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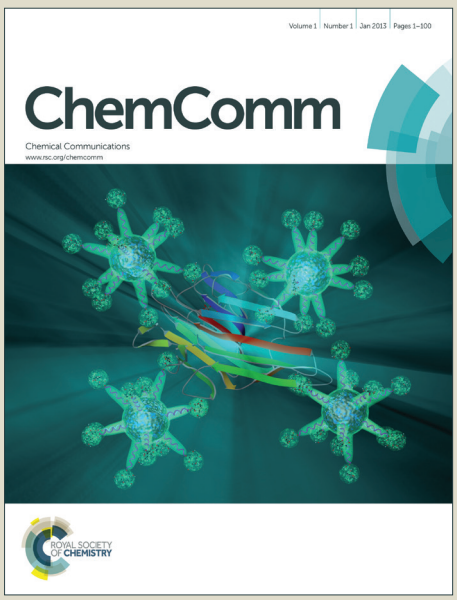

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A CuS-Based Chemical Tongue Chip for Pattern Recognition of Proteins and Antibiotics-Resistant Bacteria

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In this work we developed a CuS based sensor array for highly stablility and selectivity to complex protein analytes on a quartz chip. Our platform also exhibited the excellent discrimination ability in the complex analytes of real biological samples such as the bacteria extracts.

Proteins has been central to a variety of life sciences, such as disease diagnosis and cellular regulatory networks.[1] Convenient and rapid analysis of proteins is of great importance due to the growing demands for detecting. Large amounts of strategies have been developed for monitoring proteins including mass spectrometry, enzyme methods, electrophoresis methods, etc. Enzyme-linked immunosorbent assay,[2] which is the most extensively used detection method for protein sensing. However, when dealing with multi analytes, this method is restricted because of their high production cost and difficulties in obtaining specific receptors. Meanwhile, bacteria is the most common cause of a variety of infections ranging from mild to serious.[3] Antibiotics have been extensively used to cure infectious diseases. However, abuse of antibiotics has led to the emergence of antibiotic resistance of bacteria, which results in clinical failure.[4] Since infections associated with antibiotic-resistant bacteria have high morbidity and mortality rates, the detection of antibiotic-resistant bacteria was of great important.[5] Traditional identification and susceptibility testing methods are dependent on the growth of microorganisms, and hence require an incubation period of 48 h.[3] Therefore, rapid and simple method for identifying antibiotic-resistant bacteria is challenging.

Currently, an array-based sensing which utilizes differential receptors has been actively exploited as "artificial tongue".[6] An artificial tongue is a device that mimics the discrimination of the animal gustatory system for tastes.[7] Compared to "key-and-lock" methods, strategically, the array featuring multiple nonselective receptors is able to present chemical diversity in response to a variety of analytes.[8] So far, this approach has been used to detect a wide range of analytes, including proteins, organic compounds, volatile agents, and carbohydrates[9]. Recently, Rotello et al. utilized the variance of interactions between proteins and nano-graphene to build fluorescent sensor arrays for protein analysis.[10] Fan et al. reported a new concept of adaptive aptamers based on DNA and graphene to identify molecular or cellular targets discriminatively.[11] Although these studies have successfully engendered pattern recognition on biosensing, these sensing arrays are often limited by the portability and the throughput. Therefore, the array-based sensing method can be further developed.

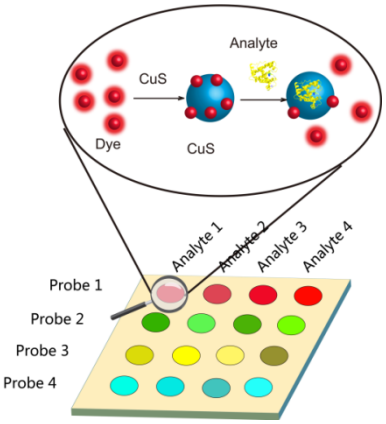

Fig. 1 Design and preparation of CuS-based chemical tongue and their application in analytes pattern recognition.

Copper sulfide nanoparticles (CuS NPs) are a type of semiconductor with excellent optical and electrical properties.[12] CuS NPs are applied as a promising platform for molecular imaging[13], photothermal therapy[14], drug delivery[15], as well as multifunctional agents[16]. Besides, based on their metal-like electrical conductivity and the ability to promote electron transfer reactions with biomolecules, CuS NPs have been widely used in the detection of biomolecules such as proteins, DNA and glucose.[17] However, specific binding probes are often required for these methods which are based on the key-and-lock approach. Since the CuS was simple synthesized without further functionalized, the sensing probe could be easily designed employing CuS nanoparticles and the small particle size provided larger specific surface area to interact with analytes. Meanwhile, compared with formation of traditional analytes-probes via π-π stacking or electrostatic interaction, CuS reacted with analytes through coordinate bond, which made CuS a broad spectrum sensing probe. Herein, we demonstrated a facile, label-free and portable optical sensing platform with high throughput ability for identification of proteins and antibiotic-resistant bacteria utilizing CuS NPs and fluorescent dyes.

It is reported that Cu(II) was able to interact with coordination group such as carboxyl and hydroxyl through coordinate bond.[18] This feature allows the formation of dye-CuS through coordinate

bond. In addition, Cu(II) is a well-known and highly efficient fluorescent quencher, which is useful for background suppression in fluorescent assays.[19] Therefore, Mixing different fluorescent dyes and CuS to form coordination complexes provides a simple way to prepare a variety of independent sensing units. The operation is relatively convenient and the interactions between dyes and CuS NPs are potentially tunable with variation of the dye structures. In the array system, CuS NPs acted as receptor, and fluorescent dyes served as transducer. In order to make the platform portable, in total, four common and cheap and easily obtained fluorescent dyes with different functional groups (Rhodamine B (RB), Fluorescein (FL), Rhodamine 6G (R6G), 7-Hydroxycoumarin (HC)) were chosen in the array. They presented emissions at 550 nm, 495 nm, 525 nm and 320 nm upon excitation at 570 nm, 512 nm, 551 nm and 461 nm, respectively. (Fig. S1a) Their colours were red, green, yellow and blue, respectively, which could identified by naked eyes clearly. Their fluorescence was quenched after forming complexes with CuS NPs. Upon addition of the proteins, the dyes were displaced from the CuS NPs surface through competitive binding towards CuS and the fluorescence was recovered. Proteins bound to CuS particles in competition with fluorescence dyes which depended on not only the affinities of fluorescence dyes but also the structure and group of the protein. In the other hand, chips present excellent performance in sensing due to their high throughput ability as well as portability.[20] By applying chip analysis in the chemical tongue array, our sensing platform can be carried conveniently and simply operated. Furthermore, the sensing result is able to be observed by naked eyes under UV irradiation. As shown in Fig. 1, the different dye-CuS affinities and the different structural features of the proteins resulted in the variety of fluorescent signals. Consequently, the suggested pattern recognition platform displayed excellent discrimination among a wide range of analytes.

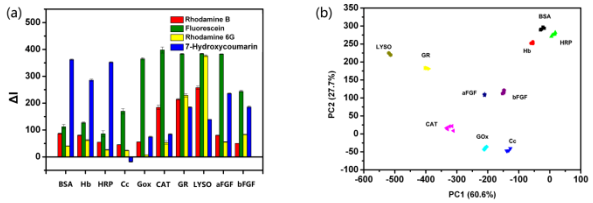

Fig. 2 (a) Fluorescence response (ΔI) of the CuS array in presence of 100 nM analyte proteins (BSA, Hb, HRP, Cc, GOx, CAT, GR, LYSO, aFGF and bFGF). Bar height represents average of five replicates. (b) The PCA score plots of 10 analytes measured five times.

The Morphology of CuS was characterized by TEM images. The CuS nanoparticles showed an average diameter of 8 nm with spherical structure (Fig. S2a, S2b). The X-ray diffraction pattern showed all of the peaks of the CuS NPs was similar to those of JCPDS no. 79-2321 and could be indexed as covellite-phase CuS with lattice parameters (Fig. S2c). A selected area electron diffraction (SAED) pattern (Fig. S2d) also confirmed the as-prepared CuS NPs structure. The optical property of the aqueous dispersion containing 0.3 mg/mL CuS NPs was studied by using UV-Vis-NIR spectroscopy (Fig. S3a). The CuS showed absorption in almost all wavelengths, which suggested that the CuS was an excellent quencher. The FTIR spectra (Fig. S3b) showed emerging absorption band at around 1600 cm^{-1} in the sample of CuS was assigned to C=O stretching of the carboxyl groups contained within the attached sodium citrate molecules. This indicated the presence of the citrate on the surface, which enabled the high stability of aqueous dispersion of CuS NPs.

To confirm the feasibility of the proposed strategy, we investigated the quenching ability of CuS NPs. RB, FL, R6G and HC with a certain concentration were added in the well plates. They presented bright red, green, yellow and blue emission under the irradiation of UV lamp. As shown in Fig. S1b, after adding the well dispersed CuS solution and incubating for 10 min, the dyes interacted with the CuS NPs and their fluorescence was quenched completely. The control group was initiated by adding phosphate-buffered saline (PBS) buffer instead of CuS solution. Little fluorescence change was observed. The changes of fluorescent intensity versus the concentration of CuS were obtained to quantify the affinities of reporters. As shown in Fig. S4 and S5, the fluorescent intensity declined with the increase of concentration of CuS. The quenching was analyzed using the Stern-Volmer equation: $F_0/F = 1 + Ksv[Q]$, where F and F_0 are the fluorescent intensity at 570 nm, 512 nm, 551 nm, 461 nm for each fluorescent dye in the presence and absence of CuS, respectively. Ksv is the Stern-Volmer quenching constant, and [Q] is the quencher concentration. The Ksv values were 0.491 L/mg, 0.32 L/mg, 0.69 L/mg and 0.326 L/mg. The Ksv suggested that CuS exhibited different quenching ability toward each fluorescent dyes. To explore the fluorescence recovery caused by analytes, proteins were first used for testing. Bovine serum albumin (BSA) was mixed with the solutions of dyes and CuS. Competitive binding between dyes and BSA occurred on the CuS surface. As a result, the fluorescence of dyes was modulated by both BSA and CuS. It turned out that a significant fluorescence enhancement of the solution was observed (Fig. S1b). In contrast, the fluorescence of wells composed of dyes and CuS remained quenched under the UV irradiation. These results indicated that the quenching-recovery of the dye-CuS system could realize a protein sensing array.

In order to demonstrate the performance of the platform, the diversity responses of the sensing array were investigated. The dye-CuS was treated with and without BSA followed by measuring the fluorescent spectra. Due to the diversity of the molecular structure of dyes, the rates of displaced dyes by BSA were distinctly different. As a result, the fluorescence recovery demonstrated variety. As exhibited in Fig. S6, the fluorescent intensity could recover to about 17%, 8%, 22% and 72% of the original one of RB, FL, R6G, HC, respectively. For different dyes, the recovery extents were different when the concentration of BSA was the same. To exam the ability of distinguish multi-analytes, we then utilized the sensing array to identify multiple samples. We selected 10 proteins which have different molecular weights and isoelectric points (Table S1), including bovine serum albumin (BSA), hemoglobin (Hb), horseradish peroxidase (HRP), cytochrome c (Cc), glucose oxidase (GOx), catalase (CAT), glutathione reductase (GR), lysozyme (LYSO), acid fibroblast growth factor (aFGF) and basic fibroblast growth factor (bFGF). These proteins were diluted with PBS to an even concentration. Fig. 2a showed the fluorescence response on the sensing array for

proteins followed by incubated with the dye-CuS solution. The relatively fluorescence changes for proteins were significantly different due to the difference interaction between the CuS and proteins. The unique pattern of the fluorescence changes for each protein was used to identify the proteins.

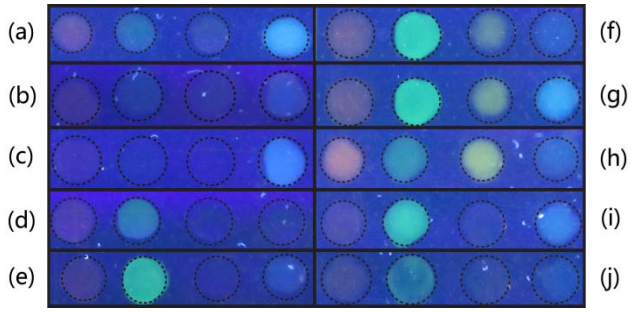

Fig. 3 The visual identification of ten kinds of proteins using probe chips array. The proteins were incubated with probes on the probe chips for 5 min before measured. These proteins are (a) BSA, (b) Hb, (c) HRP, (d) Cc, (e) GOx, (f) CAT, (g) GR, (h) LYSO, (i) aFGF and (j) bFGF.

The pattern response data were quantitatively analyzed using the method of PCA, which visualized the extracted feature for proteins identification. The original data of responses were compiled into a matrix. Five replicates were obtained for each protein in each sensor, producing 200 data points (4 sensors × 10 proteins × 5 replicates) for each array. Four principal components were generated, which represented linear combinations of the fluorescence response matrix. The first two most significant principal components accounted for 60.6% and 27.7% of variance in the detection, respectively. A 2D plot was presented in Fig. 2b in which each point represents the response pattern for an individual protein sample against the sensor array. The protein targets were clearly classified in clusters in this pattern recognition using the method of PCA. To validate the recognition efficiency of the 10 different proteins, the PCA results were utilized as training sets ("training matrix") for identification of unknown specimens. We tested 30 unknown samples against the training sets, achieving 87% of identification accuracy for a randomly selected group of samples (26 out of 30, Table S2). The results indicated that the protein targets were clearly classified and identified in this pattern recognition using PCA identified. The limit of detection (LOD) of the sensor array could identify is determined by the dose−response experiment. For BSA, different concentrations of analytes were detected with the RB-CuS sensor. Fluorescence change upon the increasing concentration of BSA was shown in Fig. S7, suggesting the interaction between BSA and CuS depended on the concentration of BSA. The LOD reached 10 nM for BSA, at least comparable to the previously reported method.[9d] This indicated the sensor array was sufficiently sensitive to identify proteins at nanomolar concentrations.

After the ability of identifying proteins of this array was investigated, we expected to carry out the experiments on quartz chips to develop a portable and high throughput chemical tongue platform. As showed in Fig. S8a, the quartz chips were sized of 3 cm × 1 cm × 1 cm with smooth surface. To exam whether the fluorescence could be observed by naked eyes, 5 μL of 4 kinds of dyes were dropped on different position of the surface of a quartz chip. Under UV irradiation, the fluorescence could be readout in the location of each dye (Fig. S8b). In order to perform the pattern recongnition array on the chips, we replaced the dyes by 4 kinds of dye-CuS probes to generate a probe chip. The sample proteins were dropped on the corresponding position of the 4 probes. After incubation, the probe chip was irradiated by a UV transillumination and the images of the probe chip were taken. Ten chip images were presented in Fig. 3 in which each chip represented an individual protein sample of the ten proteins we test above. The fluorescent spots could be read out and distinguished by naked eyes resulted in a visual identification of proteins. What's more, several probe chips could be read out simultaneously, which indicated this chemical tongue with probe chip array was a high throughput platform These probe chips with dye-CuS probes were also handy and convenient for easy carry and derectly sensing.

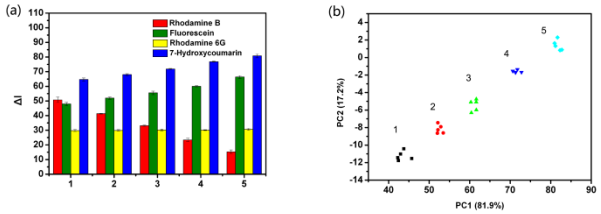

Fig. 4 (a) Fluorescence response (ΔI) of the CuS array in presence of mixtures of extracts. Ratios of MSSA:MRSA of sample 1-5 were 100%:0, 75%:25%, 50%:50%, 25%:75%, 0%:100%, respectively. (b) The PCA score plots of 5 mixture of extracts measured five times. Ratios of MSSA:MRSA of sample 1-5 were 100%:0, 75%:25%, 50%:50%, 25%:75%, 0%:100%, respectively.

Since this platform exhibited excellent performance in distinguishing proteins, we applied the proposed system in identification of antibiotic-resistant bacteria. Proteins existed widely in bacteria and were variety between bacterial species and even between different strains of the same species. For example, the extract of MRSA contains β-lactamase enzyme which was the main cause for the antibiotic resistance of SA. In contrast, in MSSA which do not show antibiotic resistance, theβ-lactamase enzyme cannot be expressed.[21] CuS nanoparticles were expected to identify bacteria via interacting with the proteins in bacteria and the bacteria extracts were employed as sensing samples. Methicillin-resistant Staphylococcus aureus (MRSA) was used as an example of antibiotic-resistant bacteria and methicillin-susceptible Staphylococcus aureus (MSSA) was used as antibiotic-susceptible bacteria in this work. The bacteria extracts of MSSA and MRSA were obtained by ultrasonic degradation. The extracts were firstly investigated by the dye-CuS sensor array by recording the fluorescence change. Fig. S9 indicated that the extracts of MSSA and MRSA showed different interaction to the Dye-CuS system owing to the dissimilarity their component. The fluorescence data were also quantitatively analyzed using PCA (4 sensors × 2 extracts × 5 replicates). The first two most significant principal components accounted for 86.1% and 13.4% of variance in the detection, respectively. The PCA score plots showed that the extracts of MSSA and MRSA were clearly distinguished in clusters (Fig. S10). We then tested mixtures of extracts of MSSA and MRSA with different molar ratios (concentration rate = 75/25,

25/75, and 50/50). The fluorescence responses were differentiated with each other obviously (Fig. 4a). The PCA score plots showed that not only the extracts but also mixtures were clearly distinguished from each other (Fig. 4b). The plots properly arranged with the order of ratios in the dimension of the two principal components. As a result, our sensor array presented highly stability and selectivity for identification of antibiotic-resistant bacteria and hold great promise for practical application. It also indicated that our pattern sensor array has potential applications in identification in complex system such as whole blood for biomedical diagnostics..

Conclusions

In summary, we develop a facile and portable sensor array for high sensitive identification of proteins and antibiotic bacteria based on the CuS NPs. The CuS NPs with four different fluorescent dyes are employed to construct a label-free 4-sensor array, which is simply in design and low cost. The fluorescent signals can be measured conveniently by fluorescent spectroscopy without any complex instruments. More importantly, CuS NPs are a broad spectrum sensing probe when compared with traditional π-π stacking or electrostatic interaction probes. The design principle denotes the universal application of the approach. This platform provides high-precision identification of a variety of proteins with different molecular weights and isoelectric points. The limit of detection is better than or at least comparable to the previously reported method. The application of the quartz chips in the pattern recognition array provides a visual identification for proteins which can be read out by naked eyes. Meanwhile, the probe chips are not only facile to prepare and easy to carry but also high throughput. Furthermore, it exhibits excellent discrimination ability between antibiotic-resistant and antibiotic-susceptible bacteria extracts. As many biomolecules can form stable complexes with CuS Ns, it offers a potential approach to the detection of a wild spectrum of analytes. We expect this strategy may have important applications in biosensing, biomedicine and nanotechnology in the future.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experiment section and Supporting figures. See DOI: 10.1039/b000000x/

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