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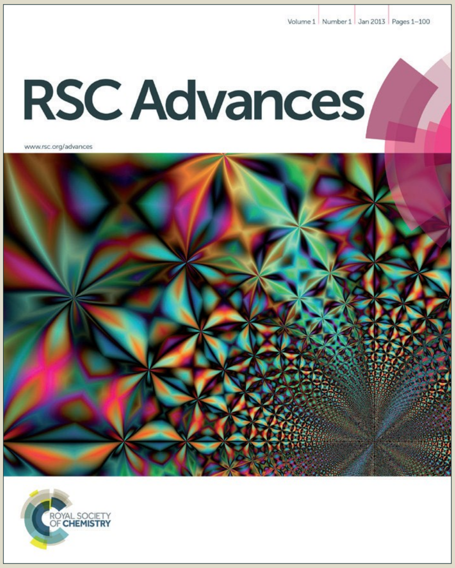

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# Squaramide-based lab-on-a-molecule for the detection of silver ion and nitroaromatic explosives

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**A squaramide based chemosensor SA was developed as a quantitatively operating lab-on-a-molecule, for the detection of silver ions and nitroaromatic explosives in aqueous solution, performing significant emission quenching and absorption enhancement in dual channel along with distinct solution color change.**

The field of lab-on-a-molecule[1] was proposed by de Silva[2] as a molecular logic gate producing an output signal (generally fluorescent emission) in response to various chemical input signals, which has been employed to overcome the boundary of single analyte detection and to initiate highly selective orthogonal signaling protocols for multi channel ions sensing in large varieties[3].

Dramatic improvements in the detection of nitroaromatic compounds such as 2, 4, 6-trinitrotoluene (TNT) and its derivatives picric acid (TNP), 1-methyl-4-nitrobenzene (NT), 1-methyl-2,4-dinitrobenzene (DNT) have aroused wide public concern since they are highly explosive and environmentally deleterious substances[4]. Similarly, trace amount of toxic metal ions such as $Ag^+$ can pose serious threats to human health and environment.

The presently used detections of nitroaromatics are usually time-consuming with the employment of cumbersome[5] and expensive gas chromatography coupled to a mass spectrometer[6], ion mobility spectrometry[7] and surface acoustic wave method[8], which requires frequent instrument calibration, sophisticated vapor sampling and preconcentration procedures[9]. In contrast, the fluorescence-based chemosensors have been of significant importance with remarkable advantages over the other newly developed detection technologies owing to high sensitivity, portability, short response time[10], low cost and dual compatibility in solid and solution media[11].

Therefore, the development of robust and sensitive platforms for their real-time analytical detection has attracted considerable research efforts in recent years[12]. Fluorescence quenching-based chemosensors[13], where analyte is binding with producers and attenuates in the light emission[14], are believed to be the most suited for the detection of nitroaromatics because the electron-deficient nitro compounds are the strong quenchers of electron-rich fluorophores via an electron-transfer mechanism[15].

Until now, most of the fluorescence resonance energy transfer (FRET) based chemosensors usually employ two photoluminescent elements with an approximate energy band gap, in which the fluorescent emission of a donor is transferred to an acceptor and thus activates the fluorescence of the acceptor. However, the FRET from a photoluminescent donor to a non-emissive receptor for direct chemodetection of analyte has thus been rarely explored. In principle, if the absorption band of an analyte (or its derivatives) overlaps with the emission band of a fluorescent dye, the resonance energy transfer due to the polar-polar interaction at a spatial proximity will result in the quenching of donor fluorescence, which may provide a facile and sensitive detection for the target analyte.

On the other hand, given the toxic mental ions such as silver posing serious threats to human health and environment, there is a growing demand to develop the up-to-date technologies and methods for the qualitative and quantitative detection of silver. Although different analytical methods are developed for the detection of $Ag^+$, namely, quantum-dot-based assay[16], electrochemical[17] and fluorescent sensor[18], which can detect $Ag^+$ ion with high sensitivity and selectivity but need highly sophisticated expensive instrumentation[19], the absorption-based colorimetric chemosensors are greatly favored due to the distinct visual color change response for fast and easy detection by naked-eye[20].

Herein, we reported the resonance energy transfer-amplifying fluorescence quenching of dye for the sensitive detection of TNT and its derivatives[21] and competitve sensing of silver ions. The specific binding of TNT with the amine ligands leads to the formation of TNT-amine complexes, which strongly suppressed the fluorescence emission of the proximally positioned marker dye through the resonance energy transfer from the chosen dye to the TNT derivatives. The amplifying fluorescence quenching can sensitively detect the ultratrace TNT in the solution[22], and selectively distinguish different types of nitrocompounds.

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Notably, the title probe also performed high competitive selectivity of $Ag^+$ ion over other metal ions by its outstanding "turn-on" effect in absorption channel, even in the presence of other metal ions[23].

## Experimental

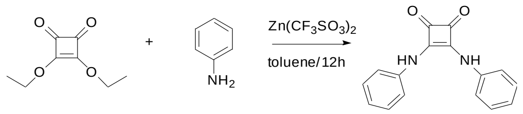
### Synthetic materials and instrumentation

All the chemicals used for the synthesis were of AR grade from Sigma-Aldrich unless otherwise stated. All the reagents and solvents were purchased from commercial sources and used without further treatment. The aqueous stock solutions of cations were prepared from their perchlorate salts. The stock solution of squaramide sensor **SA** was prepared by dissolving an accurately weighed **SA** in DMSO, and then diluted to $5\times10^{-5}$ mol/L with buffer solution (pH = 7.24, DMSO: buffer = 90 / 10, v / v, aq. buffer = 0.1 M Tris-$ClO_4$).

The $^1H$-NMR was recorded on a Varian mercury-plus 400 NMR spectrometer at room temperature using $d_6$-DMSO as solvent according to the internal standard. Absorption spectra were performed on a TU-1810 ultraviolet spectrophotometer. Fluorescence emission spectra were measure with a Hitachi F-4500 luminescence spectrometer.

### Synthesis of 3, 4-bis (phenylamino) cyclobut-3-ene-1, 2-dione (SA)

Typically, to a stirred solution of 3, 4-diethoxycyclobut-3-ene-1, 2-dione (81.3 μL, 0.55 mmol, 1.0 equiv.) and zinc trifluoromethanesulfonate (40 mg, 0.11 mmol, 20 mol %) in toluene / DMSO 19:1 (1 mL) was added aniline (92.6 μL, 1.15 mmol, 2.1 equiv.). The solution was heated to 100 ℃ and stirred for 12h. When the solution was cooled down to room temperature, a white precipitate was observed and isolated by decanting the solvent. The solid was further washed with methanol (3 × 5 mL) to acquire white solid (138 mg, 99% yield)[24]. $^1H$ NMR (400 MHz, DMSO-d6) δ 7.50 (4H, d, J = 7.3 Hz), 7.38 (4H, app t, J = 7.4 Hz), 7.90 (2H, app t, J = 7.4 Hz); $^{13}C$ NMR (100 MHz, DMSO-d6) δ 181.5, 165.6, 138.5, 129.3, 123.2, 118.4; HRMS (EI) calcd. for $C_{16}H_{12}N_2O_2$ 264, found m/z 264.



**Scheme 1** The synthetic route for the preparation of **SA**.

## Results and discussion

### Sensing of $Ag^+$ by SA

The colorimetric sensing properties of **SA** combine with various cations were investigated by UV-Vis absorption spectroscopy. In buffer solution (pH = 7.24, DMSO: $H_2O$ = 90:10, v/v), **SA** showed one characteristic peak at 422 nm (Fig. S1) which was due to the n→π* electronic transition. Upon the addition of equivalent cations into the buffered aqueous solution of **SA**, the dramatic absorption enhancement at 422 nm (Fig. S1) accompanied with the obvious solution color change from yellow to dark red could only be observed in the case of $Ag^+$, clearly indicating the formation of a complex species between **SA** and $Ag^+$. In contrast, the addition of other cations, such as $Ba^{2+}$, $Zn^{2+}$, $Ni^{2+}$, $Ca^{2+}$, $Mg^{2+}$, $Pb^{2+}$, $Cd^{2+}$, $Co^{2+}$, $Hg^{2+}$, and $K^+$ did not cause either significant UV-vis spectroscopic change or solution color change. Therefore, it can be conclude that **SA** can recognize $Ag^+$ selectively over other anions not only by naked eyes but also by absorption spectroscopy.

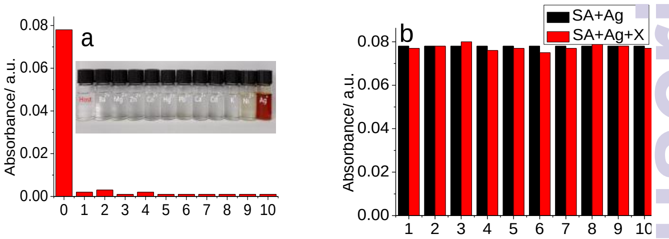


**Fig. 1** Absorption of **SA** in the presence of a) 10 equiv. of cations (Inset: naked-eye detection of $Ag^+$ ion by probe **SA** under visible) and b) 10 equiv. of $Ag^+$ + 10 equiv. of other cations at λ = 422 nm in 0.1 M Tris-$ClO_4$: DMSO = 10 / 90, v / v, pH 7.24. [$X^-$ = $Ag^+$ (0), $Ba^{2+}$ (1), $Zn^{2+}$ (2), $Ni^{2+}$ (3), $Ca^{2+}$ (4), $Mg^{2+}$ (5), $Pb^{2+}$ (6), $Cd^{2+}$ (7), $Co^{2+}$ (8), Hg (9), $K^+$ (10)].

In addition, the sensitive recognition and binding mode of SA with $Ag^+$ was demonstrated by carrying out absorption titration experiments. With the constant addition of $Ag^+$ from 0-10 equivalents to the solution of **SA** ($5\times10^{-5}$ mol/L) in aqueous buffer (pH = 7.24, DMSO: $H_2O$ = 90:10, v/v), the absorption intensity at 422 nm was increased sharply due to the formation of complexes between probe **SA** and $Ag^+$.

As a rigorous test, the selective detection of silver ion using **SA** was challenged in competition experiments (Fig. 1). In none of the cases, the presence of other anions interfered substantially. The good linear relationship between the absorption intensity and concentration of $Ag^+$ (Fig. 2 inset) allowed for a facile quantification of $Ag^+$ with a detection limit of 4.6 μM according to the literature method[24].

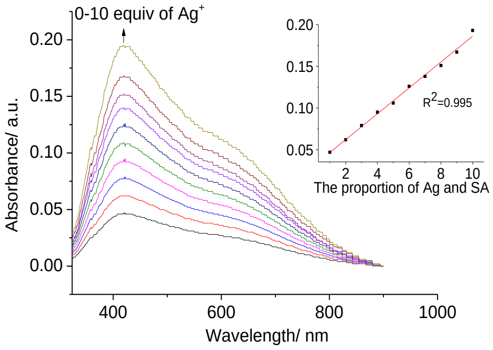


**Fig. 2** The UV-vis titration of $5\times10^{-5}$ mol/L **SA** with 1-10 equiv addition of $Ag^+$ ions. Inset: The absorption intensity of probe **SA** at λ = 422 nm as a function of mole equivalents of $Ag^+$ ions.

### FRET Mechanism between SA and TNT Derivatives

TNT is almost faint yellow in solution and does not absorb any visible light. Our work showed that the solution of TNT could change from faint yellow to deep red by adding organic amines such as **SA**. A strong charge transfer interaction, as a predominant

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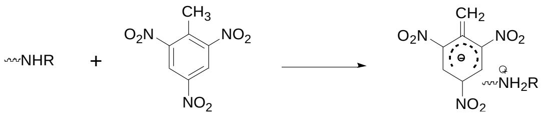
process, occurring between the electron-deficient aromatic ring of TNT and the electron-rich amine group of **SA**, could lead to the formation of a Meisenheimer complex as zwitterions of $TNT^-$ and $RNH_2^+$, which is resonance-stabilizing form due to three electron-withdraw nitro groups (Scheme 2).



**Scheme 2** The interaction between TNT and **SA** and Meisenheimer complex formed between TNT and the amine.

The absorbance spectrum of TNT solution shows a strong visible absorption with λ at 264-600 nm, and has a spectral overlapping with the fluorescence emission of **SA** solution at 285-540 nm, suggesting that the resonance energy transfer from **SA** to TNT derivatives may occur when the fluorophores and TNT derivatives are spatially close enough to each other. As a result, the non-emissive resonance transfer-based fluorescence quenching of **SA** will occur, and the quenching efficiency mainly depends on the degree of spectral overlapping between the emission of donor and the absorption of analyte acceptor and on the distance between these two, as depicted by following equation[25],

$$\Phi_T = 1/Q, \quad Q = 1+(R/R_0)^6$$

where R is the distance of the two fluorescent groups; $R_0$ is the distance of the Förster (The critical transfer distance of the donor and acceptor).

### UV/Vis response of SA and nitroaromatic explosives

The UV-vis selective response of **SA** upon the presence of nitroamine explosives can be seen from the observed color change of **SA** solution from pale-yellow to dark red (Fig. S3). In order to perform these naked-eye experiments, 100 equivalents of various nitroamine explosives ($1.5\times10^{-1}$ mol/L, NT, DNT, TNT, TNP) were added to the solution of **SA** ($5\times10^{-5}$ mol/L) in buffer solution (pH = 7.24, DMSO: $H_2O$ = 90:10, v/v). As show in Fig. 3, the distinct solution color change was only observed in the presence of TNT along with the significant absorption intensity enhancement in visible band centered at 512 nm (Fig. S4).

In addition, the sensitive recognition of **SA** with TNT was demonstrated by carrying out absorption titration experiment. With the progressive addition of TNT from 0-100 equivalents to the solution of **SA** ($5\times10^{-3}$ mol/L) in buffer solution (pH = 7.24, DMSO: $H_2O$ = 90:10, v/v), the absorption intensity at 512 nm was increased gradually. The linear relationships between the absorption intensity and amount of TNT was established for **SA** (Fig. 4) that allow for quantification over a wide range of concentration with a detection limit of 5.0 μM.

**Fig. 3** Naked-eye color changes of **SA** ($5\times10^{-3}$ mol/L) with subsequent addition of TNT from 0.5 to 100 equiv under visible.

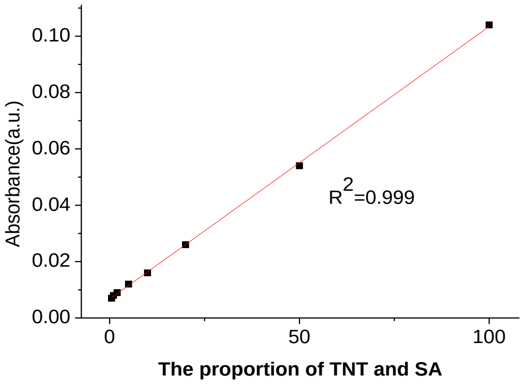


**Fig. 4** UV-vis absorption titration of **SA** (10 μM) upon addition of various equiv. of TNT in DMSO buffered solution (pH = 7.24, DMSO: $H_2O$ = 90:10, v/v). Inset: Linear correlation of absorption intensity at λ = 512 nm versus concentration of TNT.

### Fluorescence response of SA and nitroaromatic explosives

As expected, upon the addition of TNT, a charge-transfer interaction occurred between the electron-deficient aromatic ring of TNT and the electron-rich amino group, leading to the formation of a Meisenheimer complex and exhibiting a strong absorption band at approximate 338 nm. The well overlap (see Fig. S4) between the absorption of TNT and the emission band of the chosen **SA** fluorophore facilitated the FRET mechanism by intermolecular polar-polar interaction in the spatial proximity, as a result the efficient quenching effect could be found on the emission of the **SA** donor[26].

The probe **SA** also possesses the ability to react with TNT in a dose-response manner, which can be utilized for quantification of the analyte. As is shown in Fig. 5, the emission intensity of **SA** at 338 nm gradually decreased with the successive addition of TNT.

Similar quenching effect was also found in the case of other aromatic nitroamines such as NT, DNT and TNP with a relative weaker degree. Therefore, it can be concluded that **SA** could selectively response the aromatic nitroamines through emission quenching.

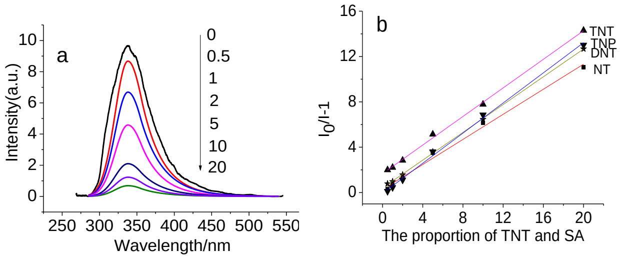


**Fig. 5** a) PL intensity of **SA** (10 μM) at $\lambda_{exc}$ = 278 nm with respect to the concentration of TNT from 0 to 20 equivalents in DMSO buffered solution (pH = 7.24, DMSO: $H_2O$ = 90:10, v/v); b) Stern–Volmer plot of **SA** treated with various explosives at different concentrations at $\lambda_{em}$ = 338 nm.

To evaluate the quenching efficiency of different kinds of nitroaromatic analytes, the Stern-Volmer equation is applied

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as follows: $(I_0/I)-1=K_{SV}[TNT]$, where $I_0$ is the initial fluorescence intensity in the absence of analyte, I is the fluorescence intensity in the presence of [TNT], and $K_{SV}$ is quenching constant with TNT. By employing the emission intensity data of **SA** at $\lambda_{exc} = 278$ nm into Stern-Volmer equation, it is calculated that the quenching efficiency of NT, DNT, TNT and TNP is $6.01\times10^4$, $6.14\times10^4$, $6.30\times10^4$ and $6.65\times10^4$ $M^{-1}$, respectively.

## Conclusions

We have successfully demonstrated a novel lab-on-a-molecule of nitroaromatic compounds and silver ions by using UV-Vis and emission channels. In UV-Vis channel, the remarkable absorption intensity enhancement was observed only in the presence of silver ions, while in emission channel the selective emission quenching effect was found towards nitroaromatic compounds such as TNT, DNT, NT and TNP through resonance energy transfer mechanism by the formation of a Meisenheimer complex. To the best of our knowledge, this is the first time reporting dual channel chemosensor for the selective detection of aromatic nitroamines and $Ag^+$ ions, even in the presences of other competing analogues, suggesting that probe **SA** could be used as dual channel lab-on-a-molecule for detecting nitroaromatic explosives and metal ions by simply achieved probes.

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# Graphical Abstract

# Squaramide-based Lab-on-a-molecule for the detection of silver ion and nitroaromatic explosives

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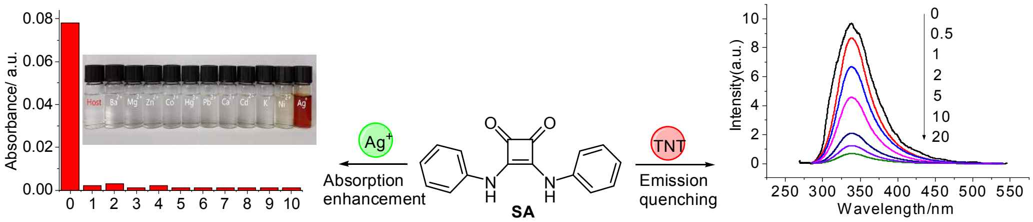

A squaramide based Lab-on-a-molecule performed selective absorption enhancement and emission quenching towards $Ag^+$ and nitroaromatic explosives, respectively in aqueous solution.