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Amide-based diarylmaleimide derivatives and polymers: Highly selective and ratiometric fluorescence sensing for anions



PIGMENTS

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1. Introduction

Fluorimetric probes and sensors have been used extensively in labeling of biomolecules and detection of ions for excellent sensitivity, good specificity, a large linear range of analysis, and ease of handling [1,2]. Particularly, fluorescent sensing for anions has attracted wide interest of researchers due to the crucial role of anions in environmental, chemical, and biological systems in forms of drugs, food additives, agricultural fertilizers, biologic metabolites, and waste liquid from chemical plant [3–7]. Among various anions, fluoride and cyanide received considerable attention in past decades for the following reason: fluoride ions are employed to prevent dental caries [8] and treat osteoporosis [9], and applied in pharmacology, anesthetics, nerve gases, drinking water analysis, and uranium refinement [10-14], while cyanide ions are widely used as raw materials or auxiliaries in the production of nylon, resins, herbicides, and in the extraction of gold [15]. However, an excess of fluoride can lead to fluorosis for renal function damage, thyroid activity depression and immune system disruption

ABSTRACT

A series of diarylmaleimide derivatives and polymers based on amide group were designed and synthesized to detect anions selectively and sensitively. Three bisindolylmaleimide derivatives (FAM-p, FAMm and FAM-o) employing trifluoroacetamide as receptor exhibited strong fluorescent quenching upon fluoride and cyanide. Trifluoroacetamide receptor was replaced by acetamide to obtain other two derivatives (AM-p and AM-o) which showed higher selectivity than FAMs with sensing for only fluoride. The diarylmaleimide derivatives were introduced into the backbone of polyfluorene by Suzuki reaction to prepare polymer PFAM and PAM with same sensing selectivity for anions as FAM and AM, respectively. PFAM and PAM were found to be ratiometric fluorescence sensors for a fluorescence resonance energy transfer (FRET) from fluorene segments to diphenylmaleimide units. The experimental and DFT/TDDFT calculation results demonstrated that the interaction mechanism of FAMs with F⁻ and CN⁻ was hydrogen bond interaction and nucleophilic addition reaction, respectively.

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[16–18]. Cyanide is among the most toxic inorganic anions [19] for even a small amount being a serious threat to human health. Therefore, to develop fluorescent sensors with high selectivity and sensitivity for F^- and CN^- anions is very necessary.

The fluorescent sensors based on NH hydrogen bond donor were widely employed in the recognition for anions due to the relatively high energy and the significant availability of hydrogen bond interaction [20–22]. Although the hydrogen bond is one of the most frequently used and effective sensing methods, it has generally displayed poor selectivity for fluoride or cyanide [23,24]. For example, Lees previously synthesized a luminescent metal complex integrated with an amide moiety, which exhibited high affinities for cyanide, halides and acetate anions with binding constants as high as $10^4 - 10^5 \,\mathrm{M}^{-1}$ in CH₂Cl₂ solution [25]. Chen and Huang designed the novel upper-rim anthracene bridged calix[4] arene and found that the fluorescence of the anthracene group was quenched upon addition of basic anions such as AcO⁻ and F⁻ in acetonitrile solution [26]. Eichen investigated the molecular recognition properties of Cyclo[2]benzimidazole containing two amide groups with fluoride, bifluoride and the oxo anions dihydrophosphate and benzoate through the inhibition of an excitedstate intramolecular proton transfer process [27]. On the other hand, most of the fluorimetric sensors detect anions through fluorescence enhancement or quenching mechanism [28]. As a



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result, the excitation power and the detector sensitivity can impose great effect on the detection. It is still a challenge to design novel fluorescent sensors for fluoride or cyanide with high selectivity and sensitivity, as well as easy identification.

Bisindolylmaleimides, one kind of the typical diarylmaleimide derivatives, are common substructures of natural products found for alkaloids from slime molds [29]. Their biological activity as potent inhibitors of protein kinase C made them widely apply in the pharmaceutical area and therapy for autoimmune diseases [30–32]. Meanwhile, most of bisindolylmaleimide compounds showed strong red luminescence in solution and in solid, which stimulated their application as electroluminescent materials in organic light-emitting diode (OLED) [33-35]. However, diary-Imaleimides have received much less attention in the field of anion recognition than naphthalimides, an analogous dye [36]. Actually, the susceptibility of diarylmaleimides in emission to external environment kept them become potential candidate as anion sensor [37,38]. In this paper, a series of diarylmaleimide derivatives and polymers based on amide group were designed and synthesized to detect anions selectively and sensitively. First, three bisindolylmaleimide derivatives (FAM-p, FAM-m and FAM-o) employing trifluoroacetamide as receptor were synthesized through changing the substituted position of trifluoroacetamide (Fig. 1). These compounds exhibited strong fluorescent quenching upon fluoride and cyanide. Second, trifluoroacetamide receptor was replaced by acetamide to obtain other two derivatives (AM-p and AM-o) which showed higher selectivity than FAMs with sensing for only fluoride, due to less electropositivity of the carbonyl group in AMs. Last, the diarylmaleimide derivatives were introduced into the backbone of polyfluorene through substituting indolyl rings with phenyl rings which are easy to be linked to fluorene by Suzuki reaction. Consequently, polymer PFAM and PAM with different receptor were prepared to improve the sensitivity of FAMs and AMs for the amplified fluorescence signal of conjugated polymers based on the "molecular wire" effects [39,40]. Furthermore, PFAM and PAM were found to be ratiometric fluorescence sensors for a fluorescence resonance energy transfer (FRET) from fluorene segments to diphenylmaleimide units, for which the reliability of the anion detection was substantially enhanced.

2. Experimental section

2.1. General

Electronic spray ion (ESI) mass spectra were recorded on a TSQ Quantum Access MAX of Thermo Fisher Scientific. NMR spectra



Fig. 1. The structure of FAMs, AMs, PFAM and PAM.

were measured in CDCl₃ on a Bruker Ascend 400 FT-NMR spectrometer; ¹H and ¹³C chemical shifts were quoted relative to the internal standard tetramethylsilane. UV–vis spectra were obtained on a Shimadzu UV-2600 spectrophotometer. The PL spectra were probed on a Shimadzu RF-5301PC fluorescence spectrophotometer. All photographs were recorded on a Canon Powershot G7 digital camera under daylight or UV light (365 nm) lamp. The fluorescence quantum yields were determined in CH₃CN solutions at 293 K against Nile red as a reference ($\Phi_p = 0.78$) [41]. The weight-average molecular weights (M_w) and the polydispersity index (PDI) of soluble polymers were determined by gel-permeation chromatography (GPC) with a Waters 1515 instrument. The polystyrene and THF were used as standards and as eluant, respectively.

All of the reagents and solvents used were obtained from commercial suppliers and were used without further purification unless otherwise noted. Thin layer chromatography was performed on G254 plates of Qingdao Haiyang Chemical. Column chromatography was performed on Sorbent Technologies brand silica gel (40–63 mm, Standard grade).

2.2. The synthetic procedure of compounds 2, 3 and 4

To a solution of **1** (0.2 g, 0.56 mmol) in THF (20 ml) were added phenylenediamine (0.18 g, 1.66 mmol) and K_2CO_3 (0.2 g, 1.45 mmol). The mixture was heated to reflux for 12 h. It was allowed to cool to ambient temperature and was filtered. The product was purified by silica gel column chromatography with CH₂Cl₂ as the eluant, affording dark red solids in 27–53% yield.

Physical data for **2** (53%). ¹H NMR (DMSO-d6, 400 MHz): δ 7.84 (s, 2H), 7.43 (d, 8 Hz, 2H), 7.04 (m, 4H), 6.81 (d, 8 Hz, 2H), 6.63 (m, 4H), 5.28 (s, 2H), 3.86 (s, 6H).

Physical data for **3** (27%). ¹H NMR (DMSO-d6, 400 MHz): δ 7.85 (s, 2H), 7.44 (d, 8 Hz, 2H), 7.13 (t, 6.8 Hz, 1H), 7.06 (t, 7.6 Hz, 2H), 6.82 (d, 8 Hz, 2H), 6.69 (t, 7.2 Hz, 2H), 6.58 (m, 3H), 5.29 (s, 2H), 3.86 (s, 6H).

Physical data for **4** (29%). ¹H NMR (CDCl₃, 400 MHz): δ 7.73 (s, 2H), 7.28 (d, 2H), 7.18 (m, 2H), 7.11 (m, 2H), 6.95 (d, 2H), 6.90 (m, 2H), 6.74 (m, 2H), 3.84 (s, 6H).

2.3. The synthetic procedure of compounds FAM-p, FAM-m and FAM-o

A dichloromethane solution of compounds **2**, **3** or **4** (0.1 g, 0.22 mmol) was placed into ice-water bath and stirred for 30 min. To the solution were slowly added trifluoroacetic anhydride (0.1 ml, 0.71 mmol) and triethylamine (0.1 ml) in succession. It was allowed to heat to ambient temperature and stirred for 2 h. The solution was successively washed with water (10 ml) and brine (10 ml). It was then dehydrated over anhydrous MgSO₄. After removing the solvent, the crude product was purified by column chromatography with CH_2Cl_2 as the eluant, affording the desired compounds.

Physical data for **FAM-p** (87%). ¹H NMR (CDCl₃, 400 MHz): δ 8.03 (s, 1H), 7.75 (s, 2H), 7.71 (d, 8.8 Hz, 2H), 7.59 (d, 7.2 Hz, 2H), 7.31 (d, 8 Hz, 2H), 7.11 (t, 7.2 Hz, 2H), 6.96 (d, 8.0 Hz, 2H), 6.75 (t, 7.6 Hz, 2H), 3.86 (s, 6H). ¹³C NMR (CDCl₃, 100 MHz): δ 170.23, 153.90, 153.53, 135.84, 133.05, 132.03, 129.25, 125.96, 125.50, 125.29, 121.30, 121.25, 119.88, 119.80, 119.13, 116.15, 113.28, 108.40, 104.66, 32.36. HRMS (ESI): *m/z* calcd for C₃₀H₂₂F₃N₄O₃ (M + H)⁺ 543.1639, found 543.1635.

Physical data for **FAM-m** (80%). ¹H NMR (CDCl₃, 400 MHz): δ 8.16 (s, 1H), 7.74 (m, 3H), 7.65 (d, 8 Hz, 1H), 7.49 (t, 7.6 Hz, 1H), 7.44 (d, 8 Hz, 1H), 7.30 (d, 8 Hz, 2H), 7.12 (t, 7.6 Hz, 2H), 6.96 (d, 8.4 Hz, 2H), 6.75 (t, 7.6 Hz, 2H), 3.84 (s, 6H). ¹³C NMR (CDCl₃, 100 MHz): δ 170.05, 153.88, 153.51, 135.84, 134.69, 132.37, 129.10, 128.77, 125.50, 125.25, 122.69, 121.29, 119.14, 118.10, 116.93, 113.20, 108.39, 104.61, 32.35.



Scheme 1. The synthesis of FAMs and AMs.

HRMS (ESI): m/z calcd for $C_{30}H_{22}F_3N_4O_3 (M + H)^+$ 543.1639, found 543.1637.

Physical data for **FAM-o** (85%). ¹H NMR (CDCl₃, 400 MHz): δ 8.93 (s, 1H), 7.96 (d, 8 Hz, 1H), 7.73 (s, 2H), 7.49 (t, 6.8 Hz, 2H), 7.42 (t, 7.2 Hz, 1H), 7.33 (d, 8 Hz, 2H), 7.14 (t, 7.6 Hz, 2H), 6.95 (d, 8 Hz, 2H), 6.77 (t, 7.6 Hz, 2H), 3.87 (s, 6H). ¹³C NMR (CDCl₃, 100 MHz): δ 170.48, 154.50, 154.13, 135.88, 133.32, 129.66, 127.83, 127.30, 126.31, 125.85, 125.16, 124.65, 121.47, 121.33, 119.30, 113.43, 108.51, 104.51, 32.43. HRMS (ESI): *m/z* calcd for C₃₀H₂₂F₃N₄O₃ (M + H)⁺ 543.1639, found 543.1637.

2.4. The synthetic procedure of compounds AM-p and AM-o

A similar procedure to the preparation of FAM was employed from compounds **2**, **4** and acetamide (0.07 ml, 0.74 mmol).

Physical data for **AM-p** (83%). ¹H NMR (CDCl₃, 400 MHz): δ 7.73 (s, 2H), 7.61 (br, 2H), 7.46 (d, 6.0 Hz, 2H), 7.35 (s, 1H), 7.30 (d, 8.4 Hz, 2H), 7.11 (t, 7.2 Hz, 2H), 6.97 (d, 8.0 Hz, 2H), 6.74 (t, 7.2 Hz, 2H), 3.84 (s, 6H), 2.17 (s, 3H). ¹³C NMR (CDCl₃, 100 MHz): δ 170.35, 167.23, 135.93, 135.83, 131.98, 127.36, 125.83, 125.49, 125.32, 121.29, 121.22, 119.06, 108.34, 104.74, 32.36, 23.73. HRMS (ESI): *m/z* calcd for C₃₀H₂₅N₄O₃ (M + H)⁺ 489.1921, found 489.1918.

Physical data for **AM-o** (82%). ¹H NMR (CDCl₃, 400 MHz): δ 8.04 (d, 8.0 Hz, 1H), 7.74 (m, 3H), 7.44 (t, 7.6 Hz, 1H), 7.34 (d, 8.0 Hz, 2H), 7.30 (d, 8.8 Hz, 2H), 7.13 (t, 7.2 Hz, 2H), 6.97 (d, 8.0 Hz, 2H), 6.77 (t, 7.2 Hz, 2H), 3.86 (s, 6H), 2.11 (s, 3H). ¹³C NMR (CDCl₃, 100 MHz): δ 170.56, 167.45, 135.86, 133.08, 132.24, 127.92, 127.34, 125.82,

125.21, 124.51, 124.40, 123.43, 121.40, 119.22, 108.48, 104.64, 32.40, 23.63. HRMS (ESI): m/z calcd for $C_{30}H_{25}N_4O_3$ (M + H)⁺ 489.1921, found 489.1920.

2.5. The synthesis of compound 7

To an aqueous solution (100 ml) of 10% KOH was added **5** (1.25 g, 3.1 mmol). The mixture was heated to reflux for 3 h and then neutralized by the addition of hydrochloric acid (2 N) until precipitates formed. It was filtered, and the filtrates were dried in vacuo. The product was purified by silica gel column chromatography with CH₂Cl₂/hexane (1:1) as the eluant, affording yellow-green solids of **6** (0.92 g) in a 73% yield.

To a solution of **6** (0.92 g, 2.25 mmol) in THF were added ophenylenediamine (1.1 g, 10.1 mmol) and K₂CO₃ (0.94 g, 6.8 mmol). The mixture was heated to 60 °C for 24 h. It was allowed to cool to ambient temperature and was filtered. The product was purified by silica gel column chromatography with CH₂Cl₂ as the eluant, affording green solids of **7** (0.73 g) in 65% yield. ¹H NMR (CDCl₃, 400 MHz): δ 7.55 (d, 8 Hz, 4H), 7.42 (d, 7.6 Hz, 4H), 7.24 (m, 1H), 7.14 (d, 8 Hz, 1H), 6.91 (m, 2H), 3.76 (s, 2H).

2.6. The synthesis of polymer P1

To a mixture of monomer **7** (75 mg, 0.15 mmol), **8** (0.2 g, 0.3 mmol), and 2,7-dibromo-9,9-dioctyl-9H-fluorene (83 mg, 0.15 mmol) under a nitrogen atmosphere was added TBAI (30 mg,



Scheme 2. The synthesis of PFAM and PAM.

 Table 1

 Physical data of compounds FAMs and AMs.

Compound	λ_{abs} (nm)	λ_{em} (nm)	$\lambda_{0-0} (nm)$	$\Phi_{ m em}$ (%)
FAM-p	485	651	558	27
FAM-m	487	652	560	33
FAM-o	504	677	587	7
АМ-р	482	652	557	15
AM-o	493	668	574	12

0.08 mmol), aqueous K₂CO₃ (2 M, 0.8 ml), and toluene (5 ml). To it was added tetrakis(triphenylphosphine)palladium (12 mg, 0.01 mmol) while under nitrogen, and then the mixture was refluxed for 48 h. The polymerization was completed by refluxing with benzeneboronic acid (55 mg, 0.5 mmol) for 6 h and with bromobenzene (0.05 ml, 0.5 mmol) for 8 h. The mixture was cooled to room temperature and poured into a methanol solution of 10% H₂O. The precipitates were collected by filtration, and dried in vacuo. The polymer was further purified by extracting with acetone for 24 h using a Soxhlet apparatus to afford green solids of **P1** in 30% yield. ¹H NMR (300 MHz, CDCl₃): δ 7.75 (m, 2H), 7.55 (m, 4H), 1.99 (4H, br), 1.23–0.86 (m, 30H). GPC: $M_w = 12800$; PDI = 1.48.

2.7. The synthetic procedure of polymer PFAM and PAM

A dichloromethane solution of P1 (0.1 g) was placed into icewater bath and stirred for 30 min. To the solution were slowly



Fig. 2. a) Absorption and emission spectra of FAMs. b) Emission spectra of PFAM and PAM.

added anhydride (0.1 ml) and triethylamine (0.1 ml) in succession. It was allowed to heat to ambient temperature and stirred for 2 h. The mixture was poured into acetone with stirring to give the desired polymer.

Physical data for **PFAM** (79%). ¹H NMR (CDCl₃, 400 MHz): δ 7.77–7.61 (m, 6H), 2.05 (br, 4H), 1.27–0.84 (m, 30H).

Physical data for **PAM** (71%). ¹H NMR (CDCl₃, 400 MHz): δ 7.77 (m, 2H), 7.68 (m, 4H), 2.04 (br, 4H), 1.26–0.90 (m, 30H).

3. Results and discussions

3.1. Synthesis of dyes and polymer

The bisindolylmaleimide derivatives were obtained readily through three steps which are shown in Scheme 1. The compounds 1 obtained by a cyclization of indolylacetic acids [42], coupled with phenylenediamine to give compounds 2, 3 and 4, followed by an acetylization with trifluoroacetic anhydride or acetic anhydride to afford the resulted compounds FAM-p, FAM-m FAM-o, AM-p or AM-o.

The synthesis of polymer **PFAM** and **PAM** is outlined in Scheme 2. Compound 5 was synthesized according to a previously reported method [43]. Monomer **7** was smoothly prepared from **5** by two reactions in succession, hydrolysis under NaOH aqueous solution and condensation with o-phenylenediamine. Suzuki coupling reaction of 7 with monomers based on fluorene was employed to



Fig. 3. a) The relative fluorescence intensity of FAM-o at 677 nm and of AM-o at 668 nm in the presence of various anions (20 eq for FAM-o and 200 eq for AM-o). b) The fluorescence intensity ratio I_{452}/I_{640} for PFAM and PAM in the presence of various anions (10 eq). Anions arrangement: 1-none, 2-HSO_{4} , 3-Br^- , 4-Cl^- , 5-OAc^- , 6-I^- , $7\text{-H}_2\text{PO}_{4}^-$, 8-NO_{3}^- , 9-CN^- , 10-F^- .

produce P1, followed by an acetylization with trifluoroacetic anhydride or acetic anhydride to afford the desired polymer **PFAM** or **PAM**.

3.2. Physical properties

The photophysical properties of the compounds FAMs and AMs in dichloromethane solution are summarized in Table 1. The bisindolylmaleimide derivatives exhibit three major absorption bands in the range of 250-550 nm regions (Fig. 2a and Fig. S1 in Supplementary content). The high energy absorption band before 330 nm is known to come from a $\pi - \pi^*$ transition localized mainly on the indole group [44]. Two featureless broad bands after 330 nm are assigned to the transitions involving both indole and maleimide moieties, because neither of them alone displays an absorption at wavelengths longer than 325 nm. The lowest energy band at ca. 490 nm exhibits a charge-transfer (CT) character, which corresponds to an electron transition from the electron donor (indole) to electron acceptor (maleimide) [45]. Charge recombination from the excited state yields an intensive red fluorescence at 651-677 nm. The quantum efficiency of these CT emissions ranges in 7-33% (Table 1). An apparent solvent effect can be observed in the emission spectra of FAMs and AM-o in various solvents, accompanied with red shift and reduced intensity of emission bands when going to more polar solvents (Fig. S2). For FAM-o, the maximum emission wavelength is red-shifted from 653 nm in toluene to 686 nm in acetonitrile.

FAM-p and FAM-m behave uniform photophysical properties in absorption, emission and quantum yield. However, FAM-o shows a large red shift (ca. 20 nm) in the spectra of absorption and luminescence. The shift is caused by the formation of intramolecular hydrogen bond between NH and carbonyl group (Fig. S3), which results in the increase of electron-withdrawing ability of maleimide unit. As a result, FAM-o shows stronger CT property, lower band gap and quantum efficiency than **FAM-p** and **FAM-m**. Similar situation is found in the absorption and emission spectra of AM-o with a small red shift (ca. 10 nm) relative to AM-p. The smaller shift of AM-o than FAM-o is attributed to the higher electron density of NH group and the weaker intramolecular hydrogen bond in AM-o. The structure of FAM-o and AM-o was optimized by B3LYP with 6-31G (d) basis set. From the optimized structure in Fig. S4, the bond length of the hydrogen bond between NH and carbonyl group in FAM-o and AM-o is 1.92 and 1.93 Å, respectively.

There are two main absorption bands longer than 300 nm in the absorption spectra of PFAM and PAM in dichloromethane (Fig. S5). Thereinto the band at 330 nm is primarily contributed by a $\pi - \pi^*$ transition of fluorene segment, while the band at 425 nm is attributed to a $\pi - \pi^*$ transition of arylmaleimide segment. It is clear that arylmaleimide fluorophore was successfully introduced into the mainchain of polyfluorene, and that the energy transfer from fluorine to arylmaleimide segment would take place for the overlap of the absorption band at 425 nm with the emission band of fluorene. Upon photo-excitation, two broad featureless bands appeared



Fig. 4. a) Emission spectra of PFAM in dichloromethane under fluoride and cyanide. b) Emission spectra of PAM in dichloromethane under fluoride.



Fig. 5. Absorption (a) and emission (b) spectra of FAM-o (5 μ M) in dichloromethane upon the addition of fluoride (0–5.5 eq). Emission spectra were taken by irradiating at 504 nm.

at 452 nm and 640 nm in the luminescent spectrum of PFAM, which are corresponding to the emission bands of fluorene and arylmaleimide fluorophores, respectively (Fig. 2b). The existence of the emission band at 452 nm shows the energy transfer from fluorene to arylmaleimide segment is incomplete. Differently, there are three observable bands at 452, 532 and 635 nm in the fluorescent spectrum of PAM. Like PFAM, the emission band at 452 and 635 nm are originated from fluorene and arylmaleimide fluorophores. The band at 532 nm is caused by the strong interaction between intramolecular and intermolecular chains.

3.3. Selectivity and interference studies

The anion sensing capability of FAMs and AMs was examined in dichloromethane solution by the addition of the tetrabutylammonium salt of various anions such as F^- , Cl^- , Br^- , I^- , $H_2PO_4^-$, HSO $_4^-$, NO $_3^-$, OAc⁻, and CN⁻ (Fig. 3a and S6). FAMs were found to have highly selective sensory properties for F^- and CN⁻. Taking FAM-o as an example, the color of sensor solution (50 μ M) changes from red to yellow along with a more than 98% of fluorescence quenching in the presence of 20 equivalents of fluoride and cyanide (Fig. S7). Replacing trifluoroacetamide with acetamide as receptor unit, the recognition selectivity is narrowed down further. Only fluoride (200 equiv) quenches the fluorescence of AM-o solution (50 μ M) at 668 nm to a degree of 95% (Fig. S8).

Polymer PFAM and PAM were designed to improve the sensing sensitivity of FAMs and AMs for the "molecular wire" effects. Due to the identical recognition group, PFAM and PAM show the same sensing selectivity for anions as FAM and AM, respectively (Figs. 3b and 4). In the presence of fluoride or cyanide, the emission band of PFAM at 640 nm disappears, accompanied with an increase in the intensity of the emission band at 452 nm. Three bands in the fluorescence spectrum of PAM are replaced by a wide and high-

intensitive band with a peak at 453 nm when fluoride is added. The ratio fluorescence properties of PFAM and PAM make their recognition for anion easier, relative to FAMs and AMs (Fig. S9 and S10).

The selective sensing of FAM-p for F^- and CN^- in the presence of other anions was also examined by a series of competitive tests (Fig. S11). The results indicated that the recognition for F^- or CN^- was not disturbed by the presence of other anions except for HSO₄ and OAc⁻. In particular, the presence of HSO₄ greatly suppressed the quenching effect of F^- or CN^- on the fluorescence of FAM-p solution, which may be resulted from a competition for proton among all the anions.

3.4. The titration of anions

The typical titration curves of fluoride and cyanide by FAM-o are shown in Fig. 5 and S12, respectively. The absorption peaks at 504 and 392 nm decrease upon addition of fluoride or cyanide along with the increasing of 457 and 363 nm bands. Three distinct isosbestic points can be found at 480, 412 and 383 nm, indicating the formation of a stable new species. In the emission spectra of FAM-o, the intensity of 677 nm band decreases gradually along with the increase in the concentration of fluoride or cyanide ion. For example, in the presence of 2 molar equivalence of fluoride or 5.5 equiv of cyanide, the emission intensity of FAM-o reduces to less than 14% of the original level. Fluoride exhibits larger influence on sensor's fluorescence intensity than cyanide. It can be explained by their different interaction mechanism with recognition group trifluoroacetamide. It is inferred that trifluoroacetamide groups interact with fluoride through hydrogen bond or deprotonation of NH, and react with cyanide by a nucleophilic addition of C=0. Nucleophilic addition reaction is not so easy to proceed as hydrogen bond or deprotonation interaction, resulting in poorer sensitivity of



Scheme 3. The possible interaction mechanism of FAM-p with F⁻ and CN⁻.

FAM-o upon cyanide than fluoride. The interaction mechanism of the sensor with F^- and CN^- would be discussed below. FAM-m and FAM-p show similar titration curves as FAM-o for fluoride or cyanide (Fig. S13–S16). However, the blue shift of absorption spectra of FAM-m and FAM-p is smaller than that of FAM-o. The large blue shift in FAM-o's absorption spectra should be caused by the interruption of intramolecular hydrogen bond between C=O and NH groups, which made the electronic density of maleimide segment increase.

The titration curves of fluoride by AM-o and AM-p are shown in Fig. S17 and S18, respectively. Due to the weak acidity of acetamide and weak interaction with fluoride, the absorption spectra of AM-o and AM-p don't appear distinct blue shift as FAMs. The absorption band of AM-o at 493 and 388 nm decrease upon addition of F^- along with the enhancement of 315 nm band. Only one isosbestic point can be observed at 368 nm. For AM-o and AM-p, the fluorescent intensity decreases to 19% and 38% upon adding 40 equiv of F, respectively. Compared with AM-p, AM-o displays a larger blue shift in titration absorption spectra for the interruption of intramolecular hydrogen bond between C=O and NH groups, Like FAM-o.

Figs. 6, S19 and S20 give absorption and emission spectra changes of PFAM and PAM in the process of titrating anions. For instance, the absorption band of PFAM at 427 nm diminishes with concomitant growth of the band at 330 nm upon addition of 3.3 equiv of fluoride, which produces an isosbestic point at 385 nm. The prominent response is observed in its emission spectra when F^- was added. Fluorescence eventually appears at 452 nm, not at 640 nm. The intensive blue fluorescence can be easily observed by the naked eye (Fig. S9). The ratio of fluorescence intensity at 452 and 640 nm (I₄₅₂/I₆₄₀) exhibits a 17 fold enhancement from 2 without F^- to 34 with 3.3 equiv of fluoride. Cyanide induced similar spectral response as fluoride with an almost eight-fold improvement in the ratio of I₄₅₂/I₆₄₀.

3.5. Sensing mechanism of fluoride and cyanide

FAM-p was chosen as a model to analyze the sensing mechanism of F⁻ and CN⁻. The binding stoichiometry of FAM-p with both F⁻ and CN⁻ was determined by Job's plots and was found to be 1:1 (Fig. S21). Based on the result, we proposed the possible interaction mechanism of FAM-p with F⁻ and CN⁻ as illustrated in Scheme 3. It is possible for fluoride to react with NH group in FAM-p to form anion I by a deprotonation, or to form species II by a hydrogen bond interaction. Both of the two interactions would make electronic density of N atom increase, resulting in fluorescence quenching of FAM-p. There are also two sensing approaches of CN⁻ by FAM-p. The one is to yield anion I through an acid-base reaction for the strong basicity of cvanide. The other is to form the carbonyl addition species III stabilized by intramolecular H-bonding, because of the strong nucleophilicity of cyanide. In order to confirm the mechanism, FAM-p was treated with a strong base N(Et)₄OH in dichloromethane, and its emission spectra were recorded (Fig. S22). The fluorescence of FAM-p is not obviously quenched in the presence of excess amount of base (10 equiv). It is clear that fluoride and cyanide with weaker basicity than OH⁻ couldn't quench FAM-p's fluorescence by acid-base reaction.

We have investigated the molecular interactions between FAMp and anions by NMR spectroscopy. The solution of FAM-p in deuterated chloroform was monitored by gradual addition of F^- or CN^- (Fig. S23). The increase in fluoride concentration resulted in the amide proton signal (H_a) disappeared at 7.94 ppm. Two sets of aromatic protons in the benzene ring are shifted toward the opposite direction with H_b toward the lower field and H_c toward higher field. These observations are consistent with the proposed mechanism given product II. The bonding of FAM-p with fluoride makes the electronic density of the benzene ring increase, which results in the upfield shift of H_c. However, the hydrogen bond interaction between fluorine atom and H_b causes its downfield shift. Although addition of cyanide also caused the amide proton signal (H_a) disappeared, both of H_b and H_c exhibit a little upfield shift. The result can be explained by the nucleophilic addition mechanism to yield species III. The bonding of FAM-p with cyanide also increases the electronic density of benzene ring. However, the effect of cyanide on the HNMR of FAM-p is smaller than that of fluoride, because of the weaker H-bonding interaction of atomic oxygen anions than fluoride. This is the reason why the recognition sensitivity of CN⁻ by FAM-p is lower than that of F^- . The ¹³C NMR titration plots show that the carbonyl carbon signal at 153.90 and 153.53 ppm diminishes with the increase in cyanide concentration with a new saturated carbon signal appeared at 64.19 ppm, which verifies the exist of the nucleophilic addition mechanism (Fig. S24).

The increasing in fluorescent intensity of polymer sensors upon F^- or CN^- was originated from the interruption of photo-induced energy transfer process [46]. For instance, the blue emission of fluorene unit in PFAM was partly quenched by arylmaleimide segment for incomplete energy transfer process as mentioned before, resulting in two emissions at 452 nm and 640 nm (Fig. 2b). After bonding with F^- or CN^- , the absorption peak of arylmaleimide unit at 427 nm disappeared (Fig. 6a or S19a). It meant that arylmaleimide no longer absorbed the energy of fluorine unit and



Fig. 6. Absorption (a) and emission (b) spectra of PFAM (5 μ M) in dichloromethane upon the addition of fluoride (0–3.3 eq). Emission spectra were taken by irradiating at 395 nm.

Table 2

Calculated excitation energies (E) and oscillator strengths (f) for FAM-p and species II, III.

	Absorption		Emission	
	E (ev, nm)	f	E (ev, nm)	f
FAM-p Species II Species III	2.27 (547) 2.50 (496) 2.48 (500)	0.1914 0.1528 0.1623	1.71 (725) 0.86 (1442) 0.87 (1428)	0.2160 0.0020 0.0026

emitted a weak red light at 640 nm. As a result, PFAM only emitted an intense blue light at 452 nm.

3.6. Molecular orbital calculations

To gain further insight into the sensing mechanism of FAM-p for F^- and CN^- in solution, molecular modeling was performed by the density function theory (DFT) with B3LYP using 6-31G (d) basis sets implanted in a Gaussian 09 program [47]. The optimized geometries of FAM-p, species II and III are shown in Fig. S25. For FAM-p, the two indole rings oriented *anti* to each other are twisted clockwise to the central maleimide ring at about 34°, while the benzene ring is twisted clockwise at 43.96°. Both F^- and CN^- don't change much the geometries of indole rings, but make the twist angle of benzene ring larger by about 10°.

The spectra of FAM-p and species II, III were simulated by timedependent DFT (TDDFT) calculations in dichloromethane with the results summarized in Table 2 and S1–S3. The wavelengths correlated remarkably well with the experimental data (Fig. 2a, S15 and S16). For example, the calculated main absorption wavelengths of FAM-p and species II, III are 547 nm, 496 nm and 500 nm, respectively, with oscillator strengths of 0.19, 0.15 and 0.16. The calculated hypsochromic shift is in good agreement with experimental results (Fig. S26). The calculated emission spectra explain the fluorescent quenching of FAM-p for a very small value of oscillator strength of species II and III (0.0020 and 0.0026). Thus, the computational results provided strong support for bonding mechanism of FAM-p with both F[–] and CN[–].

The charge-transfer nature of the electronic transitions of FAMp and species II, III is better illustrated by the drawings of molecular orbital. The electron density distributions of HOMO and LUMO can be depicted in Fig. 7. As expected the electron density in the HOMO of FAM-p is mainly populated on the two symmetrically equivalent indolyl moieties, while the LUMO is localized on the central maleimide moiety. A charge separation is expected to be produced when an electron is promoted from the HOMO to the LUMO. However, the electron distribution in Species II and III, particularly in the HOMO, is different from that of FAM-p. The electron density of HOMO in Species II and III is transferred from indole segments to phenylacetamide moiety. As a result, the CT character corresponding to the electron transition from indole to maleimide is disrupted by the introduction of F⁻ or CN⁻, which results in the quenching of the CT emissions of FAM-p. The calculated electronic nature of FAM-p and species II, III complies with their experimental observations.

4. Conclusions

Diarylmaleimide derivatives (FAMs) with trifluoroacetamide were synthesized and found sensitive to fluoride and cvanide with significant fluorescence quenching. NMR titration combined with lob plots analysis and theory calculation demonstrated that the interaction mechanism of FAMs with F⁻ and CN⁻ was hydrogen bond interaction and nucleophilic addition reaction, respectively. The substituted location of trifloroacetamide at para-position (FAM-p), meta-position (FAM-m) and ortho-position (FAM-o) of phenyl group could not impose obvious an effect on the sensing selectivity for anions. Replacing trifluoroacetamide with acetamide as recognition group, AMs displayed a remarkable selectivity for fluoride with color fading and fluorescence quenching. Cyanide could not make AMs solution change in color and fluorescence, because of lower electropositivity of carbonyl carbon, which suppressed the addition reaction of carbonyl group. However, weak acidity of acetamide cause the lower sensitivity of AM-o with 95% of fluorescent decrease in the presence of 200 eq of F⁻, than that of FAM-o with 98% of fluorescent quenching by 20 eq of F⁻. To improve the sensitivity of sensors, the diarylmaleimide derivatives were introduced into the polyfluorene mainchain to form polymer PFAM and PAM. When F⁻ was added into the solution of PFAM or PAM, the polymer sensors show a ratiometric dual fluorescence at 420 and 630 nm. The intensity of I420/I630 increases with the increasing concentration of fluoride. The dual fluorescent nature was originated from an energy transfer from fluorine to maleimide segments.



Fig. 7. The HOMO and LUMO energy levels and the orbital of FAM-p, species II and III.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http:// dx.doi.org/10.1016/j.dyepig.2014.08.006.

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