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

Rebecca M. Duke,[a] Emma B. Veale,[a] Frederick M. Pfeffer,[b] Paul E. Kruger[c] and Thorfinnur Gunnlaugsson*[a]

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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

[a] *School of Chemistry, Centre of Synthesis and Chemical Biology, University of Dublin, Trinity College Dublin, Dublin 2, Ireland. E-mail: gunnlaut@tcd.ie, +353 1 896 3459*
[b] *School of Life and Environmental Sciences, Faculty of Science and Technology, Deakin University, Waurn Ponds, 3217 Australia*
[c] *Department of Chemistry, College of Science, University of Canterbury, Christchurch, 8020, New Zealand*
† Part of a themed issue on the supramolecular chemistry of anionic species.

Rebecca M. Duke

Rebecca Duke studied chemistry at Trinity College Dublin and graduated with honours in 2005. Under the supervision of Prof. T. Gunnlaugsson (Trinity), she completed her PhD degree in 2009, where she worked on the development of colorimetric and fluorescent sensors for anions. After spending some time in pharmaceutical industry, she is currently carrying out postdoctoral research with Prof. Pauline Rudd at the National Institute for Bioprocessing Research and Training (NIBRT) University College Dublin.

Emma B. Veale

Emma Veale received her BSc from National University of Ireland, Maynooth, and a PhD in supramolecular and medicinal chemistry from School of Chemistry, Trinity College Dublin. She has been a senior research fellow in the group of Prof. Gunnlaugsson since 2007; working in the medicinal chemistry areas of anion recognition and sensing and supramolecular self-assembly formations.

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,[16] 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

Frederick M. Pfeffer

Fred Pfeffer completed his PhD in 2001 working with Professor Richard Russell on the synthesis of peptide functionalised molecular frameworks then moved to Trinity College Dublin to take up a temporary lectureship. This teaching post was followed by a postdoctoral fellowship with Thorfinnur Gunnlaugsson and Paul Kruger on the development of naphthalimide based anion sensors. He returned to Australia in 2004 to take up a lecturing position at Deakin University in the Faculty of Science and Technology where he is now senior lecturer. His current interests include the development of new antidiabetic and antimicrobial agents as well as supramolecular anion recognition; in particular the development of conformationally preorganised norbornane based hosts.

Paul E. Kruger

Paul E. Kruger completed his BSc (Hons) and PhD degrees in Chemistry at Monash University under the direction of Prof. Keith S. Murray, and then undertook postdoctoral research with Prof. Vickie McKee at the Queen's University of Belfast. He was then appointed as a Lecturer in Inorganic Chemistry at the University of Dublin, Trinity College in 1996, before moving to the University of Canterbury in 2007 where he is a Professor of Supramolecular and Inorganic Chemistry. His research interests touch upon all aspects of supramolecular chemistry ranging from coordination chemistry and metallo-helicate spin-crossover complexes, through materials and structural chemistry, to host–guest and anion sensor chemistry.

Thorfinnur Gunnlaugsson

Thorfinnur (Thorri) Gunnlaugsson currently holds a Personal Chair in Chemistry, in the School of Chemistry, Trinity College Dublin. He obtained his BSc from University of Iceland; a PhD from Queen's University Belfast and undertook a postdoctoral fellowship in Durham University before being appointed to a Lectureship in Medicinal Chemistry in Trinity College Dublin in 1998. He became a Lecturer in Organic Chemistry in 2000, being promoted to an Associate Professor in 2004 and a Personal Chair in 2008. He was an Erskine Fellow in the Department of Chemistry, University of Canterbury, New Zealand in 2009 and a Visiting Professor at NEO - Nanostructures Organiques, Institut des Sciences Moléculaires CNRS/Université Bordeaux 1, 2008 and in Deakin University, Victoria, Australia in 2005. He was awarded the Bob Hay Lectureship by the RSC Macrocyclic and Supramolecular Chemistry Group, in 2006.

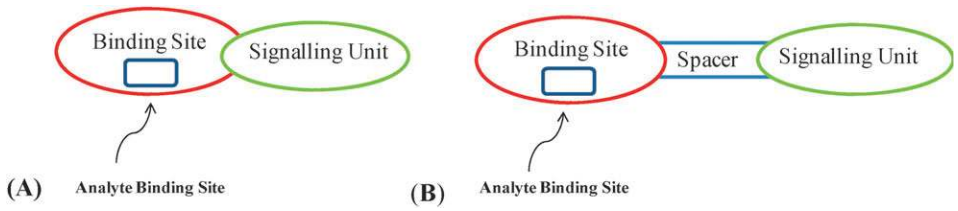


Fig. 1 Schematic representation of design concepts for the construction of colorimetric and fluorescent sensors: (A) The binding side and the signalling unit are covalently connected with π–π bonds, and anion recognition should give rise to changes in the absorption spectra and possibly, depending on 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.

solution.[24–26] 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, indoaniline, anthraquinone, *etc*.[27] 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.[28]

Alternatively, the receptor moiety and signalling subunit may be covalently linked by a 'spacer' group, as demonstrated in Fig. 1b.[29] 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.[30]

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.[31] 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.[32] This design principle, originally detailed by de Silva *et al*.[33] and Czarnik *et al*.[34] has been widely used in chemo-sensing to date.

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.[30,35,36] 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 Fabbrizzi and co-workers.[37,38] 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.

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-naphthalimides 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 λ_{max} 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,[39] as demonstrated in Fig. 3, where both the λ_{max} 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 (Φ_F) 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 Φ_F often being

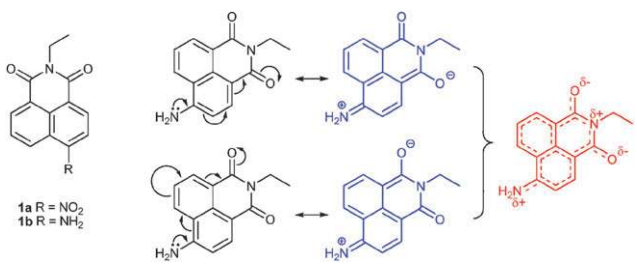


Fig. 2 4-Nitro, **1a**, and 4-amino-1,8-naphthalimide **1b**, structures, and schematic representation of the ICT excited state within the 4-amino-1,8-naphthalimide fluorophore caused by a 'push–pull' action.

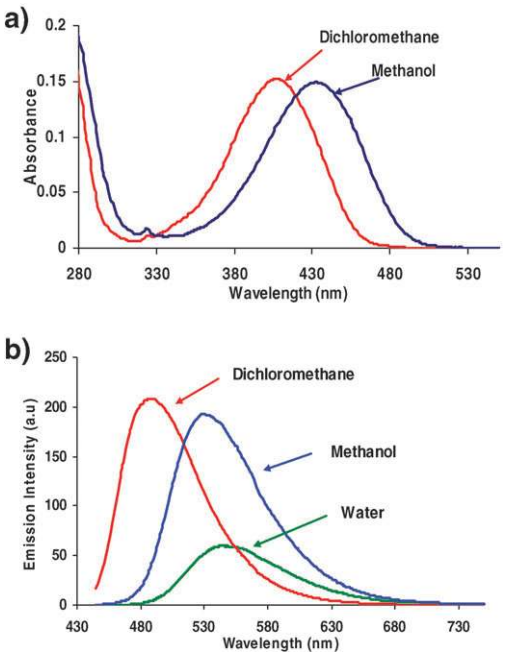
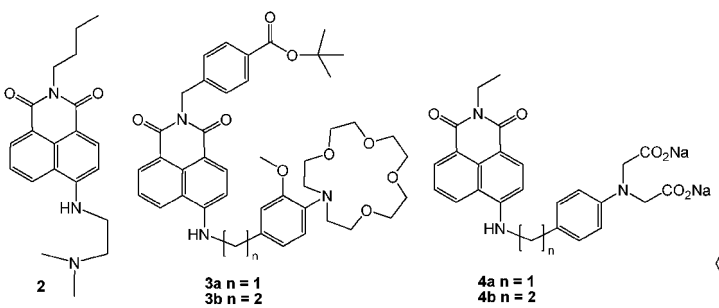
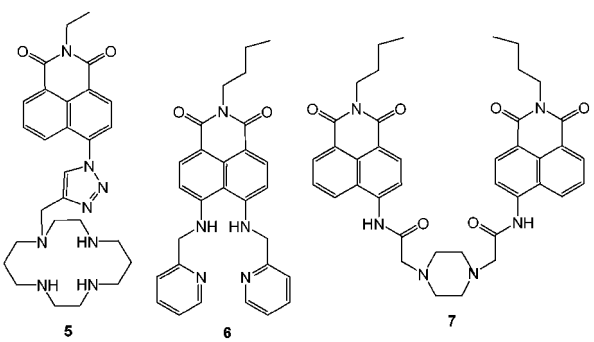


Fig. 3 (a) The absorption spectra of **1b** when recorded in dichloromethane and methanol, demonstrating the effect of the solvent on the ICT band. (b) The fluorescence emission spectra of **1b** in dichloromethane, methanol and in water.

reported to be close to unity; while in water, significant quenching is observed, *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]





In contrast, compound **3**, possessing a crown ether receptor was developed for analysis of Na^+ in blood samples,[41] while **4**[42] and **5**[43] have been developed for detecting Zn(II). Structure **4** has also recently been used for imaging of bone structures using epifluorescence microscopy.[44] The Zn(II) sensors **4a** and **4b**, are based on the fluorophore–spacer–receptor principle, and were shown to bind the Zn(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 *et al.*,[43] upon binding to Zn(II). Recently, Watkinson *et al.*, have extended their design to form naphthalimide dimers, using Cu(II) catalysed click chemistry.[45]

In contrast, compound **6**, developed as sensors for Cu(II), then upon binding of Cu(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(II). Similarly, **7** showed large changes in the absorption and the emission spectra of the naphthalimide moiety upon sensing of Cu(II),[47] while the mono-naphthalimide analogue has recently been shown to be a sensor for Zn(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 *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, *e.g.* at a receptor site, can also result in modulation of the ICT

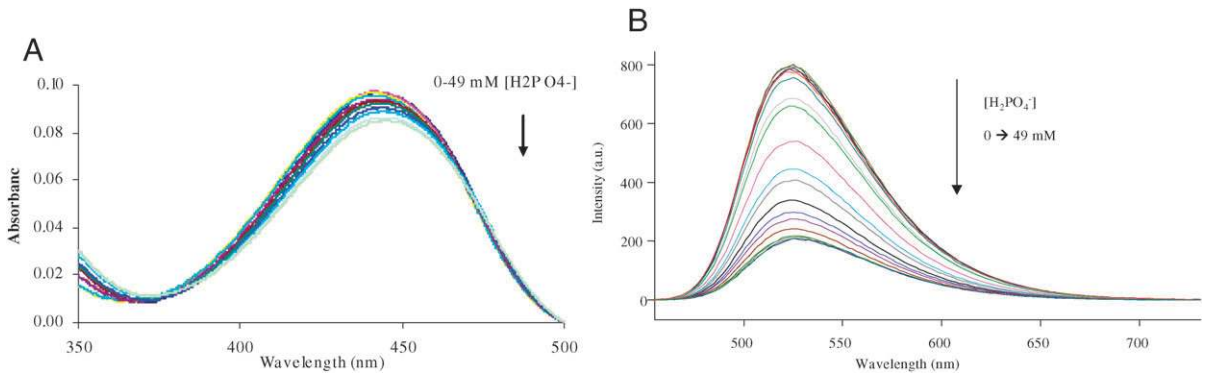


Fig. 4 The changes in the absorption spectra and emission spectra of **8b** upon titration with $H_2PO_4^-$ in DMSO.

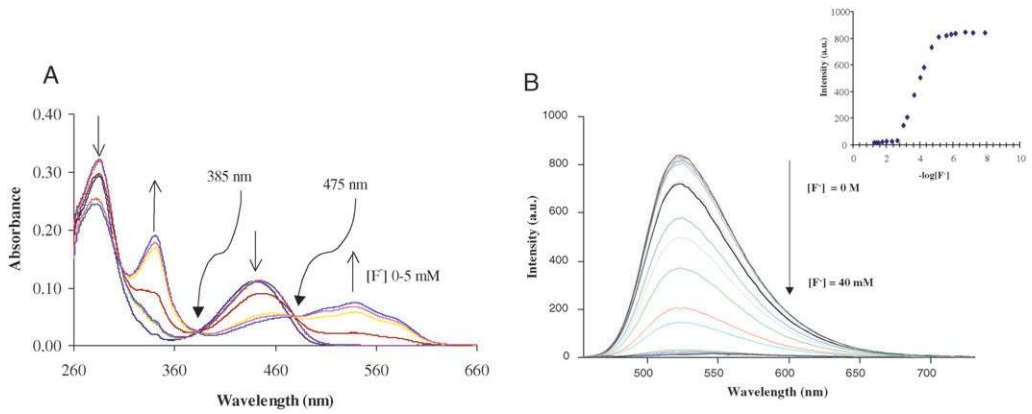
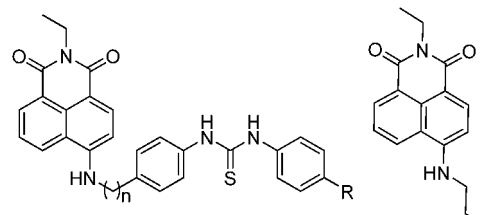


Fig. 5 (A) The changes in the absorption and emission spectra (B) of **8a** upon titration with F^- in DMSO. Inset: the changes in the λ_{max}.

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**.[50] 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.[50] 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 $H_2PO_4^-$, 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.



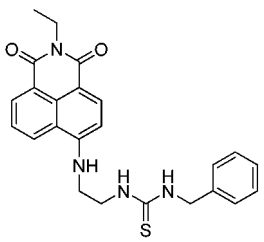
8a n = 1, R = H
8b n = 1, R = CF_3
8c n = 2, R = CF_3

9

In order to understand more fully the sensing process, derivatives **8b**, **8c**,[50] and **9**[51] 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 λ_{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 $H_2PO_4^-$ (Fig. 4A). However, significant changes were seen in the fluorescence emission spectra upon titration with $H_2PO_4^-$, (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 $H_2PO_4^-$ binding constants of $\log K_{1:1} = 2.9$ (± 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-CF_3 group present on the receptor. In contrast to the minor changes noted upon addition of AcO^-, $H_2PO_4^-$, 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

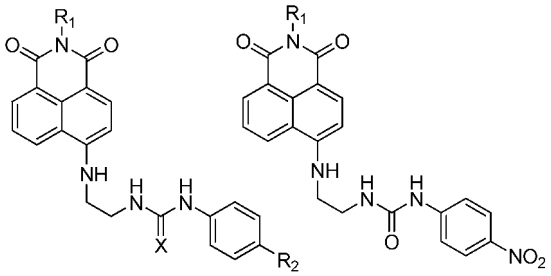
red shifted, which occurred with the formation of two clear isosbestic points. The changes were accompanied by a striking colour change from yellow–green to purple, which was reversed upon the addition of MeOH. These changes (Fig. 5) were assigned to the deprotonation of the 4-amino moiety of the naphthalimide, (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 ^1H 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 Pfeffer *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 oxyanions such as $H_2PO_4^-$. 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, ^1H NMR titrations of **10** in DMSO-d_6 clearly demonstrated that the binding of $H_2PO_4^-$ 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 $H_2PO_4^-$ 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 ^1H NMR titration data for **10** was fitted for the formation of 1 : 1 complexes, using non-linear regression analysis, for which binding constants of $\log K_{1:1} = 3.4$ (± 0.1) and $\log K_{1:1} = 3.6$ (± 0.1) were determined for $H_2PO_4^-$ and AcO^-, respectively. A lower binding constant was reported for the quenching of the emission of **8a** with $H_2PO_4^-$ ($\log K_{1:1} = 2.9$ (± 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 $H_2PO_4^-$ 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.



10

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 *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 *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.

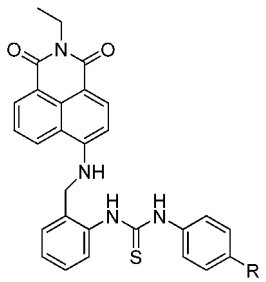


11a $R_1 = (CH_2)CH_3$, X = S, R_2 = H
11b $R_1 = (CH_2)CH_3$, X = S, $R_2 = CF_3$
11c $R_1 = (CH_2)CH_3$, X = O, $R_2 = CF_3$
11d $R_1 = (CH_2)CH_3$,

Subsequent to the reports of sensors **11a–c**, Ying-Li *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 ^1H 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 $\log K_{1:1} = 4.48$ (± 0.1) and $\log K_{1:1} = 4.16$ (± 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. ^1H 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**, Pfeffer *et al.*,[55] evaluated the anion binding capabilities of **12a** and **12b**, which were structurally modified to preserve cooperative binding of $H_2PO_4^-$ whilst restoring the fluorescent properties to that seen for **8a–c**. The electron-rich *o*-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 $H_2PO_4^-$ being observed for both **12a** and **12b**, as determined by ^{1}H NMR titrations in DMSO-d_6. Binding constants for $H_2PO_4^-$ 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 $H_2PO_4^-$ 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 $H_2PO_4^-$ 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 $H_2PO_4^-$ and AcO^-, respectively.



12a R = H
12b R = F

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_2O. 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.

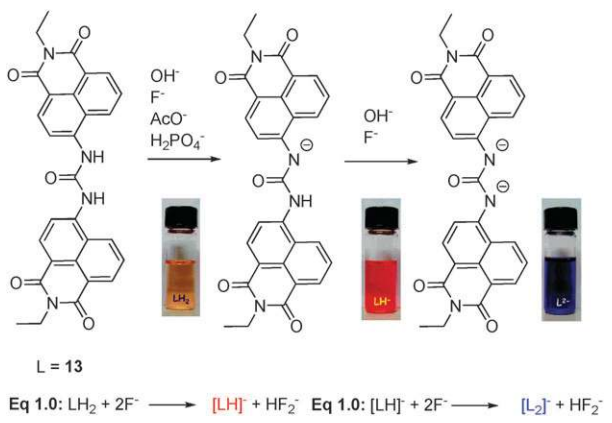
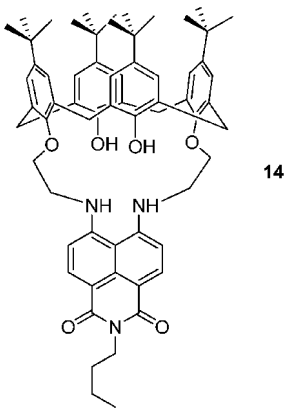


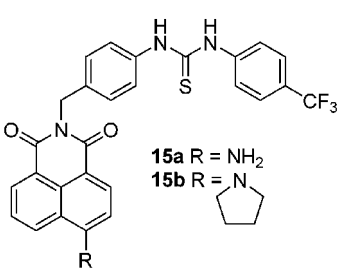
Fig. 6 The interaction of sensor **13** with various anions and the corresponding colour changes upon deprotonation. Adapted with permission from ref. 56. Copyright 2005 American Chemical Society.



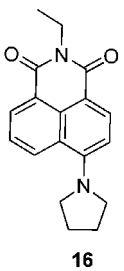
14

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 $H_2PO_4^-$, 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.



15a R = NH_2
15b R = N



16

N-Imide functionalised naphthalimide sensors

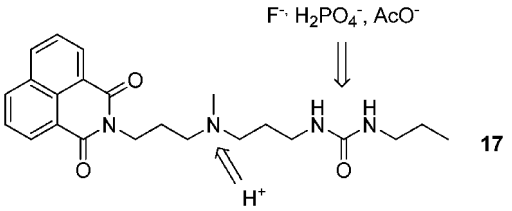
Structurally similar to sensors **8a–c**, compounds **15a** and **15b** were designed and investigated by Veale *et al.*[58,59] and PET

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 $H_2PO_4^-$ 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 $H_2PO_4^-$ 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.[59] For example, the quenching of the fluorescence of **15a** to give $\log K_{1:1} = 3.5$ (± 0.03) by $H_2PO_4^-$, 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,[17] 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.*[60] described the synthesis and photophysical characterisation of the fluorophore–spacer–receptor$_1$–spacer–receptor$_2$ 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 $H_2PO_4^-$ were determined, which gave binding constants that were considerably lower than those reported for **8a** and **8b**.

The ^{1}H NMR titrations of **17** in DMSO-d_6 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.



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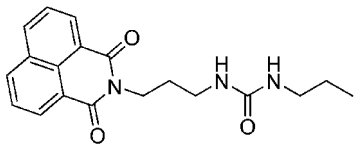
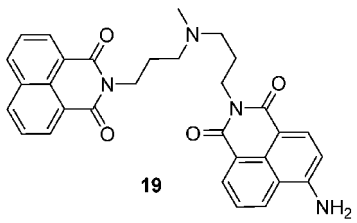


18

Pischel *et al.*[61] 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.

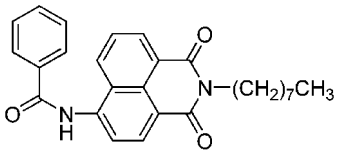


19

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.*[62] The absorption spectrum of **20** in MeCN exhibited a λ_{max} 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.



20

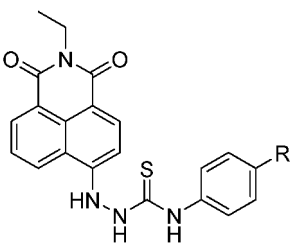
As an extension of this work, Tian *et al.*[63] have recently reported the benzoimidazole functionalised naphthalimide, **21**, for the sensing of F^-, a design that also functioned *via* deprotonation. The absorption spectrum of **21** showed a λ_{max} at 378 nm for the ICT transition, which was shifted 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.

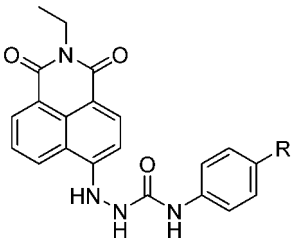
The examples outlined in this section, as well as in the previous sections, detailed the sensing of anions in organic solution such as DMSO, MeCN and THF. However, the following section describes two naphthalimide-based anion sensors, which are capable of sensing in more competitive aqueous-based solutions.

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^-, $H_2PO_4^-$ 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 $-\log[AcO^-]$, a sigmoidal curve was observed that changed over two log units, which is characteristic of 1 : 1 binding.

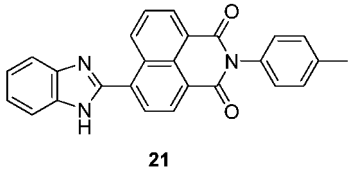


23a R = CH_3
23b R = CF_3



24a R = H
24b R = CH_3
24c R = CF_3

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 $H_2PO_4^-$ 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



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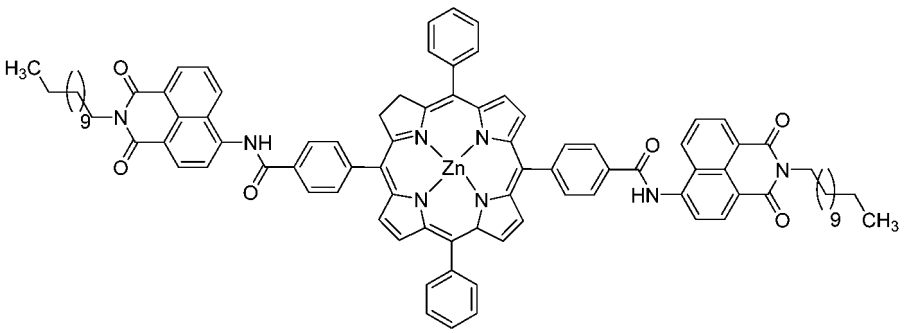
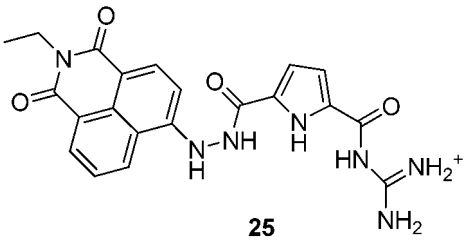


22

4-aminonaphthalimide moiety. The deprotonation was also verified using ^{1}H NMR titration in DMSO-d_6. The most important 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_2O 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^- ($\log\beta$ = 3.4 ±0.1)), over $H_2PO_4^-$ ($\log\beta$ = 2.2 (±0.1)) and F^- ($\log\beta$ = 1 (±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.

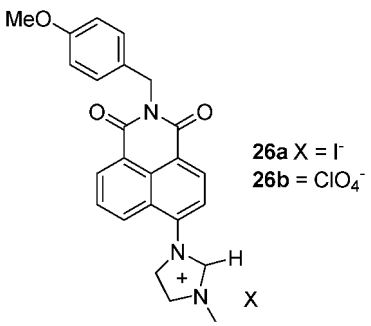
In contrast to these results, the urea-based sensors **24a–c** were only able to bind anions in aprotic solvents, such as DMSO.[66] These three sensors showed similar changes in their absorption spectra to those above upon binding to anions, such as AcO^-, $H_2PO_4^-$ and F^- in DMSO, which was clearly 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 $H_2PO_4^-$ 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 guanidiniocarbonyl 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_2O–DMSO (v/v, 10 mM HEPES, pH = 7.4) with the Na^+ salts of F^-, Cl^-, AcO^-, NO_3^-, SO_4^- 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.



25

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 downfield, whereas the N–Me group was shifted upfield.



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]arene, **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

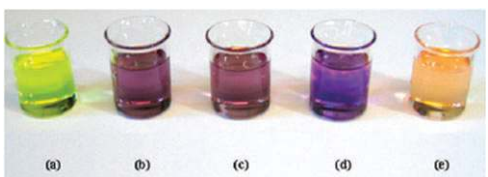
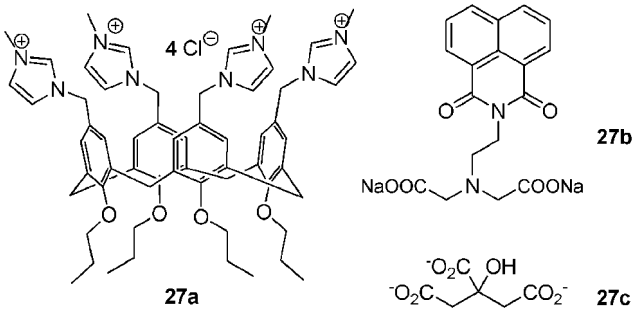
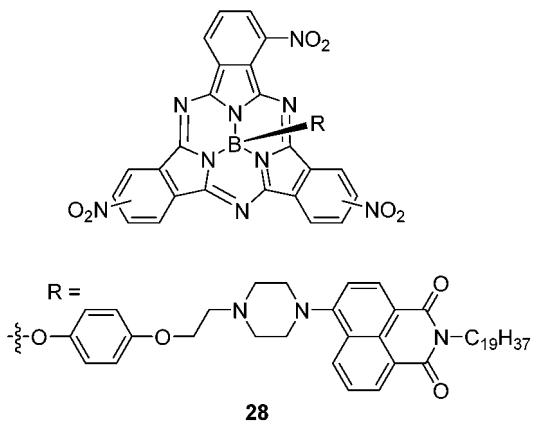


Fig. 7 The chromogenic response of DMSO solutions of sensor **24c** [1 × 10^{-3} M] upon interaction with various anions: (a) sensor **24c**; (b) **24c** + 1 eq. AcO^-. (c) **24c** + 1 eq. $H_2PO_4^-$ (d) **24c** + 1 eq. F^- (e) **24c** + F^- (excess). Ref. 66 – Reproduced by permission of the CNRS and the Royal Society of Chemistry.

amino form of **27b**, which caused a strong fluorescence quenching. The addition of citrate or tartrate to the binary complex lead to the recovery of the ammonium form of **27b**, resulting in a fluorescence enhancement.

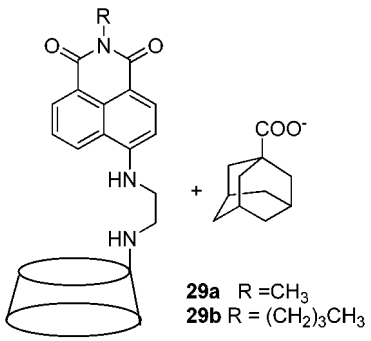


In an alternative approach to the assembly of the ternary complex above, Tian *et al.*[70] have reported the subphthalocyanine (SubPc) based F^- sensor, **28**, in which F^- resulted in dissociation of the axial coordinated 1,8-naphthalimide-based ligand. This sensing mechanism is reminiscent of indicator displacement assays.[37,38] 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.[71] The weak naphthalimide fluorescence was postulated 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^- to **28** 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,[72] which gave a high binding constant of $\log K_{1:1} = 5.11$. As the intensity of the fluorescence at 495 nm increased with a concomitant decrease at 578 nm, sensor **28** can be referred to as a ratiometric anion sensor.



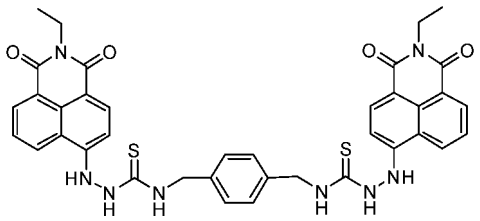
28

As for the calixarene cavity of **27a**, cyclodextrins (CD), which are a group of naturally occurring cyclic oligomers of D-glucopyranose, have been shown to form inclusion complexes with a variety of organic compounds in aqueous solution. Fu *et al.*,[73] 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 developed within the Gunnlaugsson group,[74] 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.*,[75] 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 $\log K_{1:1} = 3.4$ (±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.[75] 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.



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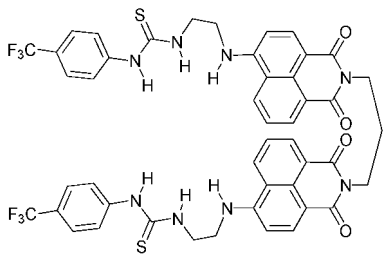

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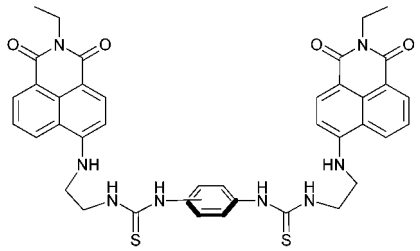
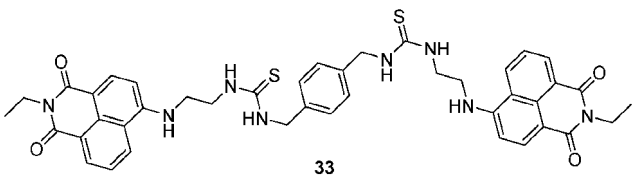


32a 1,3 disubstituted
32b 1,4 disubstituted

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 $H_2PO_4^-$, which, using ^{1}H NMR titrations, was recognised by **33** in 1 : 1 stoichiometry.[76]

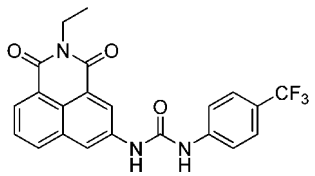


33

3-Functionalised-1,8-naphthalimide-based (thio)urea sensors for anions

The selection of naphthalimide-based sensors discussed above, have mostly been based on functionalising the 4-position of the naphthalimide ring. The 3-position can also be explored for such sensing; but the ICT character for this structural isomer is much weaker, due to lesser push–pull effect than seen for the 4-substituted analogous. Several examples of urea- and thiourea-derived 3-amino-1,8-naphthalimide structures have been developed to date. However, these were synthesised with the view of exploring the anticancer properties of such structures and their anion recognition properties were not investigated.

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}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 (λ_{em} 530 nm for **8a**), clearly demonstrating the weakening ICT character for this structure.

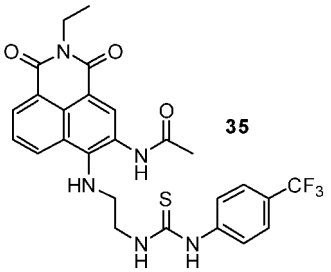


34

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 AcO^- and $H_2PO_4^-$. However, upon titration of **34** with F^- ($TBAF{\cdot}3H_2O$) a minor increase was observed in its absorption spectrum at short wavelengths indicating the interaction of 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 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 F^- for **34**. Nevertheless, **34** can be described as being a highly selective sensor for 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 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-acetamide to participate in strong cooperative binding of the anions.

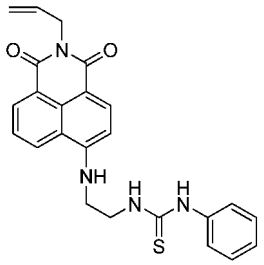


35

Polymeric-based naphthalimide sensors for anions

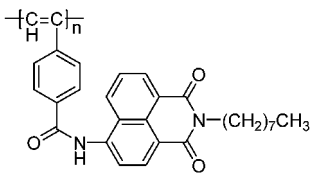
The above examples have all centred on the use of anion sensors in solutions; where changes in colour or emission properties can monitor the presence or the concentration of certain anions. The incorporation of such sensors into polymeric matrixes to facilitate sensing of environmental, biological and industrial samples containing anions *in situ* has also been recently explored. Examples of such systems includes those of Gunnlaugsson *et al.*,[79] 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-(hydroxylethylmethacrylate) (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 at the polymerisation stage. In general, these systems have shown significant changes in the photophysical properties of the naphthalimide structure, where the sensing of anions in aqueous solutions can be achieved.

In a related study, Callan *et al.*[80] have recently reported the incorporation of the polymerisable 1,8-naphthalimide structure **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^-, $H_2PO_4^-$ were also fitted ($\log \beta_{1:1}$ = 3.10 and 2.98, respectively) and higher binding constants were determined than for those obtained for the free sensor, **11e** ($\log \beta_{1:1}$ = 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.



36

Tian *et al.*[81] 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 λ_{max} 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^{-5} M] to sense anions in MeCN was investigated and the changes observed were similar to those observed for the free sensor **20**.



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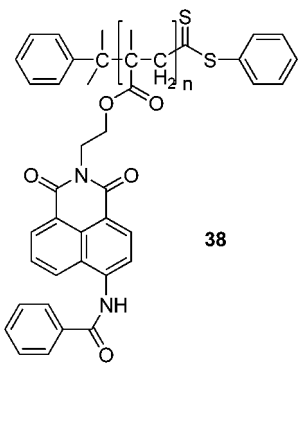

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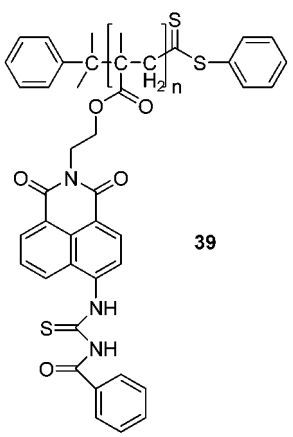


39

Tian *et al.*, has also recently developed another analogue of **37**, by using reversible addition–fragmentation chain transfer (RAFT) polymerisation.[82] 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_3CN solution.

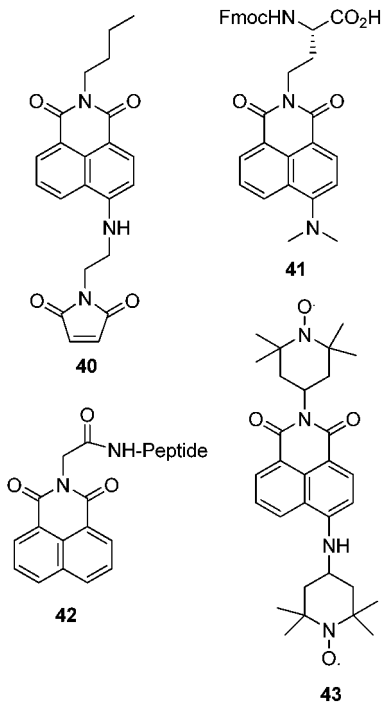
Sensor **39** is a modified version of this design, and was also investigated by Tian *et al.*[83] This example was able to detect several anions, such as phosphate and acetate which gave rise to blue to orange colour changes at low concentration of these anions. Fluoride also gave rise to such a colour 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

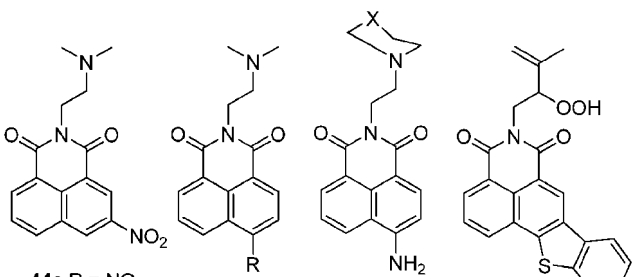
In addition to being a highly versatile structure for use in both cation and anion sensing, the 1,8-naphthalimide structure has been employed in sensing of neutral molecules as well as biomolecules such as thiols or thiol containing amino acids, *e.g.* **40** developed by de Silva *et al.*[84] based 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 Brãna *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 diimide 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]



44a R = NO_2
44b R = NH_2

45a R = NO_2
45b R = NH_2

46a X = O
46b X =NCH_3

47

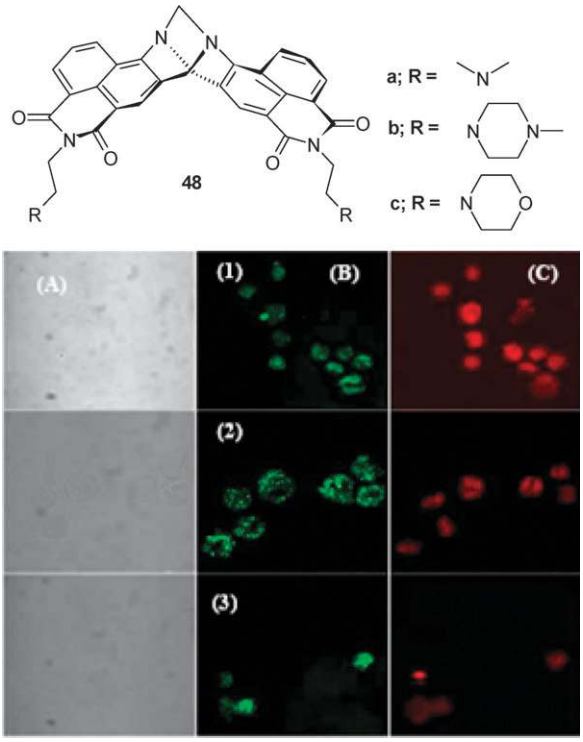
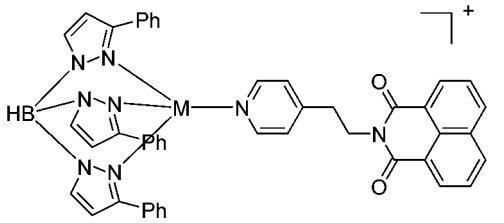


Fig. 8 The naphthalimide-based Tröger's bases **48a–c**, and the emission arising from the three structures within cancer cells, imaged by using confocal fluorescence spectroscopy: (A) phase, (B) fluorescence arising from **48a–c** (10 μM), (C) co-stained with propium iodide. Reprinted with permission from ref. 92. Copyright 2009 American Chemical Society.

Compounds **48a–c** (Fig. 8) were recently developed by Veale *et al*., and shown to bind strongly to DNA, with binding constants resembling those seen for polypyridyl Ru(II) metal complexes.[92] These Tröger'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, *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 cellar death in drug-resistant K562 leukaemia cell lines.

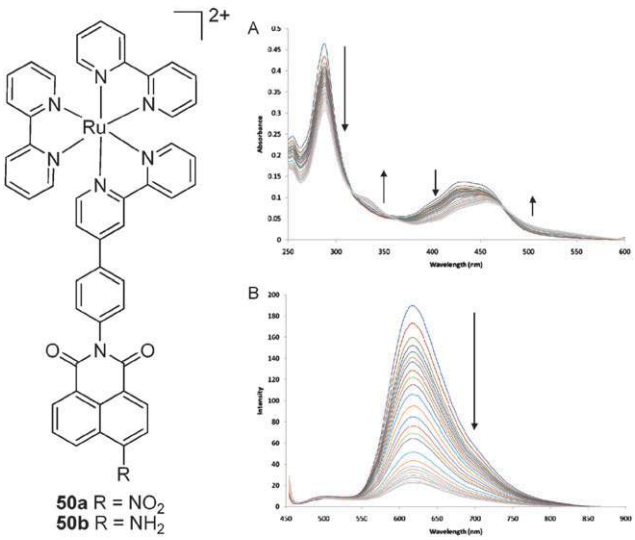
The use of naphthalimides in conjunction with metal complexes has also been demonstrated to be an affective way of enhancing the binding of such structures to DNA where the metal centre can give additional affinity for the negatively charged phosphate backbone. An example of the use of such design in **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.[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 *et al*.[94] 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 cantered emission of **50b** was enhanced by *ca*. 50%. Furthermore, both compounds were found to be effectively cleave plasmid DNA under photoirradiation.



49a M = Co(II)
49b M= Cu(II)
49b M= Zn(II)

Compound **50b** has recently been further investigated as a MLCT luminescent sensor for anions such as acetate, phosphate and fluoride in CH_3CN.[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^-, 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^-, as demonstrated in Fig. 9B, where the emission was almost fully switched off. Moreover, upon titration of **50b** with Cl^- the emission was also quenched. This was postulated to be also due to a potential anion exchange mechanism,



50a R = NO_2
50b R = NH_2

Fig. 9 The structure of the Ru(II) naphthalimide conjugates **50a** and **50b** and the changes (A) in the absorption spectra and (B) in the emission spectra of **50b** upon recognition of F^- in CH_3CN.

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 matrixes. 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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