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ARTICLE

Ratiometric fluorescent detection of silver ions using thioflavin T-based organic/inorganic hybrid supraparticles

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In this work, we present a new type of functional organic/inorganic hybrid supraparticles that spontaneously assemble from silver ions (Ag^+), iodide ions (I^-) and thioflavin T (ThT) under an aqueous solution condition. ThT alone in aquous solution was weakly fluorescent with emission band at 494 nm, which was related to the monomer. However, in the above-mentioned hybrid supraparticles (i.e., ThT@AgI SPs) structure, the ThT monomer can form dimer with a new emission band. The new band shifted to 546 nm and the emission intensity increased. We further present a facile strategy of reversible fluorescence switch of ThT by simple cation (Ag^+) and anions (I^- and S^{2-}), which can be employed for ratiometric fluorescent detection of Ag^+ with high sensitivity and selectivity. The linear range of detecting Ag^+ was from 100 nM to 10 μM, with a limit of detection as low as approximately 50 nM. Morever, it can be successfully applied for operation of a logic gate system and to the sensing of Ag^+ in real water samples.

Introduction

Smart supraparticle assemblies that can respond to one or more external stimuli, such as pH, ionic strength, temperature, etc., have great potentials to be applied in a wealth of fields, including drug delivery, and design of new sensors and catalysts.[1] Organic molecules are reported to be integrating functional inorganic components (e.g., metal nanoparticles, silica, quantum dots, and transition metal ions) for making functional organic/inorganic hybrid supraparticles (SPs) with interesting properties, which represents an interesting new direction.[2] Silver-based nanomaterials (AgNMs) are a large group of structurally well-defined inorganic particles with diversified structures and properties. The marriage of AgNMs and organic components will endow the as-prepared hybrid SPs with appealing properties of potential utility. For example, biomolecule-scaffolded fluorescent silver nanoclusters (AgNCs) have recently gained considerable attention due to their good biocompatibility and facile synthesis, which have found a wide range of biomedical applications.[3] Takata et al. reported the facile synthesis of variable-size AgI nanoparticles (AgI NPs) coated with poly-N-vinyl-2-pyrrolidone (PVP), and this PVP/AgI hybrid SPs can realize the highest ionic conductivity for a binary solid at room temperature.[4] However, the applications of certain silver-based hybrid SPs are still limited owing to

their inborn nature of instability. In this regard, if the synthesis process rather than the product of silver-based hybrid SPs can be directly applied in the area of sensing, the instability of such hybrid SPs after a long time no longer matters. To the best of our knowledge, few studies have been performed to exploit the formation process for sensing application. Moreover, to open new avenues for smart silver-based hybrid SPs, the synergistic combination of AgNMs and fluorescent dyes or probes would be worthy of consideration.

Thioflavin T (ThT), a very important dye molecule, can bind to amyloid fibrils with dramatic fluorescence enhancement.[5] ThT has been widely used to study several neurodegenerative disorders such as Alzheimer's, Parkinson's, Huntington's and prion diseases.[6] Over the years, it has been found that the "off-on" fluorescence regulation of ThT can be induced by different certain confining systems like cyclodextrin, cucurbiturils, polymer membranes, micelles, porous silicon, mesoporous silica xerogels, DNA, etc.[7-13] However, little is known about the mechanism even over the molecularity responsible for the fluorescence enhancement of ThT in a restricted environment.[7] To develop novel strategies for the "off-on" fluorescence regulation of ThT would be beneficial for illustrating its fluorescence enhancement mechanism by spatial confinement and furthermore expanding its application in diverse fields, such as biosensors.

Fluorescence-based sensors have applied to sensitively and selectively detected small organic molecule, metal ions, and gases in recent years.[14] Some of them have been reported about fluorescent Ag^+ sensors derived from benzenamine, ethanamine, pyrene and cyanine.[15-18] However, most of Ag^+ sensors used complex methods to synthesize their fluorescent probe by using organic solvent and even toxic chemicals, so that they are not cost-effective and environmental-friendly.

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†Electronic Supplementary Information (ESI) available: Reagents, instrumentation and supporting data. See DOI: 10.1039/x0xx00000x.

Therefore, it is highly desirable to develop a facile, green (low toxicity and consumption reagent) and environmental-friendly probe for the detection of Ag+. It is reported that Ag+ is capable of coordinating with the sulfur atom of the ThT benzothiazolering,[19] and also Ag+ can covalently bonds with I− to generate AgI NPs.[20] Enlightened by the above facts, we herein prepare a novel silver-based hybrid SPs that spontaneously assemble from Ag+, I− and ThT under an aqueous solution (Scheme 1A). The fluorescence of ThT can be enhanced with another emission band accompanied by a red shift in such hybrid supraparticle structure (ThT@AgI SPs), which offers a new opportunity to regulate the fluorescence of ThT and also provides a potential to form a novel fluorescent material. As mentioned above, ThT is a well-known fluorescent marker used in biomedical research, but the mechanism of its "off-on" fluorescence regulation induced by confining system is poorly understood, and few reports have been unveiled on expanding its application in varied areas. In this respect, a facile strategy is further presented to reversible fluorescence "off-on" switching of ThT by simple cation (Ag+) and anions (I− and S2−), which can be readily employed for ratiometric fluorescent detection of Ag+ with high sensitivity and selectivity, and furthermore for the operation of a logic gate system (Scheme 1B).
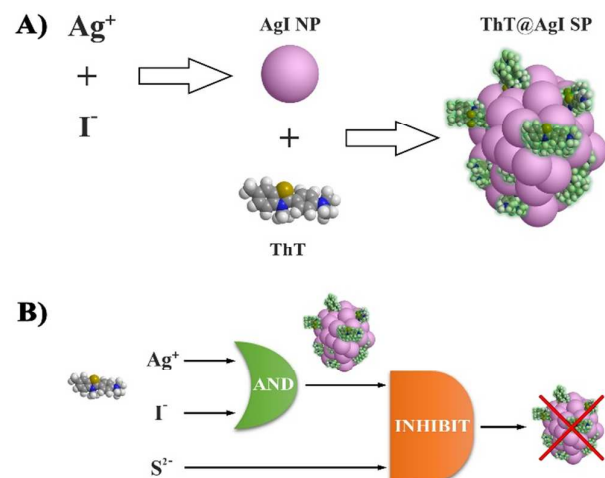
Scheme 1. (A) The preparation of our proposed hybrid SPs that spontaneously assemble from Ag+, I− and ThT under an aqueous solution; (B) The construction of a logic gate system based on the reversible fluorescence switching of ThT by Ag+, I− and S2−.

Experimental

Reagents and materials. Thioflavin T was obtained from the Sigma Chemical Company. Silver nitrate was purchased from Shanghai Chemical Reagent Co. Ltd. Other chemicals were of analytical reagent grade and came from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All chemicals used were bought from commercial sources and directly used without further purification. All aqueous solutions were prepared with ultrapure water (>18 MΩ•cm).

Instrumentation. Fluorescence spectra were recorded in a fluorescence microplate reader (Infinite M200 pro, TECAN, Switzerland) using a black 384-well microplate (Fluotrac 200, Greiner, Germany). The excitation wavelength used was 425 nm for the emission spectra. BioScope atomic force microscope (AFM) (NanoScope IIIa SPM System, Digital Instruments, Inc., U.S.A) was used to study the morphology of the as-prepared AgI NPs and ThT@AgI SPs. Dynamic light scattering (DLS) was performed on a Malvern Instruments Zetasizer Nano-ZS instrument for characterization of the size distribution of materials in solution. The elemental analysis was performed with energy-dispersive X-ray spectrometer (EDX, X-Max Oxford, U.K). X-ray diffraction (XRD) patterns were obtained using X-ray powder diffraction (XRD, Bruker D8 Advance X-ray diffractometer, Bruker AXS, Inc., Madison, WI, USA).

Measuring the fluorescence spectra of varied concentrations of ThT. A solution of varied concentration of ThT (0.5 μM, 1 μM, 10 μM, 25 μM, 50 μM, 250 μM, and 500 μM) were prepared at room temperature. Then, the fluorescence emission spectra were recorded.

Assays for Ag+. The detection of Ag+ was realized based on the following operation. Different concentrations of Ag+ (0, 0.1, 0.5, 1, 2, 4, 6, 8, 10, 20, 30, 40, and 50 μM) was mixed well with ThT–I− solution (1 μM ThT and 100 μM I− used). Subsequently, the fluorescence intensity of the solution was measured. The selectivity analysis for Ag+ was confirmed by adding other competing metal ions instead of Ag+ in a similar way, and the final concentrations of the cations were 1000 μM, while the concentration of Ag+ was 20 μM.

Logic operation. A logic gate system was set up consisting of 1 μM ThT, 20 μM Ag+, 100 μM I− and 50 μM S2− as inputs. The fluorescence emission spectra of corresponding mixed solution were recorded.

Results and discussion

Ag+ can covalently bond with I− to generate hardly soluble AgI NPs due to that I− in the anionic state is highly polarizable. When excess I− is added, the solubility increases dramatically attributed to the formation of complexes, a cluster containing AgI NPs and excess I−. When the total Ag+ concentration increases, the concentration of the cluster of AgI NPs rises.[21] The ThT molecule consists of a pair of benzothiazole and dimethylaminobenzene rings linked with a C–C bond.[22] Because of its ability to gradually twist around the single C–C bond, ThT behaves as a rotor molecule. Such a bond twisting process introduces a highly efficient internal non-radiative channels, resulting in an extremely low emission yield. In solution conditions, the positively charged ThT molecule dissociates completely into chlorion and a cationic derivative of benzothiazole aniline.[12] Ag+ can form a weak coordination complexe with the sulfur atom of the ThT's benzothiazole ring. Meanwhile, Ag+ and excess I− can form a negatively charged AgI NPs cluster. In this case, a new type of small molecule–NP hybrid supraparticles (i.e., ThT@AgI SPs) would be formed by the spontaneously assemble of Ag+, I− and ThT.
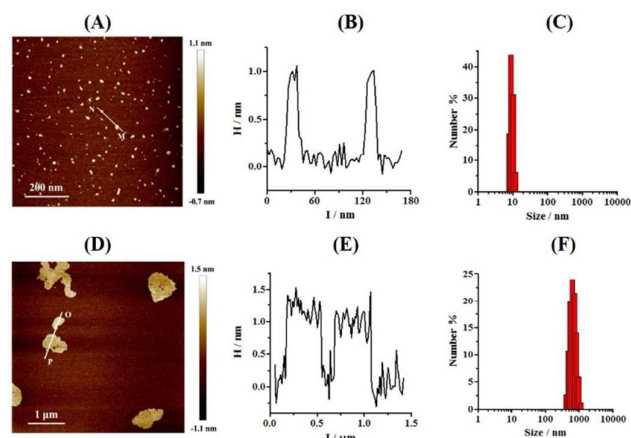
Figure 1. Atomic force microscope (AFM) image of (A) AgI NPs and (D) ThT@AgI SPs. (B) The height profile along the line MN indicated in (A), and (E) The height profile along the line OP indicated in (D). Size distribution of (C) AgI NPs and (F) ThT@AgI SPs by dynamic light scattering (DLS).

In the absence of ThT, the assembly of Ag^+ and I^- can form AgI NPs, which were characterized by atomic force microscopy (AFM) and dynamic light scattering (DLS). The AFM image showed that the as-prepared AgI NPs are highly dispersed in aqueous solution (Figure 1A), and the DLS measurement indicated that the average diameter of AgI NPs is estimated to 9 nm (Figure 1C). The AFM image indicated that the average topographic height of AgI NPs is about 1 nm (Figure 1B). The chemical compositions of AgI NPs were analyzed by an energy-dispersive X-ray (EDX) spectrograph. The peaks of Ag^+ and I^- were shown in the spectra of AgI NPs (Figure S1). To demonstrate ThT@AgI SPs, we integrated Ag^+, I^- with ThT and observed spontaneous formation of supraparticles. The AFM image in Figure 1D and DLS measurement in Figure 1E confirm the successful assembly of ThT@AgI SPs with an average diameter of 654 nm, which are obviously larger than AgI NPs in diameter. We also carried out XRD analysis to investigate the crystallite structures of ThT@AgI SPs and AgI NPs, and the corresponding XRD patterns are shown in Figure S2. The diffraction peaks located at 2θ values of 22.32°, 23.71°, 25.35°, 39.2°, 42.63°, and 46.31° were indexed as the (1 0 0), (0 0 2), (1 0 1), (1 1 0), (1 0 3), and (1 1 2) planes of the standard hexagonal β-AgI (JCPDS No. 09-0374). Moreover, the peaks obtained were intense and sharp, demonstrating that the two samples were well crystallized meanwhile no other impurities were detected. In addition, it was clearly observed that ThT@AgI SPs kept much the same crystal structure as AgI NPs in the progress of assembly from ThT and AgI NPs.

As shown in Figure 2, ThT in solution conditions was weakly fluorescent with an emission band at 494 nm, and no significant change is observed in the fluorescence spectra of ThT upon the addition of Ag^+ or I^- alone. However, in the presence of Ag^+ and I^-, a fluorescence enhancement of ThT with another new emission band at 546 nm accompanied by a red shift was observed. It has been reported that ThT can form dimer with fluorescence enhancement in a restricted environment or at high concentrations.[8, 9, 19] In order to

illustrate the above fact, we monitored the fluorescence emission spectra of ThT with varied concentrations in solution. The fluorescence spectral features of ThT significantly depend on its concentration (Figure S3). At low concentration, ThT showed only one emission band at 494 nm. With the increase in the concentration of ThT, the fluorescence intensity gradually increased while the emission band did not shift at the low ThT concentration range. However, as the concentration of ThT increased over 250 μM, ThT became dimer at higher concentrations and another emission band at 546 nm can be noticed. As a result, the emission band at 494 nm should be related to the monomer and another emission band observed at 546 nm was caused by the formation of the dimer. In this respect, it is reasonable to believe that the as-prepared ThT@AgI SPs in our work, resulting from the spontaneously assemble of Ag^+, I^- and ThT, can provide a restricted environment to readily confine the involved ThT for its fluorescence enhancement. Moreover, the Ag^+-induced formation of ThT@AgI SPs has great potential to develop a ratiometric fluorescent assay for Ag^+ based on the regulating of ThT fluorescence, in which the simultaneous measurement of two fluorescence signals at 546 nm and 494 nm followed by calculation of their intensity ratio should be applied. Ag^+ plays an important role in the electronics industry, photography/imaging industry, as well as pharmaceutical industry.[23] Ag^+ can inactivate sulfhydryl enzymes and is able to bond with amine, carboxyl groups and imidazole of different metabolites.[24] Excessive Ag^+ accumulating in human body will result in pernicious effects on body health, causing argyria and inducing severe symptoms.[23] Thus, rapid and accurate detection of trace Ag^+ is of great significance in environmental monitoring, biomedical research and so on. [25]
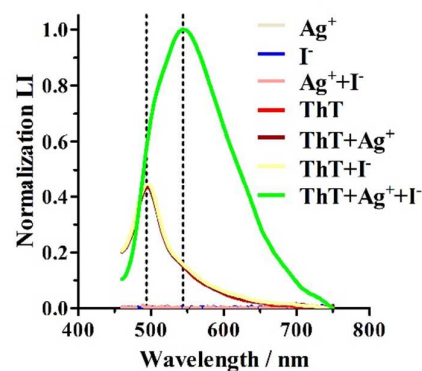

Figure 2. Fluorescent spectra of ThT in different conditions, including the presence of Ag^+, I^- and $Ag^+ + I^-$, respectively.

To demonstrate the feasibility of our proposed approach, the experiments begin with the use of ThT–I^- solution for Ag^+ sensing based on the concept demonstrated above. The different concentrations of Ag^+ from one stock solution were added to the prepared ThT–I^- solution. Figure 3A illustrates how the addition of increasing concentrations of Ag^+ in the range of 0 to 30 μM result in the gradual fluorescent enhancement of ThT–I^- solution, while a new emission band

red shifted to 546 nm appears. It indicates the existence of the ThT dimer according to the above fact. The sensitivity of the ThT–I$^-$ solution for Ag$^+$ was investigated. From Figure 3B, it can be seen that the fluorescence ratio (F_{546}/F_{494}) is sensitive to the concentration of Ag$^+$, the fitting range is from 0 to 30 μM with a Boltzmann sigmoidal equation and a linear equation can be obtained from the concentration range of 0-10 μM. A series of 8 repetitive measurements with 4 μM Ag$^+$ was used for investigating the precision of ThT–I$^-$ solution response, and obtained a relative standard deviation (RSD) of 1.3% (Figure S4), demonstrating an excellent reproducibility of the assay. The limit of detection of Ag$^+$ using ThT–I$^-$ solution was approximately 50 nM based on 3σ. Compared to the detection limit of the reported triphenylmethane dye/G-quadruplex complexes-based method of 80 nM[26] and carbon nanodots-based method of 320 nM[27], our presented sensing platform is one of the most sensitive Ag$^+$ detection methods (Table S1). These reported methods are a single-channel output based on the fluorescence quenching or enhancing of the sensing unit by the Ag$^+$, while our proposed method can perform with the advantages of ratiometric fluorescence for the detection of Ag$^+$. The ratiometric measurement allows the simultaneous measurement of two fluorescence signals at 546 nm and 494 nm, followed by the calculation of their intensity ratio (F_{546}/F_{494}) to minimize the ambiguities on fluorescence signals, thus giving greater analytic accuracy relative to single-channel detection.
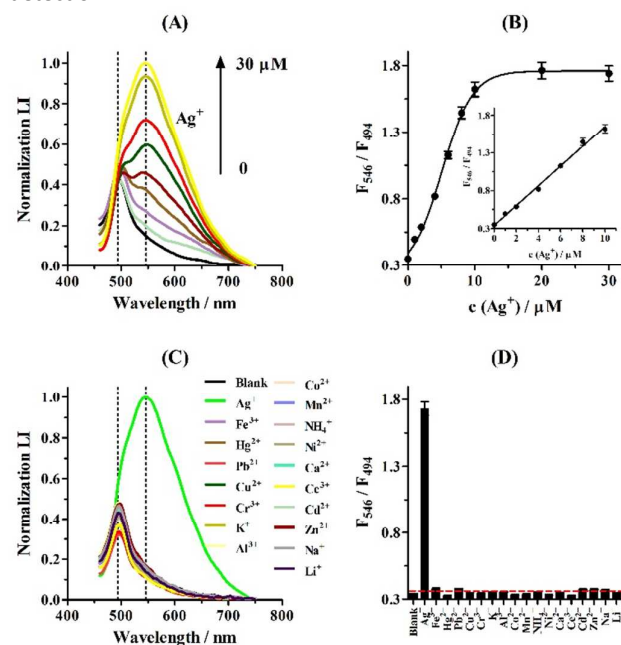
Al^{3+}, Co^{2+}, Mn^{2+}, NH$_4^+$, Ni^{2+}, Ca^{2+}, Ce^{3+}, Cd^{2+}, Zn^{2+}, Na$^+$ and Li$^+$, each at a concentration of 1000 μM, were tested under the same conditions as in the case of 20 μM Ag$^+$. It was found that none of these competing metal ions, except for Ag$^+$, could lead to a significant fluorescence response, demonstrating the excellent selectivity of this approach applied in Ag$^+$ detection over other metal ions (Figure 3C, 3D).

In order to test the feasibility of our proposed method in real applications, we evaluated its ratiometric fluorescent response to Ag$^+$ in tap water. Before spiking Ag$^+$ in real water samples, no ratiometric fluorescent response of the ThT–I$^-$ solution was observed in the real water samples, indicating Ag$^+$ in these real water samples was not detected (below the limit of detection of concentration in the proposed method). While upon the addition of Ag$^+$ in the real water samples, the ThT–I$^-$ solution was induced with a ratiometric fluorescent response. The concentrations of Ag$^+$ in different samples were measured by the standard addition method using ThT–I$^-$ solution. Recovery of added known amount Ag$^+$ to the samples was in general larger than 95%, which indicated that the present method has a promise in practical application with great accuracy and reliability (Table S2).

In this work, the formation of ThT@AgI SPs can be devised as a logic gate system, in which the AgI NPs-induced fluorescence enhancement of ThT enabled the design of a AND logic gate, while AgI NPs and S^{2-}-mediated reversible fluorescence changes of ThT can carry out a INHIBIT logic gate based on Boolean logic (Scheme 1B and Figure 4). For input, we defined the presence of Ag$^+$, I$^-$ or S^{2-} as 1 and their absence as 0. Fluorescence change of ThT (FI$_{546}$) was defined as the output (1 or 0) for the logic gate. The eight possible input combinations were listed in the truth table (Figure 4B). In the AND logic system, only inputting Ag$^+$ and I$^-$ at the same time without S^{2-}, the formation of the dimer can be induced by AgI NPs and the fluorescence intensity FI$_{546}$ obviously increased, resulting in the "on" state and giving an output signal of 1. The rest of other input modes into the system would not create the dimer, so the fluorescence outputs signal were all 0. The principle of the INHIBIT logic system is based on the stronger binding affinity between S^{2-} and Ag$^+$ than I$^-$. Thus, Ag$_2$S replace the AgI NPs when I$^-$ competes against with S^{2-}. At this moment, due to the destruction of AgI NPs, the ThT would not come into being the dimer, leading to the quenching of the fluorescence, displaying the fluorescence "off" state anew and output 0. The operation reversibility of this described logic gate was investigated. Figure 4C shows the repeated switching behaviour through the alternating addition of Ag$^+$, I$^-$ and S^{2-}. After cyclic treatment, the variation of fluorescence intensity gradually decreased. The observed loss in fluorescence switching efficiency can be possibly due to the dilution effect, owing to the increase in the total solution volume with the alternating addition of Ag$^+$, I$^-$ and S^{2-}.
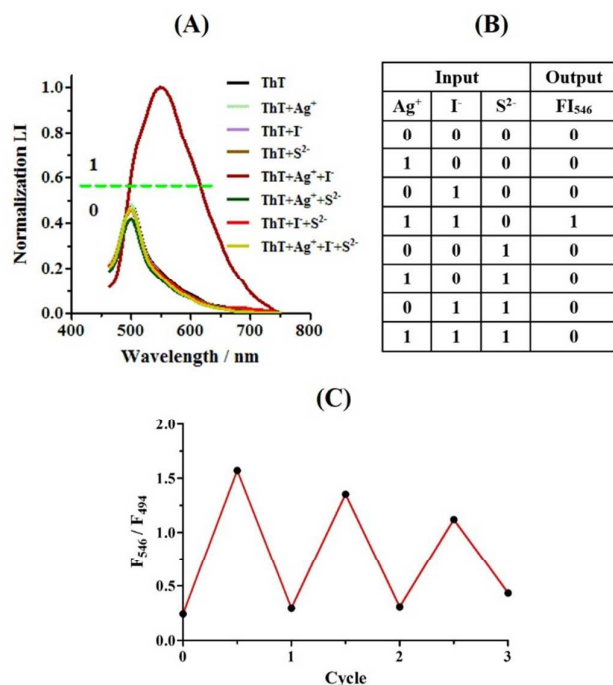

Figure 3. (A) The fluorescence emission spectra are shown for ThT–I$^-$ solution to various Ag$^+$ concentrations from 0 to 30 μM. (B) Plot of fluorescence ratio (F_{546}/F_{494}) with the different concentrations of Ag$^+$ from 0 to 30 μM. (C) Fluorescence emission spectra of ThT–I$^-$ solution for the other tested cations with concentrations of 1000 μM, while Ag$^+$ with concentrations of 20 μM. (D) Bars represent the F_{546}/F_{494} of ThT–I$^-$ solution for the tested cations.

To validate the selectivity of this approach for Ag$^+$, competing metal ions including Fe^{3+}, Hg^{2+}, Pb^{2+}, Cu^{2+}, Cr^{3+}, K$^+$,

Figure 4. A logic gate system consisting of ThT using Ag^+, I^- and S^{2-} as inputs.

Conclusions

In summary, a novel type of small molecule–NP hybrid supraparticles (ThT@AgI SPs) is prepared by spontaneously assembling Ag^+, I^- and ThT under an aqueous solution condition. The as-prepared ThT@AgI SPs can provide a restricted environment to readily confine the involved ThT for its fluorescence enhancement with another emission band accompanied by a red shift. We have firstly demonstrated that Ag^+, I^- and S^{2-} can be used to switch reversible fluorescence of ThT for inexpensive, quick, sensitive and selective detection of Ag^+ and furthermore for a logic operation. The formation of dimer is responsible for the fluorescence enhancement with another emission band accompanied by a red shift in a restricted ThT@AgI SPs. This is a new scope of control strategy for "off-on" fluorescence regulation of ThT, and opens an opportunity for design of more methods based on simple cation-anion strategy.

Acknowledgements

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