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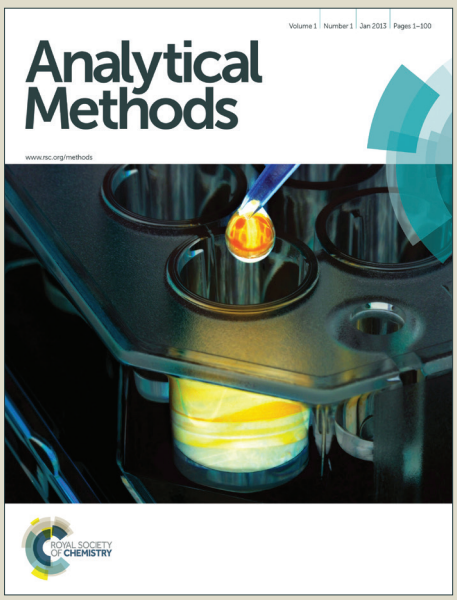

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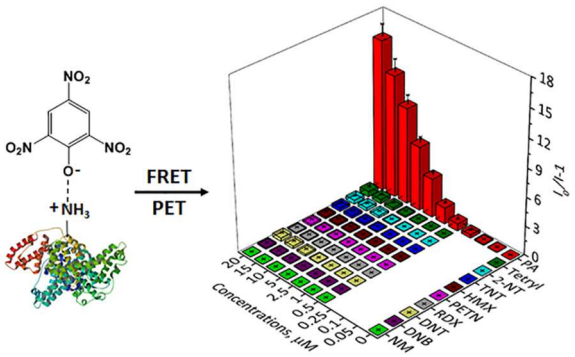

A novel protein based fluorophore for sensitive, selective and fast detection of picric acid in aqueous phase due to electron transfer, Förster resonance energy transfer and electrostatic interactions.

Analytical Methods

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Protein-based Sensitive, Selective and Rapid Fluorescence Detection of Picric Acid in Aqueous Media

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Xiangcheng Sun,[a] Xiaoyu Ma,[b] Challa Vijaya Kumar,[c] and Yu Lei[a*]

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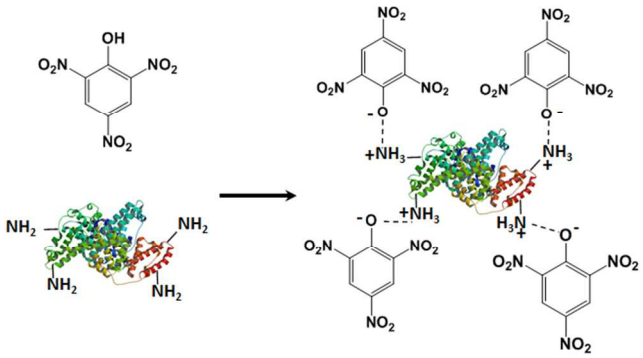
Highly-sensitive, ultra-selective and rapid detection of picric acid (PA) in aqueous media is accomplished using a protein fluorophore bovine serum albumin (BSA). This extraordinary sensing performance is ascribed to electron transfer and Förster resonance energy transfer (FRET) mechanisms as well as acid-base pairing interactions between amino groups of BSA and picrate anions of PA in aqueous solution.

Rapid, cheap, sensitive and selective detection of explosives is of paramount importance due to its implications in homeland security, global demining and environmental protection.[1-3] 2,4,6-trinitrophenol (picric acid, PA) has been employed in the preparation of lethal weapons for a long time, whose explosive power is superior to that of 2,4,6-trinitrotoluene (TNT).[4] PA is also widely used in the manufacture of rocket fuels, dyes, fireworks, matches and leather.[4-7] In addition, the wide use of PA has made it highly possible to be released into the environment and thus contaminate the soil and aquatic systems, but PA is harmful to wildlife and humans and has been recognized as an environmental contaminant.[8] Therefore, there is an urgent need to develop picric acid sensors.

Fluorescent sensing technology represents one of the most promising approaches for trace explosives detection due to the combination of a number of advantages: short response time, excellent sensitivity, and instrumental simplicity.[9-11] A majority of research has been focused on the development of TNT and 2,4-dinitrotoluene (DNT) sensors, but less attention has been paid to picric acid detection.[5,6,12] Even though several reports on fluorescence sensors for PA detection are available in literature, most of them lack the combined merits of high selectivity, low detection limit and quick response which are highly desirable for practical applications.[6,8,13-18]

In addition, several challenging issues still exist in current fluorescent materials for explosive detection. First, although popular, the conjugated polymers are often hampered by their time-consuming, low-yield and multi-step synthetic routes.[2,19,20] Second, other fluorescent materials are toxic and their potential leakage into the environment has increased much concern recently. For example, the toxicity of pyrene and related compounds discourage their routine use;[21] heavy metals such as Cadmium which have been widely used in quantum dots and metal-organic frameworks are also toxic to the environment and thus hamper their wide application.[6,22,23] Furthermore, limited aqueous stability/solubility of current fluorescent materials is another impediment that limits real-world applications.[6,24,25]

Bovine serum albumin (BSA), a protein derived from cows, has numerous applications due to its medical importance, low cost, ready availability, stability, unusual ligand-binding properties, etc.[26-29] BSA has two tryptophan residues (Trp-134 and Trp-212) that contribute to its intrinsic fluorescence. However, the application of BSA as a fluorescent protein for explosive sensing has not been fully investigated. The work reported herein demonstrates, for the first time, that ordinary BSA is capable of sensitive and fast detection of picric acid with unprecedented selectivity (Table S1).



Scheme 1 Schematic illustration of the selective electrostatic interactions between BSA and picric acid.

BSA in water has an absorption peak at 279 nm and exhibits a broad strong emission peak at ~ 348 nm when excited at 279 nm (Fig. S1). Fluorescence is strongly quenched upon incremental addition of PA to 0.5 μM BSA solution (Fig. 1). Due to the curvature noted in the corresponding Stern-Volmer plot (0.5 μM BSA, red triangles, Figure 1b), ground state association between BSA and PA was suspected (Scheme 1), and hence, the effect of BSA concentration on PA detection has been examined (Fig. 1b).

Trading-off the sensitivity and detection range, 0.5 μM of BSA was used in the later study. The detection limit for PA is 17.2 nM (3.9 ppb) using 0.5 μM of BSA fluorophore. This sensitivity is better than those of most current fluorescent PA sensors (See Table S1). In addition, the response time of BSA sensors to PA was investigated by monitoring the fluorescence intensity as a function of time at different concentrations of the analyte. The response to PA is almost instantaneous, and the equilibrium is also reached rapidly. The response and equilibrium time is less than 1 min, which is much better than the reported response time for PA fluorescence sensors.[8]

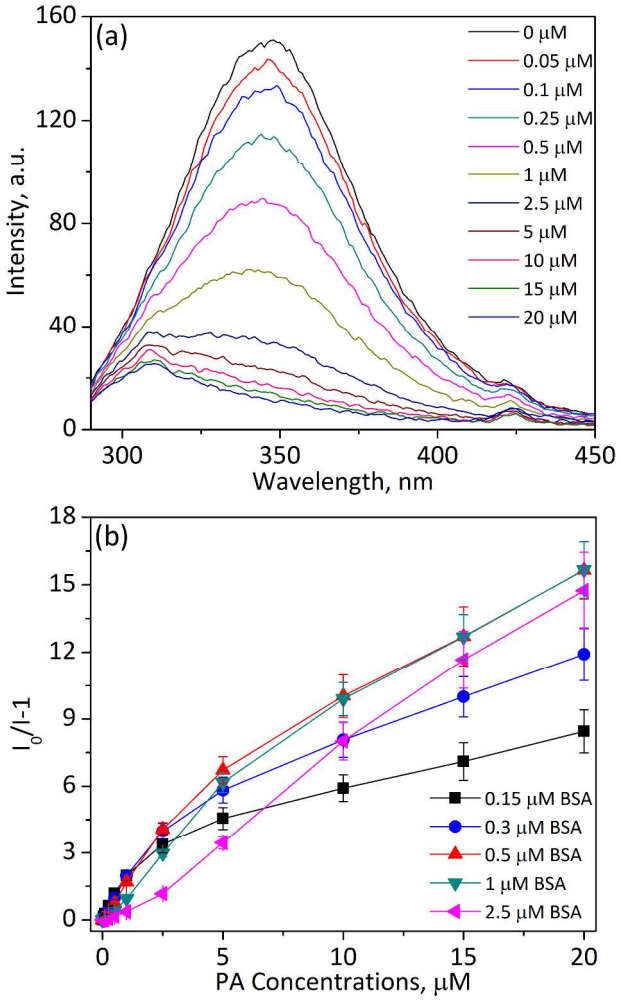


Fig. 1 (a) Fluorescence spectra of 0.5 μM BSA upon addition of various PA concentrations in water. (b) Stern-Volmer Plots for PA detection using various concentrations of BSA.

Fluorescence quenching titrations were also conducted with other nitro explosives. All the other analytes showed little effect on the fluorescence intensity of BSA (Fig. 2 and Fig. S2-S10). One can see from Fig. 2a that the fluorescence quenching percentage for 2.5 μM PA is 80.1%, while the same concentration of all the other analytes only quenches less than 8%. These results suggest that BSA fluorescent sensor possesses high selectivity toward PA against other nitro explosives. The quenching behaviour is also characterized by the quenching efficiency (I/I_0) and quenching constant (K_{SV}) using the Stern-Volmer (S-V) equation, $\boldsymbol{I_0/I = K_{SV}[A] + 1}$, where I_0 is the initial fluorescence intensity in the absence of analytes, I is the fluorescence intensity in the presence of analytes, [A] is the molar concentration of analytes, and K_{SV} is the quenching constant (M^{-1}). The intensity for BSA emission peak at ~ 348 nm are plotted versus the concentration of various analytes and shown in Fig. 2b. As shown in Fig. S11, the S-V plot for PA shows two linear regions at low and high concentration ranges, respectively, and bends downward at high concentrations. The phenomenon can be presumably attributed to the presence of two fluorophore populations, self-absorption or energy transfer processes.[3,6,30] For BSA intrinsic fluorescence, Trp-212 is located within a hydrophobic binding pocket of the protein and Trp-134 is located on the surface of the molecule.[28] The Trp fluorescence quenching is regarded as a sensitive technique for measuring ligand binding affinities.

The quenching constant for PA is 1.65×10^6 M^{-1} in the low concentration range, which is almost the largest value during the reported fluorescence PA sensors (Table S1).[5-8,13-18] The calculated quenching constant for PA is about two orders larger than those for other nitro-explosives (Table S2, e.g., TNT of 1.64×10^4 M^{-1}; DNT of 1.77×10^4 M^{-1}, etc.), which is one of the highest value known, suggesting an unprecedented selectivity to PA of the as-developed sensor.[5-8,14,16]

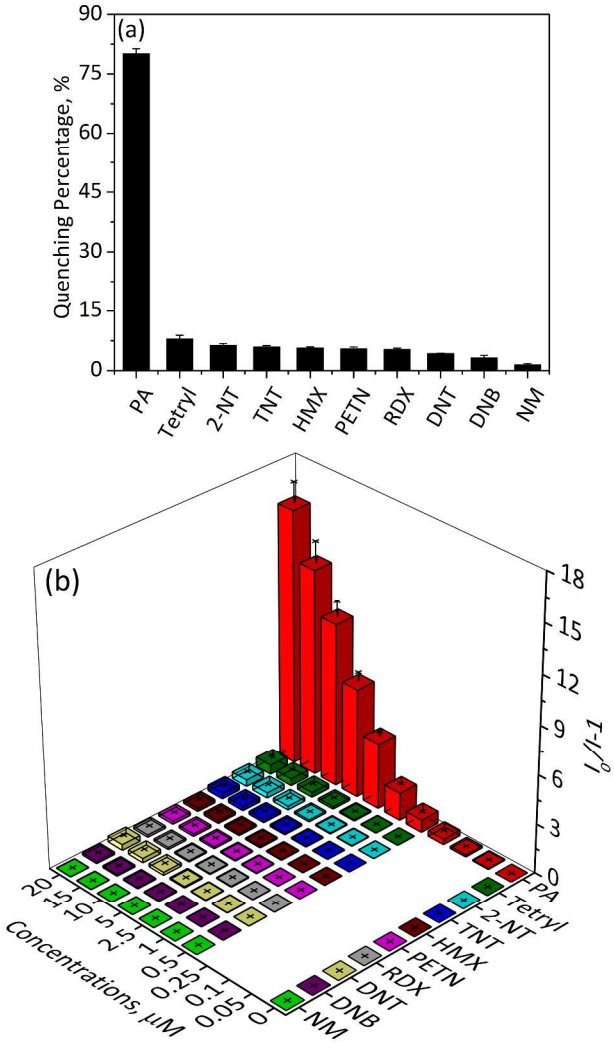


Fig. 2 (a) Percentage of fluorescence quenching obtained for BSA fluorescent sensors upon addition of 2.5 μM of different nitro-explosives (BSA concentration is 0.5 μM BSA), indicating excellent selectivity for picric acid. (b) Stern-Volmer plots of BSA fluorescent sensors with different nitro-based explosives at 0.5 μM BSA.

Generally, photo-induced electron transfer (PET) plays a key role in explosive quenching process due to the electron-deficient nature of explosives.[6,11,31] The driving force is the energy difference between the lowest unoccupied molecular orbital (LUMO) of fluorophore and LUMO of analytes. The LUMO values for different nitro-explosives are summarized in Table S3. As expected, because of its small LUMO value, the PET driving force for PA is larger than that of other analytes. This may partly explain the sensitivity and selectivity of BSA sensors to PA detection. However, the order of fluorescence quenching percentage is not in complete accordance with LUMO energy sequences; in addition, although PA and Tetryl have very similar LUMO values, the quenching performance demonstrates a large difference. These findings suggest that PET is not the sole mechanism for the observed fluorescence quenching.

The non-linearity of the S-V plot for PA possibly suggests ground state association of the donor and the acceptor followed by an energy transfer from the excited donor to the ground state acceptor. Energy transfer mechanism has been used to develop a number of elegant fluorescence sensors, and can dramatically enhance the fluorescence-quenching efficiency and also improve sensitivity.[6,10] According to Förster resonance energy transfer (FRET) theory,[1,29,32] the rate of energy transfer depends on: 1) the relative orientations of the donor and acceptor dipoles, 2) the extent of overlap of fluorescence emission spectrum of the donor (the fluorophore) and absorption spectrum of the acceptor (the analyte), and 3) the average distance between the donor and the acceptor. The probability of resonance energy transfer depends upon the extent of spectral overlap between these molecules. Fig. 3 depicts the absorption spectra of various analytes and emission spectrum of BSA.

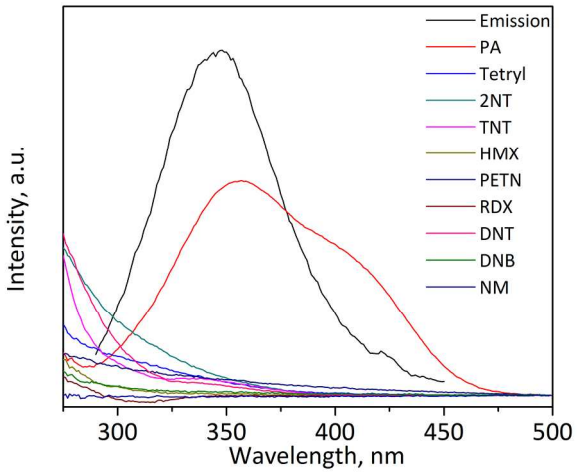


Fig. 3 Absorption spectra of various explosive analytes and emission spectrum of BSA in aqueous solutions.

The large overlap between absorption spectrum of PA and emission spectrum of BSA was observed, while almost no overlap for other analytes. Degree of spectra overlap integral J(λ) values for various explosive analytes were calculated and shown in Table S4. Thus the efficient quenching at ~ 348 nm for PA demonstrates a much higher quenching response because of higher probability of resonance energy transfer. In addition, the donor-to-acceptor distance r_0 is obtained as 2.70 nm (in Supporting Information), on the 2-8 nm scale, which greatly satisfies FRET requirements.

Furthermore, as a protein, BSA has a large number of amino groups, which might function as receptors for the nitroaromatic compounds. In the aqueous solution, PA behaves as a strong acid due to the three nitro groups affixed on the benzene ring, thus it can easily form a charged anion. As expected, BSA with high content of electron-rich amino groups could effectively bind to PA by the acid-base pairing interactions, and the charge transfer complexing interactions would lead to BSA fluorescence quenching.[5,6,8] Since the electron-withdrawing effect of the nitro group plays a crucial role in the interaction between PA and BSA, further fluorescence quenching titrations were conducted using 2,4-dinitrophenol (DNP), 4-nitrophenol (4-NP) and 3-nitrophenol (3-NP). As shown in Fig. S12, the quenching efficiency follows the order PA>DNP>4-NP>3-NP, which is in complete agreement with the order of acidity of these analytes, where the pKa values of the quenchers are 0.38 for PA, 4.89 for DNP, 7.15 for 4-NP, and 8.4 for 3-NP. In addition, the quenching efficiency of PA and DNP are much higher than their corresponding structure-similar nitro compounds (TNT, DNT and DNB). This feature when coupled with the magnitudes of the overall integrals may explain the molecular basis for the unprecedented selectivity for PA, as other nitro compounds do not have a hydroxyl group and so they cannot interact efficiently with amino groups of BSA, thus resulting in a very low quenching efficiency. In addition, the interaction of the hydroxyl groups with the basic sites in BSA is expected to follow the order of their acidity. PA, with its highly acidic hydroxyl group interacts strongly with the fluorophore and the quenching effect is carried over long range dipolar interactions owing to energy transfer mechanism, thus resulting in an amplified response.

Circular dichroism (CD) is highly sensitive to protein conformational changes which normally accompany upon interactions with small molecules. As can be seen in Fig. 4, BSA exhibits two negative CD bands at 208 and 222 nm in the ultraviolet region, characteristic of its typical α-helix structure.[29,33] The intensities of two double minimum reflect the amount of helicity of BSA and further these indicate that BSA contains more than 50% of α-helical structure. Upon addition of PA to BSA solution, the intensities of double minimum were reduced which is directly related to the interaction between PA and BSA. Therefore, the α-helix content of the protein secondary structure decreases as PA interacts with the BSA molecule, providing direct evidence that protein secondary structural changes are responsible for the quenching of BSA fluorescence by PA. Thus, direct evidence for the origins of quenching is provided which is in support of the above proposed energy transfer mechanism for explosive detection.

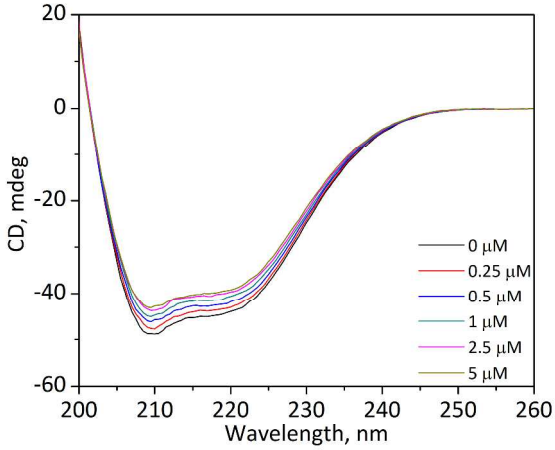


Fig. 4 CD spectra of of 0.5 μM BSA in the presence of various concentrations of PA.

In conclusion, we have successfully employed BSA as a fluorophore to detect PA in aqueous media, exhibiting high-sensitivity (as low as 17.2 nM), ultra-selectivity (about two

orders of K_{sv} values larger than other nitro-explosives), and short response time (less than 1 minute). These features can be explained by combined electron transfer and energy transfer due to large overlap between absorption spectrum of PA and emission spectrum of BSA as well as acid-base pairing interactions between amino groups of BSA and charged anion of PA. The present study provides insights into the design of a cheap and simple PA fluorescence sensor which is completely biodegradable and environmentally safe.

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Notes and references

[a]Department of Chemical and Biomolecular Engineering, University of Connecticut, Storrs, Connecticut, 06269, USA. Email: ylei@engr.uconn.edu
[b]Department of Biomedical Engineering, University of Connecticut, Storrs, Connecticut, 06269, USA.
[c]Department of Chemistry, Department of Molecular and Cell Biology, and the Institute of Materials Science, University of Connecticut, Storrs, Connecticut, 06269, USA.
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