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A graphene oxide-based AIE biosensor with high selectivity toward bovine serum albumin†

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Graphene oxide (GO) was found to effectively enhance the selectivity of aggregation-induced emission (AIE) biosensors, and a new method based on GO and AIE molecules was proposed to detect bovine serum albumin (BSA) with high sensitivity and selectivity.

The search for various biosensors to detect proteins with high sensitivity and good binding linearity has attracted more and more interest in recent years, due to the important role of proteins in the life course and their close relation to the origin, evolution and metabolism of life. Several classic detection methods were developed for sensitive protein quantification in solution or gel, including absorption spectrometry, Lowry method,[1] Biuret method,[2] Bradford method,[3] and fluorescence spectrometry. Among them, the fluorescence (FL) probe methods exhibited some advantages, such as high sensitivity, low background noises, and wide dynamic ranges, in which fluorescamine, cyanine dyes, SYPRO dyes, and Nile Red are the famous ones used.[4] As for the analytes of proteins, serum albumins are the major soluble protein constituents of the circulatory system and have many physiological functions,[5] for example, serving as transporters for a variety of compounds. Especially, bovine serum albumin (BSA) has been one of the most extensively studied proteins in this group, because of its structural homology with human serum albumin (HSA).[6] So far, different FL reagents have been used for the detection of BSA,[4e,7] however, the thorny aggregation-caused quenching of FL probes led to drastic reductions in their FL signals, accompanying with the aggregation of the dyes.

Recently, Tang *et al.* have discovered a novel photophysical effect of aggregation-induced emission (AIE): nonluminescent molecules are induced to emit efficiently by aggregate formation.[8] In order to explain and confirm the AIE phenomenon, different types of AIE molecules were synthesized for investigation, combining with theoretical calculations, the restriction of intramolecular rotation in the aggregates was found to be
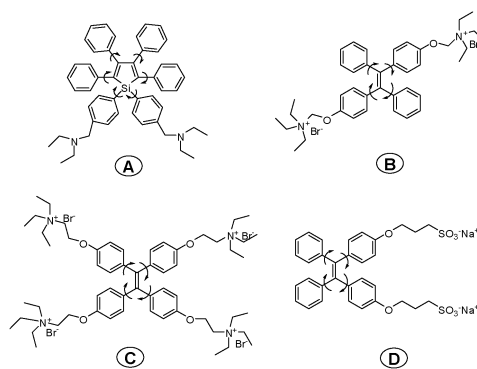
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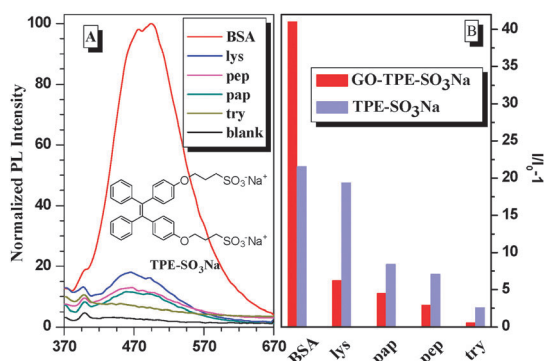
Chart 1 Different molecular structures of some water soluble AIE derivatives for the detection of biomacromolecules.

the main reason.[8] In view of such unusual fluorescence behaviors of AIE molecules, a variety of new AIE-based biosensors has been developed. Chart 1 shows some examples of AIE molecules for the detection of biomacromolecules (DNA, RNA, and proteins), including BSA.[9] Taking TPE–SO_3Na (D in Chart 1) for example, it is nonluminescent in solution, upon the addition of BSA, the molecules are caught by BSA, and the binding site of BSA prevents the free rotor motions in the molecule moiety, thus, TPE–SO_3Na becomes highly emissive.[9b] It is a pity that although high sensitivity is achieved, nearly no selectivity is obtained. Besides BSA, similar response was observed in the presence of other proteins (B in Fig. 1),[9b] which badly limits its practical application in the biological and related fields. Other AIE biosensors demonstrate the same behavior, *i.e.* nearly no selectivity for biomacromolecules.[9] Thus, if this problem could be resolved, AIE biosensors will exhibit more advantages over other biosensors.

Graphene (G), a single layer of carbon atoms in a closely packed honeycomb two-dimensional lattice,[10] is a new star nanomaterial due to its unique properties.[11] Graphene oxide (GO), the oxidized counterpart of G,[12] has been used as a platform for the detection of DNA,[13] proteins,[14] metal ions,[15] drug delivery,[16] and so on,[17] by utilizing its water dispersibility, versatile surface modification and photoluminescent quenching effect. And it was reported that there were some π–π interactions between the aromatic fluorophores and GO, which directly quenched the fluorescence. As mentioned above, after AIE biosensors are caught by proteins, the binding site of proteins

Fig. 1 (A) Fluorescence spectra of GO–TPE–SO$_3$Na upon the addition of BSA, lysozyme, pepsin, papain and trypsin at the same concentration of 200 μg mL^{-1}. (B) Fluorescence intensity changes (I/I_0-1) of GO–TPE–SO$_3$Na and TPE–SO$_3$Na towards different proteins, including BSA, lysozyme, papain, pepsin and trypsin, at the same concentration of 200 μg mL^{-1}. Excitation wavelength (nm): 350.

prevent the free rotor motions of the aromatic rings in the AIE molecules, leading to the induced fluorescence. Thus, if GO was introduced into the AIE sensing system, there should be mainly two different interactions to our concern: the π–π interactions between the AIE sensors and GO, and the binding effect of AIE molecules and the preferred protein. Only in the case where the binding effect is stronger than the π–π interactions, some fluorescent signals could be detected. If not, none. Thus, due to the different binding effects of AIE molecules toward different proteins, it is possible that GO can be used to enhance the selectivity of AIE-based biosensors. In this communication, we report a new method for the detection of BSA with good sensitivity and selectivity, with the aid of GO.

Fig. 2 shows a schematic representation of this detection platform. TPE–SO$_3$Na can be bound to GO to form a GO–TPE–SO$_3$Na complex through π–π stacking interaction. Upon the addition of BSA, TPE–SO$_3$Na might be possibly dragged out to bind with BSA through hydrophobic interaction, the non-emissive TPE–SO$_3$Na becomes highly emissive as a result of the restriction of its phenyl rotors, providing the detection of BSA sensitively and selectively, since the binding effect of TPE–SO$_3$Na for BSA is a little higher than other proteins (Fig. 1B).

TPE–SO$_3$Na (Fig. 1) was synthesized as reported previously.[9b] GO was prepared using the modified Hummers method.[18] After the preparation of the complex of GO and TPE–SO$_3$Na, BSA was added to its solution, fluorescence signals were observed at the concentration of 5 μg mL^{-1}, and the fluorescence intensity increased with the increase of the concentration of BSA (Fig. S1, ESI†). In the concentration range of 0–60 μg mL^{-1}, the plot of the BSA concentration is nearly a linear line with a correlation coefficient of 0.981 (Fig. S2, ESI†), and the detection limit was determined to be 0.4 μM (signal-to-background ratio higher than 3). At the concentration of 200 μg mL^{-1}, the PL intensity enhanced 40 times, showing high sensitivity towards

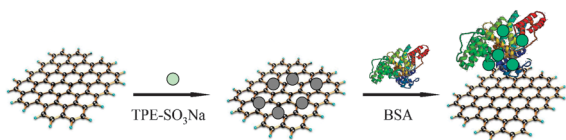BSA (Fig. 1). A control experiment was conducted to see the sensitivity of TPE–SO$_3$Na towards BSA without GO (Fig. S3, ESI†). The solution of TPE–SO$_3$Na in the absence of BSA is almost nonluminescent, the fluorescence of TPE–SO$_3$Na switched on instantly by the addition of BSA. At the concentration of 200 μg mL^{-1}, the PL intensity enhanced only 22 times (Fig. 1). As we know, GO can quench fluorescence signals of fluorescent molecules.[13,14] In our case, even though the fluorescence of TPE–SO$_3$Na is weak in solutions, the quenching efficiency of GO on TPE–SO$_3$Na still leads to the low background, thus, the signal-to-background ratio can be greatly improved. So, it is noteworthy that GO greatly amplifies the fluorescence signal and enhances the sensitivity of AIE biosensors.

In order to investigate whether this new method can enhance the selectivity of AIE-based biosensors, control experiments were carried out by using lysozyme, pepsin, papain and trypsin instead of BSA under the same conditions. Fig. 1A shows the normalized PL intensities of GO–TPE–SO$_3$Na towards different proteins at the same concentrations. Besides BSA, nearly no obvious fluorescence turn-on was observed. Fig. 1B and Fig. S4 (ESI†) demonstrate the fluorescence intensity changes (I/I_0-1) of GO–TPE–SO$_3$Na complexed with proteins, the fluorescence-enhancing capability of BSA is seven to seventy times than that of other proteins. For comparison, in the absence of GO, the selectivity of TPE–SO$_3$Na itself towards different proteins was also studied (Fig. S5–S7, ESI†). The fluorescence of TPE–SO$_3$Na was light-up immediately with the addition of lysozyme or papain. Taking lysozyme as a typical example, at the concentration of 200 μg mL^{-1}, the PL intensity was enhanced 19 times, nearly identical to BSA (Fig. 1B and Fig. S8, ESI†). So, it is evident that TPE–SO$_3$Na shows bad selectivity towards proteins, similar to that reported in the literature.[9b]

Generally, BSA consists of two binding sites, namely, site I and site II. The binding affinity offered by site I is mainly through hydrophobic interactions, while, site II involves hydrophobic, hydrogen bonding, and electrostatic interactions.[7a,19] For our case, TPE–SO$_3$Na is negatively charged, so the electrostatic interactions should be ignored, and hydrophobic interaction plays an important part. To partially confirm this point, and also investigate the possible influence from charged molecules, we further conducted some control experiments by using some inorganic salts, tetra-butylammonium iodide (TBAI), and sodium dodecylbenzenesul-fonate (SDBS) as possible interferents. As shown in Fig. S9–S11 (ESI†), the addition of these charged molecules did not cause any apparent change in the fluorescent intensity of GO–TPE–SO$_3$Na, and GO–TPE–SO$_3$Na could still report BSA selectively in the presence of these charged molecules. Based on the results, we assume that there are perhaps two reasons for the high sensitivity and selectivity of TPE–SO$_3$Na towards BSA in the presence of GO: first, the hydrophobic interactions between BSA and TPE–SO$_3$Na are high enough to disturb the π–π interactions between GO and TPE–SO$_3$Na; secondly, the distance between GO and BSA is far enough to hold up the energy transfer.

We used atomic force microscopy (AFM) to characterize GO and GO–TPE–SO$_3$Na–BSA. Fig. 3 (left) shows the AFM image of GO prepared by a modified Hummers method, the thickness of GO was about 1 nm, in good accordance with that reported in the literature.[20] Upon addition of TPE–SO$_3$Na, the height was about 1.25 nm (Fig. S12, ESI†), no obvious change

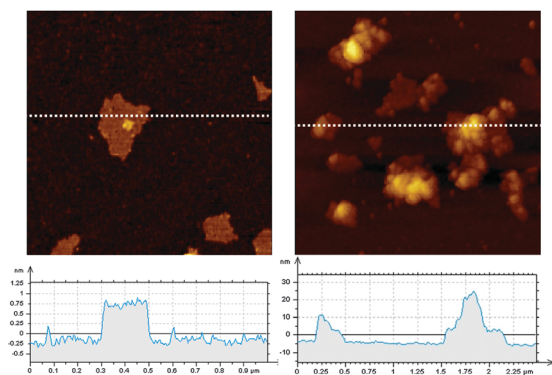**Fig. 2** Schematic representations of the detection for BSA.

Fig. 3 AFM images of GO (left) and GO–TPE–SO$_3$Na–BSA (right), scan size: 1.5 μm × 1.5 μm (left); 2.5 μm × 2.5 μm (right).

was observed, it can be concluded that TPE–SO$_3$Na molecules were homogenously absorbed on the surface of GO without any aggregation. After the addition of BSA to the complex of GO–TPE–SO$_3$Na (Fig. 3, right), the height increased to 10–20 nm, supporting the assumption that BSA capped TPE–SO$_3$Na aggregates at both sides of the GO sheet. Transmission electron microscope (TEM) was also used for the characterization of GO–TPE–SO$_3$Na before and after the addition of BSA (Fig. S13–S14, ESI†). After BSA was added, the GO sheet tends to aggregate, in well accordance with the AFM results (Fig. 3).

In conclusion, we present a new method for the detection of BSA with high sensitivity and selectivity. With the aid of GO, the defect of bad selectivity of AIE sensors can be resolved. We suggest that this method offers several advantages. First, GO can be easily chemically synthesized with large quantities, and GO can decrease the background fluorescence signals, therefore enhance the sensitivity. Second, the AIE biosensor is a new type of FL turn-on biosensor, there are several AIE-based biosensors for the detection of DNA or proteins, though all of them show high sensitivity but nearly no selectivity, however, by using this method, the problem can be successfully solved. Moreover, our studies of GO modified AIE biosensors will provide new insights for protein bioanalysis.

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