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Tandem copper and gold nanoclusters for two-color ratiometric explosives detection

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Abstract

We report a sensory platform for the determination of common explosive species (*e.g.*, TNT, PETN, RDX) based on the differential response from two different luminescent metal nanoclusters. In particular, whereas the red emission from bovine serum albumin-protected gold nanoclusters was strongly quenched by nitro-, nitrate-, and nitroamine-containing explosive organic molecules, blue-emitting glutathione-capped copper nanoclusters proved inert to quenching by these same analytes, instead showing evidence for aggregation-induced emission enhancement (AIEE). As a result, this discrete gold/copper nanocluster pairing provides a dual-probe, ratiometric (red-to-blue) system signaling the presence of TNT and other common explosives. This strategy opens up new potential for nanocluster-based analyte signaling, with implications to fluorescence resonance energy transfer (FRET) strategies as well.

Graphical abstract

A dual-emission platform comprising discrete gold and copper metal nanoclusters is introduced for the ratiometric fluorescence detection of explosives.

The sensitive and selective detection of powerful explosive compounds has moved to the forefront of analytical sensing due to their growing use in deadly terrorist attacks,¹ as well as the negative environmental effects they exert on soil and ground water.² Of the many currently available detection techniques, luminescent sensors are particularly attractive due to their short response times, simplicity in both sample preparation and instrumentation, and high sensitivity.³ Recently, nanoscale fluorescent reporters such as quantum dots⁴ and nanocrystals (*e.g.*, Mn²⁺-doped ZnS, silicon)^{5, 6} have shown promise in explosives detection

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which benefits from their facile tunability in terms of size, surface chemistry, nanoarchitecture (*e.g.*, core–shell), and doping to favorably modulate their photoluminescence properties.

One emerging class of nanoscale reporter that has received surprisingly little attention for explosives detection strategies is metal nanoclusters (MNCs).^{7–11} MNCs are typically smaller than 3 nm, generally containing several to a few dozen metal (*e.g.*, Au, Ag, Pt, Cu) atoms, a size regime giving rise to “molecule-like” optical properties, such as discrete electronic states and photoluminescence.^{12, 13} Due to their photoemissive nature, MNCs have shown promise as candidates for the fluorescence sensing of analytes capable of quenching their emission, including heavy metal ions^{9, 10, 14} and hydrogen peroxide (H₂O₂).¹⁵ Of key relevance to the current work, select MNCs have also been recently applied as single-color quenchometric (on-off) probes in the intensity-based detection of explosive compounds,^{16–19} although ratiometric (*i.e.*, dual-color) sensing using MNC pairs is still awaited.

Specifically, employing ratiometric analysis by simultaneously exploiting signals from two different probes (or a dual-emissive single probe) for example, is more analytically sound than strictly intensity-based measurements from a lone probe. Indeed, ratiometric analysis is less error-prone, being less subject to instrumental fluctuations (*e.g.*, excitation source intensity, detector efficiency, sample geometry), and variations in probe concentration (*e.g.*, dilution, localization, photobleaching).^{4, 14, 20, 21} The difficulty in constructing ratiometric fluorescence sensing schemes lies in identifying suitable probe pairs which yield spectroscopically distinguishable signals (minimal spectral overlap in their emission bands) and produce a differential response to the target analyte(s). For example, using intensity as an example (other parameters are also possible, such as polarization/anisotropy and emission wavelength), one probe may be efficiently quenched by an analyte whereas the other probe remains essentially unquenched or, ideally, shows an enhanced signal in the presence of analyte.

In this work, we have devised a ratiometric fluorescence sensing strategy that couples the well-studied red-emitter comprising bovine serum albumin-capped gold nanoclusters (AuNCs@BSA) with cyan-emitting glutathione (GSH)-capped copper nanoclusters (CuNCs@GSH) as internal reference (Fig. 1C and 1B, respectively). This dual MNC probe system allows for the sensitive detection of the explosive molecule 2,4,6-trinitrotoluene (TNT), and other common nitro-, nitrate-, and nitroamine-containing explosives, including 2,4-dinitrotoluene (DNT), 4-nitrotoluene (4-NT), nitrobenzene (NB), pentaerythritol tetranitrate (PETN), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). This tandem MNC system offers a convenient two-color, analytically-robust response and delivers an unoptimized limit of detection (LOD) for these explosives down to the few-micromolar regime (2.5 μ M for TNT). By way of comparison, the Veinot group recently described the single-color, intensity-based detection of TNT, PETN, and RDX using Si nanocrystals, reporting LODs in the millimolar (mM) regime.⁶

The possibility of developing a ratiometric, two-color dual-MNC tactic for sensing TNT and other explosives was inspired by observations that although the fluorescence from BSA-

templated AuNCs is efficiently quenched by mercury ion (Hg^{2+}),¹⁴ H_2O_2 ,¹⁵ and TNT,¹⁹ GSH-capped CuNCs appear largely unaffected by heavy metal ions and are virtually resistant to quenching by H_2O_2 , suggesting that they might also be relatively unaffected by nitroaromatic explosives. It is important to note that CuNCs@GSH already satisfy the criterion of showing fluorescence emission that is spectroscopically divergent from AuNCs@BSA (Fig. 1).

Interestingly, while single-MNC studies on as-prepared CuNCs@GSH clearly show a complete lack of response to TNT (see Fig. S1 in the ESI[†]), as we will show later, dual-MNC studies of CuNCs@GSH conducted in the presence of AuNCs@BSA reveal varying degrees of enhanced emission, depending upon the nature of the analyte. We tentatively attribute this effect to aggregation-induced emission enhancement (AIEE), a phenomenon well-established amongst thiolate-protected AuNCs,^{22, 23} and one recently proposed to occur for CuNCs as well.^{24, 25} It is important to recognize two implications of the AIEE effect specific to our dual-MNC nanoprobe system: (i) because an AIEE response to nitro-containing explosives was not observed in isolated CuNCs@GSH experiments, it appears that the protein scaffold of the AuNC@BSA cluster actually plays an important templating role; and (ii) the AIEE effect is actually analytically fortuitous as it improves the red-to-blue ratiometric response to nitro-, nitrate-, and nitroamine-containing explosives.

The synthesis of AuNCs@BSA, details of which are provided in the ESI[†], followed previous reports by our group^{15, 26} which were based on the seminal original report by Ying and co-workers.²⁷ For the synthesis of CuNCs, the tripeptide glutathione (GSH) was employed as a dual reducing/stabilizing agent, alongside ascorbic acid (AA) as a weak co-reductant, to yield CuNCs@GSH from copper nitrate.

Individual AuNCs@BSA and CuNCs@GSH solutions were optically characterized by UV-Vis spectrophotometry and excitation and emission fluorescence spectroscopy, as summarized in Fig. 1A. The AuNCs@BSA showed an absorption shoulder near 280 nm and produced an intense red emission with a maximum near 640 nm when excited at 435 nm, consistent with previous reports.^{26, 27} The CuNCs@GSH displayed a less distinct absorption shoulder near 250 nm and produced cyan emission centered at 500 nm upon 435 nm excitation. The lack of apparent surface plasmon resonance in the visible region and the emissive nature of both samples are consistent with MNC formation, rather than nanoparticle growth.

While the optical features are in line with MNC formation, their successful synthesis requires detailed mass spectrometric confirmation. Because the synthesis of AuNCs@BSA is extremely robust²⁷ and they have already been tremendously well-characterized by myriad groups, they were not further characterized by mass spectrometry. Previously-reported GSH-reduced and -stabilized CuNCs contained 6 or 15 Cu atoms with 3 or 4 oxidized GSH molecules within the cluster,^{13, 28} respectively. However, the addition of AA as a co-reductant may produce structural changes to yield a new cluster composition. In order to elucidate structural information on the new CuNCs@GSH, electrospray ionization mass

[†]Electronic Supplementary Information (ESI) available: ESI figures and experimental details. See DOI: 10.1039/x0xx00000x

spectrometry (ESI-MS) coupled with Fourier-transform ion cyclotron resonance (FT-ICR) was employed due to its ultrahigh mass accuracy capabilities, which is especially important for the characterization of unknown molecular species such as emergent MNCs.

Additionally, negative-ion mode ESI was used as it has been previously shown to be highly suitable for the characterization of glutathione-metal complexes²⁹ and CuNCs@GSH.^{28, 30}

A representative negative-ion mode ESI FT-ICR mass spectrum of our CuNCs@GSH is shown in Fig. 2. Oxidized glutathione (GSSG, $M = C_{20}H_{32}N_6O_{12}S_2$) is one of two peaks dominating the mass spectrum, and is detected as a singly deprotonated ion $[M-H]^-$ at m/z 611.14482 (0.2 ppm mass error). In addition, $[M+Na-2H]^-$ and $[M+2Na-3H]^-$ adducts of GSSG are detected at m/z 633.12519 (−2.3 ppm) and 655.10595 (−4.0 ppm), respectively. The dominant ion in the mass spectrum at m/z 672.05796 corresponds to GSSG incorporating copper $[M+Cu-3H]^-$ (−1.0 ppm error) and matches a peak observed in the ESI mass spectra of GSH mixed with Cu(I).²⁹ The inset of Fig. 2 provides the isotopic distribution for this species, with experimental isotope abundances in purple and the theoretical abundances overhead in black, showing superb agreement for abundances as well as exact mass agreement. A doubly-charged anion isotope distribution overlaps with this one, as peaks at m/z 674.57247 and 675.57407 in the inset are most likely isotope peaks of $[2M+2Cu-H]^{2-}$ with −5.1 ppm and −1.4 ppm error, respectively, indicating that the smallest possible MNCs (*i.e.*, two atom-containing) were generated. Additionally, as shown in Fig. S2[†], there are a number of doubly-charged peaks between m/z 900 and 1100. While many of these are sodiated oligomers of GSSG, there are several that arise from cuprous-GSSG complexes. For example, the series of isotope peaks starting at m/z 958.67168 corresponds to $[3M+Cu+Na-5H]^{2-}$, with additional sodium ions displacing protons at higher m/z . Most interesting is the cluster shown in the inset at m/z 1011.11093, corresponding to $[3M+3Cu-3H]^{2-}$ (0.9 ppm error). Another cluster containing three copper atoms is observed at m/z 1022.10234 ($[3M+3Cu+Na-4H]^{2-}$). Note that in these doubly-charged clusters, since the exact composition is unequivocally known, one can deduce that a mixed-valence cluster exists; that is, one copper is Cu(I) and the other two must be Cu(0). These results show that employing the present synthetic protocol results in the formation of two or three Cu atom containing MNCs wherein one GSSG ligand is likely associated with each atom, giving MNC formulas of $[Cu_2GSSG_2-3H]^{2-}$, $[Cu_3GSSG_3-3H]^{2-}$, and $[Cu_3GSSG_3+Na-4H]^{2-}$. Beyond m/z 1040, a series of cuprous-GSSG complexes are observed that contain five GSSG molecules, a single copper atom, and multiple sodium atoms. A corresponding positive-ion ESI FT-ICR mass spectrum of the CuNCs@GSH sample is provided in Fig. S3[†]. While the mass spectra are dominated by sodiated cuprous-GSSG complexes, the presence of these species reinforces the notion that the observed enhanced fluorescence of CuNCs@GSH in the presence of select explosives might tentatively be attributed to AIEE arising from explosive-mediated aggregation of these small CuNCs onto the BSA protein scaffold provided by the partner MNC AuNC@BSA (*vide infra*).

The fluorescence from AuNCs@BSA was highly responsive to the addition of TNT (Fig. 3A), showing efficient fluorescence quenching similar to previously reported results.^{18, 19} Meanwhile, the CuNCs@GSH appeared to be spectroscopically inert, showing no apparent fluorescence quenching by similar quantities of TNT (Figs. 3B and S1[†]). Fluorescence quenching plots showing the responses of the individual MNCs to the presence of increasing

amounts of TNT are provided in Fig. 3C, highlighting the differential responses for the two MNCs to TNT. Significantly, these divergent responsivities, in conjunction with the spectrally separated emission colors of the two MNCs, can be exploited to develop a dual-probe, ratiometric fluorescence-based sensing system for TNT. Indeed, as evidenced by the spectral series shown in Fig. 4D, an aqueous system containing both AuNCs@BSA and CuNCs@GSH yields a smooth ratiometric fluorescence response to TNT in which the relative intensity of the AuNCs@BSA fluorescence decreases monotonically with TNT concentration. It should be noted that, to avoid interference from the Raman scatter of water (see Fig. S4[†]), the excitation wavelength was shifted to 390 nm in these dual-MNC experiments.

In addition to TNT, the tandem MNC system was also responsive to other common explosives (*i.e.*, NB, 4-NT, DNT, PETN, and RDX) with varying sensitivities, as summarized in Fig. 4A and B. The spectra used to generate these plots can be found in Figs. S5 and S6 of the ESI[†]. In Fig. 4A, F_{650} and F_{500} indicate the fluorescence signals contributed by AuNCs@BSA and CuNCs@GSH, respectively, at the indicated wavelengths. In similar fashion to observations made by Tu *et al.*⁵ with amine-capped ZnS-Mn²⁺ nanocrystals, quenching tests using different nitrotoluene congeners (4-NT, DNT, TNT) revealed a strong correlation between the number of nitro groups attached to the benzene ring and the ability to quench AuNC@BSA emission. Namely, the quenching efficiency is progressively enhanced as the number of nitro groups increases due to the greater electron accepting ability of multi-nitro aryl compounds. Interestingly, the non-aromatic explosive PETN (the nitrate ester of pentaerythritol) also strongly quenches AuNC@BSA emission with an efficiency intermediate between that of DNT and TNT. In contrast, the nitroamine explosive RDX (which, along with PETN is the main ingredient of the general-purpose plastic explosive Semtex) is a much less potent quencher of AuNC@BSA fluorescence, showing a lower quenching efficiency than DNT, despite containing three nitroamine groups. Not surprisingly, these results clearly demonstrate that not only the number of functional groups but also the *nature* of the pendant electron-accepting chemistry (*e.g.*, organic nitro, nitrate, or nitroamine groups) dictates the strength of the explosive molecule as a quencher of AuNC@BSA fluorescence.

As shown in Fig 4A, TNT produces the greatest ratiometric signal response within the dual-MNC AuNC@BSA plus CuNC@GSH sensory system. The limits of detection (LODs) based on F_{650}/F_{500} response curves using the three-sigma (3σ) convention increase in the following order: TNT ($2.5 \pm 0.8 \mu\text{M}$), PETN ($4.4 \pm 1.0 \mu\text{M}$), DNT ($7.6 \pm 1.2 \mu\text{M}$), RDX ($25.0 \pm 7.2 \mu\text{M}$), NB ($55.8 \pm 6.3 \mu\text{M}$), and 4-NT ($63.7 \pm 6.7 \mu\text{M}$). In Fig. 4 C, the appearance of a mixture of AuNCs@BSA and CuNCs@GSH under UV-A excitation is shown for progressive amounts of TNT. What is initially a distinctly pink sample under a back light becomes purple in appearance and finally cyan as the emission from AuNCs@BSA is selectively quenched by TNT. Due to the well-resolved emission colors in this dual-MNC ratiometric system, the changes in the relative extent of quenching are discernible to the naked eye as an apparent luminescence color change, a phenomenon which can be exploited for fast, simple, instrument-free preliminary explosives detection on-site or in the field, or developed as a cheap paper-supported fluorescence sensor.

Surprisingly, while the emission from the AuNCs@BSA was quenched to various extents by the different explosives tested, we observed a notable increase in CuNC@GSH emission in the dual-MNC system, particularly in the cases of TNT, PETN, and DNT, as illustrated in Fig. S5[†]. We tentatively ascribe this peculiar photophysical behavior to aggregation-induced emission enhancement (AIEE), a phenomenon in which restricted molecular motion blocks nonradiative decay pathways, resulting in a higher fluorescence quantum yield. Notably, the absence of explosive-mediated AIEE for the lone CuNCs@GSH system (Fig. 3B) suggests that the presence of the AuNC@BSA partner is essential to this process. Given this scenario, the most plausible explanation is that these model explosive molecules coordinate with CuNCs@GSH, followed by deposition onto the protein scaffold of AuNC@BSA. The BSA apparently provides a suitable platform for the stabilization of agglomerated CuNCs@GSH, resulting in enhanced emission due to a reduction in CuNC vibrational, rotational, and torsional movements. We note that the observation of AIEE is well documented in relation to thiolated AuNCs,^{22, 23} with recent precedent for such behavior for thiol-capped CuNCs as well,³¹ generally within the context of cuprophilic interactions, such as those involving Cu(I)_x-GSSG_y complexes (*e.g.*, surface etching).³¹ In the dual-MNC system, the CuNC@GSH emission peak intensity increases monotonically with explosive concentration (*e.g.*, TNT, PETN, DNT) alongside a blue-shifted emission (Fig. S5[†]), providing preliminary evidence for an AIEE effect. We note that Rogach and co-workers recently reported the observation of confinement-assisted emission enhancement for GSH-capped CuNCs incorporated within zeolitic-imidazolate metal-organic frameworks (ZIF-8).³² In contrast to our results, however, the emission from ZIF-8-incorporated CuNCs was selectively quenched by TNT, rather than enhanced. The origin behind this discrepancy in response to TNT remains unclear, although the impact of the ZIF-8 electronics and redox behavior surely plays a role in light of the reported stabilization of the CuNCs against oxidation.

Further evidence for the existence of AIEE is provided in Fig. S7[†] in the form of second-order Rayleigh scatter plots where I_S and $I_{S,0}$ denote the integrated scattering intensity in the presence and absence of quencher, respectively. Expressly, since Rayleigh scattering intensity is proportional to the sixth power of the radius of a particle, the observed increase in $I_S/I_{S,0}$ with explosive concentration (S7A[†]) indicates an apparent increase in the size of the scattering body. The increase in second-order Rayleigh scatter is most evident for PETN, although TNT also shows a pronounced increase as well. This effect may arise from assembly of CuNCs@GSH onto the surface of BSA to increase the effective particle size during the AIEE process. Referring to Fig. S7B[†], for the explosives PETN, TNT, and DNT, the relative increases in scattering intensity are highly correlated with the relative enhancement in fluorescence signal, further reinforcing the notion of an AIEE effect.

In the simplest case of collisional quenching of fluorescence, the classical Stern–Volmer (SV) relationship holds:

$$\frac{F_0}{F} = 1 + K_{sv} [Q] \quad (1)$$

where F_0 and F are the steady-state fluorescence intensities of a fluorophore in the absence and presence of quencher, $[Q]$ is the concentration of quencher, and K_{SV} is the Stern–Volmer quenching constant. For this ideal case, a plot of F_0/F versus $[Q]$ will thus be linear with a slope equal to K_{SV} and an intercept of unity. It is also useful to note that, intuitively, $(K_{SV})^{-1}$ is the quencher concentration at which $F_0/F = 2$ (i.e., 50% of the intensity is quenched). Stern–Volmer quenching plots for AuNCs@BSA (Fig. S8[†]) reveal that eqn. (1) is able to adequately describe the quenching by TNT and the other explosives, a result which is generally indicative of a single class of fluorophore equally accessible to a quencher which operates by a single quenching mechanism (either static or dynamic).^{33, 34} The recovered K_{SV} values using TNT, PETN, DNT, RDX, NB, and 4-NT as quencher were 9.1×10^3 , 4.0×10^3 , 3.1×10^3 , 3.4×10^3 , 1.1×10^3 , and $1.5 \times 10^3 \text{ M}^{-1}$, respectively, a trend that coincides well with the observed ratiometric responses and the corresponding LODs (Fig. 4). It is instructive to note that $(K_{SV})^{-1}$ for TNT is 110 μM , the concentration of TNT which should manifest in loss of half of the fluorescence signal from AuNCs@BSA.

In summary, we have developed the first ratiometric fluorescence sensing strategy to signal the presence of important explosive molecules (e.g., TNT, PETN, RDX) in an aqueous milieu based on the differential response of two different fluorescent metal nanoclusters. In particular, the fluorescence from protein-templated gold nanoclusters was quenched with wide-ranging efficiencies by various nitro, nitrate, and nitroamine explosives. Meanwhile, thiolate-protected copper nanoclusters were not quenched and instead showed evidence for aggregation-induced emission enhancement. These opposing responses generated a ratiometric (red-to-blue) dual-cluster fluorescent reporter system that was analytically simple and robust, even showing the potential for naked-eye screening for the presence of TNT and other explosives in resource-limited settings. Although the analytical platform is responsive to all explosives tested, it is selectively responsive to TNT (followed by PETN), displaying an unoptimized limit of detection of 2.5 μM . Given the diversity of luminescent metal nanoclusters currently available, our findings suggest the potential for their wider use as signaling units in other ratiometric fluorescence sensing motifs, bioimaging strategies, and multi-input logic gates, to name a few.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

(A) UV-Vis spectra (dotted profiles) and steady-state excitation (dashed lines) and emission (solid lines) fluorescence spectra of AuNCs@BSA (pink) and CuNCs@GSH (cyan). The excitation wavelength for the emission spectra was set at 435 nm, while the excitation spectra were collected by setting the emission monochromator at the respective emission maxima for the two MNCs. Photographs of (B) CuNC@GSH and (C) AuNC@BSA solutions under ambient lighting (left) and for 366 nm UV lamp excitation (right). Note that the cartoon representations of the MNC structures provided are only illustrative and are not properly scaled to one another.

Fig. 2.
Full negative-ion ESI FT-ICR mass spectrum of glutathione-capped copper nanocluster (CuNC@GSH) sample.

Fig. 3.

(A) Normalized fluorescence emission spectra of AuNCs@BSA in the presence of increasing quantities of TNT from 0–44 μM (0–10 ppm) ($\lambda_{\text{ex}} = 490 \text{ nm}$). (B) Normalized fluorescence emission spectra of CuNCs@GSH in the presence of increasing TNT from 0–44 μM ($\lambda_{\text{ex}} = 435 \text{ nm}$). (C) Quenching plots showing the fluorescence responses of AuNCs@BSA and CuNCs@GSH (monitored at 650 and 500 nm, respectively) to TNT. F and F_0 denote the integrated fluorescence in the presence and absence of TNT, respectively, and the fluorescence emission was integrated over 10-nm slices centered at the respective MNC emission maximum. (D) TNT-dependent fluorescence emission spectra of the dual-MNC system, normalized to the CuNC@GSH peak intensity ($\lambda_{\text{ex}} = 390 \text{ nm}$). The narrow peaks near 447 nm (indicated by *) arise from the Raman scatter of the medium (water).

Fig. 4.

(A) Fluorescence ratios for the dual AuNC@BSA + CuNC@GSH system in response to addition of 0–10 ppm TNT, PETN, DNT, RDX, NB, and 4-NT. In this plot, F_{650} and F_{500} represent the experimental fluorescence intensities of the AuNC@BSA and CuNC@GSH components determined by spectral integration over 50-nm slices centered at the respective MNC emission maximum (650 and 500 nm, respectively) ($\lambda_{\text{ex}} = 390$ nm). (B) Limits of detection (LODs) determined for the different explosives investigated. (C) Photograph of a succession of dual-MNC samples containing 0, 2, 4, 6, 8 and 10 ppm TNT (left to right) under black light illumination (UV-A lamp, 365 nm).