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# **Guest Editor: Anthony Harriman**



## Editorial

## **Electronic energy transfer**

Anthony Harriman, *Phys. Chem. Chem. Phys.*, 2010 DOI: <u>10.1039/c0cp90032j</u>

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## Quantum coherence and its interplay with protein environments in photosynthetic electronic energy transfer

Akihito Ishizaki, Tessa R. Calhoun, Gabriela S. Schlau-Cohen and Graham R. Fleming, *Phys. Chem. Chem. Phys.*, 2010 **DOI:** <u>10.1039/c003389h</u>

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Bo Albinsson and Jerker Mårtensson, *Phys. Chem. Chem. Phys.*, 2010 **DOI:** <u>10.1039/c003805a</u>

# Physical origins and models of energy transfer in photosynthetic light-harvesting Vladimir I. Novoderezhkin and Rienk van Grondelle, *Phys. Chem. Chem. Phys.*, 2010 DOI: <u>10.1039/c003025b</u>

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Hu Xu, Eugeny A. Ermilov, Beate Röder and Dennis K. P. Ng, *Phys. Chem. Chem. Phys.*, 2010 **DOI:** <u>10.1039/c004373g</u>

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Muhammad N. Nordin, Konstantinos N. Bourdakos and Richard J. Curry, *Phys. Chem. Chem. Phys.*, 2010 **DOI:** <u>10.1039/c003179h</u>

# Superexchange-mediated electronic energy transfer in a model dyad

Carles Curutchet, Florian A. Feist, Bernard Van Averbeke, Benedetta Mennucci, Josemon Jacob, Klaus Müllen, Thomas Basché and David Beljonne, *Phys. Chem. Chem. Phys.*, 2010 **DOI:** 10.1039/c003496g Switching off FRET by analyte-induced decomposition of squaraine energy acceptor: A concept to transform turn off chemodosimeter into ratiometric sensors Haibo Yu, Meiyan Fu and Yi Xiao, *Phys. Chem. Chem.* 

Phys., 2010 DOI: <u>10.1039/c001504k</u>

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Jaykrishna Singh and Eric R. Bittner, *Phys. Chem. Chem. Phys.*, 2010 **DOI:** <u>10.1039/c003113e</u>

#### Photophysics of conjugated polymers: interplay between Förster energy migration and defect concentration in shaping a photochemical funnel in PPV Sangoota Saipi and Riman Bagchi, Phys. Chom

Sangeeta Saini and Biman Bagchi, *Phys. Chem. Chem. Phys.*, 2010 **DOI:** <u>10.1039/c003217d</u>

## Electronic energy harvesting multi BODIPY-zinc porphyrin dyads accommodating fullerene as photosynthetic composite of antenna-reaction center

E. Maligaspe, T. Kumpulainen, N. K. Subbaiyan, M. E. Zandler, H. Lemmetyinen, N. V. Tkachenko and F. D' Souza, *Phys. Chem. Chem. Phys.*, 2010 **DOI:** <u>10.1039/c002757j</u>

# Switching off FRET by analyte-induced decomposition of squaraine energy acceptor: A concept to transform 'turn off' chemodosimeter into ratiometric sensors

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FRET cassette-type sensors, SN-1 and SN-2, which contain two naphthalimide donors and a squaraine acceptor, have been developed on the concept of switching off FRET through removing the spectral overlap by the analyte-induced decompositions of acceptor chromophore. SN-2 shows sensitive responses to fluoride anion and cyanide through both naked-eye detectable colour changes and ratiometric fluorescence changes. As the first example, SN-2 confirms that transformation of the common 'turn-off' chemodosimeter into ratiometric sensors can be realized conveniently based on the novel 'switching FRET off' approach.

# 1. Introduction

Forster resonance energy transfer (FRET) is one of the major mechanisms<sup>1</sup> widely used in biological labeling,<sup>2</sup> fluorescence probes,<sup>3</sup> and molecular beacon in biology,<sup>4</sup> due to its excellent characteristics such as a single excitation wavelength, a large pseudo-Stokes shift and proportional changes of two emission bands. The proportional changes of two emission bands make FRET more widely used in ratiometric approaches. Particularly, a approach called 'spectral overlap-modulated FRET strategy' has been applied to modulate the spectra overlap between the donor emission and acceptor absorption within FRET systems, which proves to be an efficient method to develop ratiometric sensors for small molecular analytes.<sup>5</sup> Rhodamine spirolactams, which are colorless and non-fluorescent leuco compounds and can readily undergo ring-opening reactions to generate a rhodamine fluorophore with strong absorption and emission, play important roles in the popularization of the above method.<sup>6</sup> For example, we have developed a FRET-based ratiometric sensor by connecting a rhodamine spirolactam to a BODIPY.<sup>7</sup> It exhibits clear Hg<sup>2+</sup>-induced changes in the intensity ratio of the two well-separated and comparably strong emission bands of BODIPY and rhodamine. Considerable efforts have been directed toward the transformation of rhodamine spirolactams, the typical fluorescence 'turn-on' type chemodosimeters, into FRET-based ratiometric sensors. Theoretically, other types of chemodosimeters can also be as usable as rhodamine spirolactam. However, to our knowledge, only very limited attempts have been reported.

Different from rhodamine spirolactam, squaraine is an important fluorescence 'turn-off' type chemodosimeter for the recognition of nucleophiles.<sup>8</sup> Due to significant electron-deficiency, squaraine can be readily attacked by nucleophiles

such as  $F^-$ ,  $CN^-$  and thiol compounds, to generate colorless and non-fluorescent products. Unfortunately, 'turn-off' sensors are not very desirable for biological sensing or imaging, for the lower accuracy in quantitative detection, compared with ratiometric sensors. Thus, it is interesting (important for biological imaging) to transform the 'turn-off' squaraine chemodosimeter into a ratiometric one. On the other hand, squaraine's strong absorption and emission in the visible region make it an ideal candidate for the energy acceptor.<sup>9</sup> However, to the best of our knowledge, only very few FRET cassettes using squaraine as acceptor have been reported, <sup>10</sup> and they were not applied as chemodosimeters.

Herein, as illustrated in Scheme 1, we proposed a new design concept to transform the 'turn-off' chemodosimeters into ratiometric ones. The nucleophilic analytes attacked the electrondeficient squaraine center and thus the FRET process was switched off due to complete cancellation of the spectral overlaps. This resulted in the recovery of the donor's emission and the quenching of the acceptor's emission, simultaneously, which provided the basis of ratiometric detection. In order to reveal the feasibility of the above concept, we constructed FRET systems SN-1 and SN-2 composed of one squaraine and two naphthalimide moieties. Noticeably, the chemically reactive recognition of a nucleophilic analyte was a FRET 'switching-off' process, which was a new mode different from the known FRET 'switching-on' sensors based on the platform of rhodamine spirolactam. The synthetic routes were shown in Scheme 2.

# 2. Experimental

# 2.1 General

3,4-Dihydroxy-3-cyclobutene-1,2-dione (squaric acid) was purchased from commercial suppliers. Other reagents and solvents were of analytical grade. The squaraine reference compound is synthesized according to the previous procedure.<sup>11</sup> <sup>1</sup>H NMR and <sup>13</sup>C NMR were measured on an AV-400 spectrometer with chemical shifts reported as ppm. Mass

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**Scheme 1** 'Switching-off' FRET model: design concept of squarainebased ratiometric sensors for nucleophilic analytes.



Scheme 2 Synthetic routes of FRET sensor SN-1 and SN-2.

spectra were measured on HP 1100 LC-MSD and GC-Tof MS spectrometers. Fluorescence spectra were measured on a PTI-700 fluorescence lifetime spectrophotometer. Absorption spectra were determined on a TU-1901 UV-vis spectrophotometer.

The stock solution of probe (SN-1 and SN-2) was  $10^{-3}$  mol L<sup>-1</sup> in dichloromethane. In a typical experiment, test solutions were prepared by placing 50 µL of the probe stock solution into a test tube, and diluting the solution to 5 mL with dichloromethane or acetonitrile. Stock solution of fluoride and cyanide was 0.01 mol L<sup>-1</sup> tetrabutylammonium fluoride and sodium cyanide, respectively. Tetrabutylammonium fluoride was dissolved in dichloromethane and sodium cyanide was dissolved in water.

#### 2.2 Synthesis of compound 4

Compound 3 (400 mg, 1.0 mmol), N-methylaniline (3 mL, 27.8 mmol) and KI (5 g, 30.1 mmol) were dissolved in DMF (5 mL). The mixtures were then refluxed for 12 h. After completion of the reaction (monitored via thin-layer chromatography), the mixtures were cooled down to room temperature and extracted with dichloromethane. The organic phase was dried over MgSO<sub>4</sub>. The solvent was evaporated and the residue was purified by silica gel column chromatography using eluent (CH<sub>2</sub>Cl<sub>2</sub>-CH<sub>3</sub>OH = 100:1 v/v). 300 mg of a vellow solid was obtained. Yield: 70%; mp: 188.2-189.5 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 8.59 (d, 1H, J = 7.2 Hz), 8.54 (d, 1H, J = 7.6 Hz), 8.43 (d, 1H, J = 8.4 Hz), 7.72 (t, 1H, J = 7.6 Hz), 7.24 (t, 2H, J = 8.4 Hz), 6.92 (d, 2H, J =7.85 Hz), 6.65 (t, 2H, J = 8.25 Hz), 4.40 (t, 2H, J = 6.8 Hz), 4.02 (s, 4H), 3.66 (t, 2H, J = 4 Hz), 3.26 (s, 4H), 3.08 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ (ppm) 164.6, 164.2, 155.9, 132.7, 131.3, 130.3, 130.1, 129.4, 126.3, 126.0, 123.3, 117.1, 115.1, 112.3, 67.1, 53.6, 50.1, 38.5, 36.9.

#### 2.3 Synthesis of SN-1

A mixture of compound 4 (200 mg, 0.5 mmol), squaric acid (22.4 mg, 0.2 mmol) in a mixed solution of toluene and butanol (1:1, v/v) was heated to reflux with an azeotrope removing water under stirring for 12 h to obtain the condensation product as a dark blue solid. The reaction mixture was washed with n-hexane and filtered to give a green solid which was dissolved in CH<sub>2</sub>Cl<sub>2</sub> and purified by column chromatography on silica gel with a mixture of dichloromethane and methanol (v/v, from 50:1 to 35:1) as the eluent to give 23 mg of a green solid. Yield: 4%; mp: > 300 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 8.55 (d, 2H, J = 7.05 Hz), 8.47 (d, 2H, J = 7.95 Hz), 8.38 (d, 2H, J = 8.15 Hz), 8.10 (d, 2H, J = 8.95 Hz), 7.71 (t, 2H, J = 7.95 Hz), 7.20 (d, 2H, J = 7.85 Hz), 6.85 (d, 2H, J)J = 8.25 Hz), 4.49 (t, 4H, J = 6 Hz), 3.91 (t, 8H, J = 4 Hz), 3.49 (t, 4H, J = 6 Hz), 3.28 (s, 6H), 3.23 (t, 8H, J = 4 Hz); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ (ppm) 164.6, 164.2, 155.9, 148.4, 132.7, 131.3, 130.3, 130.1, 129.4, 126.3, 126.0, 123.3, 117.1, 115.1, 112.3, 67.1, 53.6, 50.1, 38.5, 36.9.

#### 2.4 Synthesis of 4'

The synthesis of compound 4' was similar to compound 4.

Yield: 91%; mp: 119–120 °C; MS (TOF): 459.2150, calc.: 459.2158; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 8.58 (d, 1H, J = 7.2 Hz), 8.52 (d, 1H, J = 8.0 Hz), 8.42 (d, 1H, J =8.4 Hz), 7.72 (t, 1H, J = 8.0 Hz), 7.23 (d, 1H, J = 8.0 Hz), 7.15 (t, 2H, J = 7.6 Hz), 6.65 (m, 3H), 4.43 (t, 2H, J =5.6 Hz), 4.02 (s, 4H), 3.78 (t, 2H, J = 5.6 Hz), 3.68 (t, 2H, J =5.6 Hz), 3.47 (t, 2H, J = 5.6 Hz), 3.26 (s, 4H), 2.89 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 164.5, 164.0, 155.7, 149.4, 132.7, 131.3, 130.2, 130.1, 129.1, 126.3, 125.9, 123.4, 117.3, 116.2, 115.1, 112.2, 68.4, 68.2, 67.1, 53.6, 52.5, 39.3, 38.9.

#### 2.5 Synthesis of SN-2

The synthesis of SN-2 was similar to that of SN-1.

Yield: 5.9%; mp: 210–212 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 8.52 (d, 2H, J = 6.8 Hz), 8.46 (d, 2H, J = 8.0 Hz), 8.37 (d, 2H, J = 8.4 Hz), 8.13 (d, 4H, J = 8.8 Hz), 7.69 (t, 2H, J = 8.0 Hz), 7.19 (d, 2H, J = 8.0 Hz), 6.64 (d, 4H, J = 8.8 Hz), 4.41 (t, 4H, J = 5.2 Hz), 3.94 (s, 8H), 3.84 (t, 4H, J = 5.2 Hz), 3.74 (d, 4H, J = 5.2 Hz), 3.64 (d, 4H, J = 4.4 Hz), 3.22 (s, 8H), 3.11 (s, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 183.1, 164.6, 164.1, 155.9, 154.6, 132.9, 132.8, 131.3 130.4, 129.9, 126.1, 125.9, 123.1, 119.8, 116.8, 115.0, 112.5, 68.3, 67.1, 53.5, 52.5, 39.5, 39.1.

## 3. Results and discussion

As we know, there are two main factors which determine the efficiency of FRET. One is overlap between the donor's emission and the acceptor's absorption, and the other is distance between the two fluorophores. In the construction of FRET systems SN-1 and SN-2, naphthalimide fluorophore was chosen as an energy donor because it has strong emission in the visible range and its broad emission (450-650 nm) covers a part of squaraine's absorption band, as shown in Fig. 1. The absorption spectra of both SN-1 and SN-2 showed two bands characteristic of naphthalimide and squaraine, respectively. For example, as shown in Fig. 1, the absorption bands at 402 nm ( $\epsilon = 14000 \text{ L mol}^{-1} \text{ cm}^{-1}$ ) and 636 nm  $(\varepsilon = 186000 \text{ L mol}^{-1} \text{ cm}^{-1})$  clearly indicated the existence of naphthalimide and squaraine within SN-2. SN-1 and SN-2 were different in the terms of the spacer which connected the naphthalimide unit to the squaraine fluorophore. In SN-1, a short ethylene group was employed for higher energy transfer efficiency. A short ethylene group was employed as spacer in the FRET system. However, a short distance between donor and acceptor may also facilitate photoinduced electron transfer (PET) whose competition would be excluded. Thus, we also designed SN-2, within which a little longer and flexible ester chain (diethyoxyl group) was used as the spacer. Before the reactive recognition of nucleophiles, within SN-1 and SN-2, FRET processes were in the 'on' state, as illustrated by the fluorescence properties. For example, upon excitation of SN-2 at 402 nm, only one emission band was observed at 659 nm. The emission of naphthalimide was not found at 525 nm, which indicated that the efficiency of energy transfer would be high. The efficiency of energy transfer (EET) was calculated according to the literature.<sup>12</sup> The Forster critical radius was calculated using the following equation:

$$R_0 = 0.211 [k^2 n^{-4} \Phi_{\rm D} \int_0^\infty I_{\rm D}(\lambda) \varepsilon_{\rm A}(\lambda) \lambda^4 d\lambda]^{1/6}$$
(1)

with  $R_0$  in Å, where  $k^2$  is the orientational factor,  $\Phi_D$  is the quantum yield of fluorescence emission of the donor, *n* is the average refractive index of the medium in the wavelength range where spectral overlap is significant,  $I_D(\lambda)$  is the normalized fluorescence spectrum of the donor,  $\varepsilon_A(\lambda)$  is the molar absorption coefficient of the acceptor (in dm<sup>3</sup> mol<sup>-1</sup> cm<sup>-1</sup>) and  $\lambda$  is the wavelength in nanometres. As already done, the orientation factor was assumed to be equal to the dynamic average, which is 2/3. In the frame of Forster's theory, the transfer efficiency is given by the following equation:

$$EET = R_0^{6} / [R_0^{6} + R^{6}]$$
 (2)

where  $R_0$  is the Forster critical radius and R is the donoracceptor distance. The calculation data of energy transfer for SN-1 and SN-2 are shown in Table 1.



Fig. 1 The normalized absorption of SN-2 and emission spectra  $(\lambda_{ex} 402 \text{ nm})$  in CH<sub>2</sub>Cl<sub>2</sub>. The emission spectra of naphthalimide 4'  $(\lambda_{ex} 402 \text{ nm})$  and squaraine reference  $(\lambda_{ex} 610 \text{ nm})$ .

As mentioned above, squaraine can be used as a sensor for fluoride ions. For SN-1, the F<sup>-</sup> recognition experiment was carried out in dichloromethane due to its poor solubility in polar solvents, such as acetonitrile and ethanol, etc. It should be stated that dichloromethane might not be very suitable because such media did not favour nucleophilic reactions. The responses of SN-1 to fluoride in its absorption and emission spectra are shown in Fig. 2. The fluoride concentration titration was conducted using a 10 µM solution of SN-1 in dichloromethane. With fluoride addition to the solution of SN-1, the absorption band of naphthalimide at 402 nm was not changed, but the absorption band of the squaraine section at 636 nm gradually decreased. When the concentration of fluoride ion was increased to 5 mM, the squaraine absorption band disappeared. Meanwhile, the squaraine emission peak at 659 nm disappeared too. However, the emission band of the naphthalimide unit did not rise as previously anticipated, which meant that there existed some fluorescence-quenching factors within the mixture of SN-1 and fluoride ion. As shown in Fig. 3, the spectral responses of squaraine to fluoride ion was similar to that of the squaraine part within SN-1. The absorption spectra of compound 4 were not affected by addition of fluoride ion, which indicated that there was no interaction between the naphthalimide part and fluoride ions, and product of the nucleophilitic reaction between squaraine and fluoride would be the major fluorescence-quenching factor.

Fluorescence properties of **2** and **4**, as reference compounds, were studied, as shown in Table 2. Similarly, the fluorescence of compound **4** ( $\Phi = 0.02$ ) was much weaker than intermediate **2** ( $\Phi = 0.31$ ). PET was a reasonable explanation.<sup>13</sup> Upon addition of fluoride ions, the generated anilino fragments were very strong electron donors from which to the electron-deficient naphthalimide PET could efficiently occur owing to the favorable driving forces.<sup>14</sup> Actually, in previous investigations of our group, the aromatic amino type electron donors were introduced to the imide site of the naphthalimide fluorophore, and excellent PET sensors were obtained.<sup>15</sup> Since there was only a single-emission decrease in response to fluoride ion, SN-1 was a 'turn-off' sensor, but not the expected ratiometric sensor.

To overcome the major problems (solubility and PET) encountered in SN-1, we made a modification to the spacer and developed SN-2. The spacer was lengthened to eliminate

Table 1 Calculation data of energy transfer for SN-1 and SN-2

| Parameter                                     | Compounds                               |                            |   |
|---|---|----------------------------|---|
|   | SN-1 in CH <sub>2</sub> Cl <sub>2</sub> | SN-2 in CH <sub>3</sub> CN | SN-2 in CH <sub>3</sub> CN-H <sub>2</sub> O (9:1) |
| $J^{a}/M^{-1} \text{ cm}^{-1} \text{ nm}^{4}$ | $9.13 \times 10^{14}$                   | $1.61 \times 10^{14}$      | $3.2 \times 10^{13}$                              |
| $R_0^{\prime b}/\text{\AA}$                   | 39.5                                    | 29.1                       | 20.3  |
| $\vec{R^{c}}/\dot{A}$                         | 10.04                                   | 12.83                      | 12.83   |
| EET   | 99.8%                                   | 99.2%                      | 93.9%   |
|   |   |                            |   |

<sup>*a*</sup> The overlap integral of the donor emission and the acceptor absorption spectra.  $J = \int_0^\infty I_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda$ . <sup>*b*</sup> Calculated according to the eqn (1);  $k^2 = 2/3$ ,  $n(CH_2Cl_2) = 1.4240$ ,  $n(CH_3CN) = 1.3441$ . <sup>*c*</sup> Distance between the D and A groups (estimated using standard bond lengths).



Fig. 2 The changes of absorption spectra (a) and emission spectra (b) of SN-1 (10  $\mu$ M) with addition of F<sup>-</sup> in CH<sub>2</sub>Cl<sub>2</sub>.



Fig. 3 The spectral responses of compound 4 and squaraine to  $F^-$ , respectively.

Table 2 Fluorescence quantum yield of reference compounds

| Compound                            | Fluorescence quantum yield ( $\Phi^a$ ) |
|-------------------------------------|---|
| 2                                   | 0.31                                    |
| 4                                   | 0.02                                    |
| 4'                                  | 0.21                                    |
| <sup>a</sup> Determinated by rhodam | ine 6G as a reference.                  |

PET, and the higher polar nature of the ester chain could improve the solubility and facilitate reactive recognitions in polar solvents. The reference compound 4' showed strong

fluorescence. Its quantum yield ( $\Phi = 0.21$ ) is much higher than that of compound **4**, although it is a little lower than that of compound **2**. The PET process within compound **4'** was weakened, which indicated that within the products of reactions between SN-2 and nucleophiles, the effect of PET could be removed to large extents. This was also proved by the response of SN-2 to fluoride ions investigated in acetonitrile. The changes of absorption and emission spectra were shown in Fig. 4.

The fluoride concentration titration was conducted using a 10  $\mu$ M solution of SN-2 in acetonitrile. Upon addition of fluoride ion, the changes of absorption spectra were similar to SN-1, but the emission spectra were different: the emission band of the squaraine section at 636 nm decreased, and the emission band of naphthalimide at 525 nm gradually increased. So a ratiometric response based on the FRET 'switching-off' principle was realized. Due to the polar solvent acetonitrile, which was favourable to the nucleophilicity of the fluoride, the sensitivity of SN-2 was higher than sensor SN-1. The detection limit of SN-2 was 0.2  $\mu$ M in acetonitrile. The ratios of absorption and emission with the concentration of F<sup>-</sup> could be obtained, and is shown in Fig. 4.



Fig. 4 The absorption (a) and emission (b) changes of 10  $\mu$ M SN-2 in acetonitrile with increasing concentration (2  $\mu$ M–1.2 mM) of fluoride anion. Inset: the ratio plot of absorption (a) and emission (b) with the concentration of F<sup>-</sup>.



Fig. 5 Absorption spectra (a) and emission spectra (b) of (5  $\mu$ M) SN-2 with addition of CN<sup>-</sup> (0.4  $\mu$ M–0.76 mM) in a mixture solvent of water and acetonitrile (1/10, v/v). Inset: the ratio plot of emission at 537 nm and 667 nm.

Cyanide is one of the most dangerous poisons because of its extreme toxicity, inhibiting cytochrome oxidase of the mitochondrial respiratory chain.<sup>16</sup> Since cyanide was extensively used in industry as the raw material to produce organic chemicals, polymers and for gold extraction, industrial waste water has been the main environmental pollution source for cyanide anions. So, it is necessary to find a convenient, accurate, quantitative and real-time detection method for cyanide anions. Although many 'turn-on' fluorescent sensors based on the chemodosimeter approach have been developed to detect the cyanide anion,<sup>17</sup> the ratiometric method based on 'switching FRET off' has not been reported. Moreover, the color and fluorescence changes of this sensor would be clearly distinguished by the naked eye. The electron deficient center of squaraine dyes could be successfully used to design the cyanide ratiometic sensor, because cyanide is as nucleophilic as fluoride. Hence, the sensor SN-2 was also chosen to detect cyanide. The changes of spectra were shown in Fig. 5.

In order to illustrate the potential practical application of SN-2, a mixed solvent of water–acetonitrile was used in the response of SN-2 to the cyanide ion. In the solvent of water and acetonitrile (1/10, v/v), SN-2 showed three obvious absorption peaks at 400 nm, 575 nm and 641 nm. The emission band at 400 nm belonged to naphthalimide but, different to SN-1, the other two emission bands at 575 nm and 641 nm were ascribed to the squaraine unit. Upon addition of cyanide anions, the absorption bands at 575 and 641 nm had obviously decreased. When the concentration of cyanide was increased to 0.76 mM, the two peaks had totally disappeared, but the absorption peaks at 400 nm had not changed. These results indicated that the cyanide anion attacked the electron deficient center of squaraine to break the conjugation system whilst,

simultaneously, the naphthalimide fluorophore was not affected in recognition process.

The fluorescence properties are shown in Fig. 5b. When the naphthalimide was excited at 400 nm, the SN-2 showed two emission bands with peaks at 537 and 667 nm, which could be ascribed to naphthalimide and squaraine, respectively. Obviously, the emission at 667 nm resulted from the FRET process from naphthalimide to squaraine. However, there remained a weak emission of naphthalimide, which indicated that energy transfer was incomplete. It might be explained by the fact that in the highly polar solvent of water-acetonitrile mixtures, the energy transfer was not efficient due to the high polarity of the solvent.<sup>18</sup> With the addition of the cyanide, the emission at 667 nm decreased sharply, followed by the increases of the emission of naphthalimide at 537 nm, which indicated that the chemical reaction between the cyanide and the squaraine blocked the FRET process between the two dyes, after which the fluorescence of naphthalimide recovered. The ratio changes of the two fluorescence peaks  $(I_{537}/I_{667})$  produced a linear function with the concentration of cyanide between 0.5 mM and 500 mM as shown in Fig. 5b.

The selectivity of SN-2 was investigated. As shown in Fig. 6, we selected some anions, such as  $F^-$ ,  $Br^-$ ,  $I^-$ ,  $OH^-$ ,  $PO_4^{3-}$ ,  $SCN^-$ ,  $CH_3COO^-$ ,  $ClO_4^-$ ,  $NO_3^-$ ,  $C_2O_4^{2-}$ ,  $SO_4^{2-}$ ,  $S^{2-}$  and  $CN^-$ . For the absorption and emission spectra, apart from  $F^-$  and  $CN^-$ , the anions did not make any dramatic changes. Although  $OH^-$  could also slightly affect the absorption and emission spectra, there were no significant changes of the pH of SN-2's solution before and after addition of  $F^-$  and  $CN^-$ . From Fig. 6c, the sensor SN-2 had good selectivity for  $F^-$  and  $CN^-$  because  $F^-$  and  $CN^-$  have stronger nucleophilicity than other anions.



Fig. 6 The absorption changes (a) and the ratio intensity at 528 nm and 659 nm for different anions (b). Insert: emission spectra changes in the presence of some usual anions (100 eq.) in the mixture of acetonitrile and water (v/v 10:1)

In conclusion, for the first time, the approach of squaraine chemodosimeter for nucleophilic analytes was coupled with the FRET principle in the construction of cassettes SN-1 and SN-2. In our design concept, the nucleophilic attacks on electron-deficient squaraine center switched off the FRET process by completely canceling the spectra overlap, and thus resulted in the recovery of emission from the naphthalimide donor and the quenching of emission from the squaraine acceptor, simultaneously. SN-2 proved to be an efficiently ratiometric sensor of the fluoride and cyanide anions, supporting our tentative idea. It should be noted that, 'turn-off' chemodosimeters are common, and not just confined to squaraine. Thus, the chemically 'switching FRET off' strategy is of universal importance and should find more applications in the transformation of various 'turn-off' chemodosimeters into ratiometric sensors.

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