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Colorimetric and fluorescent anion sensors: an overview of recent developments in the use of 1,8-naphthalimide-based chemosensors†

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This critical review focuses on the development of anion sensors, being either fluorescent and/or colorimetric, based on the use of the 1,8-naphthalimide structure; a highly versatile building unit that absorbs and emits at long wavelengths. The review commences with a short description of the most commonly used design principles employed in chemosensors, followed by a discussion on the photophysical properties of the 4-amino-1,8-naphthalimide structure which has been most commonly employed in both cation and anion sensing to date. This is followed by a review of the current state of the art in naphthalimide-based anion sensing, where systems using ureas, thioureas and amides as hydrogen-bonding receptors, as well as charged receptors have been used for anion sensing in both organic and aqueous solutions, or within various polymeric networks, such as hydrogels. The review concludes with some current and future perspectives including the use of the naphthalimides for sensing small biomolecules, such as amino acids, as well as probes for incorporation and binding to proteins; and for the recognition/sensing of polyanions such as DNA, and their potential use as novel therapeutic and diagnostic agents (95 references).

Introduction

Anions play a major role in our daily life; being crucial to physiological function as well as various industrial process. Consequently, in the environment, anionic species can be either essential to sustain growth or act as harmful pollutants. It is therefore not surprising that in the last decade the development of colorimetric and luminescent sensors for anions, where the function, concentration and location of the negatively charged species can be monitored, has become a very active area of research.1–4

While earlier examples of anion sensors focused on the proof of various principles, often using structurally simple hydrocarbon-based chromophores/fluorophores, furnished with one or more charged or charge-neutral recognition moieties, recent anion sensing research has become more focused.2,5 Such targeted investigations include the development of more specific sensors (e.g. for the recognition of a particular anion, or family of anions); more potent sensors (e.g. that can target a particular anion within a given concentration range); sensors that function in more competitive media (e.g. aqueous media, for use in...
environmental, biological or medical applications); and more economical sensors (e.g., that can be formed in few synthetic steps using inexpensive starting materials). 6–13

For practical applications, these sensors also need to satisfy strict photophysical criteria, such as being able to absorb and emit at long wavelengths to facilitate the use of naked eye detection or the use of inexpensive optics, posses relatively long lived excited states, 12 and have high quantum yields.

Due to its unique photophysical properties, the naphthalimide structure has found application in many areas of chemistry.14,15 Its absorption and fluorescence emission spectra lie within the UV and visible regions, and the various photophysical properties can be easily fine tuned through judicious structural design. Synthetic modifications are readily accommodated on either the aromatic ‘naphthalene’ moiety itself, or at the ‘N-imide site’, allowing for varieties of functional groups and structural motifs to be incorporated.

Consequently, the 1,8-naphthalimide structure has been extensively used within the dye industry, as strongly absorbing and colourful dyes, in the construction of novel therapeutics, 15 as well as in the formation of chemical probes, 17–20 particularly for the sensing of biologically relevant cations. 21–23 It is thus no surprise that this structure has found application in the field of anion recognition and sensing, and in the last seven years or so many excellent examples of naphthalimide-based anion sensors have been published, clearly demonstrating the versatility of this structure within this fast growing field of research.

This review will focus on this recent development; beginning with a short introduction on the design of colorimetric and fluorescence sensors, followed by a discussion on the photophysical properties of naphthalimides which can be easily tuned through synthetic design. This discussion is followed by a summary of the use of the naphthalimide structure for sensing of structurally simple anions and biologically relevant polyanions such as DNA. The examples presented herein are mainly grouped by the location of the anion receptor moiety on the naphthalimide fluorophore (commonly the 4-position or the 1,8-imide position) and by the structural similarities of the various anion receptor moieties employed. Finally, alternative anion sensing mechanisms and the potential use in other related areas of research involving the naphthalimide fluorophore will be discussed.

**General design principles employed in colorimetric and fluorescence sensing**

There are two main strategies used in the design of colorimetric and fluorescent sensors for the detection of analytes in...
solution. The binding site may be directly attached, or integrated, into the signalling moiety, such as shown in Fig. 1a. In such an instance the mechanism for signal transduction involves interaction of the analyte with a receptor that is part of the π-system of the signalling moiety. Chromophores which are directly attached to the receptor mainly consist of organic dyes such as azobenzene, nitrobenzene, indoline, anthraquinone, etc. Many chromophores can also be fluorescent and can therefore give rise to dual responses in both their absorption and fluorescence emission spectra upon analyte interaction.

Alternatively, the receptor moiety and signalling subunit may be covalently linked by a ‘spacer’ group, as demonstrated in Fig. 1b. When covalently linked, the binding event in colorimetric sensors is in many instances communicated from the receptor to the chromophore via conjugation with aromatic compounds such as quinoxaline, oxadiazole and porphyrin, to name just a few, and this design has been extensively employed in supramolecular analytical chemistry.

However, covalently linked fluorescent probes mainly consist of a receptor and fluorophore, which are electronically independent. The covalent linkage which separates the receptor and the fluorophore units is typically a short aliphatic spacer that minimises any ground-state interactions. Photo-induced electron transfer (PET) sensors are examples of such ‘fluorophore–spacer–receptor’ designs and ‘ideally’ only changes in their quantum yield or fluorescence intensity occur upon recognition of the analyte. This design principle, originally detailed by de Silva and co-workers, and Czarnik et al., has been widely used in fluorescent cation sensing.

An alternative is the use of indicator displacement assays (IDAs) in which a receptor–chromophore or receptor–fluorophore ensemble is selectively dissociated by the addition of an appropriate competitive analyte. The analyte interacts efficiently with the receptor resulting in a detectable response of the chromophore or fluorophore. Such systems have been, in particular, developed by Anslyn and co-workers as well as Fabbri and co-workers. Also, prevalent in the design of chemosensors (particularly for practical purposes), is the need for reversibility in order to provide continuous monitoring of the analyte. However, in the case of ‘once-off’ measurements, such reversibility is not necessary.

![Schematic representation of design concepts for the construction of colorimetric and fluorescent sensors](image)

**Fig. 1** Schematic representation of design concepts for the construction of colorimetric and fluorescent sensors: (A) The binding side and the signalling unit are covalently connected with π–π* bonds, and anion recognition should give rise to changes in the absorption spectra and possibly, depending on the nature of the chromophore employed also in the emission spectra. (B) The two parts are separated by a covalent spacer that does not allow for any ground-state π–π* or n–π* interactions to occur. Hence, such systems usually give rise to significant changes in the emission spectra upon anion recognition with only minor or no changes in the absorption spectrum.

**The basic photophysical properties of the 1,8-naphthalimide structure and their earlier use in fluorescent cation sensing**

The photophysical properties of the naphthalimide structure are governed by the nature of the substituent and the substitution pattern employed. For instance, 3- or 4-nitro-1,8-naphthalimidines such as 1a, shown in Fig. 2, possess high energy excited states, as the nitro functional group and the imide moiety are electron withdrawing. Hence, general structures such as 1a possess a broad absorption band, with λmax centred at about 360 nm, and emit at short wavelengths. In contrast, reduction of this moiety to give the 4-amino-1,8-naphthalimide derivative 1b, would give a ‘push–pull’ based, internal charge transfer (ICT) excited state, caused by the electron donating amine and the electron withdrawing imide. This ICT character, which gives rise to a large excited-state dipole and, in turn, broad absorption and emission bands centred at ca. 450 and 550 nm, respectively, when recorded in water (Fig. 1 for 1b). The ICT transition is highly solvent dependent, as demonstrated in Fig. 3, where both the λmax of the absorption and the emission are affected; where polar protic solvents stabilise the ICT character more than apolar solvents, e.g. Fig. 3a. This same effect can also be clearly seen in the fluorescence excitation spectra, Fig. 3b, which also demonstrates that the fluorescence quantum yield (ΦF) is also highly solvent dependent.

The 4-amino-1,8-naphthalimide derivatives are usually found to be highly emissive in organic solvents such as dichloromethane and chloroform, with ΦF often being.

![4-Nitro, 1a, and 4-amino-1,8-naphthalimide 1b, structures, and schematic representation of the ICT excited state within the 4-amino-1,8-naphthalimide fluorophore caused by a ‘push–pull’ action](image)
reported to be close to unity; while in water, significant quenching is observed, \textit{i.e.} Fig. 3b. Nevertheless, the use of 4-aminonaphthalimide for sensing in water is well established, particularly for the sensing of cations. Sensors 2–7 are just a few recent examples where the receptors are connected to the fluorophore via the amino functionality of the aryl ring. In these, depending on the design principles employed, the naphthalimide emission spectra alone (as is usually the case for PET-based sensors) or the absorption and the emission spectra were highly modulated upon binding of cations at the respective binding sites (receptors). Of these, 2 was developed for the sensing of H\(^+\), where through an electron-transfer mechanism, the excited state of the naphthalimide is quenched upon protonation of the tertiary aliphatic amine.\(^{40}\) Many other examples of such pH dependent PET naphthalimide sensors have been developed to date, for use in solution or within, or on, solid supports.\(^{18}\)

\[4^{42}\text{ and } 5^{43}\] have been developed for detecting Zn(\(\text{II}\)). Structure 4 has also recently been used for imaging of bone structures using epifluorescence microscopy.\(^{44}\) The Zn(\(\text{II}\)) sensors 4a and 4b, are based on the fluorophore-spacer–receptor principle, and were shown to bind the Zn(\(\text{II}\)) ion in a highly selective manner at the iminodiacetate moiety, in competitive media at pH 7.4; this increases the oxidation potential of the receptor, preventing photoinduced electron transfer quenching taking place from the receptor to the excited state of the fluorophore, and hence, this caused the naphthalimide fluorescence to be ‘switched on’. Whether the emission is switched ‘off’ or ‘on’ for such PET sensors depends on the changes that occur in the oxidation or the reduction potential of the receptor, in comparison to that of the fluorophore, upon analyte recognition. Here, the 4-amino moiety does not participate directly in ion binding and hence, the absorption spectrum was not significantly affected. Similarly, large enhancements were seen in the emission spectra of 5, developed by Watkinson \textit{et al.},\(^{43}\) upon binding to Zn(\(\text{II}\)). Recently, Watkinson \textit{et al.}, have extended their design to form naphthalimide dimers, using Cu(\(\text{II}\)) catalysed click chemistry.\(^{45}\)

In contrast, compound 6, developed as sensors for Cu(\(\text{II}\)), then upon binding of Cu(\(\text{II}\)) does engage the two aryl amines in the binding.\(^{46}\) Hence, the absorption spectrum is significantly affected, and indeed so much so that the binding is visible to the naked eye; hence 6 is a colorimetric as well as a fluorescent sensor for Cu(\(\text{II}\)). Similarly, 7 showed large changes in the absorption and the emission spectra of the naphthalimide moiety upon sensing of Cu(\(\text{II}\)).\(^{47}\) while the mononaphthalimide analogue has recently been shown to be a sensor for Zn(\(\text{II}\)).\(^{48}\)

These examples are all based on the use of 4-amino-1,8-naphthalimide structures, where the focus has been on the detection of cations, but the 3-amino-1,8-naphthalimide structures have also been employed in such sensing, as demonstrated elegantly by de Silva \textit{et al.}.\(^{49}\)

4-Amino-1,8-naphthalimide-based urea and thiourea sensors for anions

The selection of examples discussed above, clearly demonstrate the potential of such a structure in sensing technology. In a similar manner, anion interaction either with or in the vicinity of such amino-naphthalimide moieties, \textit{e.g.} at a receptor site, can also result in modulation of the ICT

![Fig. 3](image-url)
character of the naphthalimide structure. This, as the examples above demonstrated, can also lead to changes in either or both the absorption and fluorescence emission.

Modification of the 4-amino-1,8-naphthalimide moiety to include a thiourea anion receptor led to 8a. Sensor 8a was developed in our laboratories and is based on the fluorophore–spacer–receptor principle and was the first example of a naphthalimide-based anion sensor to include a hydrogen-bonding receptor, Fig. 4 and 5. The naphthalimide fluorophore was connected, through a short methylene spacer, to a charge neutral diarylthiourea anion receptor. The spacer was used to prevent any ground-state interactions between the receptor and the fluorophore and the absorption spectrum of 8a was not affected by the presence of anions, such as AcO− or H2PO4−, to any great extent. In contrast to this, the fluorescence emission of 8a was switched ‘off’ upon anion recognition due to enhanced PET from the electron-rich thiourea to the naphthalimide. Hence, the changes in the emission intensity resulted from excited-state reduction of the fluorophore by the electron-rich anion complexed receptor.

In order to understand more fully the sensing process, derivatives 8b, 8c, and 9 were synthesised and analysed in the presence of various anions. The absorption spectra of all the sensors in DMSO showed the presence of the naphthalimide ICT transition with \( \lambda_{\text{max}} \) at ca. 444 nm, which upon excitation gave rise to long wavelength emission at ca. 527 nm. As observed for 8a, only minor changes were seen in the absorption spectra of 8b and 8c upon titration with AcO− and H2PO4− (Fig. 4A). However, significant changes were seen in the fluorescence emission spectra upon titration with H3PO4−, (Fig. 4B for 8a). The degree of fluorescence quenching differed depending on the nature of the substituent on the aryl receptor. The quenching of 8a and 8c was to a lesser extent than for 8b due to the less acidic receptor (8a) and the longer spacer (8b). From the fluorescence titrations of 8a and 8b against H3PO4− binding constants of log \( K_1 = 2.9 \) (±0.1) and 3.7 (±0.1) were determined, respectively, using non-linear regression analysis. The higher binding constant of 8b reflects the increased acidity of the thiourea protons due to the electron withdrawing phenyl-CF3 group present on the receptor. In contrast to the minor changes noted upon addition of AcO−, H3PO4−, Cl− or Br−, sensors 8a–c showed a dramatic response in their absorption spectra (Fig. 5A) when excess F− was added (> 30 mM). New transitions developed at both short and long wavelengths when the ICT transition was
red shifted, which occurred with the formation of two clear isosbestic points. The changes were accompanied by a striking colour change from yellow–green to purple, which was reversed upon the addition of MeOH. These changes (Fig. 5) were assigned to the deprotonation of the 4-amino moiety of the naphthalimide, (verified by titration of the model compound 9 with F\(^{-}\)).\(^{51}\) Interestingly, addition of one equivalent of F\(^{-}\) to 9 resulted in the formation of a HCO\(_3\)\(^{-}\) complex of 9, which was characterised by X-ray crystallographic analysis. The anionic form of 9, resulting from deprotonation of the 4-amino moiety, fixed CO\(_2\) as bicarbonate in quantitative yield. Furthermore, the \(^1\)H NMR spectrum of 9 in DMSO-\(d_6\) after the addition of two equivalents of F\(^{-}\) showed the formation of a triplet at 16 ppm. This was assigned to the formation of bifluoride (HF\(_2\)\(^{-}\)), and confirmed that deprotonation had occurred. For sensors 8a–c, titrations with F\(^{-}\) were accompanied by almost complete quenching of the emission in DMSO. Excitation of the new long wavelength absorption at ca. 540 nm, which appeared at high concentrations of F\(^{-}\), as shown in Fig. 4, resulted in the formation of a new long wavelength emission band, which increased in intensity with the addition of further F\(^{-}\).

The dramatic colour change from yellow to purple observed upon deprotonation with F\(^{-}\) prompted the design of 10 by Pleffer \textit{et al.}.\(^{52}\) It was anticipated that the acidic 4-amino moiety would cooperatively take part in hydrogen bonding with the N–H protons of the thiourea receptor resulting in stronger anion–receptor interactions, particularly for tetrahedral oxanions such as H\(_3\)PO\(_4\). Therefore, in order to introduce flexibility into the receptor, the design of receptors 8a–c was modified by the introduction of an ethylamine spacer and also a benzylthiourea receptor. Indeed, \(^1\)H NMR titrations of 10 in DMSO-\(d_6\) clearly demonstrated that the binding of H\(_3\)PO\(_4\) was significantly enhanced due to cooperation between all three of the N–H protons. The 4-amino proton was shifted by ca. 1.4 ppm in the presence of H\(_3\)PO\(_4\) where little or no shift was seen upon titration with AcO\(^{-}\). In the case of F\(^{-}\), significant broadening of all resonances was observed and, as seen for 9, the addition of 2.5 equivalents showed the formation of HF\(_2\)\(^{-}\), which indicated that deprotonation had occurred. The \(^1\)H NMR titration data for 10 was fitted for the formation of 1:1 complexes, using non-linear regression analysis, for which binding constants of \(\log K_{1:1} = 3.4 (\pm 0.1)\) and \(\log K_{1:1} = 3.6 (\pm 0.1)\) were determined for H\(_3\)PO\(_4\) and AcO\(^{-}\), respectively. A lower binding constant was reported for the quenching of the emission of 8a with H\(_3\)PO\(_4\) (\(\log K_{1:1} = 2.9 (\pm 0.1)\)), therefore, supporting the formation of a complex in which all three N–H’s cooperatively bind. In contrast, the changes in the fluorescence emission spectra of 10 upon addition of AcO\(^{-}\) and H\(_3\)PO\(_4\) in DMSO were only minor when compared to those observed for 8a, showing that the flexible ethyl chain resulted in a reduced rate of PET, caused by the greater distance between the components of the sensor.

In an attempt to achieve more significant changes in the fluorescence emission spectra of 10 upon addition of anions, sensors 11a–c were also designed by Gunnlaugsson \textit{et al.}\(^{53}\) These sensors possess the same spacer between the thiourea receptor and the naphthalimide moiety as 8. Hence, it could be expected that any PET from the receptor to the naphthalimide excited state might be affected as the rate of PET is distance dependent. In comparison to 8, compounds 11a–c only differ in the nature of the receptor moiety, e.g. urea \textit{vs.} thiourea, and the nature of the substituent. These sensors were titrated with the same anions as discussed for 8–10, and were shown to be highly selective for the detection of F\(^{-}\), where the naphthalimide emission was fully quenched in the case of 11b. Moreover, the long wavelength shift of the ICT band was also observed at higher concentrations of F\(^{-}\). Of these systems, the urea-based sensors 11c gave rise to the least changes in the emission spectra.

Subsequent to the reports of sensors 11a–c, Ying-Li \textit{et al.}\(^{54}\) reported the anion binding capabilities of the nitro derivative, 11d, in DMSO. Its recognition for various anions was investigated by UV-Vis absorption, fluorescence and \(^1\)H NMR (DMSO-\(d_6\)) spectroscopy. These studies showed that receptor 11d could selectively recognise F\(^{-}\) and AcO\(^{-}\) over Cl\(^-\), Br\(^-\), I\(^-\), HSO\(_4\)\(^-\) and ClO\(_4\)\(^-\). In contrast to that seen for sensor 10, larger changes were observed in the absorption spectra of 11d upon addition of only eight equivalents of F\(^{-}\) and AcO\(^{-}\). This was most likely due to the presence of the electron withdrawing chromogenic p-nitrophenyl group. These changes occurred with a colour change from greenish yellow to bright yellow. These were also followed by a concomitant change in the fluorescence emission, which was quenched by 81% and 55% upon the addition of 0.17 mM F\(^{-}\) and AcO\(^{-}\), respectively. The changes in the absorption spectra were fitted as above and resulted in high binding constants of \(\log K_{1:1} = 4.48 (\pm 0.1)\) and \(\log K_{1:1} = 4.16 (\pm 0.1)\) for F\(^{-}\) and AcO\(^{-}\), respectively. As for 8a–c, large changes in the absorption spectra were also observed upon the addition of large concentrations of F\(^{-}\) (ca. 70 equivalents) and were assigned to deprotonation of the 4-amino moiety. \(^1\)H NMR titrations of 11d with F\(^{-}\) were also carried out in DMSO-\(d_6\) and, interestingly, it was reported that the formation of the HF\(_2\)\(^{-}\) triplet at 16 ppm was not observed, even after the addition of three equivalents of F\(^{-}\). Therefore, it was concluded that deprotonation...
did not occur at low concentrations of F\(^-\) and that cooperative binding between F\(^-\) and 11d occurred in DMSO-d\(_6\).

In an attempt to further improve on the design of 10, Pfeiffer et al.,\(^{55}\) evaluated the anion binding capabilities of 12a and 12b, which were structurally modified to preserve cooperative binding of H\(_2\)PO\(_4\)^- whilst restoring the fluorescent properties to that seen for 8a-c. The electron-rich \(\sigma\)-substituted amino benzyl amine spacer was introduced to facilitate pre-organisation and as for sensors 11a-d, a phenyl receptor was also employed to increase the acidity of the thiourea N-H protons. Indeed, this design proved somewhat successful with cooperative binding of H\(_2\)PO\(_4\)^- being observed for both 12a and 12b, as determined by \(^1\)H NMR titrations in DMSO-d\(_6\). Binding constants for H\(_2\)PO\(_4\)^- of log \(K_{1:1}\) = 3.7 (12a) and 4.1, (12b) respectively, were estimated from these titrations, as after the addition of three equivalents of H\(_2\)PO\(_4\)^- the N-H resonances disappeared. As anticipated, the binding constant for 12a was larger than that reported for the precursor 10. However, only minor quenching of the fluorescence of 12a was observed (6.5\% and 31\% with H\(_2\)PO\(_4\)^- and AcO\(^-\), respectively). In contrast, the fluorescence emission of the fluoro-based receptor, 12b, was quenched by 36\% and 59\% after the addition of only five equivalents of H\(_2\)PO\(_4\)^- and AcO\(^-\), respectively.

Fabbrizzi et al.\(^{56}\) have reported the symmetrical bis-naphthalimide-based sensor, 13, which does not contain a spacer, but consists of two naphthalimide fluorophores connected at the 4-position via a urea moiety, Fig. 6. In the presence of excess F\(^-\), 13 underwent stepwise deprotonation in DMSO of both urea N-H protons, which gave rise to two distinct sets of changes in the visible region of the absorption spectra and therefore, large colour changes, which were clearly visible to the naked eye, as shown in Fig. 6. These results are not surprising, as the urea protons are located directly at the aryl moiety. The changes in the absorption spectra at the different concentrations ranges of F\(^-\) were reversed upon addition of H\(_2\)O. Additionally, the formation of [L]^2^- was supported by titration of sensor 13 with TBAOH and also using \(^1\)H NMR spectroscopy in DMSO-d\(_6\).

The first naphthalimide-based calixarene for anion sensing, 14, was reported by Qian and Yoon et al.\(^{57}\) The naphthalimide fluorophore was connected to the calixarene scaffold via the 4-amino and the 5-amino ethyl spacers and therefore, provided a hydrogen-bonding pocket, in which four hydrogen atoms were available to interact with anions. Sensor 14 exhibited a typical naphthalimide absorption spectrum in MeCN, which upon excitation at 435 nm, gave rise to a broad fluorescence emission spectrum centred at ca. 490 nm. Upon titration with anions such as H\(_2\)PO\(_4\)^-, HSO\(_4\)^-, AcO\(^-\), I\(^-\), Br\(^-\) and Cl\(^-\), only quenching of the emission was observed for F\(^-\). These changes were attributed to hydrogen bonding and consequent deprotonation, in which the latter was supported by the appearance of the HF\(_2\)^- triplet at 16 ppm in the \(^1\)H NMR spectrum of 14 in DMSO-d\(_6\) upon titration with F\(^-\).

The sensors discussed in this section contain an anion receptor moiety at the 4-position of the naphthalimide fluorophore. However, as previously mentioned, sensors have been designed which contain an anion receptor moiety connected to the naphthalimide via the imide moiety. These will be discussed in the following section.

**N-Imide functionalised naphthalimide sensors**

Structurally similar to sensors 8a-c, compounds 15a and 15b were designed and investigated by Veale et al.\(^{58,59}\) and PET...
quenching was noted upon the addition of anions. As for sensors 8a–c, no significant changes were observed in the absorption spectra upon titration with the various anions. The reference compound, 16, which lacks an anion receptor, showed no changes in its absorption or emission spectra upon titration with anions. As in the case of 8a–c, strong colorimetric detection of F− by 15a was also achieved, but at lower concentrations (ca. 0.2 mM). However, no strong colour changes were observed for either 15b or 16 upon titration with F−, both of which lack the 4-amino proton, indicating that the colour changes were indeed due to deprotonation of this moiety. The fluorescence emission of 15a was quenched upon titration with AcO− and a second binding interaction with the 4-amino moiety was observed at high concentrations of anion. Both H2PO4− and F− also gave rise to quenching of the fluorescence of 15a and 15b, where for 15a the emission was quenched by ca. 60% and 95% upon addition of 44 mM H2PO4− and 32 mM F−, respectively. The changes in the emission of 15a and 15b upon titration with anions were analysed using non-linear regression analysis. Comparison of the binding constants determined with those previously obtained for 8a–c showed that the location of the anion receptor did not affect the sensitivity of the anion sensing to any great extent. For example, the quenching of the fluorescence of 15a to give log $K_{1:1} = 3.5$ (±0.03) by H2PO4−, was of the same magnitude as that observed for 8b, log $K_{1:1} = 3.7$ (±0.1), under identical conditions.

It has been previously shown that the fluorescence emission of such naphthalimide H+ sensors were only switched on when the receptor moiety was located at the 4-amino moiety. Therefore, these compounds are the first examples of naphthalimide-based sensors that enable bi-directional PET quenching of their emission by anions.

Pischel et al. described the synthesis and photophysical characterisation of the fluorophore-spacer–receptor1–spacer–receptor2 system, 17, in which PET also occurs through the imide of the naphthalimide moiety in MeCN, upon interaction with cations and anions. The absorption spectrum of sensor 17 in MeCN exhibited transitions at 332 and 345 nm, which upon excitation at 325 nm, gave rise to a fine-structured blue emission bands at 364 nm and 380 nm, and a shoulder at 400 nm. Protonation of the amino group of 17, with one equivalent of trifluoroacetic acid (TFA), lead to a 20 times enhancement in the fluorescence of 17, due to blocking of the PET pathway. In contrast to this, anion binding to the urea lead to quenching of the fluorescence emission by PET. Additionally, titration of mono protonated 17 with F− lead to quenching of the emission and the simultaneous binding of cations and anions also resulted in quenching of the emission, most likely through electrostatic interactions between the protonated ammonium amine and the anions. Due to the observed fluorescence quenching of 17 by the amino function, binding constants were obtained by titrating 18 with various anions. Significant fluorescent quenching of 18 was observed and, hence, values for the 1:1 complexation of 18 with F−, AcO− and H2PO4− were determined, which gave binding constants that were considerably lower than those reported for 8a and 8b.

The 1H NMR titrations of 17 in DMSO-d6 were also carried out and the binding constants determined were slightly lower than those obtained from the fluorescence titrations with 18. The authors speculated that the lower affinity was possibly due to self-aggregation phenomena and competition of the binding site with DMSO. The above changes in the luminescent properties were described by the authors as an example of functionality integrated two-input inhibit (INH) logic at the molecular level, where the proton and anions function as chemical inputs and the fluorescence of 1,8-naphthalimide fluorophore as the output.

Pischel et al. extended their design of such logic gates with the bis-naphthalimide sensor 19, in which different fluorescent responses were triggered by protonation and deprotonation of the sensor with H+ and F− ions in MeCN. In contrast to 17, which has a urea as the anion binding receptor, 19 was designed to function solely via protonation or deprotonation of the acidic N–H moieties. The naphthalimide fluorophores were linked by an aliphatic spacer chain, which consisted of a central tertiary amine, the site of protonation and subsequent deprotonation. Upon protonation, PET quenching of the 1,8-naphthalimide fluorophore was blocked in 19. This resulted in concomitant electronic energy transfer (EET) between both fluorophores. The protonated tertiary amine could then be deprotonated by F− (<20 μM), which reactivated PET and resulted in fluorescence emission quenching of ca. 41%. Higher concentrations of F− (>1 mM) also effected deprotonation of the 4-amino moiety, which lead to further quenching (ca. 93%). Therefore, the interplay of PET, EET and ICT gave rise to a one-channel fluorescence output with three signal levels (low, medium and high), which the authors classified as a ternary NOR logic gate mimic.

Urea and thiourea-based 4-amido-1,8-naphthalimide-based anions sensors

The 4-amido-based naphthalimide sensor, 20, was designed and investigated by Tian et al. The absorption spectrum of 20 in MeCN exhibited a λmax at 360 nm, which upon excitation gave rise to a broad emission spectrum. It was found that upon
titration of 20 with the TBA salts of F\(^-\), Cl\(^-\), Br\(^-\) and I\(^-\), large changes were observed in both the absorption and the emission spectra upon titration with F\(^-\), with concomitant colour changes from colourless to yellow, while the fluorescence emission was changed from blue to orange.

As an extension of this work, Tian et al.\(^{63}\) have recently reported the benzoimidazole functionalised naphthalimide, 21, for the sensing of F\(^-\), a design that also functioned via deprotonation. The absorption spectrum of 21 showed a \(\lambda_{\text{max}}\) at 378 nm for the ICT transition, which was shifted by ca. 100 nm and also decreased in absorbance with the formation of a new band at ca. 476 nm, upon the addition of F\(^-\) in THF. A concomitant colour change from colourless to magenta was also observed. Upon excitation at 378 nm, 21 exhibited a broad fluorescence emission band centred at 478 nm. Upon titration with F\(^-\), quenching of the emission and a red shift in the spectrum to 603 nm, was observed. The deprotonation was confirmed using \(^1\)H NMR titration in DMSO-\(d_6\), for which the resonance pertaining to the N–H proton of compound 21 disappeared as the concentration of F\(^-\) was increased and the resonances for adjacent protons on the benzoimidazole moiety were also shifted upfield.

Further utilising this deprotonation mechanism, Tian et al.\(^{64}\) have also covalently attached two 4-amido-1,8-naphthalimide fluorophores to a zinc porphyrin core resulting in a novel F\(^-\) ion triggered dual fluorescent molecular switch, 22. The absorption spectrum of 22 consisted of a naphthalimide absorption at ca. 360 nm and the typical Soret and Q bands of the porphyrin at 417, 550 and 590 nm, respectively.\(^{64}\) However, the addition of F\(^-\) resulted in the formation of a new absorption band at 500 nm, significant of deprotonation of the 4-amino moieties and a colour change from pale to dark orange occurred. The deprotonation also resulted in enhanced PET to the porphyrin, which quenched its fluorescence emission. Furthermore, in the presence of F\(^-\), the fluorescence emission of the porphyrin could be regulated ‘on-off’ upon excitation at 365 nm or ‘off-on’ upon excitation at 504 nm, due to changes in the energy pathways present.

The examples outlined in this section, as well as in the previous sections, detailed the sensing of anions in organic solution such as DMSO, MeCN and THF. However, the following section describes two naphthalimide-based anion sensors, which are capable of sensing in more competitive aqueous-based solutions.

**4-Hydrazine-based urea and thiourea and related naphthalimide sensors for anions**

Gunnlaugsson et al. have reported the novel colorimetric hydrazine-based naphthalimide anion sensors, 23a–b\(^{65}\) for use in aqueous solution. In order to gain an understanding of the anion binding ability of the thiourea sensors, 23a and 23b, titrations were initially carried out in DMSO solution. Sensor 23b showed a broad absorption band centred at 441 nm and a smaller shoulder at 560 nm, which upon addition of anions, such as of AcO\(^-\), H\(_2\)PO\(_4\)\(^-\) and F\(^-\), increased in intensity at the expense of the 441 nm absorption. Furthermore, a new band at 350 nm and two clear isosbestic points at 465 and 380 nm were also observed. These changes occurred with a yellow to purple colour change, which was clearly visible to the naked eye. By plotting the changes at 560 nm, as a function of \(-\log[\text{AcO}^-]\), a sigmoidal curve was observed that changed over two log units, which is characteristic of 1:1 binding.

It was postulated that the 4-amino moiety may aid in the binding, hence, giving rise to such colour changes, in a similar manner to that seen for 13. The addition of a large excess of AcO\(^-\) or H\(_2\)PO\(_4\)\(^-\) to 23a and 23b did not give rise to any additional changes in absorption spectra. However, when excess F\(^-\) was used, additional colour changes were observed that were attributed to deprotonation of the
4-aminonaphthalimide moiety. The deprotonation was also verified using $^1$H NMR titration in DMSO-$d_6$. The most positive result from the studies of 23a and 23b was that upon addition of competitive hydrogen-bonding solvents, such as MeOH, the colour changes were not reversed. Therefore, titrations were carried out in 1:1 (v/v) EtOH–H$_2$O buffered solutions at pH 7.1 and pH 7.3 for 23a and 23b respectively. On both occasions, titrations with anions gave rise to the same general spectral and colour changes as described in DMSO above. These changes were fitted and it was shown that 23a had a higher affinity for AcO$^-$ (log $\beta_1 = 3.4 \pm 0.1$), over H$_2$PO$_4^-$ (log $\beta_1 = 2.2 \pm 0.1$) and F$^-$ (log $\beta_1 = 1 \pm 0.2$). These results clearly demonstrated the feasibility of the use of charge neutral anion receptors in mixed aqueous media, which was a significant stepping stone towards real application of such sensors in competitive media.

In contrast to these results, the urea-based sensors 24a–c were only able to bind anions in aprotic solvents, such as DMSO.$^{66}$ These three sensors showed similar changes in their absorption spectra to those above upon binding to anions, such as AcO$^-$, H$_2$PO$_4^-$ and F$^-$ in DMSO, which was clearly visible to the naked eye (Fig. 7). Also, upon excitation of the ICT transition, the addition of anions resulted in quenching of the fluorescence emission or a shift to longer wavelengths. Additionally, $^1$H NMR titrations in DMSO-$d_6$ demonstrated that AcO$^-$ was recognised through ‘pure’ hydrogen bonding while the interactions for H$_2$PO$_4^-$ and F$^-$ were due to initial hydrogen bonding followed by full or partial deprotonation.

Fu et al.$^{67}$ have also utilised the colorimetric and fluorescent properties of 4-hydrazine-based naphthalimides in the design and synthesis of sensor, 25, which showed highly selective fluorescent sensing of pyrophosphate (PPI) in aqueous solution, as determined from fluorescence titrations with anions. The guanidino-carbonyl pyrrole receptor, which was designed specifically to satisfy the geometrical requirements of PPI, was attached to the 4-amino position via a hydrazine spacer. Molecular modelling studies showed the appropriate size and geometry of the PPI anion with the receptor of 25. The titrations were carried out in the competitive media of 90% H$_2$O–DMSO (v/v, 10 mM HEPES, pH = 7.4) with the Na$^+$ salts of F$^-$, Cl$^-$, AcO$^-$, NO$_3^-$, SO$_4^{2-}$ and HCO$_3^-$, as well as the biologically important anions, ATP, AMP, ADP and PPI. Excitation of the naphthalimide ICT absorption band of 25 (460 nm) resulted in fluorescence emission at 510 nm, which was enhanced only upon addition of PPI, with a concomitant bathochromic shift from 510 to 514 nm. Job’s plot analysis indicated the formation of a 1:1 complex with PPI and a binding constant of log $K_{1:1}$ = 1.9 (±0.2) was obtained by a nonlinear least-squares fitting method.

Recently, Mashraqui et al.$^{68}$ have developed highly selective sensors for fluoride base on the use of an imidazolium ring directly connected to the 4-position of the naphthalimide ring, 26a and 26b. This design was shown to give rise to changes in both the absorption as well as in the emission spectra of the sensor, where significant changes were observed at long wavelengths in DMSO solution. The binding interaction of F$^-$ was also investigated by using $^1$H NMR in DMSO-$d_6$ where a significant broadening was observed for the imidazolium C(2)–H proton, which was also shifted down-field, whereas the N–Me group was shifted upfield.

![Fig. 7](image)

**Fig. 7** The chromogenic response of DMSO solutions of sensor 24c $[1 \times 10^{-7} \text{M}]$ upon interaction with various anions: (a) sensor 24c; (b) 24c + 1 eq. AcO$^-$; (c) 24c + 1 eq. H$_2$PO$_4^-$; (d) 24c + 1 eq. F$^-$; (e) 24c + F$^-$ (excess). Ref. 66 – Reproduced by permission of the CNRS and the Royal Society of Chemistry.

Alternative sensing mechanisms involving the naphthalimide fluorophore: assemblies, displacement assays and inclusion complexes

The imidazolium moiety has also been used in displacement based anion sensing involving the naphthalimide fluorophore. Compounds 27a–c were utilised by Pischel et al.$^{69}$ in the assembling of a novel citrate sensing ternary complex. The components of the ternary complex were the water-soluble imidazolium-substituted calix[4]arenes, 27a, the fluorescent aminodiacetate derivative of 1,8-naphthalimide, 27b, and the trianion, citrate, 27c. The addition of citrate and tartrate to the host–guest complex 27a:27b resulted in the formation of ternary complexes with values of log $K_{1:1} = 4.79$ (±0.01) and log $K_{1:1} = 3.99$ (±0.05), respectively, which gave rise to variation in the fluorescence of the 27b, while AcO$^-$, SO$_4^{2-}$, NO$_3^-$ and Cl$^-$ resulted in only minor changes. The mechanism of the intricate sensor was elucidated by fluorescence titrations, pH titrations, establishment of binding constants and structural information as obtained by $^1$H NMR spectroscopy. Overall, the authors described the photophysical observations at pH 7 as follows: complexation of the ammonium form of 27b by 27a gave rise to significant amounts of the
changes in the absorption spectra for F
visible region of the absorption spectra of the SubPc. The
turn resulted in the quenching of the fluorescence emission of
naphthalimide moiety, which restored its emission, which in
addition of F
consequence of spectral overlap. It was reported that the
addition of F
resulted in a weak emission at 495 nm, while excitation at
500 nm gave rise to a characteristic SubPc fluorescence at
578 nm. The weak naphthalimide fluorescence was postu-
lated as being due to excitation energy transfer (EET) from the
naphthalimide chromophore to the SubPc chromophore, as a
consequence of spectral overlap. It was reported that the
addition of F
resulted in the displacement of the
naphthalimide moiety, which restored its emission, which in
turn resulted in the quenching of the fluorescence emission of
the SubPc. These events also gave rise to large changes in the
visible region of the absorption spectra of the SubPc. The
changes in the absorption spectra for F
were fitted using the
Benesi–Hildebrand equation, which gave a high binding
constant of log
= 5.11. As the intensity of the fluores-
cence at 495 nm increased with a concomitant decrease at
578 nm, sensor 28 can be referred to as a ratiometric anion
sensor.

In an alternative approach to the assembly of the ternary
complex above, Tian et al. have reported the subphthalocyranine (SubPc) based F
sensor, 28, in which F
resulted in
dissociation of the axial coordinated 1,8-naphthalimide-
based ligand. This sensing mechanism is reminiscent of
displacement assays. Excitation of the ICT absorption maxima of the naphthalimide (390 nm) in THF
resulted in a weak emission at 495 nm, while excitation at
500 nm gave rise to a characteristic SubPc fluorescence at
578 nm. The weak naphthalimide fluorescence was postu-
lated as being due to excitation energy transfer (EET) from the
naphthalimide chromophore to the SubPc chromophore, as a
consequence of spectral overlap. It was reported that the
addition of F
resulted in the displacement of the
naphthalimide moiety, which restored its emission, which in
turn resulted in the quenching of the fluorescence emission of
the SubPc. These events also gave rise to large changes in the
visible region of the absorption spectra of the SubPc. The
changes in the absorption spectra for F
were fitted using the
Benesi–Hildebrand equation, which gave a high binding
constant of log
 = 5.11. As the intensity of the fluores-
cence at 495 nm increased with a concomitant decrease at
578 nm, sensor 28 can be referred to as a ratiometric anion
sensor.

As for the calixarene cavity of 27a, cyclodextrins (CD),
which are a group of naturally occurring cyclic oligomers of β-glucopyranose, have been shown to form inclusion
complexes with a variety of organic compounds in aqueous
solution. Fu et al. have recently described the fluorescent
behaviour of a 4-amino-1,8-naphthalimide derived β-cyclodextrins, 29a and 29b, in which the latter displayed
large enhancements in its fluorescence upon complexation
with various cyclic and acyclic aliphatic acids, such as
adamantane-1-carboxylic acid. In contrast, the guest-induced
fluorescent change of 29b was minor. The conformations of the two systems were also investigated using induced circular
dichroism (ICD), 2D NOESY NMR and molecular modelling
experiments. From these studies, it was concluded that 29b
adopted the self-inclusion conformation while 29a did not,
which is most likely due to the hydrophobic butyl group of
29b. Upon anion binding, exclusion of the fluorophore of 29b
from the CD cavity gave rise to an enhancement in its
fluorescence emission spectra.

Bis-naphthalimide sensors for anions

The naphthalimide-based systems 13 and 19 discussed
above are examples of bis-naphthalimide sensors for anions,
where the two fluorophores are connected via a covalent
spacer, possessing the anion recognition moiety. Compound
30 has recently been described within the Gunnlaugsson
group, possessing a xylene spacer, and was shown to bind
anions in a similar manner to that seen for 23 in organic
solution, with significant changes being observed in the
absorption spectra. Pfeffer et al. have also developed
such bis-naphthalimide systems. An example of their design
is 31, a 4-amino-1,8-naphthalimide dimer formed by
connecting two equivalents of 11 by a propyl spacer. The
sensing ability of 31 was investigated in DMSO solution by
monitoring the changes in both the absorption and the
emission spectra of the naphthalimide moieties, which in the
case of pyrophosphate gave rise to ca. 40% quenching,
while ions such as acetate, gave rise to the expected 2:1
(anion:sensor) binding. For pyrophosphate, the binding was
however, found to bind in a 1:1 stoichiometry with a binding
constant of log
 = 3.4 (±0.6). Pfeffer et al., has also
developed other analogues of this design by connecting the
two naphthalimide fluorophores via the thiourea moieties
derived from the 4-amino moieties of the naphthalimide ring
as shown for 32a and 32b, using a 1,4-phenyl or 1,3-phenyl
spacers, respectively. As in the case of 31, significant changes
were also seen in both the absorption and the emission
spectra of these sensors upon interactions with several
divalent-anions.
The Gunnlaugsson group has also explored the use of this design, and recently synthesised 33, possessing a phenylene-dimethanamine spacer, and studied the anion sensing of this structure in DMSO solution, in a similar manner to that described above, which demonstrated that 33 showed good selectivity for $\text{H}_2\text{PO}_4^-/\text{CO}_3^-$, which, using $^1\text{H}$ NMR titrations, was recognised by 33 in 1:1 stoichiometry. 76

The Gunnlaugsson group have recently synthesised 34, which is structurally related to 13, and is, to the best our knowledge, the first example of a 3-urea-based 1,8-naphthalimide systems to be evaluated for their anion binding, and investigated its anion sensing properties using absorption, fluorescence and $^1\text{H}$ NMR titrations in DMSO solutions. 77

As in the case of 13, the urea sensor 34 both the absorption and the emission spectra were effected upon interacting with anions. In the free form, the sensor exhibited three main absorption bands at ca. 285, 340 and 394 nm, for which the latter transition at longer wavelengths was assigned to the ICT transition of the fluorophore; being clearly blue shifted in comparison to the 4-urea analogue 13, or other 4-amino derivatives discussed earlier, such as 8a–c. Excitation of 34 at both 394 nm and 340 nm gave rise to a broad emission centred at 453 nm, which was similar in structure to that observed for the 4-amino-functionalised systems (such as 8a) but in contrast to that seen for 8a, was almost 100 nm blue shifted ($\lambda_{\text{em}}$ 530 nm for 8a), clearly demonstrating the weakening ICT character for this structure.

In a similar manner to the behaviour of sensor 8a, preliminary titrations on 34 showed little or no changes in absorption and fluorescence emission spectra with $\text{AcO}^-$ and $\text{H}_2\text{PO}_4^-$. However, upon titration of 34 with $\text{F}^-$ (TBAF:3H$_2$O) a minor increase was observed in its absorption spectrum at short wavelengths indicating the interaction of $\text{F}^-$ at the receptor site. These changes also occurred with a bathochromic shift in the ICT transition, which after the addition of ca. 25 equivalents of $\text{F}^-$, was significantly shifted. These were similar in magnitude to those changes observed for many of the systems discussed above upon deprotonation of the 4-amino moiety (e.g. 8a) indicating that a deprotonation of the urea proton adjacent to the naphthalimide occurred at high concentrations of $\text{F}^-$ for 34. Nevertheless, 34 can be described as being a highly selective sensor for $\text{F}^-$, and clearly demonstrating the potential of using the 3-amino position as a potential primary, or secondary binding side for anions.

This was indeed the motivation behind the design of 35 within the Gunnlaugsson group, a naphthalimide sensor for anions where both the third and the fourth positions of the ring were functionalised. The basic structure was that of 11b, which had been shown to be an excellent fluorescent sensor for $\text{F}^-$ in organic solvents, with an acetamide group in the third position, but Gunnlaugsson et al. 78 have recently shown that placing a hydrogen-bonding donor ‘adjacent’ to the main anion binding site can give rise to significant enhancement in binding affinity. The synthesis of 35 was achieved in six steps from 4-bromo-1,8-naphthalic anhydride and the anion recognition was investigated in DMSO and the anion recognition ability of 35 was found to be not in anyway superior to that observed for 11b, which could be due to the nature of the ethyl spacer which
is possibly too long, and hence not allowing the 3-acetamido to participate in strong cooperative binding of the anions.

![Image](image_url)

**Polymeric-based naphthalimide sensors for anions**

The above examples have all centred on the use of anion sensors in solutions; where changes in colour or emission properties can monitor the presence or the concentration of certain anions. The incorporation of such sensors into polymeric matrices to facilitate sensing of environmental, biological and industrial samples containing anions in situ has also been recently explored. Examples of such systems includes those of Gunnlaugsson et al., who have incorporated sensors such as 23 and 24, and several other related derivatives, non-covalently into hydrophilic polymers, or hydrogels, made from mixing such structures at the polymerisation stage with monomers of methylmethacrylate (MMA) and 2-(hydroxyethylmethacrylate) (HEMA) in different ratios. Depending on the composition of the resulting hydrogels, both the mechanical and the sensing properties of the resulting ‘sensogels’ could be tuned. These sensors were shown to be quite sensitive to several anions, where a colorimetric change occurred that were visible to the naked eye in similar manner to that observed for 23 and 24. The Gunnlaugsson group has developed many other analogues of such sensing systems, where various naphthalimide-based anion sensors possessing polymerisable group, have also been covalently incorporated at the diimide side, similar to that seen in 36, into a poly(mercaptopropylmethyl)siloxane (PMPMS) based polymer. This sensor is related to 11a–11d, where the polymerisable group was placed at the diimide site, a distance away from the phenyl-thiourea anion receptor, at the 4-amino moiety. The polymer-bound sensor showed substantial changes in its absorption and fluorescence emission spectra upon titration with F−, with other anions having virtually no effect. An increase in the absorption of the ICT band was observed and it was concluded that these changes were due to a solvent effect with the polymer, as no such absorption increase was observed in 100% MeCN. Concomitant quenching of the fluorescence emission (ca. 53% with 70 mM F−) was also observed. The changes in the fluorescence emission spectra of the polymer upon titration with AcO−, H2PO4− were also fitted (log β1:1 = 3.10 and 2.98, respectively) and higher binding constants were determined than for those obtained for the free sensor, 11e (log β1:1 = 2.55 and 2.07, respectively). Furthermore, it was concluded by the authors that upon titration of the polymer with TBAOH the observed spectral changes were not due to deprotonation of the acidic 4-amino moiety.

Tian et al., have also recently synthesised a polymeric version of 20 discussed above, for use in the development of fluorescent sensing polymers based on polyphenylacetylene. Polymer 37 consists of polyphenylacetylene monomer units which are functionalised with the F− selective sensor, 20, in the side chains of the 4-amido moiety. The absorption spectrum of 37 exhibited a λmax at 360 nm, in the same manner as 20. However, a slight shoulder was present at ca. 490 nm which the authors assigned to the π−π* transitions of the polymer backbone. The fluorescence spectrum of 37 was of lower intensity than that observed for 20, possibly due to PET from the polymer backbone to the excited state of the naphthalimide fluorophore. Similarly to that seen for 20, the ability of 37 [1 × 10−3 M] to sense anions in MeCN was investigated and the changes observed were similar to those observed for the free sensor 20.

Tian et al., has also recently developed another analogue of 37, by using reversible addition–fragmentation chain transfer (RAFT) polymerisation. Compound 38 has the polymer group at the diimide side, similar to that seen in 36 designed by Callan et al. As for 37, this compound functioned as a naked eye sensor for F−, where colour changes occurred from blue to
yellow that were visible to the naked eye. Other anions such as Cl\(^-\), Br\(^-\) and I\(^-\) did not give rise to such changes, as they are not basic enough to deprotonate the 4-amide moiety in the same manner as F\(^-\) when investigated in CH\(_2\)CN solution.

Sensor 39 is a modified version of this design, and was also investigated by Tian et al.\(^85\) This example was able to detect several anions, such as phosphate and acetate which gave rise to blue to orange colour changes at low concentration of these anions. Fluoride also gave rise to such a colour change at low concentration of the anion. However, as for all the examples discussed in this section, at higher concentration of F\(^-\), more pronounced colour changes occurred, with a clear change from orange to purple, demonstrating the ability of the anion to function as a strong base in organic solutions.

**Naphthalimide sensors for binding to small biomolecules, proteins and polyanions such as DNA**

In addition to being a highly versatile structure for use in both cation and anion sensing, the 1,8-naphthalimide structure has been employed in sensing of neutral molecules as well as biomolecules such as thiols or thiol containing amino acids, e.g. 40 developed by de Silva et al.\(^84\) based on PET sensing, and for the incorporation into polypeptides as potential probes for studying dynamic protein interactions, e.g. 41.

Sensor 41, developed by Imperiali et al.\(^85\) is based on the well known 4-N,N-dimethylaminophthalimide structure, which due to the ICT character of the fluorophore is highly sensitive to the local polar environment of the proteins, many of which are highly negatively charged. The 1,8-naphthalimide structure has also been incorporated into a polypeptide, via a glycine link, by Gunnlaugsson et al.\(^86\) for the use as a sensitizing antenna for populating lanthanide ions such as Eu(III) and Tb(III), which can then be employed to bind to other biomolecules. Their use for potentially detecting radicals has also recently been demonstrated by using structures such as 43, possessing two TEMPO moieties.\(^87\)

The above examples clearly show the versatility of the naphthalimide structure for potential use in biology. However, their use more precisely for the detection of biological relevant anions, and in particularly, DNA is well known and has been documented for some time. As DNA is a polyanion, consisting of a negatively charged phosphate backbone, the use of positively charged naphthalimide structures to bind to DNA via electrostatic, hydrogen bonding and π-π intercalating binding interactions has been explored. Such binding, usually gives rise to significant changes in the photophysical properties of the naphthalimide structure, and the DNA binding affinity can be easily quantified from those changes, and in many cases, the binding to DNA by such naphthalimide compounds can also lead to DNA damage, and/or initiated programmed cell death. While it is not the main aim of this review to discuss this area of research, which has become highly topical in the last few years, it is nevertheless, necessary to highlight some of the recent advantages in this field.

Some of the earlier examples of the use of the naphthalimide structure as DNA binding molecules was initiated by Brána et al.\(^88\) These included the synthesis of the 3-nitro and 3-amino-1,8-naphthalimide structures 44a–b possessing a 3-(dimethylamino)ethyl group at the dimide site. These were shown to be active anticancer agents in various cancer cell lines and it was proposed that these molecules interacted with DNA directly, and this was confirmed by Wilson et al.\(^89\) who studied the 4-amino and 4-nitro analogues 45a–b. The 3-nitro analogue had a greater binding affinity for DNA compared to the 4-nitro derivative. A rationale for this is steric effects where the nitro group in position 4 rotates significantly out of the monoimide plane, leading to perturbed stacking in the intercalated complex. However, all of these compounds were found to interact with DNA electrostatically, as the tertiary amine at the imine site was protonated at physiological pH.

This mode of binding has recently also been demonstrated by Veale et al.\(^90\) who made 46a–b and several other analogues, and showed that the pK\(_a\) of the tertiary amine was crucial to ensure high binding affinity of such compounds for calf-thymus DNA, as the morpholine structure 46a (pK\(_a\) ~ 6.5) gave rise to much weaker binding than the pyrazine analogue 46b (pK\(_a\) ~ 9) when measured at pH 7.4. Many other analogues of such naphthalimides have been developed recently for targeting DNA. An example is 47, developed by Qian et al. who have been very productive within this field of research.\(^91\)
Compounds 48a–c (Fig. 8) were recently developed by Veale \textit{et al.}, and shown to bind strongly to DNA, with binding constants resembling those seen for polypyridyl Ru(II) metal complexes.\textsuperscript{92} These Troger’s base compounds interact with DNA via electrostatic and hydrogen-bonding interactions through the tertiary amines, which under physiological pH are protonated (with the exception of 48c which is only partially protonated, \textit{cf.} 46a above, the precursor to 48c). These compounds also have the ability to bind via intercalation to DNA; moreover, and as demonstrated in Fig. 8, these structures can also function as dual imaging–therapeutic agents as biological imaging studies showed that the structures accumulated within the nucleus (Fig. 8 column B) and gave rise to cell death in drug-resistant K562 leukaemia cell lines.

The use of naphthalimides in conjunction with metal complexes has also been demonstrated to be an affective way of enhancing the binding of such structures to DNA where the metal centre can give additional affinity for the negatively charged phosphate backbone. An example of the use of such design in 49a–c, developed by Chakravarty and co-workers, who made Co(II), Co(II) and Zn(II) complexes of 49, and investigated their binding to calf-thymus DNA using various spectroscopic techniques.\textsuperscript{93} These complexes all displayed enhancement in their emission properties upon binding to DNA which the authors described as a light switch effect.

Another example of the use of d-metal ion complexes possessing a naphthalimide moiety for the binding of DNA is that of Gunnlaugsson \textit{et al.} who developed the Ru(II)-based polypyridyl conjugate 50a–b. The absorption spectra of the two complexes differed greatly as the 4-nitronaphthalimide component of 50a absorbed at significantly higher energy than the naphthalimide part of 50b, which partially overlaps with the MLCT absorption band. These systems also showed large changes in their respective emission spectra upon binding to DNA, where the MLCT centered emission of 50b was enhanced by ca. 50%. Furthermore, both compounds were found to be effectively cleave plasmid DNA under photoirradiation.

Compound 50b has recently been further investigated as a MLCT luminescent sensor for anions such as acetate, phosphate and fluoride in CH\textsubscript{3}CN.\textsuperscript{95} Possessing the same amino naphthalimide moieties as seen in structures such as 8 and 9, it was anticipated the MLCT absorption and the emission would be affected by either a hydrogen bonding or deprotonation of the 4-amino moiety by these anions. Indeed, this was found to be the case where the MLCT absorption was red shifted, Fig. 9A for F\textsuperscript{−}, and the MLCT emission quenched, in the presence of these anions. Of the anions tested, the most significant changes were seen upon titration of 50b with F\textsuperscript{−}, as demonstrated in Fig. 9B, where the emission was almost fully switched off. Moreover, upon titration of 50b with Cl\textsuperscript{−} the emission was also quenched. This was postulated to be also due to a potential anion exchange mechanism.
where the PF_{6}^{-} counter ions were being exchanged for Cl^{-}; indicating that several possible ‘binding modes’ could contribute to the overall sensing mechanism of such a d-metal ion coordination complex. Nevertheless, this is, to the best of our knowledge, the first example of the use of 1,8-naphthalimide conjugated d-metal ion complex as a luminescent anion sensor for small biologically relevant anions, and clearly demonstrated that the use of the 1,8-naphthalimide moiety in anion sensing has a very bright future.

Conclusions

Herein we have attempted to summarise the use of the 1,8-naphthalimide structure in the field of colorimetric and fluorescent anion sensing. This account has demonstrated the versatility of this chromophore, which in the past has also been extensively employed in the sensing of cations. By simply conjugating the fluorophore to known anion recognition moieties, either directly or via a short covalent spacer, has given rise to a large number of anion sensors, which upon anion recognition give rise to changes in the photophysical properties of the fluorophore. With minimal structural modifications the naphthalimide unit can be employed for the selective sensing of anions, in both organic and in competitive media, either in solution, or after incorporation into polymeric matrices. We have also shown that the anion receptor can be incorporated at the imide side, which is usually not possible when designing such sensors for cations. The naphthalimide structure can be tailored for binding to polyanions such as DNA and with simple design changes, such polyanions can be damaged or cleaved, by using light irradiation in the presence of the naphthalimide structures.

It is clear from this short summary that the field of anion sensing is rapidly growing and that the development of functionalised naphthalimide sensors has been central to progress in this area of research to date. The authors ‘sense’ that the future of the field of anion recognition and sensing will continue to blossom and that the 1,8-naphthalimide structure will continue to play a crucial role in its development. The development of anion sensors based on the naphthalimide structure is only emerging; the scope for further expanding on the use of this photophysical rich structure is very promising, where the substitution pattern, the nature of such substituent’s, etc. can all be modulated. Above, we have discussed some of the unpublished results from our own laboratories, with the view of giving some flavour of the many possibilities available to us as researchers. But the future is bright, and other related structures, such as the diimide and the perylene analogues, can also be employed for such sensing, either in conjunction with the naphthalimide unit or as independent entities themselves. Hence, it is clear to us that the future on the anions sensing using the naphthalimide family is very colourful and bright!

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Notes and references

75 F. M. Pfeffer, L. Henderson and N. Lewkenko, unpublished results.