VIP Biosensors

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The Biochemiresistor: An Ultrasensitive Biosensor for Small Organic Molecules**

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We introduce a new type of biosensor, which we call a biochemiresistor, that can provide exquisitely low detection limits, rapid response times, and good specificity in biological samples. Wohltjen and Snow[1] introduced the concept of chemiresistors that measure changes in resistance across a nanoparticle film owing to the presence of small organic molecules. The nanoparticles are modified with an organic layer to allow moderate selectivity for a given analyte. The penetration of the analyte molecules into the nanoparticle film causes an increase in separation between the particles in the film and thus an increase in film resistance. Wohltjen and Snow demonstrated that this novel concept has a detection range of $\delta = 2.7$ to 11 000 ppm for organic molecules such as toluene and tetrachloroethylene.[1] Other groups have further developed this idea for detecting other vapor-phase analytes by modifying particles with alkane dithiols[2] and alkane thiols with various functional groups.[3] Successful examples of arrays of chemiresistors and neural networks include the detection of volatile markers from the breath of cancer patients.[4] Furthermore, chemiresistors have recently been shown to be able to detect small organic molecules in aqueous solution, including seawater,[5] which greatly extends the utility of this sensing concept.

The attractiveness of chemiresistors is their simplicity and rapid response time. The disadvantage is that they are only partially selective for the analyte. Traditionally, such a disadvantage is overcome using biological recognition to provide specificity. The challenge for chemiresistors however is that for a measurable change in resistance to occur, the analyte must penetrate into the interstices in a nanoparticle film to change the separation between nanoparticles. It is difficult to envisage how this could be achieved with large protein recognition elements, which are typically used in biosensors. Herein, we demonstrate how this can be done by temporally and spatially separating the biorecognition of the analyte from the measurement of the resistance using gold-coated magnetic nanoparticles modified with antibodies (Ab-Au@MNPs) and magnetically assemble nanoparticle films over interdigitated electrodes (IDEs; Figure 1 a). This process cannot be achieved using a conventional immunoassay where the sensing surface is modified with antibodies that capture the analyte. This is because Ab-Au@MNPs are already well-separated such that the film would be highly resistive and thus the capture of analyte would have little or no impact on the film resistance. As a consequence, we designed an antibody displacement assay as depicted in Figure 1.

To use Au@MNPs as chemiresistors, we must first show that a chemiresistor can be readily formed by magnetically driven assembly. Typically, chemiresistors are characterized by measuring the resistance of a nanoparticle film deposited across a pair of interdigitated electrodes as a function of the separation between the particles. The separation is defined by the length of the alkyl chain of an alkanedithiol that is used to link the particles together. In sensing, organic matter that absorbs into this network further increases the spacing of the particles with a concomitant increase in resistance. To mimic the usual approach in characterizing chemiresistors, different batches of Au@MNPs were modified with different mercaptoalkyl alcohols with alkyl chain lengths ranging from 3 to 14 carbon atoms. Upon magnetic assembly over interdigitated electrodes with a gap width of 5 μm, followed by expunging excess fluid, and compacting the Au@MNPs network with a glass coverslip, the resistance between the interdigitated electrodes was measured.

As expected, the longer the alkyl chain of the mercapto alkyl alcohol, the greater the resistance of the Au@MNP network (Figure 2 a). The resistance increases exponentially with the length of the alkyl chain as predicted by the relationship:

$$R = R_0 e^{-\beta d}$$

where R is the resistance, R_0 is the resistance when bare particles are in contact, d is the spacing between particles, and β represents the attenuation factor. However, the β value was not the usual 0.8 to 1 $Å^{-1}$ observed for saturated alkyl chains in molecular junction and electrochemical experiments,[6] but rather a significantly lower value of 0.14 $Å^{-1}$. This deviation

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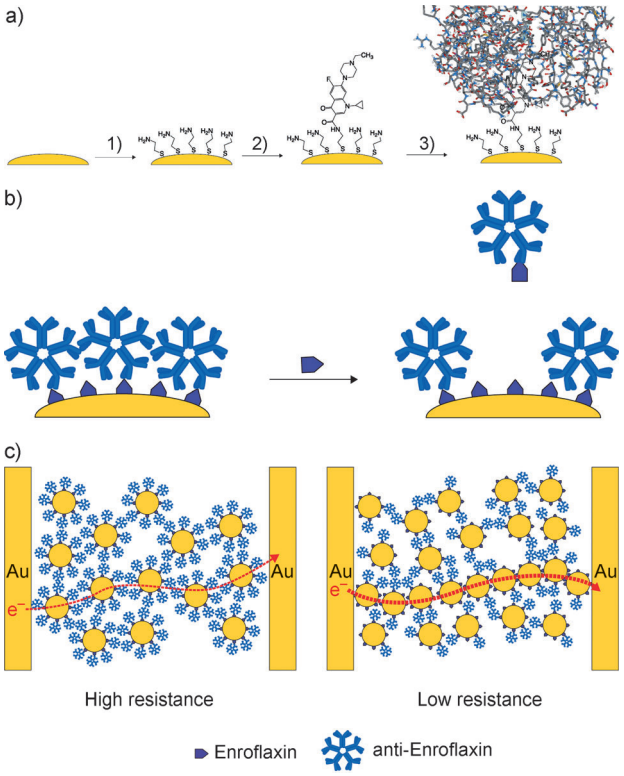

Figure 1. The biochemiresistor concept. a) Functionalization of Au@MNPs (yellow) with a self-assembled monolayer of cysteamine (step 1) followed by the attachment of enrofloxacin (2). The surface-bound enrofloxacin serves as selective binding sites for anti-enrofloxacin IgM antibodies Ab-Au@MNPs (3). b) The Ab-Au@MNPs are the biosensing particles. When distributed into a sample solution that contains enrofloxacin, some of the anti-enrofloxacin dissociates from the Ab-Au@MNPs to bind with the enrofloxacin in solution. c) Ab-Au@MNPs magnetically assembled between two interdigitated electrodes. Such films have high resistance. After exposure to the solution sample, and dissociation of antibodies, some particles in the nanoparticle film can approach each other more closely, and the resistance of the film decreases. The greater the amount of analyte, the more antibodies are displaced from the surface of the Au@MNPs, and the lower the resistance.

from the expected β value for simple electron-transfer systems can be explained by percolation theory, as derived for nanoparticle films by Muller et al.[7] In this theory, local disorder in the form of fluctuations in the separation gaps between adjacent nanoparticles can enhance the film conductivity and reduce the dependence of the conductivity on the length of the intervening molecules. Essentially, this suggests that the spatial disorder in the films creates some high-conductance pathways. The result is the variation in conductance is linear with the exponential of the average spacing between particles, but that the attenuation factor (β value) is less than that observed for conventional electron transfer across organic monolayers or single molecular junction experiments. Such spatial disorder in the Au@MNP films formed by magnetic assembly is verified by cryogenic transmission electron-microscopy images (Supporting Information, Figure S1). The resistance measured from different mercaptoalkyl alcohols show that the Au@MNP networks,

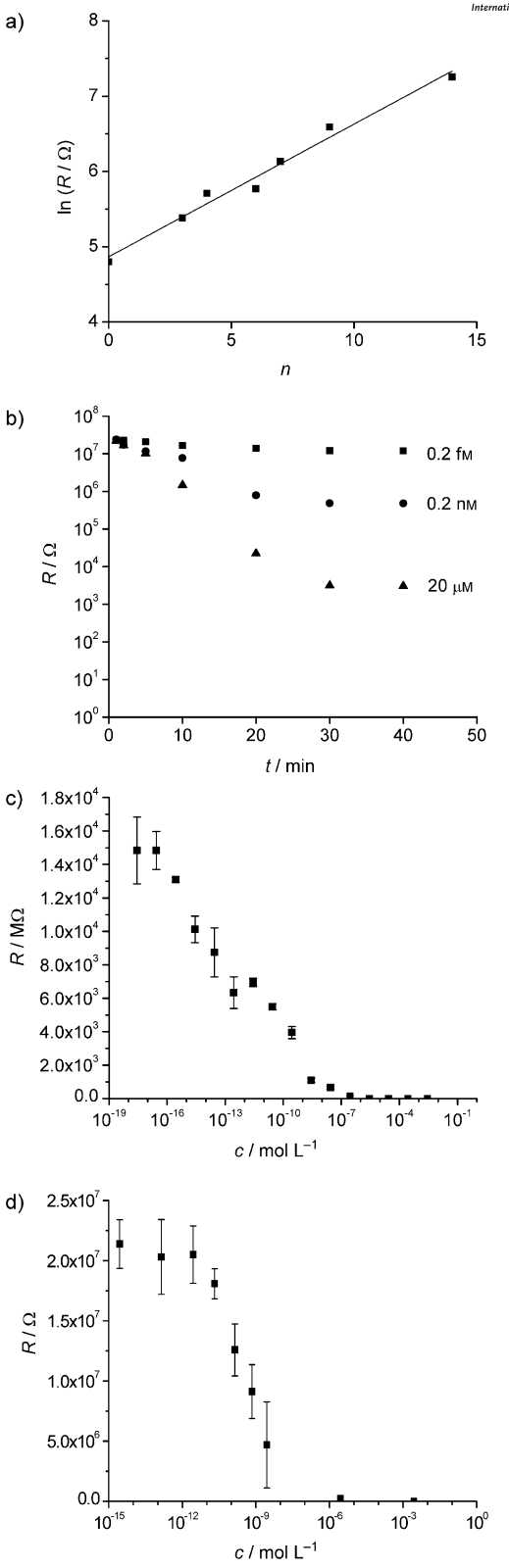

Figure 2. a) Natural logarithm of resistance R versus number of carbon atoms n for mercapto-alcohols of different lengths. A linear dependence between the length and the resistance measured is shown. b) Response time plot of three different concentrations of enrofloxacin (28 μM, 0.28 nM, and 0.28 fM). The response time seen was less than 40 min after the addition of enrofloxacin. c) Plot of measured resistance versus the concentration of enrofloxacin used. A detection limit of 0.28 fM was seen with this Au@MNP biochemiresistor sensor system. d) Calibration curve for the detection of enrofloxacin in milk using Au@MNPs in a biochemiresistor setup procedure.

formed by magnetic assembly, behave in a similar manner to chemiresistors (Figure 2a).

To create a biochemiresistor, Au@MNPs with an average diameter of 100 nm were synthesized using a method we previously developed.[8] The Au@MNPs were then modified with a self-assembled monolayer of mercaptoethanolamine followed by covalent attachment of the veterinary antibiotic, enrofloxacin, (Figure 1a) to the amine-terminated self-assembled monolayer using carbodiimide coupling (see the Supporting Information for full details). The steps in the formation of this interface were characterized by X-ray photoelectron spectroscopy (XPS; Supporting Information, Figure S2). The immobilized enrofloxacin was used as an epitope to which anti-enrofloxacin IgM antibodies can bind. As the Au@MNPs are now coated with proteins, the resistance of a resultant Au@MNP film is expected to be high. Placing these IgM-modified Au@MNPs in a sample solution containing enrofloxacin causes competition between the surface-bound enrofloxacin and enrofloxacin in solution for the antibodies. If some of the IgM antibodies dissociate from the Au@MNP surface then the resistance of the resultant films will decrease. However, to characterize this novel detection system, a number of questions need to be addressed: 1) Do proteins and other non-specific antibodies foul the nanoparticles causing an increase in resistance? 2) What is the response time of the system? And most importantly, 3) how much analyte can the system detect? We will answer each of these three questions in turn.

To answer the first question, the enrofloxacin-modified nanoparticles were exposed to three different types of proteins (BSA, anti-biotin, and anti-enrofloxacin in concentrations of 1 mM). These proteins each served to investigate the antifouling properties of the modified Au@MNPs and also the specificity of the antibody to the analyte coupled to the surface. The particles exposed to no protein had a measured resistance of $(212 \pm 20)\ \Omega$ when assembled into a film. When the particles were exposed to proteins, such as BSA and anti-biotin IgG antibodies, similar resistances of $(214 \pm 19)\ \Omega$ and $(350 \pm 89)\ \Omega$, respectively, were measured. The slightly higher resistance observed with anti-biotin IgG antibodies was attributed to minor amounts of nonspecific adsorption of protein onto the particles. However, this was not significant compared to the dramatic increase in resistance (to $(23.0 \pm 1.7) \times 10^6\ \Omega$) when the anti-enrofloxacin antibody was complexed with the Au@MNPs to give Ab-Au@MNPs. This dramatic change of resistance is due to the complexation of the specific antibodies onto the nanoparticle surface, creating a much thicker resistive layer between each particle in the film.

Next, we explored the ability of the anti-enrofloxacin antibodies to be displaced from the Au@MNP surface and the timescale over which this displacement occurred. This was explored for three concentrations of enrofloxacin and a constant concentration of enrofloxacin modified Au@MNPs in solution ($8\ g\,L^{-1}$). The change of resistance over time with the three different concentrations (Figure 2b) shows that the response reached a maximum level after 40 min incubation, even for femtomolar concentrations of analyte. Henceforth measurements were taken after 40 minutes incubation.

Thereafter, the detection limit of the Au@MNPs biochemiresistor sensor was tested by exposing the sensor to 2.8 mM to 2.8 aM of enrofloxacin. After the displacement, the Au@MNPs were washed and resuspended in PBS buffer. The particles were then deposited over the IDEs and the resistance of the film measured (Figure 2c). The calibration response curves had a sigmoidal trend, which is typical of displacement assay response. The detection limit of the system is 0.28 fM of analyte in solution. This detection limit is 10^6 times lower than the same setup with a flat electrode surface and using Faradaic electrochemistry as method of detection (30 pM).[9] The detection limit is also orders of magnitude lower than for other techniques, such as HPLC (310 nM),[10] immunoassays (3 nM),[11] and LC-MS (1 nM).[12] Furthermore, the signal detected with the Au@MNP biochemiresistor sensor had a range of Ω to MΩ, for 2.8 μM and 0.28 fM of analytes, respectively, which is important for clear discrimination between different concentrations and allows for the development of an ultrasensitive system.

Finally, the biochemiresistor sensor was exposed to a complex matrix of milk to validate the detection of enrofloxacin in real-life practical situations. We spiked neat milk with various amounts of enrofloxacin and performed the measurements with no further purification or extraction. As seen in Figure 2d, the calibration curve was reproduced in milk with a detection range of 1 nM to 1 pM and a lowest detection limit of 2.8 pM. This detection range and limit is higher than observed in buffer, which was previously shown to be due to calcium ions and possibly proteins in the milk binding to enrofloxacin and preventing detection.[9] An unknown spiked solution of milk was analyzed using the biochemiresistor in which the amount of enrofloxacin was determined to be $(21.9 \pm 3.7)\ pg\,mL^{-1}$. When the same blind sample was measured using a commercial ELISA kit, the measured amount of enrofloxacin was $(23.2 \pm 3.4)\ pg\,mL^{-1}$. These two systems showed good agreement; however, the time and preparation of analysis of the biochemiresistor sensor compared to the ELISA was far superior. With no requirement of sample preparation, and a detection time of merely 40 min, the biochemiresistor sensor proves to be a better alternative in detection of small molecules in complex media, such as milk.

In conclusion, a Ab-Au@MNPs biochemiresistor sensor has been developed for the detection of an antibiotic, namely enrofloxacin. To achieve this, a displacement assay concept was employed, which changes the distance between the nanoparticles by the displacement of antibodies off the surface of the nanoparticle. A change of the distance between the nanoparticle therefore results in a change in measured resistance. The magnetic assembly of the nanoparticles allows most of the nanoparticles to be positioned between the IDE, and thus a continuous nanoparticle network can be formed to provide a conductive pathway for resistance measurements. This system yields a detection limit of 0.28 fM, which is 10^6 times more sensitive compared to flat Faradaic electrochemical devices using the same concept. Furthermore, a detection range of 2.8 μM to 0.28 fM was observed with a response time of 40 min reported. Reproducibility of the measurements can be easily achieved by ensuring constant

amount of Au@MNPs were used and uniform suspension of the particles in solution were attained throughout the sample preparation steps. The application of Ab-Au@MNP in the biochemiresistor sensor setup opens up the application of nanoparticles in the collection and detection of non-electroactive species in solution. The system was also tested with real-life samples of milk, and a detection limit of 2.8 pM was demonstrated.

The biochemiresistor described is a generic concept that can be applied to a wide range of analytes by simply changing the antibodies coupled onto the Au@MNPs. Thus, the biochemiresistor is a low-cost sensor concept that provides an entire new avenue for facile and rapid detection of biologically relevant species, such as hormones, drugs, metabolites, and other physiologically relevant analytes, in biological fluids.

Experimental Section

Au@MNPs were synthesized according to Goon et al.[8] and subsequently modified with enrofloxacin and anti-enrofloxacin. The displacement assay was performed by incubating the modified Au@MNPs into a sample solution containing free enrofloxacin molecules. After an incubation period of 40 min, the Au@MNPs were magnetically separated and washed with PBS and then deposited over IDEs (CSIRO, Australia) and the resistance measured with a voltmeter (BK Precision, Yonda Linda, USA). Detailed experimental and characterization methods are provided in the Supporting Information.

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