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Detection of copper ions in drinking water using the competitive adsorption of proteins

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ABSTRACT

Heavy metal ions, i.e., Cu^{2+}, are harmful to the environment and our health. In order to detect them, and circumvent or alleviate the weaknesses of existing detecting technologies, we contrive a unique Surface Plasmon Resonance (SPR) biosensor combined with competitive adsorption of proteins, termed the Vroman effect. This approach adopts native proteins (albumin) as bio-receptors that interact with Cu^{2+} to be denatured. Denaturation disrupts the conformation of albumin so that it weakens its affinity to adsorb on the sensing surface. Through the competitive adsorption between the denatured albumins and the native ones, the displacement occurs adjacent to the sensing surface, and this process is real-time monitored by SPR, a surface-sensitive label-free biosensor. The affinities of native albumin is significantly higher than that of denatured albumin, demonstrated by measured K_D of native and denatured albumin to gold surafce, $5.8 \pm 0.2 \times 10^{-5}$ M and $5.4 \pm 0.1 \times 10^{-4}$ M, respectively. Using our biosensor, Cu^{2+} with concentration down to 0.1 mg/L is detected in PBS, tap water, deionized water, and bottled water. The SPR biosensor is characterized for 5 different heavy metal ions, Cu^{2+}, Fe^{3+}, Mn^{2+}, Pb^{2+}, and Hg^{2+}, most common heavy metal ions found in tap water. At the maximum contaminant level (MCL) suggested by the United States Environmental Protection Agency (EPA), the SPR biosensor produces 13.5 ± 0.4, 1.5 ± 0.4, 0, 0, and 0 mDeg, respectively, suggesting the biosensor may be used to detect Cu^{2+} in tap water samples.

1. Introduction

Heavy metal ions refer metallic elements, having a relative high density, such as iron, cobalt, copper, manganese, molybdenum, zinc, mercury, plutonium, and lead. Heavy metals are dangerous because they tend to bioaccumulate. Among them, copper ions is considered to be one of critical heavy meal ions contained in drinking water as it is a common contaminant produced by corrosion of household plumbing systems and erosion of natural deposits, and may induce gastrointestinal distress, even liver or kidney damage (Brewer, 2010).

A number of techniques have been developed over the years for heavy metal ion analysis, including atomic absorption spectrometry (Bannon and Chisolm, 2001), inductively coupled plasma mass spectrometry (Liu et al., 1999), anodic stripping voltammetry (ASV) (Yang et al., 2003; Baldo et al., 2004), X-ray fluorescence spectrometry (Eksperiandova et al., 2002), and microprobes (Arai et al., 2003). For instance, ASV is a powerful established technology for detection of metal ions and other electrochemically active substances (Wang, 1985, 1990). However, ASV has several limitations, including the need of the use of supporting electrolyte, the use of mercury electrode, high background current challenging to detect small stripping current associated with oxidation of analytes, incapability of detecting non-redox-active species, and formation of intermetallic compounds to disturb individual stripping peaks (Wang et al., 2007).

To design highly selective metal ion sensors, several innovative recognizing elements based on organic chelators (Burdette et al., 2003), organic polymers (Wu et al., 2000), proteins (Darwish and Blake, 2002), cells (Shetty et al., 2001), DNA/RNA and peptides (Yang et al., 2003, 2001; Deo and Godwin, 2000; Shults et al., 2003; Mlynarz et al., 2002) have been reported. However, most of these approaches are perhaps challenged by the design and synthesis of sensors capable of specific and strong binding of the target metal ions but not other competing metal ions (Lu et al., 2003). Besides, peptide biosensors are limited by the fact that many toxic metal ions do not have specific probes (Wang et al., 2007; Forzani et al., 2005). DNA/RNA biosensors must be used under rigorous condition of measurement which must allow survival of the cell (narrow pH and temperature ranges, lack of other toxic compounds, etc.) (Rouch et al., 1995). Metal-binding proteins are synthesized by many cell types in response to the presence of specific metals and play important roles in metal homeostasis and detoxification mechanisms (Beveridge et al., 1996). However, many technical challenges exist including complex overexpression and purification of proteins and labor-intensive, time-consuming processes (Bontidean et al.,

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2000, 1998). Therefore, a rapid, less-complex and inexpensive method that permits real-time detection of metal ions has been demanded for a variety of applications. In addition, due to the danger that the heavy metal ions pose to operators, minimal sample handling is desirable.

In this work, we report a unique sensing method of copper ions by combining Surface Plasmon Resonance (SPR), a surface-sensitive optical instrument, with competitive adsorption of proteins, termed the Vroman effect. SPR sensitively responds to minute change of refractive index at the vicinity of a sensing surface that may be produced by the conformation change of proteins, induced by copper ions. SPR biosensor is label-free so as to circumvent the labeling process that may interfere with the behavior of proteins (Haab, 2003; Yu et al., 2006). When albumins are engineered as the receptor of copper ions on the sensing surface, their conformation change is caused by disruption of ionic interactions (salt bridge) or disulfide bonds by copper ions (Bontidean et al., 1998; Sharma et al., 2008). Consequently, these denatured albumins possess weaker affinity than native ones (Renwrantz and Werner, 2008; Jung et al., 2003). By comparing the affinity of denatured albumins with that of native albumin it may be possible to distinguish one from the other. However, such direct comparison method suffers from fluctuation of measurement (Supplementary Table 1). Here we propose a unique method to obviate the measurement accuracy challenge by adopting the competitive adsorption between them. The weaker denatured albumins pre-adsorbed on sensing surface can be replaced by native ones possessing stronger affinity. This competitive adsorption process may be real-time monitored by SPR, as shown in Fig. 1a.

In traditional methods, bio-receptors, i.e., antibodies, protein lysates, peptides, and aptamers, are used to capture target proteins via specific binding affinity. However, these capturing techniques are often limited by their weak binding affinity with specific analyte, non-specific adsorption, low reproducibility, and difficulties integrating onto the surface of transducers (Chaerkady and Pandey, 2008; Shankaran and Miu, 2007; Choi et al., 2008; Choi and Chae, 2009b; Yang and Chae, 2008). One potential method to alleviate these challenges is to utilize a nature characteristic of proteins, the competitive adsorption, termed the Vroman effect that is reported by Vroman and Adams, (1969). Since then many studies have been performed to study the fundamental mechanisms of this phenomena (Slack and Horbett, 1989; Leonard and Vroman, 1992; Turbill et al., 1996). The competitive adsorption targeted here occurs between the different proteins competing to adsorb to the surface, when more than one type of protein is present in solution. When lower-affinity proteins are adsorbed on the surface first, they can be displaced by higher-affinity proteins arriving at the surface at a later point in time (Latour, 2005). Moreover, only low-affinity proteins can be displaced by high-affinity proteins (usually possessing larger size and higher molecular weight), yet the reverse sequence does not occur (Choi and Chae, 2009a; Wang et al., 2011; Choi et al., 2010).

In this work, we engineer the affinity of native albumins by introducing copper ions to disturb bonds of the albumins. Albumins are denatured by copper ions mainly due to the disruption of tertiary structure, in which copper ions disrupt ionic interactions

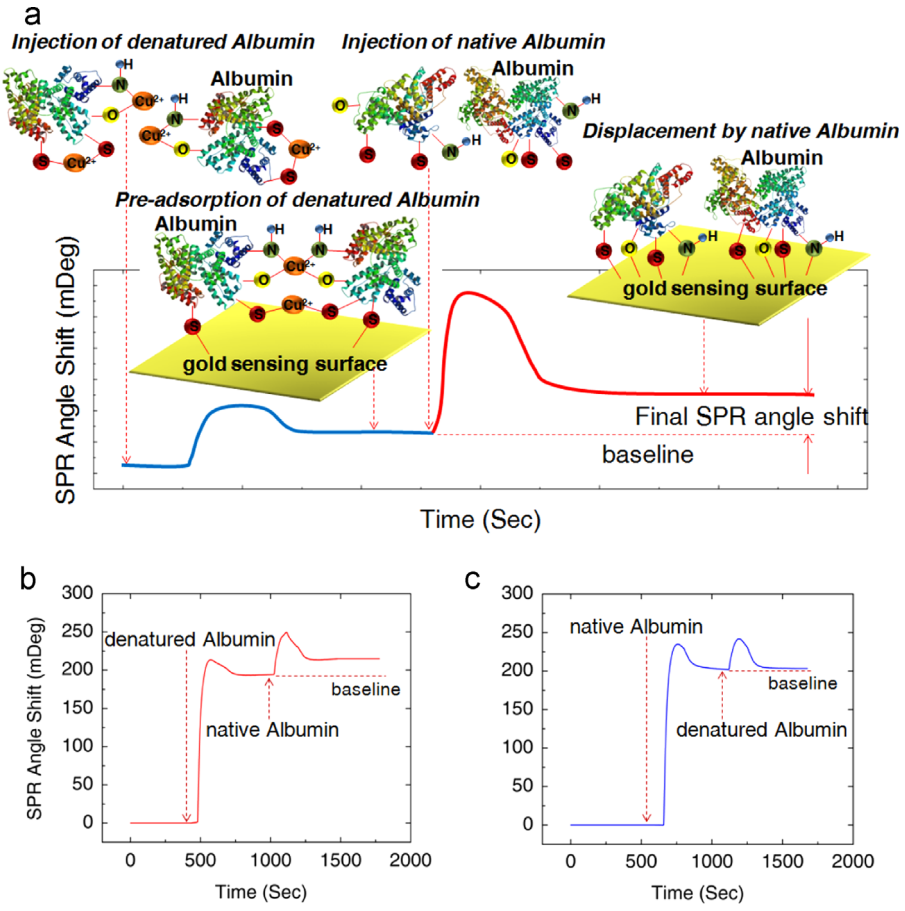


Fig. 1. The SPR angle profile describes the competitive adsorption of albumins. (a) Albumin is denatured by Cu^{2+}, through the disruption of ionic interactions and disulfide bonds. Due to the weaker affinity, the pre-adsorbed denatured albumin is displaced by native albumin. Consequently, the conformation change adjacent to the gold sensing surface generates the change of SPR angle shift. (b) Consistent with a, the denatured proteins are replaced by native ones, which generates the increase of SPR angle shift. (c) However, the reverse sequence does not occur, and change of SPR angle shift is negligible.

(salt bridge) held together by ionic charges and disulfide bonds due to heavy metal ions' higher affinity and attraction for sulfur (Bontidean et al., 1998; Sharma et al., 2008; Renwrantz and Werner, 2008; Jung et al., 2003). The affinity of proteins to adsorb on a gold surface is believed to rely on, except for hydrophobic effect, the bonds of Au–COO, Au–N and Au–S (Capanni et al., 2004; Hoefling et al., 2011; Boujday, 2008). Among these bonds, Au–COO and Au–N may be disrupted due to the disruption of ionic interactions (salt bridge), and Au–S may be disrupted due to the disruption of disulfide bonds. Therefore, the affinity of albumins adsorbed on the gold surface may be compromised. The discrepancy of protein affinity may be compared through the competitive adsorption. In this competitive process, weaker denatured albumins pre-adsorbing on the sensing surface should be replaced by native ones. This hypothesis is experimentally demonstrated through a sequence of SPR biosensing, as shown in Fig. 1b and c. Accordingly, the larger SPR angle shift represents the larger change of albumins' affinity caused by more disruption of conformation. The outcome is a rapid, less-complex, and inexpensive biosensor to be able to detect copper ions in drinking water.

2. Materials and methods

2.1. Agents

Cu^{2+}, Fe^{3+}, Mn^{2+}, Pb^{2+}, and Hg^{2+} (as $CuCl_2 \cdot 2H_2O$, $FeCl_3$, $MnCl_2$, $Pb(No_3)_2$, and $HgCl_2$, respectively) are received as powder from Sigma-Aldrich. Albumin (67 kDa) is received as lyophilized powders from Sigma-Aldrich, and used without further purification. Albumin is prepared in Phosphate Buffered Saline (PBS) solution with concentrations of 2 mg/mL. According to National Primary Drinking Water Regulations published by United States Environment Protection Agency (EPA), the Maximum Contaminant Level (MCL) of copper ions in drinking water is 1.3 mg/L. Thus, $CuCl_2 \cdot 2H_2O$ are prepared at 6 different Cu^{2+} concentrations: 2.5, 1.3, 1.0, 0.5, 0.3, and 0.1 mg/L. Other heavy metal ions are prepared following the same protocol to obtain the same concentrations.

2.2. SPR sensing surface formation

Glass slides (BK7, $n=1.517$, 150 μm thick, 18×18 mm^2) are first immersed in ethanol (Proof 200) and cleaned in an ultrasonic cleaner for 5 min. Subsequently, the slides are rinsed sequentially with ethanol and DI water, and dried thoroughly under a N_2 stream. Cr is sputtered on the glass substrates to a thickness of 2 nm followed by Au to a thickness of 47 nm. The slides are cleaned in a plasma cleaner at 10.15 W for 1 min (Renwrantz and Werner, 2008).

2.3. Experimental process

A glass slide having Cr/Au (2 nm/47 nm) is mounted on the SPR (BI-2000 from Biosensing Instrument Inc.). And then, a flow cell is coupled with the glass slide. Calibration of the SPR sensorgram is performed to minimize the discrepancy of sensitivity. The calibration coefficient, the ratio of the measured sensitivity to the theoretical sensitivity, is used to calibrate the SPR sensorgram.

2 mg/mL albumin samples are spiked by Cu^{2+}, Fe^{3+}, Mn^{2+}, Pb^{2+} and Hg^{2+}, with the concentration of 2.5, 1.3, 1.0, 0.5, 0.3, and 0.1 mg/L. These denatured albumin samples are pre-adsorbed on the gold surface on SPR. After the pre-adsorption, PBS rinses the surface to sweep excess weakly bound proteins to establish the baseline for the subsequent steps. Native albumin samples of 2 mg/mL flow across the pre-adsorbed surface. The SPR angle shift increases accordingly as the pre-adsorbed denatured albumins possess weaker affinity than the native ones so that they are replaced by the later ones via the competitive adsorption behavior. Upon rinsing with PBS, the SPR angle shift becomes stabilized to present the end of competitive adsorption behavior. By subtracting the baseline, SPR angle shifts at a given concentration of heavy metal ions are obtained, which are the function of the affinity difference between native and denatured proteins. The real time SPR sensorgram of the competitive adsorption process is shown in Fig. 1.

60 μL of sample volume flows across the sensing surface for 2 mins (flow rate of 30 μL/min), which allows proteins having ample time to adsorb on the sensing surface before and after the PBS rinsing steps. All the data obtained from SPR measurements are under kinetic equilibrium.

3. Results and discussion

Our SPR biosensor operates based upon the following two-step processes: first, Cu^{2+} ions interact with albumin, and then the interaction weakens the affinity of albumin to adsorb on a gold surface. Circular Dichroism (CD) and gold nanoparticle (AuNP) flocculation assay are adopted to visualize these 2 processes. The Cu^{2+}-denatured albumin is then pre-adsorbed on the gold surface and then native albumin flows to displace the pre-adsorbed Cu^{2+}-denatured albumin. The sensitivity of SPR biosensor is characterized by different concentration of Cu^{2+} ions spiked in 4 different media, PBS, tap water, deionized water, and bottled water. 4 different heavy metal ions, Fe^{3+}, Mn^{2+}, Pb^{2+}, and Hg^{2+} are used to characterize the selectivity of the SPR biosensor, following the same protocol to characterize the sensitivity.

3.1. Characterization of the competitive adsorption using Circular Dichroism (CD)

CD is a popular and powerful technique to study metal–protein interactions. All specific secondary structures have their characteristic spectra (i.e., proteins with high contents of α-helices have characteristic bands at about 222 and 208 nm (Boussaad et al., 2000)). If Cu^{2+} ions induce conformational change, which only disrupt the tertiary structure but not the secondary structure, the characteristic bands will remain but vary CD angle shifts or band wavelengths (shown in Fig. 2a).

We use Cu^{2+}-spiked albumin samples to perform the CD measurement. The characteristic bands, representing high contents of α-helices, are obtained at 221 and 210 nm, as shown in Fig. 2a. As the higher concentrations of Cu^{2+} ions are spiked in albumin samples, the CD angle shift increases (more negative) (in Fig. 2b) while the characteristic spectra remain unchanged. This suggests that Cu^{2+} ions interact with albumin and the interaction is primarily on the tertiary structure of albumin, not on the secondary structure.

3.2. Characterization of competitive adsorption using gold nanoparticle (AuNP) flocculation assay

The data from CD measurements support Cu^{2+} ions interact with albumin, yet they do not necessarily mean such interaction induces the affinity change on a gold surface. The characteristic spectra shown at CD measurements cannot be specifically linked to the affinity of albumin. We perform gold nanoparticle (AuNP) flocculation assay of albumin to observe the affinity change of albumin, caused by Cu^{2+} ions. AuNPs solution changes its color from pink to blue accompanied by the titration of NaCl. Added Na^+ and Cl^- reduce electrostatic repulsion of AuNPs so as to induce the aggregation of AuNPs. This aggregation changes the solution color that is decided by the size of AuNPs aggregates.

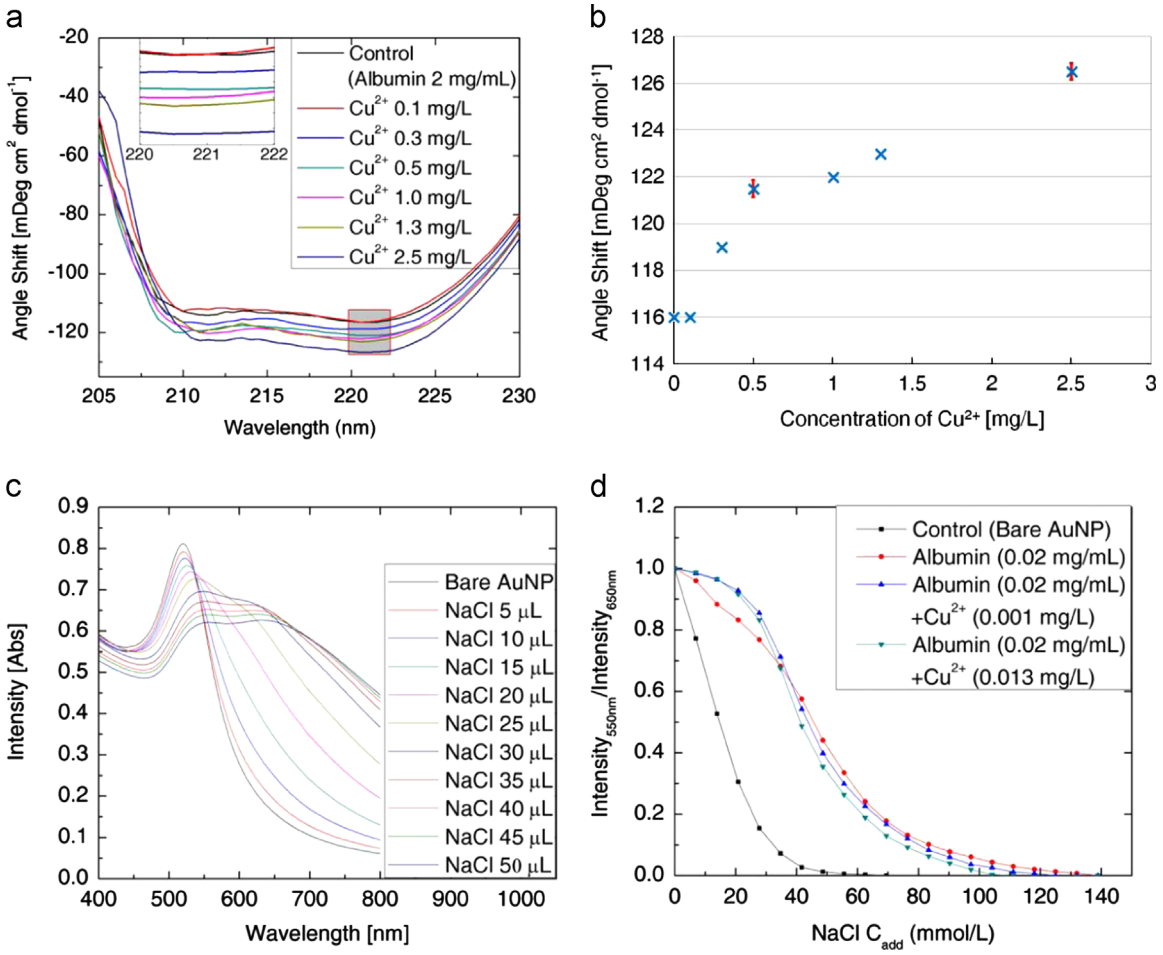


Fig. 2. Circular Dichroism (CD) test and gold nanoparticle (AuNP) flocculation assay of albumin. (a) The characteristic spectra change of α-helices in albumin with the concentration increasing of Cu^{2+}. Pure albumin with concentration of 2 mg/mL is used as the control. The red square shadow area (range of the wavelength from 220 to 222 nm) is enlarged as an inset. (b) The intensity change of the characteristic band at 221 nm as a function of Cu^{2+} concentration. The red error bars indicate relative standard deviation (RSD). (c) UV/vis adsorption spectrum shows adsorption intensity as a function of wavelength at different NaCl titration concentrations. (d) The ratio of the intensity at 550 nm to that at 650 nm as a function of titration concentrations: when no native, or denatured albumin, is added, bare AuNP (control) is readily titrated by NaCl, showing its curve at leftmost. Once native albumin is added, AuNPs are covered by albumin, consequently titration is obstructed. For instance, the ratio of 0.5 moves to rightmost, suggesting the affinity of native albumin to AuNPs is at maximum. As albumin is denatured by Cu^{2+}, the affinity of denatured albumin decreases, consequently moving the 0.5 ratio back to left. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

If the AuNPs are fully surrounded by proteins, they are protected from the titration, resulting in no color change. The protection will be stronger if the interaction of AuNPs with proteins is stronger, so that higher concentration of added NaCl (C_{add} of NaCl) is needed to execute the titration. When the albumins are denatured by heavy metal ions, C_{add} of NaCl should be lower as the weaker affinity leading to the weaker protection.

Each set of titration generates a group of spectra. Taking bare AuNP solution for example (shown in Fig. 2c), as titration continues, the peak at 550 nm becomes lower and broader, and moves to right, which represents the size of AuNPs aggregates becomes more inhomogeneous. The formation of the new peak at 650 nm represents the final flocculation with larger size. The new peak is not sharp, suggesting inhomogeneous flocculation. Every set of scanning spectra in Fig. 2c produces a data point in a curve in Fig. 2d, through calculating the ratio of the intensity at 550 nm to that at 650 nm. All samples are diluted by 100 times to visualize the titration within 1000 mmol/L NaCl. Fig. 2d illustrates the titration process with native albumin and denatured albumin as a function of Cu^{2+} concentration. The curve of bare AuNP is seen leftmost, indicating bare AuNP is readily titrated by NaCl. When native albumins are added to cover AuNPs, the high affinity native albumins obstruct titration so that high concentration of NaCl is needed to titrate at a given ratio of the spectra. Accordingly, the middle point (intensity ratio of 550–650 nm is 0.5) of this curve shifts to the rightmost. When AuNPs are covered by denatured albumin, the protection is weakened, so that curves' middle points return back to the left. The AuNP flocculation assay suggests that the affinity of albumin to a gold surface is indeed a function of the interaction of Cu^{2+} ions with albumin.

3.3. *Detection of Cu^{2+} in water using SPR biosensor*

Ionic interactions, salt bridge between positively- and negatively-charged side chains, and disulfide bonds shape the conformation of albumins and manipulate the affinity of albumins to a sensing surface of SPR. The disrupted ionic interactions, caused by Cu^{2+} ions, result in less adsorption affinity to the sensing surface, which may be replaced by native albumins via competitive adsorption of proteins. Such affinity variation can be quantitatively measured in real time by SPR. This method offers very stable and consistent SPR sensorgram as the SPR angle shift is generated by the replacement process rather than the adsorption process (Yang et al., 2003) as the displacement process is solely from the competition of 2 proteins (we have attempted to measure SPR angle shifts of Cu^{2+}-denatured albumin itself, yet the fluctuation of measurement largely compromises the accuracy of detection, as shown in Supplementary Table 1).

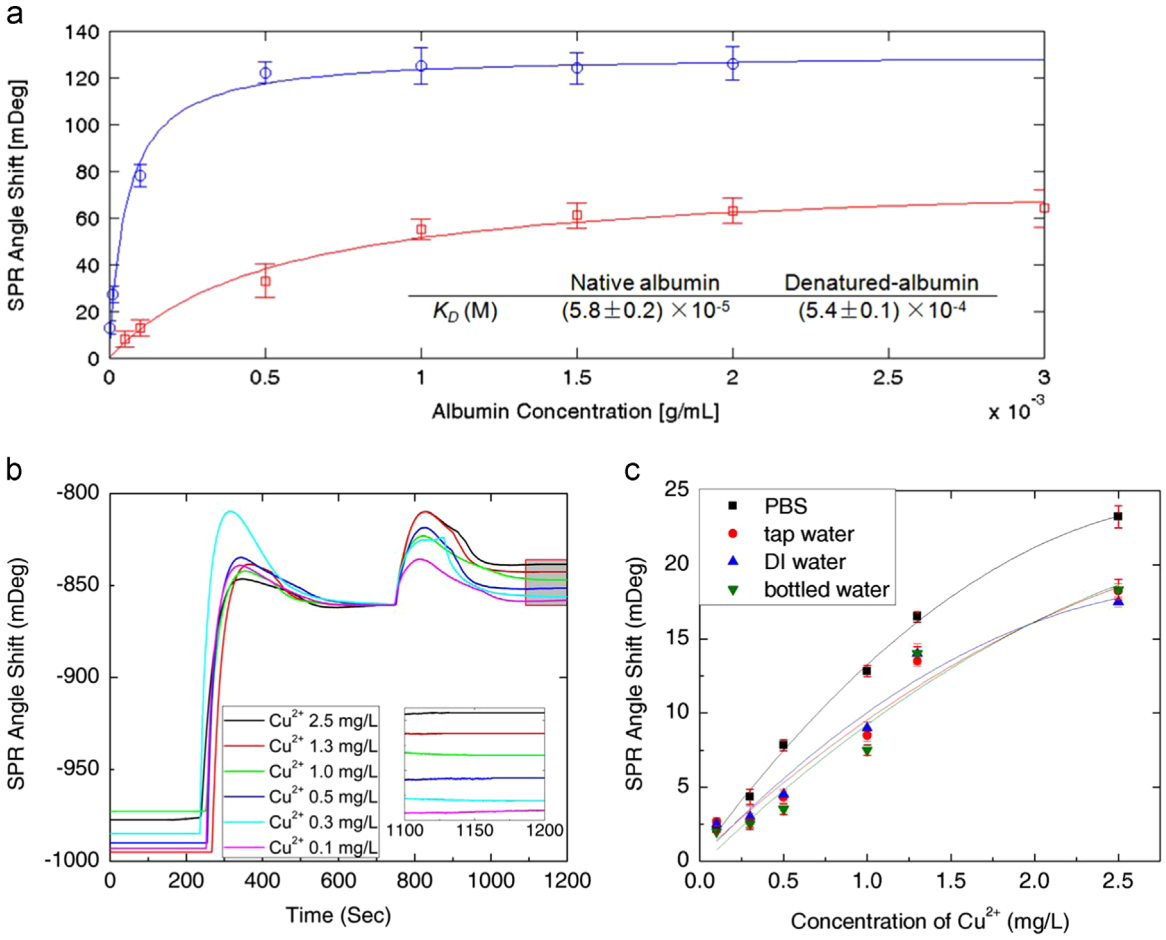


Fig. 3. Sensitivity of SPR biosensor: (a) SPR angle shift as a function of the concentrations of native (circular dots) and denatured albumin (square dots), and the dissociation constants (K_D) of native and denatured albumin on gold surface, calculated from the SPR angle shifts. (b) SPR sensorgram, describing a series of displacements of denatured/native albumin in PBS as a function of Cu^{2+} concentration. The red square shadow area, between 1100 and 1200 s, is enlarged as an inset. (c) SPR angle shift as a function of Cu^{2+} concentration of 4 different liquid samples, including PBS, tap water, deionized (DI) water, and bottled water (Evian). As the concentration of Cu^{2+} increase, denatured albumins are replaced more easily, which generates larger SPR angle shift. The data points from tap water, DI water, and bottled water, produce similar outcomes, but quite distinct from those in PBS. Solid lines are polynomial fitted curves. The error bars indicate relative standard deviation (RSD). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In order to characterize the affinities of denatured albumin and native albumin, we performed to measure K_D of denatured and native albumin using SPR (Fig. 3a). One can calculate a dissociation constant from SPR angle shifts (Forzani et al., 2007; Choi et al., 2011):

$$\Delta\theta = \Delta\theta_{max} C/(K_D + C)$$

$\Delta\theta$ is the SPR angle shift; $\Delta\theta_{max}$ is the SPR angle shift at the saturation; C is the heavy metal ions concentration; and K_D is the dissociation constant.

K_D of native albumin is approximately one order of magnitude lower than that of denatured albumin, indicating the affinity of denatured albumin is far less than that of native albumin to gold surface, allowing the adsorption/desorption behavior of native/denatured albumin on SPR sensing surface.

Fig. 3b and c shows SPR angle shifts increase as the concentration of Cu^{2+} increases. At the concentration of less than 1.0 mg/L, the polynomial fitted curves show relatively linear responses. The coefficient of determination, R^2 values of the lines are 0.9883, 0.9504, 0.9697, and 0.9628 for PBS, tap water, deionized (DI) water, and bottled water, respectively. This linear characteristic agrees well with theoretical SPR angle shift, as shown in Eq. (1) Boussaad et al., 2000,

$$\Delta\theta(\lambda) = c_1 \Delta n(\lambda) + c_2 \Delta d \quad (1)$$

$\Delta\theta$ is SPR angle shift. Δn and Δd are changes in the refractive index and average thickness of an adsorbed molecular layer, respectively. λ is the wavelength of the incident light. c_1 and c_2 are constants.

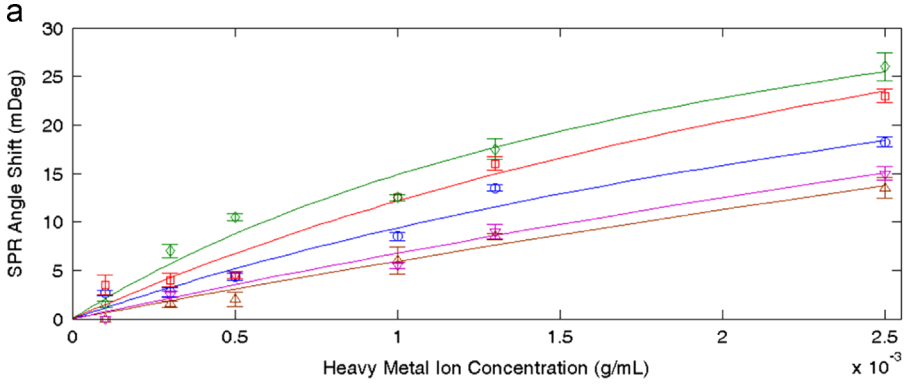
Both Δn and Δd are strongly associated with the conformation characteristics and are directly correlated to the SPR angle shift. Moreover, Δn is linearly correlated with Δd, given in Eq. (2),

$$\Delta n = -\frac{1}{6n}(n^2+2)^2\left(\frac{n^2-1}{n^2+2} - \frac{n_w^2-1}{n_w^2+2}\frac{V_p}{V}\right)\frac{\Delta d}{d} \quad (2)$$

where n_w is the refractive index of water. n and d are refractive index and average thickness of an adsorbed molecular layer, respectively. V is the volume of the protein layer and is given by $V = V_p + V_w$, where V_p and V_w are the volumes of the proteins and water molecules in the layer, respectively. Therefore, SPR angle shift is theoretically linearly dependent on conformation change.

In Fig. 3c, when the concentration of higher than 1.3 mg/L, SPR angle shift becomes off linear possibly due to the saturation of albumin denaturation. In a buffer (PBS), the SPR biosensor shows high sensitivity and the widest linear response region. We further promote the experiments under more realistic conditions, using tap water, DI water and bottled water (Evian). Data from these 3 samples show less sensitivity than those from PBS, and there is no obvious discrepancy among these data. The high sensitivity in PBS

a



b

	Cu^{2+}	Fe^{3+}	Mn^{2+}	Pb^{2+}	Hg^{2+}
SPR angle shift at 1.3 mg/L [mDeg]	13.5±0.4	8.5±0.4	9±0.7	16±0.7	17.5±1.1
Maximum Contaminant Level (MCL) [mg/L]	1.3	0.3	0.05	0.015	0.002
SPR angle shift at MCL [mDeg]	13.5±0.4	1.5±0.4	0	0	0
K_D (M)	(4.4±0.3) ×10^{-3}	(1.8±0.1) ×10^{-2}	(1.1±0.1) ×10^{-2}	(4.1±0.3) ×10^{-3}	(2.3±0.3) ×10^{-3}

Fig. 4. Selectivity of SPR biosensor. (a) SPR angle shift as a function of heavy metal ion concentration: green diamond, red square, blue circle, purple lower-triangle, and brown upper-triangle dots/lines represent Hg^{2+}, Pb^{2+}, Cu^{2+}, Mn^{2+}, and Fe^{3+}, respectively. The error bars indicate relative standard deviation (RSD). (b) SPR angle shifts at the concentration of 1.3 mg/L and the Maximum Contaminant Levels (MCL) in tap water, and K_D of various heavy metal ions. K_D is correlated to the dissociation constants of heavy metal ions to albumin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

is because PBS contains more electrolytes that provide higher ionic strength to remain proteins' native conformations and behaviors.

3.4. Selectivity characteristics of the SPR biosensor

According to National Primary Drinking Water Regulations published by United States Environmental Protection Agency (EPA), the Maximum Contaminant Levels (MCL) of heavy metal ions in tap water include copper (1.3 mg/L), iron (0.3 mg/L), manganese (0.05 mg/L), lead (0.015 mg/ L), and mercury (0.002 mg/L). The SPR biosensor is characterized by these 5 ions (Cu^{2+}, Fe^{3+}, Mn^{2+}, Pb^{2+}, and Hg^{2+}) for its selectivity as shown in Fig. 4. Direct measurement of dissociation constants of albumin to heavy metal ions, using SPR, is a challenge. This is because the SPR angle is proportional to the refractive index change at the vicinity of the sensing surface which does not directly represent the interaction of albumin to heavy metal ions, but it represents the denatured albumin to the sensing surface through the pre-adsorption. Similar to Cu^{2+}, other 4 heavy metal ions, including Fe^{3+}, Mn^{2+}, Pb^{2+}, and Hg^{2+}, are bound to albumin and lower the affinity to gold surface. Higher concentration of the ions results in weaker affinity; thus the higher concentration of ions yields easier displacement by native albumin. In other words, the higher SPR angle shift of the displacement event represents weaker affinity of denatured albumin, which is consequently caused by higher concentration of the heavy metal ions. The dissociation constants of these heavy metal ions, Fig. 4b, can be indirectly measured by a sequence of SPR angle shifts, similar to Cu^{2+}.

We set a concentration of all heavy metal ions at 1.3 mg/L, MCL of Cu^{2+}, and performed SPR measurements of a sequence of pre-adsorbing denatured albumin, PBS wash, replacing the pre-adsorbed albumin by native albumin, and PBS wash, following the same protocol we used to characterize the SPR biosensor for Cu^{2+}. The SPR angle shifts of Fe^{3+}, Mn^{2+}, Pb^{2+}, and Hg^{2+}, were 8.5, 9, 16, and 17.5, respectively. As expected, the heavy metal ions denature albumin and result in SPR angle shift. The sensitivity to these heavy metal ions at 1.3 mg/L varies, yet it is not very distinctive. Then we measured the SPR angle shift of these ions at the MCL of them (Fe^{3+}: 0.3 mg/L, Mn^{2+}: 0.05 mg/L, Pb^{2+}: 0.015 mg/L, and Hg^{2+}: 0.002 mg/L) and the SPR angle shifts of Fe^{3+}, Mn^{2+}, Pb^{2+}, and Hg^{2+} were 1.5, 0, 0, and 0 mDeg, respectively, which are significantly less than that of Cu^{2+}, 13.5 mDeg, at 1.3 mg/L (MCL of Cu^{2+}).

These data suggest both the limitation and application of our SPR biosensor. The SPR biosensor in general has the lack of selectivity to different heavy metal ions. Heavy metal ions do result in conformational changes and denaturation of albumin, resulting in SPR angle shifts. On the other hand, the SPR biosensor serves well in tap water samples to detect Cu^{2+} as the most serious threat in tap water is Cu^{2+} and the SPR responses at MCL of other heavy metal ions are significantly less than that of Cu^{2+}.

4. Conclusions

SPR is a very sensitive instrument, which detects minute refractive index change at the vicinity of gold surface. This work utilizes SPR to detect Cu^{2+}, a common contaminant in tap water, using competitive adsorption of proteins, termed the Vroman effect. Cu^{2+}-denatured albumin has lower affinity than native albumin to gold surface, which can be replaced by a sequence of adsorption/desorption phenomena. This sequence of adsorption/desorption is monitored by SPR in real time to quantify the concentration of Cu^{2+} ions in water samples, including PBS, tap water, DI water, and bottled water. The affinity change of protein denaturation by Cu^{2+} is demonstrated by Circular Dichroism and gold nanoparticle (AuNP) flocculation assay. The SPR biosensor can detect down to 0.1 mg/L Cu^{2+}, lower than the Maximum Contaminant Level (MCL), 1.3 mg/L, in National Primary Drinking Water Regulations published by United States Environment Protection Agency (EPA). The SPR biosensor is characterized for 5 different heavy metal ions, Cu^{2+}, Fe^{3+}, Mn^{2+}, Pb^{2+}, and Hg^{2+}, for its selectivity. At MCL, the SPR biosensor produces 13.5 ± 0.4, 1.5 ± 0.4, 0, 0, and 0 mDeg suggesting the biosensor may be used to detect Cu^{2+} in tap water samples. Such technique

may complement existing biosensors to add a sensing modality to facilitate the heavy metal ions detection.

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.2014.01.056.

References

Arai, Y., et al., 2003. Sci. Technol. 37, 4083–4090.
Baldo, M.A., et al., 2004. Electroanalysis 16, 360–366.
Bannon, D.I., Chisolm, J.J., 2001. Clin. Chem. 47, 1703–1704.
Beveridge, T.J., et al., 1996. Adv. Microb. Physiol. 38, 177–243.
Bontidean, I., et al., 1998. Anal. Chem. 70, 4162–4169.
Bontidean, I., et al., 2000. J. Inorg. Biochem. 79, 225–229.
Boujday, S., 2008. J. Phys. Chem. 112, 6708–6715.
Boussaad, S., Pean, J., Tao, N.J., 2000. Anal. Chem. 72, 222–226.
Brewer, G.J., 2010. Clin. Neurophysiol. 121, 459–460.
Burdette, S.C., et al., 2003. J. Am. Chem. Soc. 125, 1778–1787.
Capanni, C., et al., 2004. Cell. Mol. Life Sci. 61, 982–991.
Chaerkady, R., Pandey, A., 2008. Annu. Rev. Pathol. Mech. Dis. 3, 485–489.
Choi, S., Chae, J., 2009a. Biosens. Bioelectron. 25, 118–123.
Choi, S., Chae, J., 2009b. Biosens. Bioelectron. 25, 527–531.
Choi, S., Yang, Y., Chae, J., 2008. Biosens. Bioelectron. 24, 893–899.
Choi, S., et al., 2010. Appl. Phys. Lett. 97, 253701.
Choi, S., et al., 2011. Lab Chip 11, 3681–3688.
Darwish, I.A., Blake, D.A., 2002. Anal. Chem. 74, 52–58.
Deo, S., Godwin, H.A., 2000. J. Am. Chem. Soc. 122, 174–175.
Eksperiandova, L.P., Blank, A.B., Makarovskaya, Y.N., 2002. X-ray Spectrom. 31, 259–263.
Forzani, E.S., et al., 2005. Environ. Sci. Technol. 39, 1257–1262.
Forzani, E.S., et al., 2007. Sens. Actuators B: Chem. 123, 82–88.
Haab, B.B., 2003. Proteomics 3, 2116–2122.
Hoefling, M., et al., 2011. PLoS ONE 6, e20925.
Jung, S., et al., 2003. J. Am. Chem. Soc. 125, 12782–12786.
Latour , R.A., 2005. In: Bowlin, G.L., Wnek, G.E. (Eds.), Encyclopedia of Biomaterials and Biomedical Engineering. Taylor & Francis, New York, pp. 1–15
Leonard, E.F., Vroman, L., 1992. J. Biomater. Sci. 3, 95–107.
Liu, H.W., Jiang, S.J., Liu, S.H., 1999. Spectrochim. Acta B 54, 1367–1375.
Lu, Y., et al., 2003. Biosens. Bioelectron. 18, 529–540.
Mlynarz, P., et al., 2002. New J. Chem. 26, 264–268.
Renwrantz, L., Werner, I., 2008. J. Molluscan Stud. 74, 11–17.
Rouch, D.A., Parkhill, J., Brown, N.L., 1995. J. Ind. Microbiol. 14, 349–353.
Shankaran, D.R., Miu, N., 2007. J. Phys. D Appl. Phys. 40, 7187–7200.
Sharma, S.K., Goloubinoff, P., Christen, P., 2008. Biochem. Biophys. Res. Commun. 372, 341–345.
Shetty, R.S., et al., 2001. Abstr. Pap. Am. Chem. Soc. 221, U92 (U92).
Shults, M.D., Pearce, D.A., Imperiali, B., 2003. J. Am. Chem. Soc. 125, 10591–10597.
Slack, S.M., Horbett, T.A., 1989. J. Colloid Interface Sci. 133, 148–165.
Turbill, P., Beugeling, T., Poot, A.A., 1996. Biomaterials 17, 1279–1287.
Vroman, L., Adams, A.L., 1969. Surf. Sci. 16, 438–446.
Wang, J., 1985. Stripping Analysis: Principles, Instrumentation, and Applications. VCH Publishers, Deerfield Beach, FL
Wang, J., 1990. New approaches to metal speciation in natural waters based on modified and microvoltammetric electrodes. Summary report 251. Department of Chemistry, New Mexico State University, La Cruces, NM.
Wang, R., et al., 2011. Biosens. Bioelectron. 28, 304–307.
Wang, S., Forzani, E.S., Tao, N., 2007. Anal. Chem. 79, 4427–4432.
Wu, X.Q., Kim, J., Dordick, J.S., 2000. Biotechnol. Prog. 16, 513–516.
Yang, W.R., et al., 2001. Chem. Commun. 19, 1982–1983.
Yang, W.R., et al., 2003. Analyst 128, 712–718.
Yang, Y., Chae, J., 2008. J. Micromech. Microeng. 18, 125010.
Yu, X., Xu, D., Cheng, Q., 2006. Proteomics 6, 5494–5503.