2008. Accounts of Chemical Research 41 (7), 805–816.
Charoenthai, N., Pattanatornchai, T., Wacharasindhu, S., Sukwattanasinitt, M., Traiphol, R., 2011. Journal of Colloid and Interface Science 360 (2), 565–573.
Cui, L., Xu, H., He, P., Sumitomo, K., Yamaguchi, Y., Gu, H., 2007. Journal of Polymer Science Part A: Polymer Chemistry 45 (22), 5285–5295.
dos Santos Pires, A.C., Ferreira Soares, N.d.F., Mendes da Silva, L.H., Hespanhol da Silva, M.d.C., De Almeida, M.V., Le Hyaric, M., de Andrade, N.J., Soares, R.F., Mageste, A.B., Reis, S.G., 2011. Sensors and Actuators B—Chemical 153 (1), 17–23.
Kang, D.H., Jung, H.-S., Lee, J., Seo, S., Kim, J., Kim, K., Suh, K.-Y., 2012. Langmuir 28 (19), 7551–7556.
Kolusheva, S., Kafri, R., Katz, M., Jelinek, R., 2001. Journal of the American Chemical Society 123 (3), 417–422.
Kolusheva, S., Zadmard, R., Schrader, T., Jelinek, R., 2006. Journal of the American Chemical Society 128 (41), 13592–13598.
Lee, K., Povlich, L.K., Kim, J., 2010. Analyst 135 (9), 2179–2189.
Lu, A.-H., Salabas, E.L., Schüth, F., 2007. Angewandte Chemie International Edition 46 (8), 1222–1244.
Neuberger, T., Schöpf, B., Hofmann, H., Hofmann, M., von Rechenberg, B., 2005. Journal of Magnetism and Magnetic Materials 293 (1), 483–496.
Rubner, M.F., 1986. Macromolecules 19 (8), 2129–2138.
Schott, M., 2006. The Journal of Physical Chemistry B 110 (32), 15864–15868.
Scindia, Y., Silbert, L., Volinsky, R., Kolusheva, S., Jelinek, R., 2007. Langmuir 23 (8), 4682–4687.
Thongmalai, W., Eaidkong, T., Ampornpun, S., Mungkarndee, R., Tumcharern, G., Sukwattanasinitt, M., Wacharasindhu, S., 2011. Journal of Materials Chemistry 21 (41), 16391.
Yager, P., Domingo, G.J., Gerdes, J., 2008. Annual Review of Biomedical Engineering 10, 107–144.