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Colorimetric sensor array with unmodified noble metal nanoparticles for the naked-eyes detection of proteins and bacteria

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Abstract: Herein we report a novel strategy for the detection and identification of proteins by use of unmodified noble metal nanoparticles. Five gold nanoparticles (AuNPs) and two silver nanoparticles (AgNPs) with different sizes were utilized as sensing elements to create a colorimetric sensor array. In the presence of proteins, the UV-Vis absorbance of the noble metal nanoparticles changed due to the interaction of protein and nanoparticles, producing distinct absorbance response patterns which can be visually detected by naked eye. The color patterns of the array is a unique fingerprint for each protein sample which could be differentiated by linear discriminant analysis (LDA). Ten different proteins at the concentration of 0.5, 5 and 50 μM have be successfully discriminated. Moreover, the array was also able to discriminate different bacteria at a concentration of 0.05 OD in 200 μL, as well as cancer cells at the level of 5000 cells in 200 μL. This work demonstrates that unmodified noble metal nanoparticles-based protein detection array has the potential for applications in medical diagnostics.

Keywords: Colorimetric sensor array; Protein; Bacteria; Cancer cell; AuNPs; AgNPs

Introduction:

Protein sensing are of considerable importance in a medical diagnostics especially in proteomics, which are required to be rapid, sensitive, high throughput, and economically reliable. Over the past decades, quite a few methods have been developed to quantify proteins, including mass spectrometry (MS) technology coupled with electrospray ionization (ESI) or matrix-assisted laser desorption ionization (MALDI)[1,2] and enzyme-linked immunosorbent assay (ELISA)[3]. MS is currently considered as an important tool for analyzing and characterizing large biomolecules especially for proteins because of high accuracy, however, time-consuming, technically complex, and expensive instrumention limit its applications in multianalyte detection. Enzyme-linked immunosorbent assays (ELISA) are the most commonly used method for clinical protein quantitation. Although these specific interaction-based sensing approaches have good sensitivity and high selectivity, high cost, time-consuming, large sample volume, and impossible multianalytes detection restrict their applications.

Optical sensor array is a promising sensing protocol for a myriad of analytes or even complex

mixtures [4-7]. It has been employed in the detection and discrimination of a wide variety of chemical or biological samples, including metal ions, volatile organic compounds, amino acids, saccharides, proteins, bacteria, and even cells [8-11]. Unlike traditional "lock-and-key" approach, this method involves exploiting non-selective chemosensors (sensing elements) to generate combined response pattern which is unique for each sample. For example, Zhang *et al* used six catalytic nanomaterials as sensing elements to fabricate a chemiluminescence sensor array for protein sensing[12]. Rotello *et al* employed conjugated fluorescent polymers as sensing elements to design sensor array for protein sensing and discrimination[13]. They also utilized green fluorescent protein modified with differentially functionalized gold nanoparticles (AuNPs) as sensing elements for the identification of proteins in a complicated matrix [8, 13, 14]. Hamilton *et al* developed a functionalized porphyrins-based sensor array for protein sensing[15, 16]. Recently, Fan *et al* took advantage of nanographene oxide modified with dye-tagged DNA as identifying elements for protein sensing [17]. These types of sensor arrays are highly effective to protein sensing, but some of them are need to be heated and others should prepare a large number of receptors with fluorescent tags or fluorescence polymers as sensing elements which remains a great challenge. Therefore, the discovery of simple sensing elements is still one of the most important factors for developing robust and simple optical sensor array for proteins identification.

Colorimetric sensor arrays have been emerging as an attractive and exciting field in pattern recognition over the past decades. Suslick *et al* has utilized diverse family of chemically responsive dyes as simple sensing elements to explore colorimetric sensor arrays [18-20]. With the interaction between analytes and dyes, color changes in the dyes could be readily observed and imaged. This color change pattern can used for the discrimination of a variety of analytes, most of which are gases or volatile organic compounds (VOCs). Noble metal nanoparticles (NPs) especially gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) have been utilized as ideal sensing materials to develop colorimetric chemosensors or sensor arrays in medicine and biotechnology based on the change of colors which is related to the particle size of nanoparticles [21, 22]. They are usually served as the transducers and required to be modified with receptors on their surface, such as oligonucleotides/aptamers, antibodies or other ligands conjugating with analytes [23]. Zhang *et al* developed a colorimetric sensor array using three aptamer-protected AuNPs as sensing element for the discrimination of protein[24]. Liu *et al* used DNA-AuNPs conjugtions as sensing elements to develop a multidimensional sensor for pattern recognition of proteins[25]. Howerver, all these method required functionalized AuNPs. On the other hand, unmodified noble metal nanoparticles themselves can interact with biological molecules such as proteins non-specifically, leading to color change[26]. Therefore, they have the potential to be used as simple and the best sensing elements to fabricate the colorimetric sensor array, because they have the functions of receptors and indicators simutaneously.

It is reported that NPs with different surface properties (eg, size, shape, surface charge, and

coating material) have different interaction with proteins[27, 28]. NPs with different sizes have different surface area and curvature. The smaller size, the larger surface to volume atomic ratio, with which non-specific interaction of amino acid side chain of protein and NPs surface will increase[28]. In this study, a colormetric sensor array was designed based on five "unmodified" AuNPs and two "unmodified" AgNPs for the identification of protein. These noble metal nanoparticles were used as sensing elements which served not only as the receptors but also as the indicators, as shown in Figure 1. They could bind diversity in the presence of proteins and the distinct absorbance response patterns for proteins were created. Furthermore, the array was capable of naked-eye visualization which enable to differentiate the is simple and has the potential application in medical diagnosis.

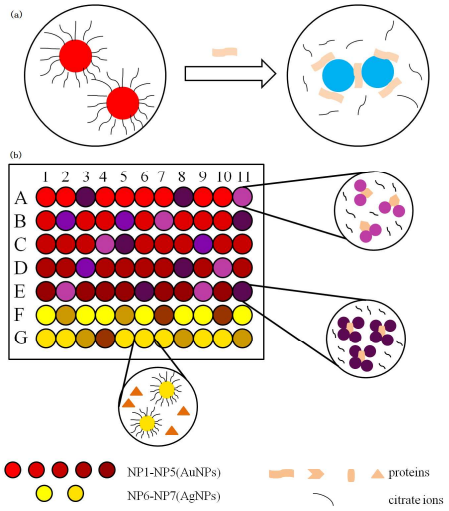

Figure 1 Schematic diagrams of colorimetric sensor array based on unmodified AuNPs and AgNPs. (a) The interaction between NPs and proteins. Once protein binds NPs, the citric acid protecting groups are replaced, resulting in the NPs aggregation and color change. (b) The sensor array based on seven nanoparticles for sensing proteins. Each row from A to G represents NP1-NP7, respectively. And each column from 2 to 11 represents a certain protein added, respectively, and the row 1 represents the blank.

Experimental Section

Materials

Chloroauric acid and silver nitrate were purchased from Shanghai Fine Chemical Research Institute (Shanghai, China) and Shanghai Chemical Reagent CO. Ltd (Shanghai, China), respectively. β-cyclodextrin was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Sodium citrate was obtained from Tianjin First Chemical Reagent Factory (Tianjin, China). The analyte proteins, cytochrome C (Cyt C), myoglobin (Mb), trypsin (Try), pepsin (Pep), lysozyme (Lys), papain (Pap), and hemoglobin (Hb) were obtained from Sigma-Aldrich Chemical Company. Casein acid(Cas), bovin serum albumin(BSA), and lipase(Lip) were purchased from Xi'an Wolsen Bio-technology Co., Ltd.(Xi'an, China). All other chemicals used were of analytical grade or higher

and were used without further purification. MilliQ water with a resistivity above 18 MΩ cm^{-1} was used throughout the experiments. Transparent 96-microplate was purchased from Corning Incorporated. Copper grid for TEM was purchased from Beijing Zhongjingkeyi Techonology Co., Ltd (Beijing, China). Seven kinds of bacteria (CRPA, acetobacter aceti, rhodospseudomonas, bacillus natto, staphylococcus, E. Coli, and bacillus) and four cancer cells (oral squamous carcinoma cells, Hela cells, PC3 cells, and A549 human lung adenocarcinoma cells) were provided by Xi'an Jiaotong University (Xi'an, China) .

Synthesis of noble metal particles

Unmodified AuNPs and AgNPs with different size were synthsized according to previously reported procedures which were described in the Supplementary Information[27]. Actually, so-called "unmodified AuNPs" are citrate-stabilized AuNPs because they are not the functionalized AuNPs. The UV-vis absorption spectra were performed in Infinite M200 PRO multimode plate reader (Tecan, Männedorf, Switzerland). The size and shape of nanoparticles were characterized using JEOL 2100 Cryo transmission electron microscopy (TEM) at 200kV (Tokyo, Japan). As shown in Fig S2, the diameter of AuNPs 1, AuNPs 2, AuNPs 3, AuNPs 4, AuNPs 5, AgNPs 1 and AgNPs 2 are 15 nm, 25 nm, 35 nm, 50 nm, 60 nm, 25 nm, 31 nm, respectively. The wavelengths of maximum absorption are 520 nm, 525 nm, 530 nm, 535 nm, 540 nm, 405 nm and 410 nm, respectively (Figure S1). With the increase in particle size, the absorption wavelength also increase, which results mainly from the strong overlap between the plasmon fields of the nearby nanoparticels. The spectrum and diameter of synthesized nanoparticles were approximately matched according to the literatures.

Fabrication of the colorimetric sensor array and sensing

Five gold NPs and two silver NPs (NP1-NP7) were utilized as sensing elements to fabricate a colorimetric sensor array. The protein identification was carried out as follows: 100 μL of the colloidal gold or silver were loaded into a Micro 96-well plate. Then 100 μL of water (blank) or protein (sample) with different concentrations were added to each well. After incubation for 20 min, the absorbance was measured using Tecan 2000 Enzyme Micro-plate Reader (Infinite M200 PRO). The differences between blanks and protein samples were used as the absorbance response (Table S1 in the Supporting Information). each sample was replicated three times. The raw data matrix was processed using classical LDA in SYSTAT (version 13.0).

For the identification of bacteria, bacteria were firstly separated from the culture medium. Then each type of bacteria strains was resuspended and diluted in ultrapure water (0.05 OD). Then 100 μL of each strain was added to each sensing element (100 μL of different NPs) in the 96-well plate, respectively. The change of absorbance at maxium absorption of NPs was recorded by Tecan 2000. Each sample was repeated six times and LDA analysis was used to differentiate quantitatively the response patterns of bacteria.

Cancer cells were grown in conventional cell culture medium, spun down, resuspended in saline medium (0.9%), and counted using a hemocytometer. The final concentration of cell suspensions is 7.5×10^4/mL. When 100 μL of cell was added into the sensor array and reacted with sensing elements for 5 min (final concentration of 5000 cells in 200 μL), the absorbance was measured by Tecan 2000. Each sample was replicated six times. The absorbance response patterns were subjected to LDA.

Results and Discussion

Visualization image

Color change is a very convenient and attractive way to be utilized in detection of analytes owning to the simplicity of detection. The aggregation of colloidal gold in the presence of protein causes color change from red to purple or blue, which can be observed by naked eyes. We selected five AuNPs with different size to investigate the interaction between AuNPs and proteins. The proteins include Cyt c, BSA, Mb, casein, trypsin, pepsin, papain, Lyso, Hb, and lipase which have different molecular weights, isoelectric points (pIs), and oligomeric states. As shown in Figure 2, the color change profiles are unique fingerprints for each specific protein at a given concentration, which can be visualized by naked eyes. Different responses can be observed when the same nanoparticles interacting with different proteins. Cyt C, lysozyme, papain, and casein give obvious responses on NP1 and the color changed from dark red to purple or blue. Whereas, the proteins have almost no color changes on NP4 except trypsin and casein. Similarly, different absorbance responses can also be obtained when same protein interacting with different nanoparticles. With the increase in the concentration of protein, obvious color changes can also be observed (Figure 2a, 2b and 2c). The simpe color change is sufficient for differentiating ten analyte proteins with naked-eye visualization and avoids use of sophisticated instruments.

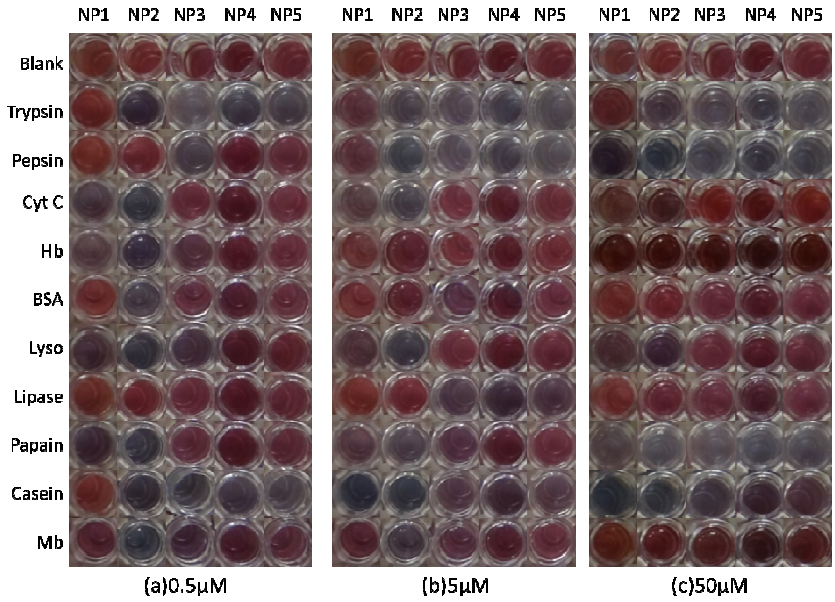

Figure 2. The color change patterns of five AuNPs of different sizes against different proteins.
NP1: 15 nm AuNPs; NP2: 25 nm AuNPs; NP3: 35 nm AuNPs; NP4: 50 nm AuNPs; NP5: 60 nm AuNPs.

Protein sensing

The chemo-selective absorbance responses provide abundant optical information which motivates us to fabricate a cross-reactive chemical sensor array for protein sensing. Differential absorption responses were obtained due to the interaction of nanoparticles with proteins, as shown in Figure 3(a). The decrease in absorbance could be observed for most of proteins, while a slight absorbance enhancement could be seen for some proteins on gold nanoparticles with the size of 50 nm (NP4). Moreover, the absorbance of silver nanoparticles (NP6 and NP7) decreased in the presence of trypsin, Cytc C, and papain. Each protein has unique response pattern which could be used for identification (figure 3(a)). Subsequently, LDA was used to differentiate the absorbance response patterns against different proteins, which is normally employed in statistics to recognize the linear combination of features that can differentiate two or more classes of objects or events. This analysis reduces the size of the training matrix (7 sensing elements × 10 proteins × 3 replicates) and transforms them into canonical factors. The three canonical factors were visualized as a well-clustered three-dimensional plot with a classification accuracy of 100%, as shown in Figure 3(b). According to the classification result of LDA, three canonical factors (60.3%, 30.4% and 6.1%) possess 96.8% of the variation. By these plots, all the proteins were clearly identified in this pattern-based recognition using noble nanoparticles. Therefore, the results demonstrate that complete differentiation of the ten analyte proteins was obtained at 5 μM by the sensor array. Furthermore, tests on other protein solutions at concentrations of 50 μM and 0.5 μM were also investigated using the proposed sensor array, respectively. The results also show that the proposed colorimetric sensor array has the ablity of

differentiating proteins of different concentrations (Figure S3 and Figure S4 in the Supporting Information).

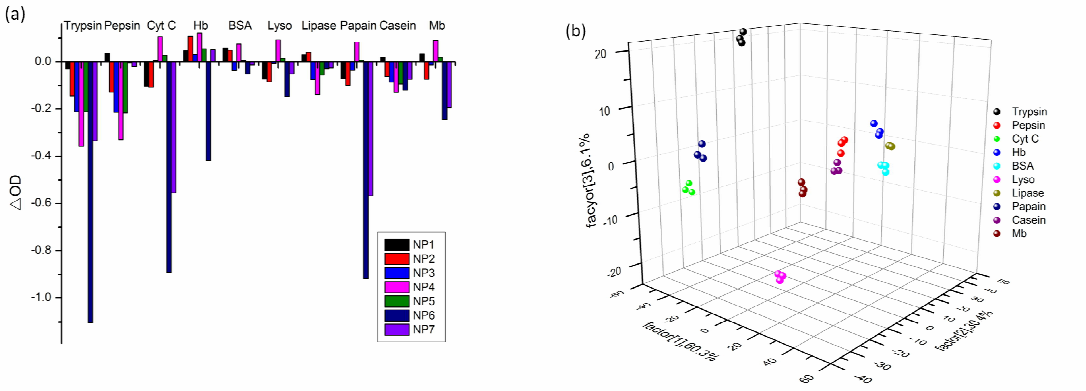

Figure 3. Protein identification by the colorimetric sensor array. (a) Absorbance response patterns of seven noble nanoparticles against ten proteins (all at 5 μM): trypsin, pepsin, cytochrome c (Cyt c), hemoglobin(Hb), bovine serum albumin (BSA), lysozyme (Lyso), lipase, papain, casein and hemoglobin (Hb). (b) Canonical score plot for three factors of simplified absorbance response patterns obtained with noble nanaoparticles-based array against 5μM proteins. All the protein were clearly identified.

Quantitative analysis

As discussed above, proteins with different concentrations give the different response patterns in the sensor array, which shows the potential of quantitative analysis. In this experiment, the linear response of protein are investigated by using certain sensors of the array. The result shows that the proposed sensor array is capable of quantifying a given protein by its UV-vis absorbance which vary linearly with the concentration of protein. Linear responses of five proteins on AuNPs 1, AuNPs 3, AuNPs 4 and AgNPs 2 were shown in supplementary Figure S5. The linear range of Mb, Pap, Pep, Cyt C and Try were 0.8-50 μM, 0.05-1.25 μM, 0.05-1.25 μM, 1.25-25 μM and 0.1-1 μM, respectively. It can be anticipated that the quantitative analysis is easy to be accomplished by a comparison of the achieved patterns from a library of patterns at different concentrations. It should be pointed out that the linear range and detection limit for each protein vary significantly with different nanoparticles.

Identification of real samples

After successful quantitative discrimination of proteins at various concentrations, we are encouraged to further evaluate the proposed sensor array for real samples. After the bacteria are incubated with sensing elements of sensor array, which include CRPA (Carbapenem-resistant pseudomonas aeruginosa), acetobacter aceti, rhodospseudomonas, bacillus natto, staphylococcus, E. Coli

(Escherichia coli), and bacillus, we confirmed that different varieties of bacteria have different absorbance patterns, as illustrated in Figure 4. It should be noted that abosorbance clearly reduces on NP5 and NP6 (50 nm & 60 nm AuNPs) for some bacteria. LDA plots for various bacteria can be completely differentiated from each other. Similarly, color change profiles could also be used for the identification of bacteria, as indicated in Figure S6. Therefore, the results demonstrate the potential of the sensor array for complex compounds with visualization.

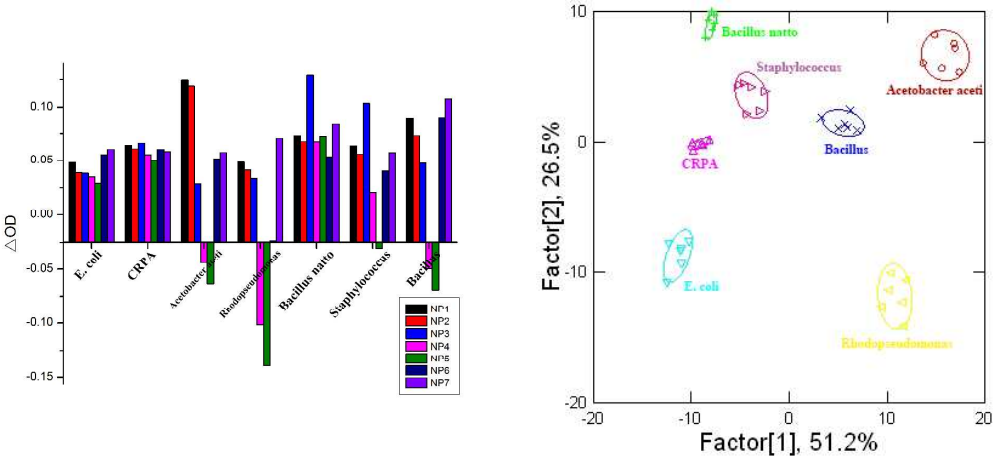

Figure 4. Identification of six microorganisms with the colorimetric sensing platform. Microorganisms include staphylococcus, rhodopseudomonas, rhodotorula, beer yeast, candida, and E. Coli, respectively. (a) Absorbance response pattern of seven nanoparticles against six microorganisms. (b) Canonical score plot for seven nanoparticles against six microorganisms with different species and strains (0.05 OD in 200 μL).

The next challenge for sensing complex compounds was to discriminate between cancer cells. Four types of cancer cells (oral squamous carcinoma cells, Hela cells, PC3 cells, and A549 human lung adenocarcinoma cells) were tested by proposed sensor array. From the Figure 5, it could be clearly seen that a well differentiated response pattern from each other were obtained. Therefore, the proposed sensor array can provide a versatile platform for sensing complex compounds including bacteria and cancerous cells.

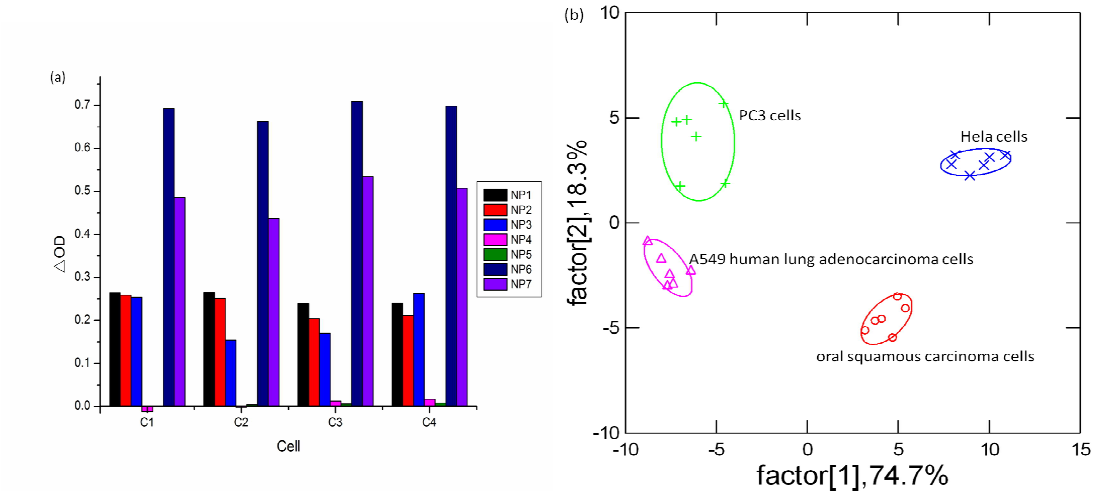

Figure 5. Identification of cancer cells with the colorimetric sensing platform. Four cancer cells are oral squamous carcinoma cells, Hela cells, PC3 cells, and A549 human lung adenocarcinoma cells, respectively. (a) Absorbance response pattern of seven nanoparticles against four cancer cells. (b) Canonical score plot for seven nanoparticles against four cancer cells (5000 cells in 200 μL).

Conclusion

Based on the interaction of proteins and nanoparticles, different response patterns for ten proteins at different concentrations have been obtained by a sensor array of metal nanoparticles which can be utilized for identification of proteins and cells. The main advantage of the proposed colorimetric sensor array is simple owing to the use of unmodified AuNPs and AgNPs of different sizes as sensing elements and naked-eyes visualization. Quantitative analysis of protein could also been carried out by the array. When the sensor array applies to real sample, senven bacteria and four cancer cells were completelly discriminated by their absorbance responses pattern, respectively. The results indicate that the colorimetric sensor array has perspective in medical diagnostics.

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