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Design principles of fluorescent molecular sensors for cation recognition

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Abstract

The main classes of fluorescent molecular sensors for cation recognition are presented: they differ by the nature of the cation-controlled photoinduced processes: photoinduced electron transfer, photoinduced charge transfer, excimer formation or disappearance. In each class, distinction is made according to the structure of the complexing moiety: chelators, podands, coronands (crown ethers), cryptands, calixarenes. The most representative examples are presented in each subclass with special attention given to selectivity.

Keywords: Fluorescent molecular sensors; Cation recognition; Photoinduced electron transfer; Photoinduced charge transfer; Excimers

1. Introduction

Detecting cations is of great interest to many scientists, including chemists, biologists, clinical biochemists and environmentalists. Sodium, potassium, magnesium, calcium are involved in *biological processes* such as transmission of nerve impulses, muscle contraction, regulation of cell activity, etc. Moreover, various metal ions belong to metalloenzymes. In *medicine*, it is important to control the serum levels of lithium in patients under treatment for manic depression, and potassium in the case of high blood pressure. Regarding aluminium, its toxicity has long been recognized and there is a controversy about its possible implication in Alzheimer's disease. In *chemical oceanography*, it has been demonstrated that some nutrients required for the survival of microorganisms in sea water contain zinc, iron, manganese as enzyme cofactors. Finally, it is well known that mercury, lead and cadmium are toxic for organisms, and early detection in the environment is desirable.

Among the numerous analytical methods that are available for the detection of cations, flame photometry, atomic absorption spectrometry, ion sensitive electrodes, electron microprobe analysis, neutron activation analysis, etc., are expensive, often require samples of large size and do not allow continuous monitoring. In contrast, the methods based on fluorescent sensors [1–6] offer distinct advantages in terms of sensitivity, selectivity, response time, local observation (e.g. by fluorescence imaging spectroscopy). Moreover, remote sensing is possible by using optical fibres with a molecular sensor immobilized at the tip [7]. Therefore, considerable efforts are being made to develop selective fluorescent sensors for cation detection.

Such fluorescent sensors consists of a fluorophore linked to an ionophore and is thus called a fluoroionophore (Fig. 1). In the design of such sensors [8], attention should be paid to both recognition and signaling moieties. The *signaling moiety* acts as a signal transducer, i.e. it converts the information (recognition event) into an optical signal expressed as the changes in the photophysical characteristics of the

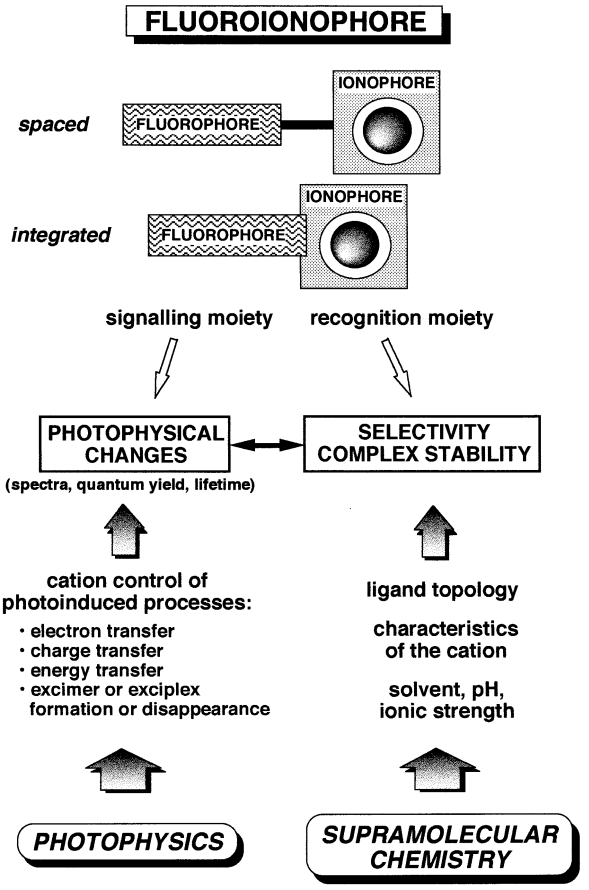


Fig. 1. Main aspects of fluorescent molecular sensors for cation recognition.

fluorophore. These changes are due to the perturbation (by the bound cation) of photoinduced processes such as electron transfer, charge transfer, energy transfer, excimer or exciplex formation or disappearance, etc. These aspects are relevant to the field of *photophysics*.

As regards the *recognition moiety*, it is responsible for selectivity and efficiency of binding which depend on the ligand topology, on the characteristics of the cation (ionic radius, charge, coordination number, hardness, etc.), and on the nature on

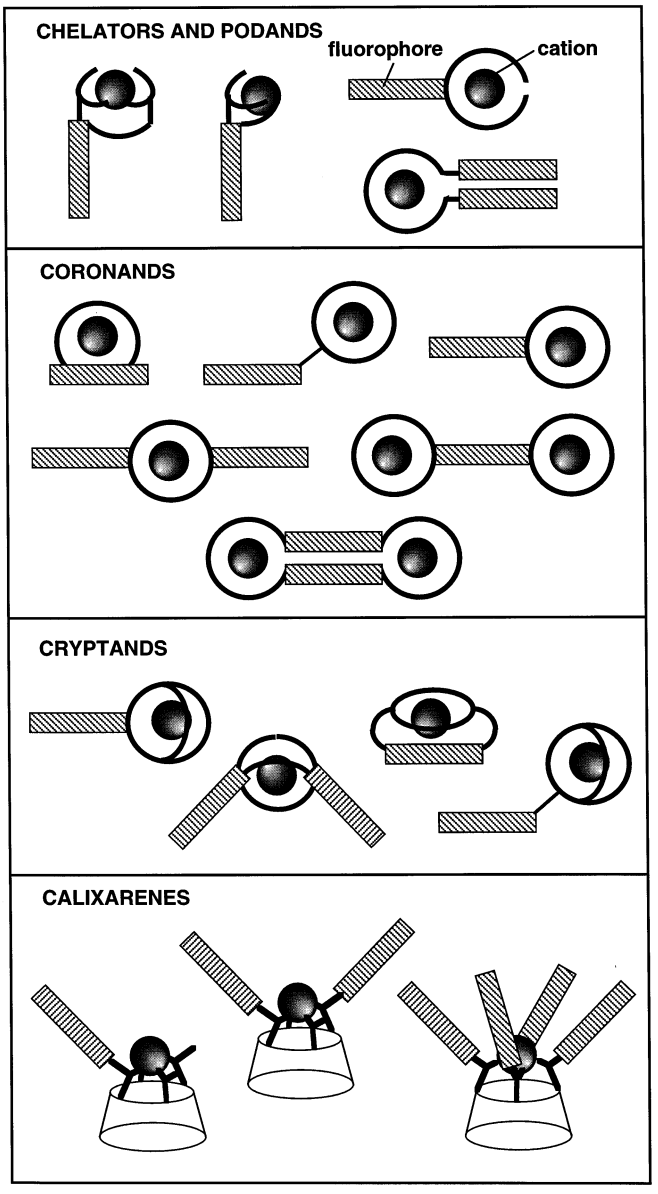


Fig. 2. Schematic illustration of various structures of fluoroionophores.

the solvent (and pH, ionic strength in the case of aqueous solutions). These aspects are relevant to the field of *supramolecular chemistry*. It should be noted that the signaling moiety can be linked to the ionophore moiety via a spacer or not. Even in the latter case, some atoms of the fluorophore may participate in the complexation. Therefore, the selectivity of binding often results from the whole structure involving both signaling and recognition moieties. The topology of some fluoroionophores is displayed in Fig. 2.

It should be emphasized that, under the conditions of detection, the dissociation constant of the complex should match the expected range of cation concentration which is very different according to the field. For instance, the concentration of calcium ion inside a living cell is in the micromolar range, whereas in blood plasma and urine it is in the millimolar range. Therefore, the well-known calcium sensors designed by Tsien (Indo-1, Fura-2, etc.) [9] are suitable for cellular biology but not for clinical diagnosis.

The present review will focus on the design of fluorescent sensors for cations with a classification according to the nature of the photoinduced process (mainly photoinduced electron or charge transfer, and excimer formation) that is responsible for photophysical changes upon cation binding. Such a classification should help the reader to understand the variations in fluorescence intensity, lifetimes and spectral changes in the numerous papers reporting cation sensing with fluorescent probes. In most of these papers, little attention is often paid to the origin of cation-induced photophysical changes.

2. Fluorescent PET (photoinduced electron transfer) cation sensors

2.1. Principles

This type of sensor has been extensively studied (ror reviews, see Refs. [5,10]). Fig. 3 illustrates how a cation can control the photoinduced charge transfer (PCT) in a fluoroionophore in which the cation receptor is an electron donor (e.g. amino group) and the fluorophore plays the role of an acceptor. Upon excitation of the

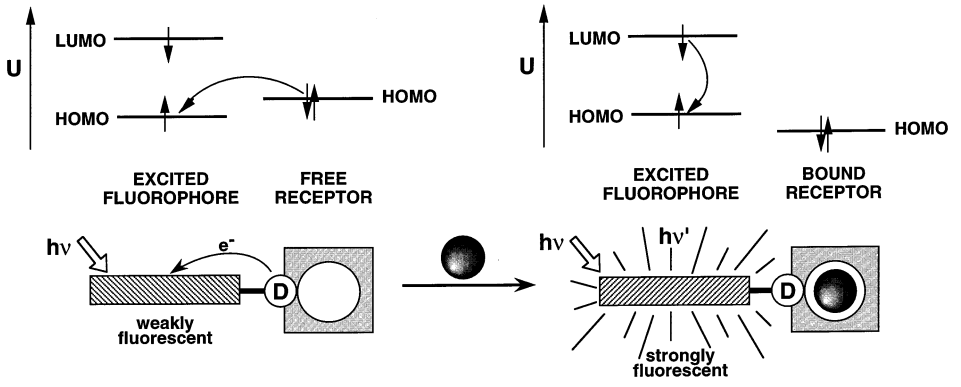


Fig. 3. Principle of cation recognition by fluorescent PET sensors.

fluorophore, an electron of the highest occupied molecular orbital (HOMO) is promoted to the lowest unoccupied molecular orbital (LUMO), which enables PET from the HOMO of the donor (belonging to the free cation receptor) to that of the fluorophore, causing fluorescence quenching of the latter. Upon cation binding, the redox potential of the donor is raised so that the relevant HOMO becomes lower in energy than that of the fluorophore; consequently, PET is not possible any more and fluorescence quenching is suppressed. In other words, fluorescence intensity is enhanced upon cation binding.

In most of PET sensors, the cation receptor involves aliphatic or aromatic amines acting as quenchers. Indeed, it has long been discovered that PET can take place from amino groups to aromatic hydrocarbons, thus causing fluorescence quenching of the latter.

Most PET fluorescent sensors are based on this scheme, but other PET mechanisms can take place with transition metal ions [11,12]. In fact, 3d metals exhibit redox activity and electron transfer can occur from the fluorophore to the bound metal ion, or vice versa. In some cases, electron exchange is possible which results in quenching of the fluorophore by nonradiative energy transfer according to the Dexter mechanism.

Various examples of PET sensors are given in Figs. 4–7; there are classified according to the chemical structure of the recognition moiety.

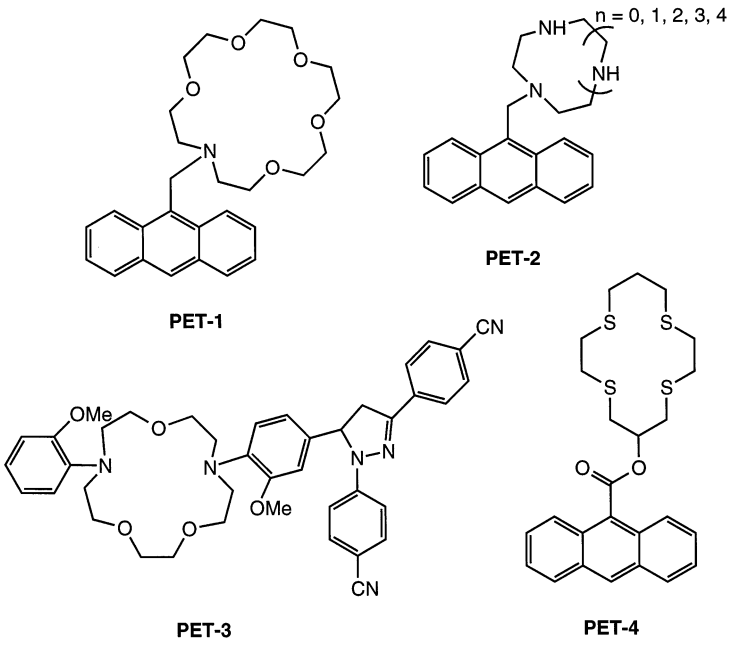


Fig. 4. Crown-containing PET sensors.

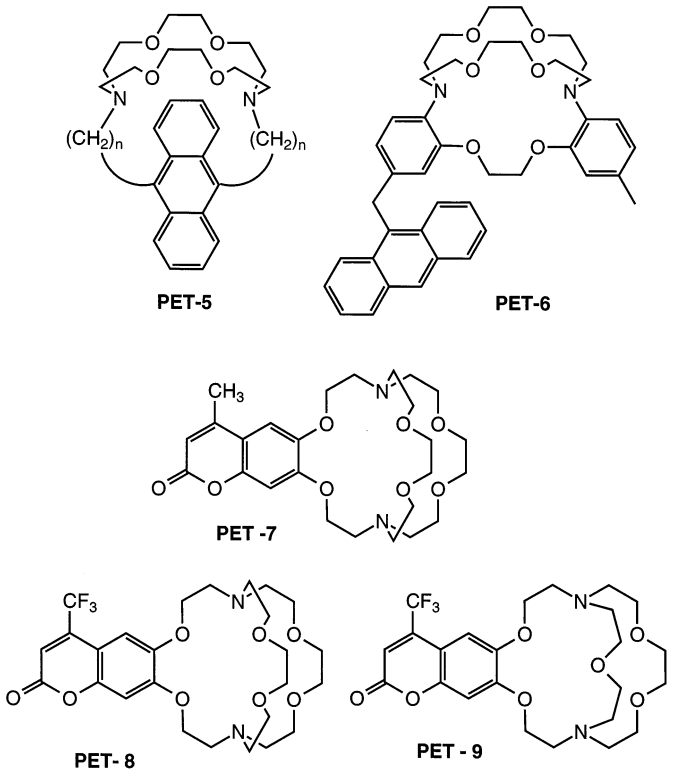


Fig. 5. Cryptand-based PET sensors.

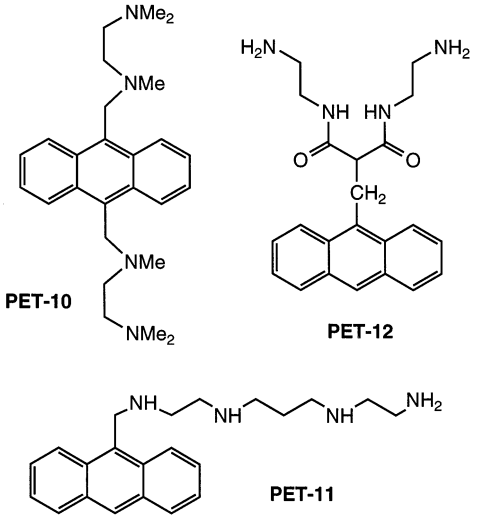


Fig. 6. Podand-based PET sensors.

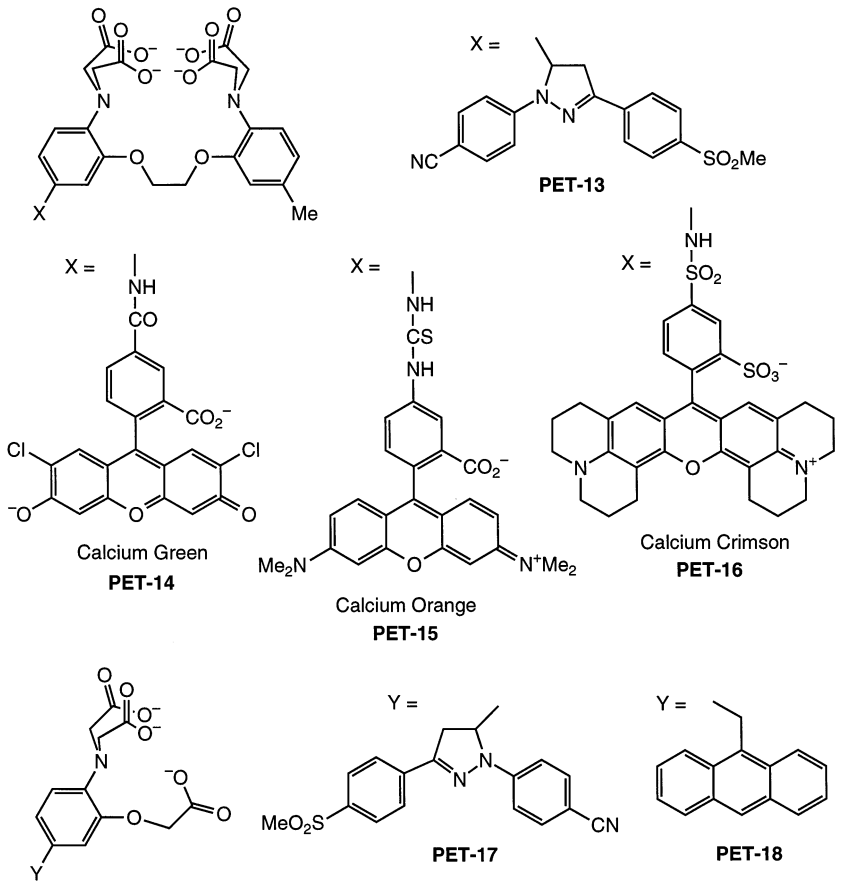


Fig. 7. Chelating PET sensors.

2.2. Crown-containing PET sensors

Examples of coronands are given in Fig. 4. **PET-1** [13] is the first and simplest coronand PET sensor. Its fluorescence quantum yield increases from 0.003 to 0.14 upon binding of K^+ in methanol. **PET-2** [14] containing a polyazamacrocycle was designed for the recognition of soft metal ions like Zn^{2+}. In **PET-3** [15], the recognition moiety is similar to Tsien's PCT fluorescent sensor called SBFI (see Section 3): the methoxy groups in *ortho* position with respect to the nitrogen atoms of the crown participate in the complexation (according to the design principle of lariat-ethers), so that strong Na^+ binding is achieved and accompanied with switching 'on' the fluorescence.

In **PET-4** [16], the crown does not contain nitrogen atoms but four sulfur atoms and is known for its strong affinity towards Cu^{II}. This sensor is also based on the PET principle, but in a different way as compared to compounds **PET-1**, **-2** and **-3**.

Quenching of fluorescence upon Cu^{II} binding arises from a PET from the fluorophore to the metal center and involves the Cu^{II}/Cu^{I} couple. It is remarkable that the other transition metal ions like Mn^{II}, Fe^{II}, Co^{II}, Ni^{II}, have negligible effect in ethanolic solutions. The crown can also efficiently bind Ag^{I}, but complexation is not signaled by a change in fluorescence intensity because the poor redox activity of this nontransition cation precludes electron transfer.

2.3. Cryptand-based PET sensors

PET-5 [17–19], **PET-6** [20], **PET-7** [21], **PET-8** and **PET-9** [22,23] (Fig. 5) are examples of macrobicyclic structures (cryptands) that are expected to be more selective towards alkali cations than macrocyclic structures. The cavity of **PET-6**, **PET-7** and **PET-8** fits well the size of K^+ whereas **PET-9** is well suited for Na^+ detection. **PET-7** has been successfully used for monitoring levels of potassium in blood and across biological membranes, but pH must be controlled because of pH sensitivity of this compound via protonation of the nitrogen atoms. This difficulty has been elegantly overcome in benzannelated cryptand **PET-6** in which the aromatic nitrogens have lower pK_a than those of aliphatic amines.

An interesting feature of **PET-5** is its capability to form exciplexes characterized by an additional band at higher wavelengths, thus allowing ratiometric measurements at two different observation wavelengths.

After these examples of fluoroionophores containing closed structures of the recognition moieties, let us consider now those which are based on open structures (podands and chelators).

2.4. Podand-based PET sensors

In Fig. 6, podands **PET-10** [24] and **PET-11** [25] contain polyamine chains and were thus aimed at Zn^{2+}, because this soft cation has a strong affinity for the soft nitrogen atoms. However, they operate in a very limited pH range and the affinity for Cu(II) is also strong. **PET-12** [26,27] can bind Cu(II) and Ni(II) and favor oxidation of these cations to the trivalent state. Fluorescence quenching of anthracene upon binding should be ascribed in this case to an electron transfer from the reducing divalent metal center.

2.5. Chelating PET sensors

Chelators with carboxylic groups are known to efficiently bind divalent hard cations like Ca^{2+} and Mg^{2+}. Many selective calcium probes have been designed by Tsien and coworkers for applications in cellular biology [28,29], i.e. for probing calcium concentrations in the micromolar range. The so-called BAPTA recognition moiety resembles EDTA, but the nitrogen atoms are linked to a phenyl group in order to avoid pH sensitivity in physiological media. Most of them are not based

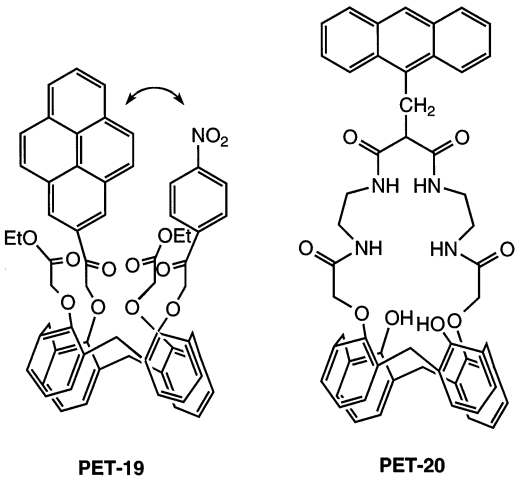


Fig. 8. Calixarene-based PET sensors.

on the PET principle (see Section 3). Nevertheless, Fig. 7 shows examples of PET calcium sensors (**PET-13** to **PET-16**). In **PET-13** [30] the spacer is the 5-methine group (within the pyrazoline), whereas it is the amide link in **PET-14** to **PET-16** [31]. However, the photophysical properties of Calcium Green appears to be more complicated than the simple PET mechanism described above because ground-state conformational changes upon calcium binding can be also involved [32]. In PET sensors, the changes in fluorescence quantum yield are accompanied with proportional changes in excited-state lifetime. Therefore, compounds **PET-14** to **PET-16** turned out to be suitable for fluorescence lifetime imaging of calcium [33].

The same design principles apply to the magnesium sensors **PET-17** and **PET-18** [34] in which the recognition moiety has a smaller 'cavity'.

2.6. Calixarene-based PET sensors

PET-19 has been designed for selective recognition of sodium [35] (Fig. 8). It contains four carbonyl functions, two of them being linked to pyrene and nitrobenzene at opposite sites on the calixarene lower rim. Complexation with Na^+ prevents close approach of pyrene and nitrobenzene and thus reduces the probability of PET. The fluorescence quantum yield increases from 0.0025 to 0.016.

Calixarene containing a dioxotetraaza unit, **PET-20** [36], is responsive to transition metal ions like Zn^{2+} and Ni^{2+}. Interaction of Zn^{2+} with the amino groups induces a fluorescence enhancement according to the PET principle. In contrast, some fluorescence quenching is observed in the case of Ni^{2+}. The authors did not comment on the latter observation: PET from the fluorophore to the metal ion is a reasonable explanation but energy transfer by electron exchange (Dexter mechanism) cannot be excluded [12].

2.7. PET sensors involving excimer formation

PET-21 [37] (Fig. 9) consists of a diazacrown ether with two pendant pyrene groups. As expected, cation binding results in a large change in the monomer/excimer ratio. There is a concomitant increase in the overall fluorescence emission as a result of the reduction of PET from the nitrogen atom to the pyrenyl groups. The monomer/excimer ratio was found to be strongly dependent on the nature of the metal ion. Among the investigated metal ions, the larger stability constants of the complexes were obtained for K^+ and Ba^{2+}, in accordance with the size of these cations with respect to the crown diameter.

In the same way, the emission spectrum of **PET-22** [38,39] existing as the protonated form in acetonitrile exhibits an excimer band whose intensity decreases upon binding of Cd^{2+} and Pb^{2+}.

Ditopic receptor **PET-23** [40,41] is a good example of special design for the recognition of α,ω-alkane diammonium ions. Upon addition of the dication H_3N^+

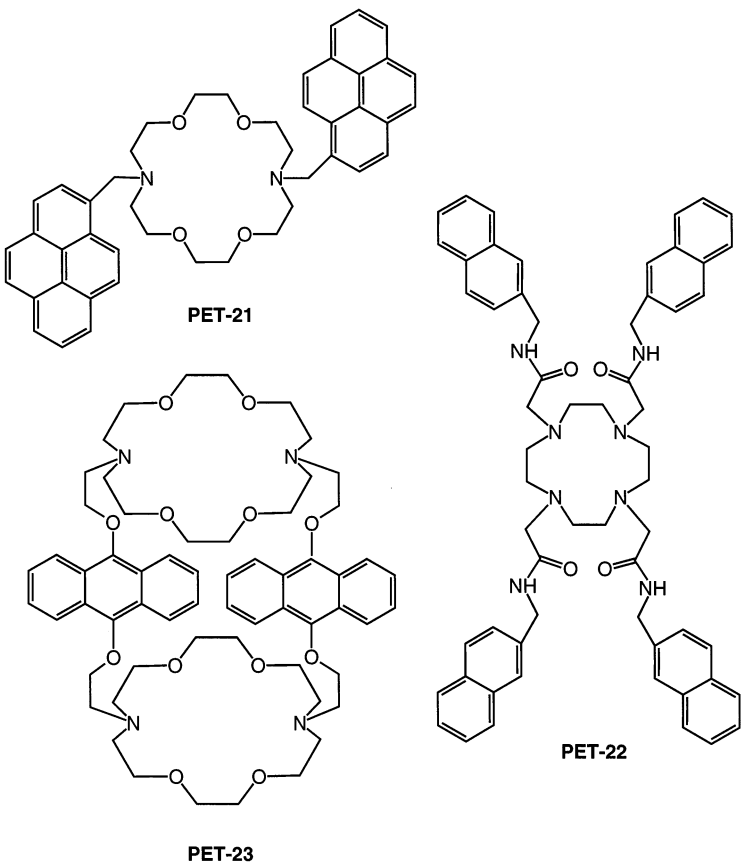
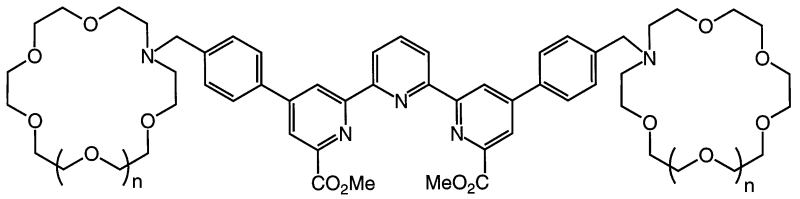


Fig. 9. PET sensors involving excimer formation.



PET-24

Fig. 10. PET sensor involving energy transfer.

$(CH_2)_7N^+H_32Cl^-$ to a solution of **PET-23** in methanol, the monomer-like emission is enhanced by a factor of ten because fluorescence quenching is hindered, and the excimer band vanishes because the two anthracene rings are parted from each other upon complexation.

2.8. PET sensors involving energy transfer

PET-24 [42] (Fig. 10) contains a terpyridyl diester that can strongly bind Eu^{III} and a crown to potentially bind K^+. Excitation energy transfer from this type of ligand to Eu^{III} is known to occur via the triplet state and should result in luminescence from Eu^{III}. However, when the crown is empty, only weak luminescence is detected because of quenching due to PET from the nitrogen atom of the crown. Binding of K^+ causes a very large enhancement of the luminescence quantum yield as expected from cation-induced reduction of the PET efficiency. **PET-24** provides the first example of metal-triggered metal-centered emission. Thanks to the long lifetime of Eu^{III} (hundreds of microseconds), time-delayed detection of the luminescence is possible which allows removal of the fast intrinsic fluorescence of biological samples.

3. Fluorescent PCT (photoinduced charge transfer) cation sensors

3.1. Principles

When a fluorophore contains an electron-donating group (often an amino group) conjugated to an electron-withdrawing group, it undergoes intramolecular charge transfer from the donor to the acceptor upon excitation by light. The consequent change in dipole moment results in a Stokes shift that depends on the microenvironment of the fluorophore; polarity probes have been designed on this basis [43]. It can thus be anticipated that cations in close interaction with the donor or the acceptor moiety will change the photophysical properties of the fluorophore because the complexed cation affects the efficiency of intramolecular charge transfer [44–46].

When a group (like an amino group) playing the role of an electron donor within the fluorophore interacts with a cation, the latter reduces the electron-donating character of this group; owing to the resulting reduction of conjugation, a blue shift of the absorption spectrum is expected together with a decrease of the extinction coefficient. Conversely, a cation interacting with the acceptor group enhances the electron-withdrawing character of this group; the absorption spectrum is thus red-shifted and the molar absorption coefficient is increased. The fluorescence spectra are in principle shifted in the same direction as those of the absorption spectra. In addition to these shifts, changes in quantum yields and lifetimes are often observed. All these photophysical effects are obviously dependent on the charge and the size of the cation, and selectivity of these effects are expected.

The photophysical changes upon cation binding can also be described in terms of charge dipole interaction [47]. Let us consider only the case where the dipole moment in the excited state is larger than that in the ground state. Then, when the cation interacts with the donor group, the excited state is more strongly destabilized by the cation than the ground state, and a blue shift of the absorption and emission spectra is expected (however the fluorescence spectrum undergoes only a slight blue shift in most cases; this important observation will be discussed below). Conversely, when the cation interacts with the acceptor group, the excited state is more stabilized by the cation than the ground state, and this leads to a red shift of the absorption and emission spectra (Fig. 11).

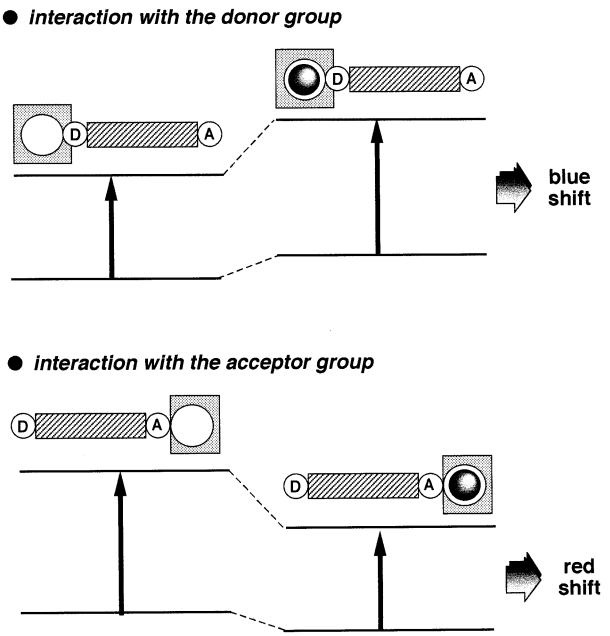


Fig. 11. Spectral displacements of PCT sensors resulting from interaction of a bound cation with an electron-donating or electron-withdrawing group.

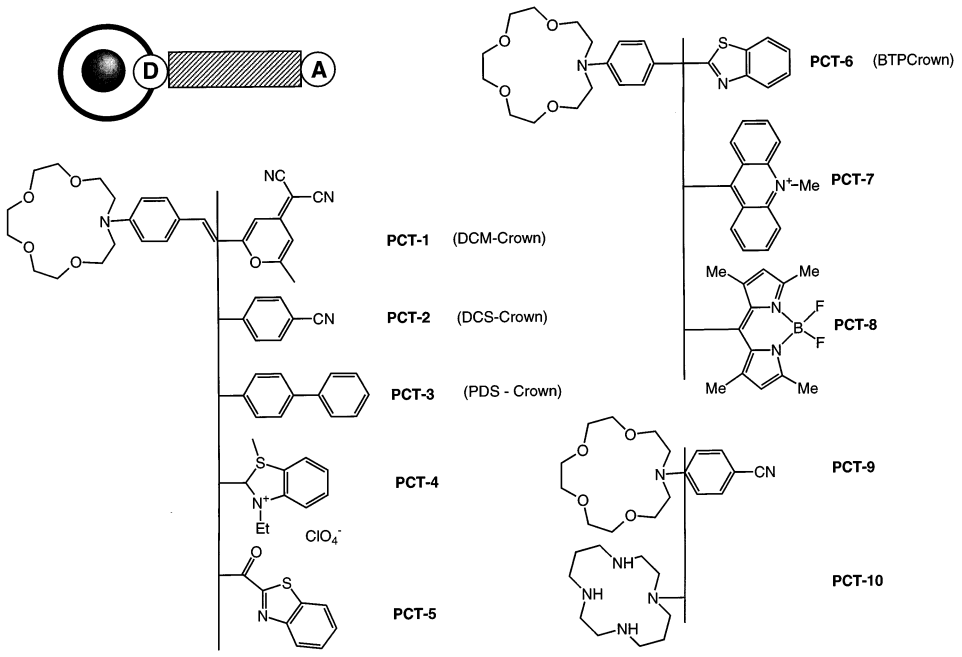


Fig. 12. Crown-containing PCT sensors in which the bound cation interacts with the donor group.

3.2. PCT sensors in which the bound cation interacts with an electron-donating group

3.2.1. Crown-containing PCT sensors

Many fluoroionophores have been designed according to the following principle [44–48]: the cation receptor is an azacrown containing a nitrogen atom which is conjugated to an electron-withdrawing group (Fig. 12). Compounds **PCT-1** to **PCT-6** exhibit a common feature: the blue shift of the absorption spectrum is much larger than that of the emission spectrum on cation binding. The wavelengths corresponding to the maximum of absorption and emission spectra in the absence and in the presence of calcium ion are reported in Table 1 for **PCT-1** [49], **PCT-2** and **PCT-3** [50], **PCT-4** [51], **PCT-5** [52] and **PCT-6** [53]. Such a small shift of the fluorescence spectrum which is surprising at first sight can be interpreted as follows. The PCT reduces the electron density on the nitrogen atom of the crown, and this nitrogen atom becomes a noncoordinating atom because it is positively polarized. Therefore, excitation induces a photodisruption of the interaction between the cation and the nitrogen atom of the crown. The fluorescence spectrum is thus only slightly affected because most of the fluorescence is emitted from species in which the interaction between the cation and the fluorophore does not exist any more or is much weaker.

This interpretation is supported by a thorough study of the photophysics of **PCT-1** and its complexes with Li^+ and Ca^{2+} [54–56]. In particular, subpicosecond pump–probe spectroscopy provided compelling evidence for the disruption of the link between the crown nitrogen atom and the cation. A photodisruption was also demonstrated in complexes of **PCT-2** and **PCT-3** [57,58]. The cation-induced spectral changes in **PCT-4** [51] and in another crowned styryl dye [59] were interpreted in the same way.

Such a photodisruption results in a lower stability of the complexes in the excited-state. Therefore, excitation of these complexes by an intense pulse of light is expected to cause some cations to leave the crown and diffuse away provided that the time constant for total release of the cation from the crown is shorter than the lifetime τ of the excited state (for a discussion on this point see Ref. [46]).

Intramolecular charge transfer in conjugated donor–acceptor molecules may be accompanied with internal rotation leading to twisted intramolecular charge transfer (TICT) states [60]. A dual fluorescence may be observed as in **PCT-9** [61] (which resembles the well-known DMABN containing a dimethylamino group instead of the monoaza-15-crown-5): the short-wavelength band corresponds to the fluorescence from the locally excited state and the long-wavelength band arises from a TICT state. The fluorescence intensity of the latter decreases upon cation binding because interaction between a bound cation and the crown nitrogen disfavors the formation of a TICT state, which leads to a concomitant increase of the short-wavelength band.

PCT-10 [62] is an analog of **PCT-9** in which the monoazacrown has been replaced by a tetraazacrown (cyclam) in order to promote complexation of transi-

Table 1
Wavelength of the absorption and emission maximum in acetonitrile for some crown-ether-linked cation sensors (the formulae are given in Fig. 12)[a]

	Absorption maximum (nm)		$\Delta\lambda$ (nm)	Emission maximum (nm)		$\Delta\lambda$ (nm)
	Free ligand	Complex with Ca^{2+}		Free ligand	Complex with Ca^{2+}	
PCT-1[b]	464	398	66	621	608	13
PCT-2[c]	392	330	62	525	503	22
PCT-3[c]	372	332	40	483	469	14
PCT-4[d]	521	411	110	600	591	9
PCT-5[e]	463	360	103	634	606	28
PCT-6[f]	360	315	45	420	~418	~2

[a] $\Delta\lambda$ represents the wavelength difference between the maximum for the free ligand and the maximum in the presence of an excess of calcium (full complexation).
[b] Ref. [49].
[c] Ref. [50].
[d] Ref. [51].
[e] Ref. [52].
[f] Ref. [53].

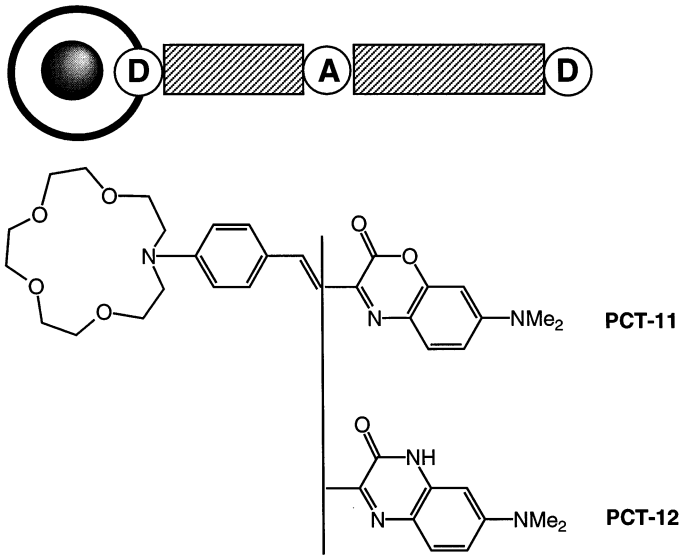


Fig. 13. Crown-containing PCT sensors in which the nitrogen of the crown plays the role of a second donor group with respect to the fluorophore.

tion metal ions. In this case, a triple fluorescence is observed: in addition to the emission from the locally excited and the TICT state, fluorescence is also emitted from an intramolecular exciplex (sandwich complex formed in the excited state thanks to the flexibility of cyclam). Such a triple fluorescence is solvent and pH dependent and is perturbed by cation binding. The relative changes of the bands depend on the nature of the cation.

The formation of a TICT state is often invoked even if no dual fluorescence is observed. For donor–acceptor stilbenes (**PCT-2**, and **PCT-3**), the proposed kinetic scheme [50] contains three states: the planar state E* reached upon excitation can lead to state P*(nonfluorescent) by double-bond twist, and to TICT state A* by single-bond twist, the latter being responsible for the main part of the emission. The existence of a fluorescent TICT state was also stated to be responsible for the photophysical properties of **PCT-5** [52]. The absence of fluorescence of **PCT-7** [63] may be due to the formation of a nonfluorescent TICT state, and an acridinium type fluorescence is recovered upon binding of H^+ and Ag^+.

In most cases, the changes in fluorescence intensity upon cation binding is not very large in these PCT molecular sensors (factors of ca. two to five) as compared to PET sensors. A remarkable exception is offered by **PCT-8** [64] whose electron-withdrawing group is boron-dipyrromethene: the fluorescence enhancement factor varies from 90 for Li^+ to 2250 for Mg^{2+}.

In contrast to the preceding PCT fluoroionophores, those shown in Fig. 13 undergo a large blue shift of the fluorescence spectrum upon cation binding. **PCT-11** [65–67] is one of the first crown-containing fluorescent PCT sensors that

has been designed. The fluorescence maximum shifts from 642 nm for the free ligand to 574 nm for the calcium complex in acetonitrile. Such a blue shift means that there is no photodisruption of the interaction between the cation and the nitrogen atom of the crown in this case in contrast to **PCT-1** to **PCT-6**, because the nitrogen atom of the crown plays the role of a second electron-donating atom with respect to the benzoxazinone moiety which is itself a donor–acceptor system. The existence of a fluorescent TICT state was shown to be likely in this compound [68]. **PCT-11** is not only responsive to alkaline-earth cations but also to divalent heavy metal ions Hg^{2+} and Pb^{2+} [69]. Environmental analytical applications are thus possible.

The structure of **PCT-12** [70] is very similar to that of **PCT-11**: the heterocyclic oxygen atom has been replaced by a nitrogen atom so that the dye is a benzodiazinone instead of a benzoxazinone. The cation-induced changes in photophysical properties and the complexing ability are comparable.

The above-described crown-containing fluoroionophores are of great interest for the understanding of cation–fluorophore interactions. They offer a large variety of photophysical changes upon cation binding that can be used for cation recognition. Their poor solubility in water precludes applications in aqueous solutions but they can be used for doping the sensitive part of optical sensor devices. However, the selectivity of azacrowns towards metal ions is not good enough when stringent discrimination between cations of the same chemical family is required.

Improvement of selectivity can be achieved by the participation of external groups as shown in Fig. 14. In **PCT-13** (PBFI) [71] and **PCT-14** (SBFI) [72], the oxygen atom of the methoxy substituent of the fluorophore can interact with a cation; binding efficiency and selectivity are thus better than those of the crown alone. SBFI has been designed for probing intracellular sodium ions and PBFI for potassium ions. In both compounds, the photophysical changes are likely to be due to the reduction of the electron-donating character of the nitrogen atoms of the diazacrown by the complexed cation. Further improvement of the selectivity towards K^+ with respect to Na^+ is desirable. **PCT-15** [73] resembles PBFI but it shows greater selectivity for potassium over sodium than PBFI.

3.2.2. Chelating PCT sensors

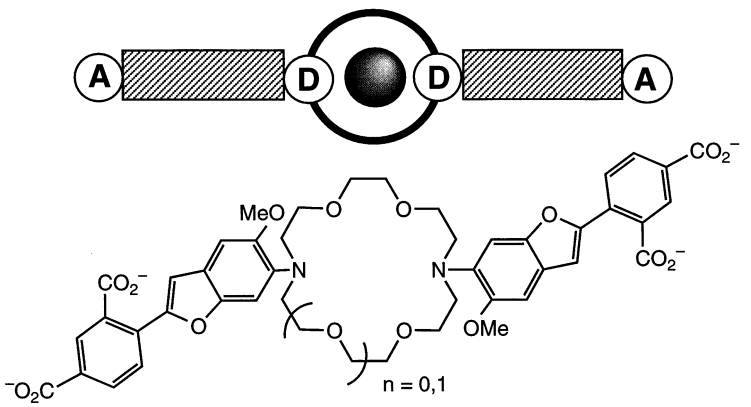
In light of the preceding considerations on crowned charge-transfer compounds, it is worth examining the photophysical properties of well-known chelators in the BAPTA series used for the recognition of cytosolic cations and in particuliar calcium ions [74]. Examples are given in Fig. 15. For instance **PCT-16** (Indo-1) and **PCT-21** (Fura-2) are widely used as calcium indicators. In this series, the fluorophore is a donor–acceptor molecule with an amino group as the electron-donating group which participates in the complexation, the ionophore being a chelating group of the BAPTA type. Upon complexation by Ca^{2+} in water, the absorption spectrum is blue-shifted, whereas there is almost no shift of the fluorescence spectrum, except for Indo-1 (see Table 2).

The same interpretation as for the above-described crown-ether-linked compounds can be proposed: the electron density of the nitrogen atom conjugated with

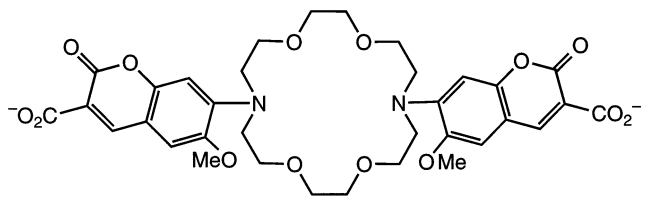
the electron-withdrawing group of the fluorophore is reduced upon excitation and might even become positively polarized; this causes disruption of the interaction between this nitrogen atom and a bound cation. Consequently, fluorescence emission closely resembles that of the free ligand. The possibility of photoejection has not been examined yet. Along this line, information on the stability of the complexes in the excited state, as compared with the ground state, is again of the utmost importance. A study of the fluorescence decay of Fura-2 with global compartmental analysis [75] showed that the stability constant of the calcium complex in the excited state is more than three orders of magnitude smaller than in the ground state.

Regarding the exception of Indo-1, the PCT may not be sufficient to cause nitrogen–Ca^{2+} bond breaking. This interpretation is consistent with the fact that the fluorescence maximum of free Indo-1 is located at a shorter wavelength than all the other ligands by ~ 30–60 nm, thus indicating a less polar charge-transfer state.

By keeping the same fluorophores, but reducing the 'cavity' size of the ionophore, one obtains **PCT-22** (Mag-Indo1) and **PCT-23** (Mag-Fura2) (Fig. 15) which are selective for magnesium [76]. Most of the chelators of Figs. 12 and 13 are commercially available in the nonfluorescent acetoxymethylester form so that they



n=1 : PBFI **PCT-13**
n =0 : SBFI **PCT-14**



PCT-15

Fig. 14. Crown-containing bifluophoric PCT sensors.

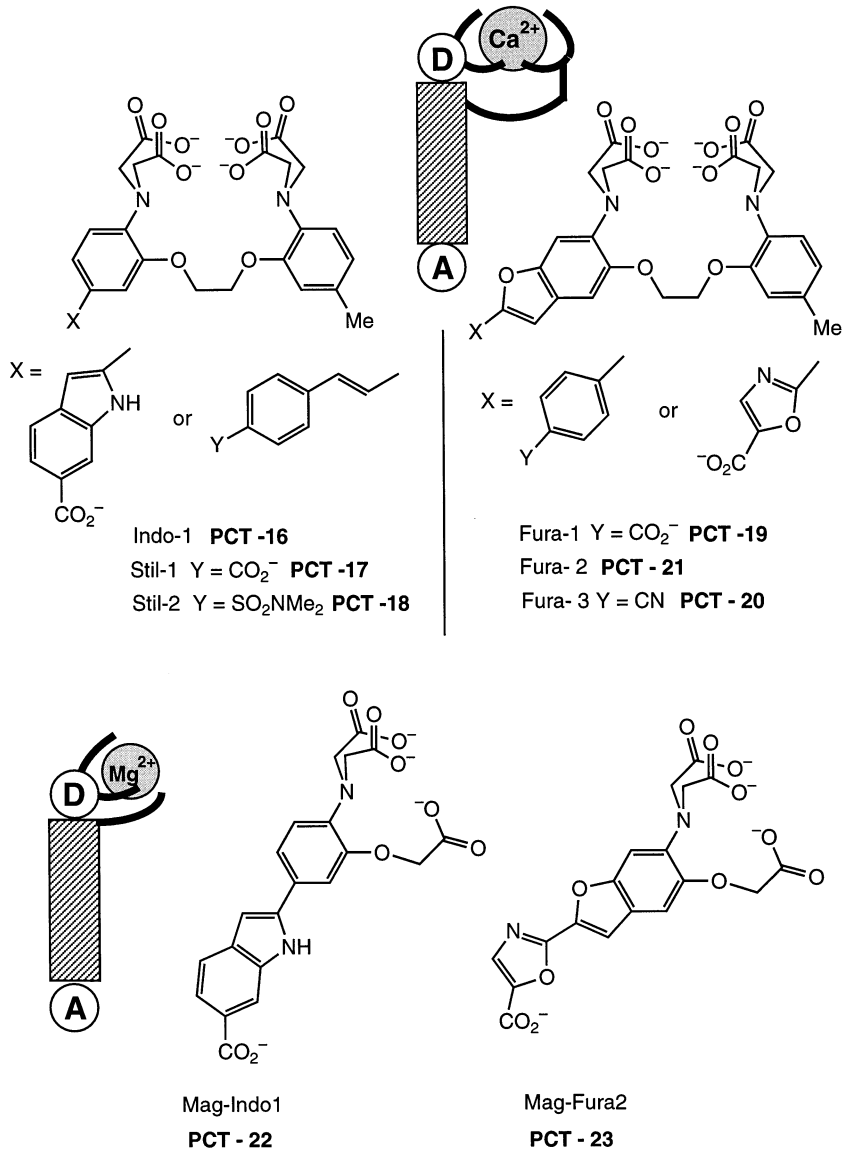


Fig. 15. Chelating PCT sensors.

are cell permeant and they recover their fluorescence upon hydrolysis by enzymes [76].

3.2.3. Cryptand-based PCT sensors

The above-described chelators are well suited for the detection of alkaline-earth cations but not alkali cations. In contrast, cryptands are very selective towards the latter.

PCT-24 (FCryp-2) [77] (Fig. 16) is a nice example of a fluorescent signaling receptor in which the ionophore moiety has been specially designed for determination of intracellular sodium free concentration. An indole derivative acts as the fluorophore: upon sodium binding, the emission maximum shifts from 460 to 395 nm and the fluorescence intensity increases 25-fold. The origin of these photophysical changes have not been studied so far. The large Stokes shift of the free ligand may be accounted for by PCT with concomitant internal rotation in the excited state leading to a TICT state. The blue shift of the emission spectrum upon sodium binding is likely to be due to the reduction of the electron-donating character of the dye-bound nitrogen atom of the cryptand.

Another example of a cryptand is **PCT-25** [78] (Fig. 16) which has potential applications as an extracellular probe of potassium. The dissociation constant in water is 1 mmol dm^{-3}, i.e. slightly lower than that of the coumarocryptand **PET-7** (see Section 2.3).

3.2.4. Calixarene-based PCT sensors

Examples of PCT sensors based on calixarene are given in Fig. 17. **PCT-26** [79] (Fig. 17) containing a benzothiazole group linked to calix[4]arene was found to be very selective towards Li^+. In a medium such that the phenolic group is not deprotonated, complexation with Li^+ induces a red shift of the emission spectra as a result of cation-induced proton ejection leading to a phenolate-cation pair. In the complex, Li^+ is also coordinated to two methoxy groups. No change of the emission spectra was observed with Na^+ and K^+.

Table 2
Wavelength of the absorption and emission maximum in water for various calcium chelators whose formulae are given in Fig. 15[a]

	Absorption maximum (nm)		$\Delta\lambda$ (nm)	Emission maximum (nm)		$\Delta\lambda$ (nm)
	Free ligand	Complex with Ca^{2+}		Free ligand	Complex with Ca^{2+}	
PCT-16 (Indo-1)	349	331	18	485	410	65
PCT-17 (Stil-1)	362	329	33	537	529	8
PCT-18 (Stil-2)	352	326	26	564	560	4
PCT-19 (Fura-1)	350	334	16	534	522	12
PCT-20 (Fura-2)	362	335	27	512	505	7
PCT-20 (Fura-3)	370	343	27	564	551	14

[a] $\Delta\lambda$ represents the wavelength difference between the maximum for the free ligand and the maximum in the presence of an excess of calcium (full complexation) (adapted from Ref. [74]).

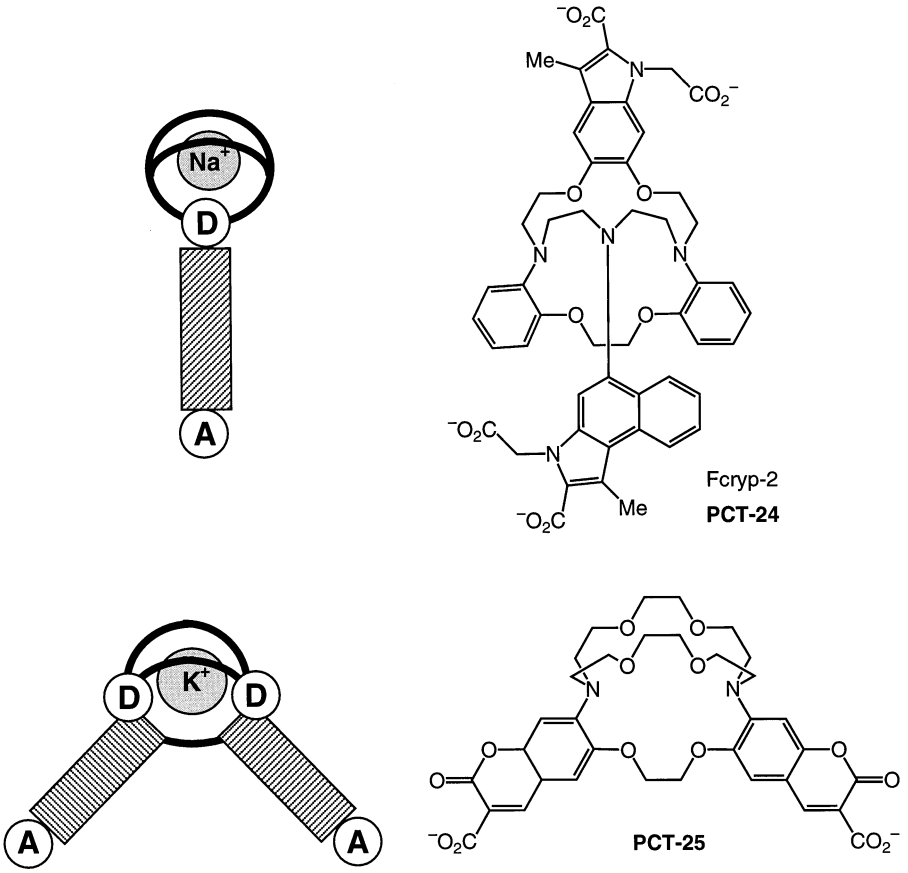


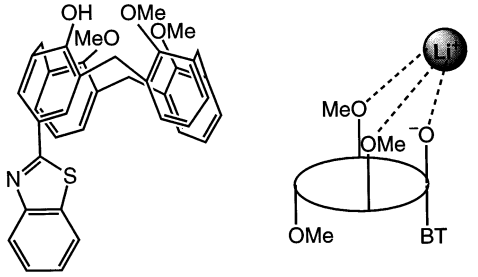
Fig. 16. Cryptand-based PCT sensors.

The fluorescence of thiacalix[4]arene **PCT-27** [80], with two appended dansyl moities, was shown to increase upon complexation of Cd^{2+}. Significant effects are also observed with Al^{3+}, Cr^{3+}, Zn^{2+} and Cu^{2+}. The dansyl fluorophore is well known for its sensitivity to polarity of the medium owing to intramolecular PCT. Consequently, the authors explained the cation-induced enhancement of fluorescence intensity by a change in polarity of the microenvironment of the dansyl moieties which are supposed to move from the outside bulk water towards the hydrophobic interior of the thiacalix[4]arene cavity. However, this explanation is not compatible with the small size of the cavity. The observed effects are more likely to be due to a cation–dipole interaction but further investigations are necessary to understand the excited-state processes.

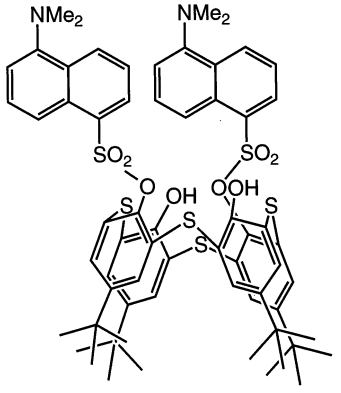
3.3. PCT sensors in which the bound cation interacts with an electron-withdrawing group

In contrast to the above described systems, there are only few systems in which the bound cation can interact with the acceptor part of charge-transfer probes. The case of coumarins linked to crowns is of special interest because the cation interacts directly with the electron-withdrawing group, i.e. the carbonyl group [81–84], in spite of the spacer between the fluorophore and the crown (Fig. 18). An important consequence is the increase in stability constant of the complexes with respect to the same crown without external complexing atoms.

Regarding the cation-induced photophysical changes, it should be kept in mind that the dipole moment of aminocoumarins in the excited state is larger than in the ground state because of the PCT occurring from the nitrogen atom of the julolidyl ring to the carbonyl group. Therefore, when a cation is coordinated with the carbonyl group, the excited state is more stabilized than the ground state so that both the absorption and emission spectra are red-shifted. The spectral red shifts observed with **PCT-30** on addition of cations are shown in Fig. 19.



PCT-26



PCT-27

Fig. 17. Calixarene-based PCT sensors.

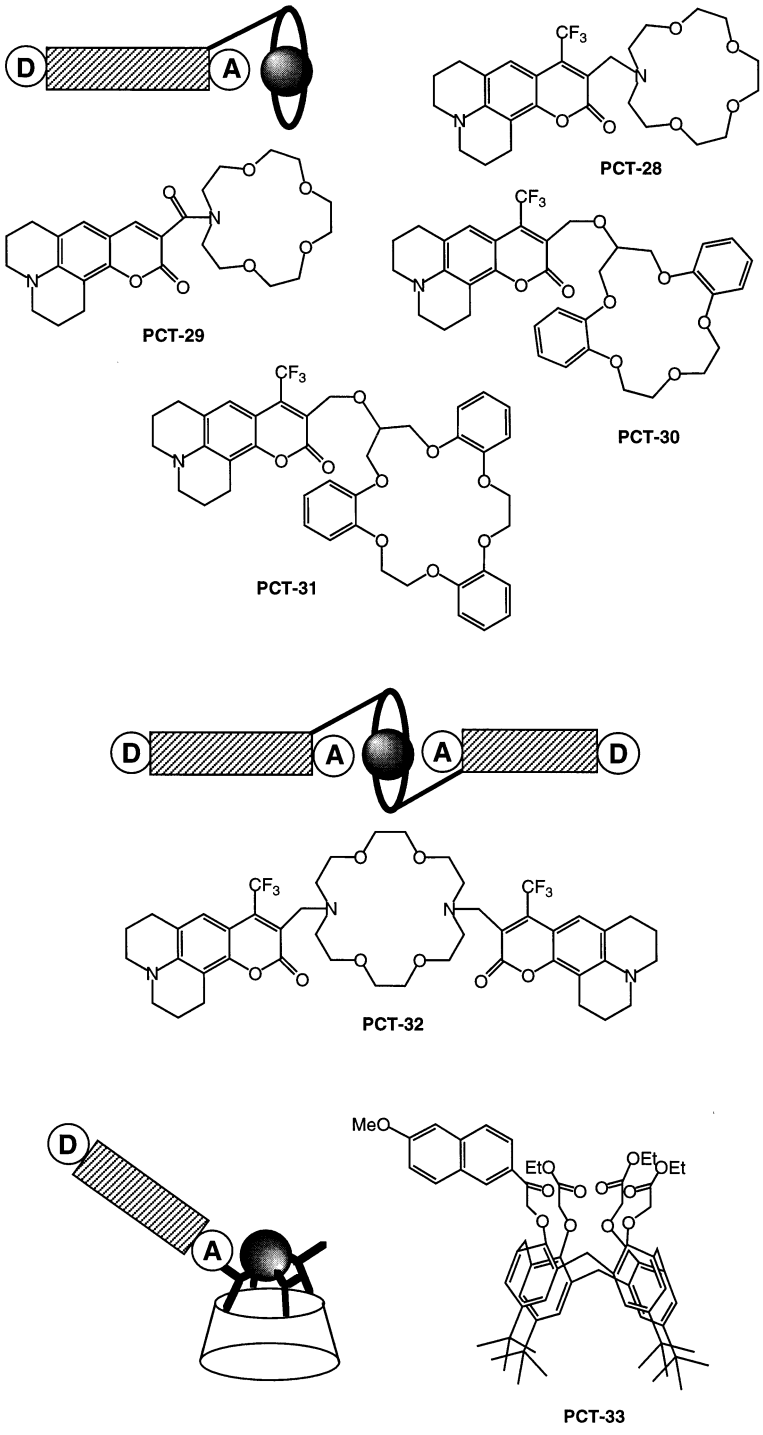


Fig. 18. PCT sensors in which the bound cation interacts with the acceptor group.

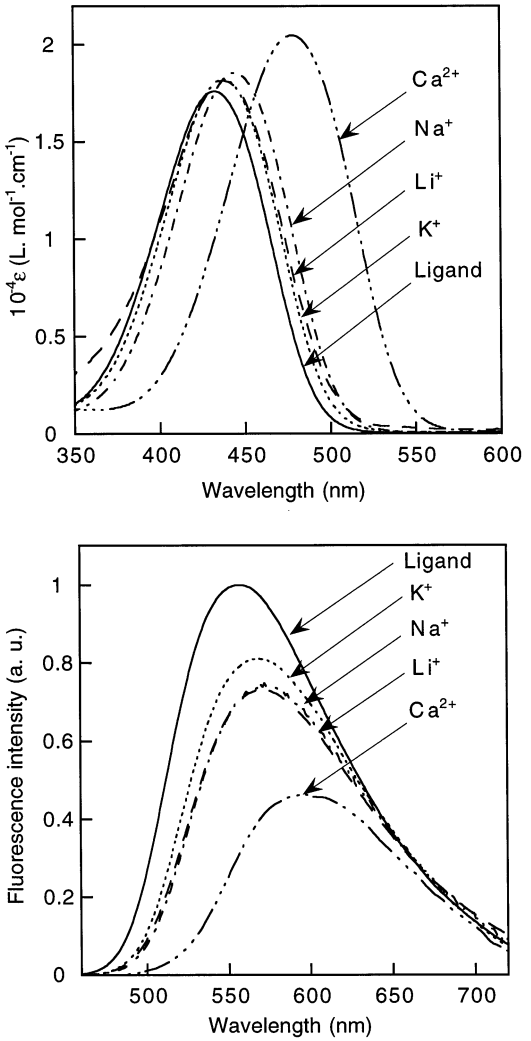


Fig. 19. Absorption (top) and fluorescence (bottom) spectra of **PCT-30** and its complexes in acetonitrile. The spectra of the magnesium complex is not shown because full complexation cannot be achieved upon addition of a reasonable amount of magnesium perchlorate.

The selectivity of **PCT-28** was in general found to be poor owing to the flexibility of the crown as well as around the spacer between the dye and the crown. Moreover, the nitrogen atom of the crown is easily protonable. The replacement of the methylene bridge of **PCT-28** by an amide bridge in **PCT-29** precludes pH sensitivity and leads to an improvement of the selectivity towards alkaline earth-metal ions with respect to alkali cations because the amide bridge is more rigid. Another way to improve the selectivity is to use a more rigid crown containing phenyl groups like dibenzocrown or tribenzocrown as expected from previous work

[85]. This can be achieved by replacing monoaza15-crown-5 by the more rigid dibenzocrown to give **PCT-30** (Valeur et al., to be published). In acetonitrile, this system was found to be selective for some cations, i.e. the selectivity expressed as the ratio of the stability constants is 12 500 for Ca^{2+}/Mg^{2+} and 16 for Na^{+}/K^{+}. The spectral shifts with cations are larger with **PCT-30** as compared to those observed with **PCT-28** and **PCT-29** and could be explained by the length of the bridge. One might expect that **PCT-30** adopts a conformation where the bound cation is closer to the carbonyl group as compared to the other systems.

A tribenzocrown has been covalently linked to coumarine to give **PCT-31** (Valeur et al., to be published). This complexing moiety is expected to be selective for K^{+}. The selectivity K^{+}/Na^{+} is only 2 in acetonitrile, but 20 in ethanol.

These examples show that the most important parameters responsible for selectivity in these crowned coumarins are (i) the rigidity of the link beween the fluorophore and the crown, (ii) the rigidity the crown itself and (iii) the size of the crown.

Additional photophysical effects can be observed when the fluoroionophore contains two fluorophores. In **PCT-32** [82], the carbonyl groups of the two coumarin moieties participate in the complexes: direct interaction between these groups and the cation explains the high stability constants and the photophysical changes. In addition to the shifts of the absorption and emission spectra, an interesting specific increase in the fluorescence quantum yield upon binding of K^{+} and Ba^{2+} ions has been observed: in the complexes with these ions that fit best into the crown cavity, the carbonyl groups of the two coumarins are preferentially on the opposite sides with respect to the cation and self quenching is thus partially or totally suppressed, whereas, with cations smaller than the cavity size of the crown, the preferred conformation of the relevant complexes may be such that the two carbonyls are on the same side and the close approach of the coumarin moieties accounts for static quenching.

Calixarene-based compound **PCT-33** [86] containing three ester groups and an appended naphthalenic fluorophore exhibits very interesting photophysical and complexing properties. Interaction of the cation with the carbonyl group of the fluorophore leads to a red shift of the absorption and emission spectra. A large enhancement of the fluorescence quantum yield has been observed under cation binding which can be explained in terms of the relative locations of the singlet $\pi\pi^*$ and $n\pi^*$ states. In the absence of the cation the lowest excited state has $n\pi^*$ character which results in an efficient intersystem crossing to the triplet state and consequently a low fluorescence quantum yield. In the presence of a cation which strongly interacts with the lone pair of the carbonyl group, the $n\pi^*$ state is likely to be shifted to higher energy so that the lowest excited state becomes $\pi\pi^*$. An outstanding selectivity Na^{+} versus K^{+} was found: the ratio of the stability constants is 1300 in a mixture of ethanol and water (60:40, v/v). Such a selectivity in the presence of water is very promising for practical applications in aqueous samples.

4. Excimer-based cation sensors

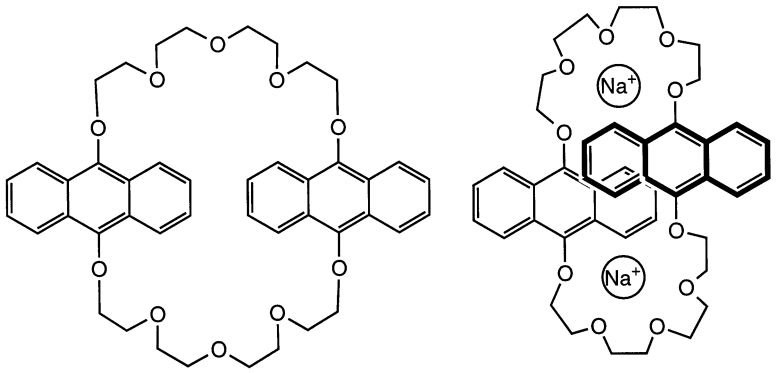
4.1. Principles

Several fluorophores like anthracene and pyrene can form excimer (excited dimer) when an excited molecule can come in close approach to another one during the lifetime of the excited state. Dual fluorescence is then observed with a monomer band and, at longer wavelengths, a structureless broad band due to excimer formation. The ratio of the fluorescence intensities corresponding to monomer and excimer emission on molecular mobility and 'microviscosity' [43].

When a fluoroionophore contains two fluorophores whose mutual distance is affected by cation complexation, recognition of this cation can be monitored by the monomer/excimer fluorescence–intensity ratio. Cation binding may favor or hinder excimer formation. In any case, such a ratiometric method allowing self-calibration measurement is of great interest for practical applications.

4.2. Coronands

The bisanthraceno-crown ether **E-1** [87–89] (Fig. 20) exhibits a fluorescence spectrum composed of the characteristic monomer and excimer bands. Gradual addition of sodium perchlorate to a solution in methanol induces a decrease in the monomer band and an increase in the excimer band. Complexation is indeed expected to bring closer together the two anthracene units which favors excimer formation. It was demonstrated [88] that a 1:2 complex is formed with Na^+ in methanol and acetonitrile with a positive cooperative effect. Interestingly, the overall stability constant obtained from absorption data was found to be lower than that from fluorescence data, which means that the complexing ability is larger in the excited state. **E-1** forms only a 1:1 complex with K^+.



E-1

Fig. 20. Excimer-forming bisanthraceno-crown sensor.

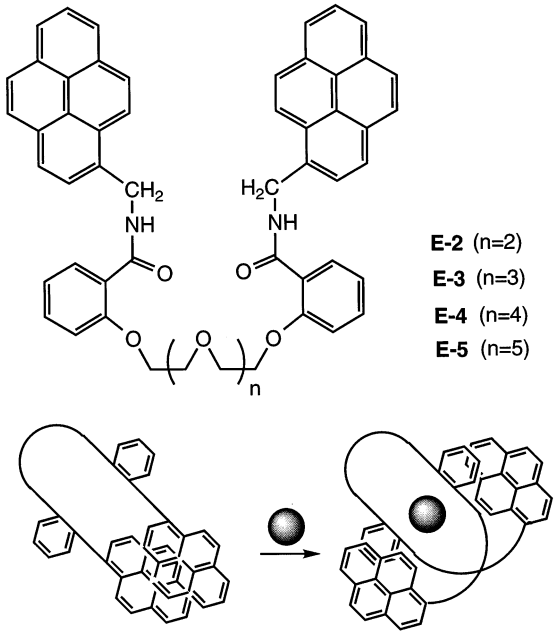


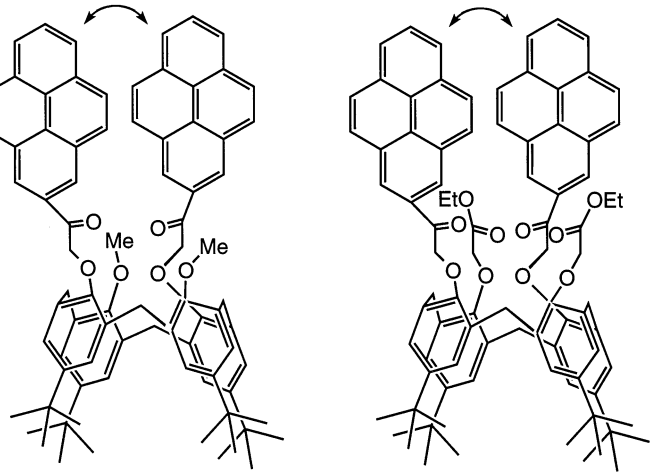
Fig. 21. Pseudocyclic structure of an excimer-forming podand.

4.3. Podands

The noncyclic ethers **E-2** to **E-5** [90] (Fig. 21) with two pyrenes linked at both ends of the chain show strong intramolecular excimer formation. Addition of alkaline-earth metal ions leads to an increase of monomer emission at the expense of the excimer band. Helical structure of the 1:1 complexes is supported by ^{1}NMR spectra. Thanks to the pseudocyclic structure, the stability constants of the complexes with Ca^{2+}, Sr^{2+} and Ba^{2+} in acetonitrile are quite high (ca. 10^6–10^7 for **E-5**), but the selectivity is poor as a consequence of the flexibility of the oxyethylene chain.

4.4. Calixarenes

E-6 [91] (Fig. 22) is the first example of an ionophoric calixarene with appended fluorophores showing the interest for this new class of fluorescent sensor. The lower rim contains two pyrene units that can form excimers in the absence of cation. Addition of alkali metal ions affects the monomer versus excimer emission (Fig. 23). According to the same principle, **E-7** [92] was designed for the recognition of Na^+; the Na^+/K^+ selectivity, as measured by the ratio of stability constants of the complexes was indeed found to be 154, while the affinity for Li^+ was too low to be determined.



E-6 E-7

Fig. 22. Excimer-forming calixarene-based sensors.

4.5. Cyclodextrins

Detection of cationic surfactants in environment is of major interest; their large toxicity is linked to a slow biodegradation owing to their bactericidal nature. Very sensitive detection is possible by means of a β-cyclodextrin derivative **E-8** [93], called CD-NA, bearing seven negatively charged naphthoate fluorophores (Fig. 24). Interaction with a cationic surfactant leads to the drop of excimer emission. The ratio of the fluorescence intensities of the monomer and excimer bands is directly

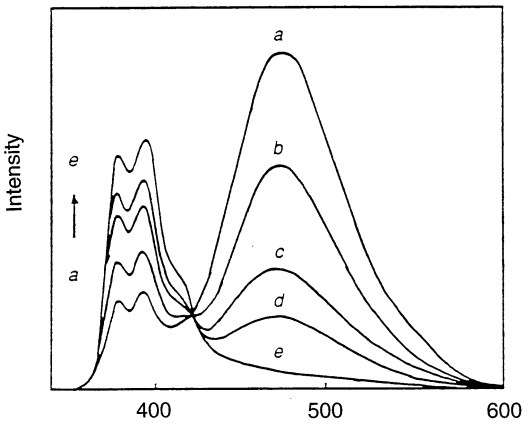


Fig. 23. Fluorescence spectra of **E-6** in diethyl ether at different LiSCN concentrations. [LiSCN] (mol l^{-1}) = (a) 0; (b) 1.0×10^{-5}; (c) 4.0×10^{-5}; (d) 1.0×10^{-4}; (e) 2.0×10^{-3} (from Ref. [92]. Reproduced by permission of the Royal Society of Chemistry).

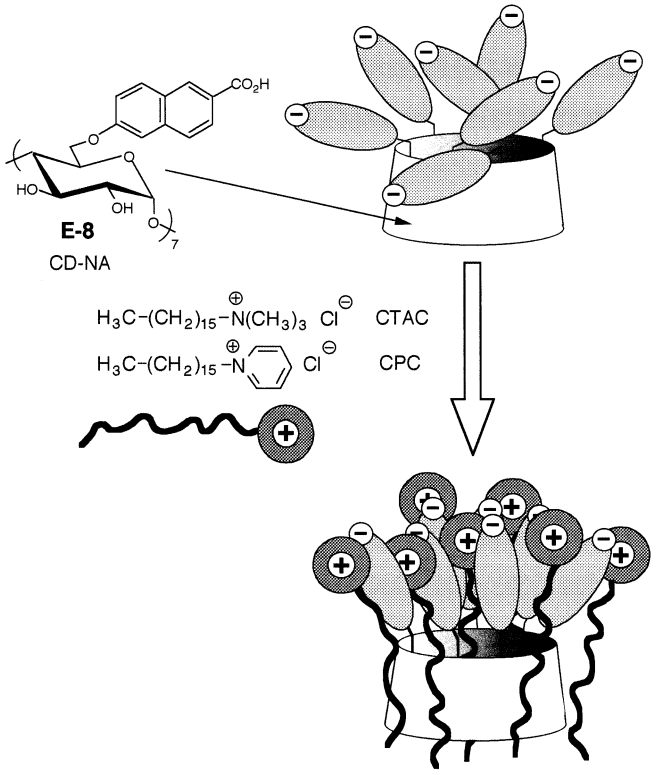


Fig. 24. Schematic illustration of the detection of cationic surfactants by a β-cyclodextrin with seven appended naphthoate fluorophores.

related to the concentration of the surfactant. In the case of electroactive surfactants such as cetylpyridinium chloride (CPC), the fluorescence quenching arising from PET can be additionally used for sensing. CD-NA permits detection of cetyltrimethylammonium chloride (CTAC) and cetylpyridinium chloride in an aqueous solution at concentrations as low as a few micromoles per liter and up to about 50 μmol l^{-1}. Interaction between CD-NA and cationic surfactants can be interpreted by a micellization process (Fig. 24) induced by CD-NA rather than by the formation of 1:1 inclusion complexes. It should be noted that addition of the anionic surfactant sodium dodecylsulphate does not induce any photophysical effect.

5. Miscellaneous

Many fluorescent probes exhibit cation-induced photophysical changes that cannot be explained along the same lines as those described in the preceding sections. Interaction of a cation with a fluorophore often leads to changes in

radiative, nonradiative and/or intersystem crossing rate constants, and consequently, changes in fluorescence quantum yield and lifetime (whereas shift of spectra is not a general rule).

5.1. Oxyquinoline-based fluorescent sensors

Various fluorescent chelators, podands and coronands containing oxyquinoline fluorophores are shown in Fig. 25. The first of them is 8-hydroxyquinoline **M-1** (8-HQ), often called oxine, and its derivatives, mainly 8-hydroxyquinoline-5-sulfonic acid **M-2** (8-HQS). 8-HQ is considered as the second chelating agent in importance after EDTA [94]. The most interesting feature of 8-HQ is its fluorogenic character, i.e. its very low quantum yield in aqueous or organic solutions and the fluorescence enhancement arising from cation binding. The nonfluorescent character of 8-HQ in neutral water has been satisfactorily explained only recently [46,95] by the occurrence of excited-state proton transfer reactions coupled to an intramolecular electron transfer. In fact, photoinduced deprotonation of the –OH group and protonation of the heterocyclic nitrogen atom can occur either with surrounding water molecules or intramolecularly, depending on the possible existence of H-bonding between the two functions. Deexcitation of the resulting tautomer (cetonic form) occurs mainly via a nonradiative pathway.

In contrast, many metal chelates of 8-HQ and 8-HQS (e.g. Cd, Zn, Mg, Al, Ga, In) exhibit intense yellow–green fluorescence because the above-described photoprocesses are impaired by a bound cation. However, the changes in electronic distribution upon excitation are likely to weaken the bond between the oxygen atom and the metal ion, thus allowing some charge transfer from the phenolate–O^- to the nitrogen atom of the adjacent ring. Weakening of this bond should be favored by the presence of water. It has been indeed observed that the metal chelates with 8-HQ or 8-HQS are more fluorescent in micellar media than in hydroorganic solvents [96] and even more fluorescent [97] in reverse micellar systems at low water content.

The selectivity of 8-HQ and 8-HQS is poor, but it can be improved by appropriate substitution on the oxygen atom to form acyclic polyethers containing two oxyquinoline fluorophores, as in compounds **M-3**, **M-4** [98], and **M-5** (Kryptofix 5, Merck) [99,100] shown in Fig. 25. The geometrical constraints in compounds **M-3** and **M-4** explain the excellent selectivity for the small lithium ion.

M-6 (Quin-2) was the first practical fluorescent indicator for cytosolic calcium with a simple 6-methoxyquinoline as its fluorophore (for a review, see Ref. [101]). Ca^{2+}-binding increases the fluorescence intensity about sixfold (without spectral displacement in contrast to Fura-2: see Section 3.2.2). The fluorescence lifetime of Quin-2 is highly sensitive to calcium concentration; Quin-2 can thus be used as a probe in the technique of fluorescence lifetime imaging [102].

Diaza-18-crown-6 substituted with 5-chloro-8-hydroxyquinoline exhibits very interesting complexing properties. For instance, **M-7** [103] is very selective for Ba^{2+} over other alkaline-earth cations and for K^+ over Na^+ in methanol. Unfortunately, investigation of fluorogenic effects with other cations has not been reported by the

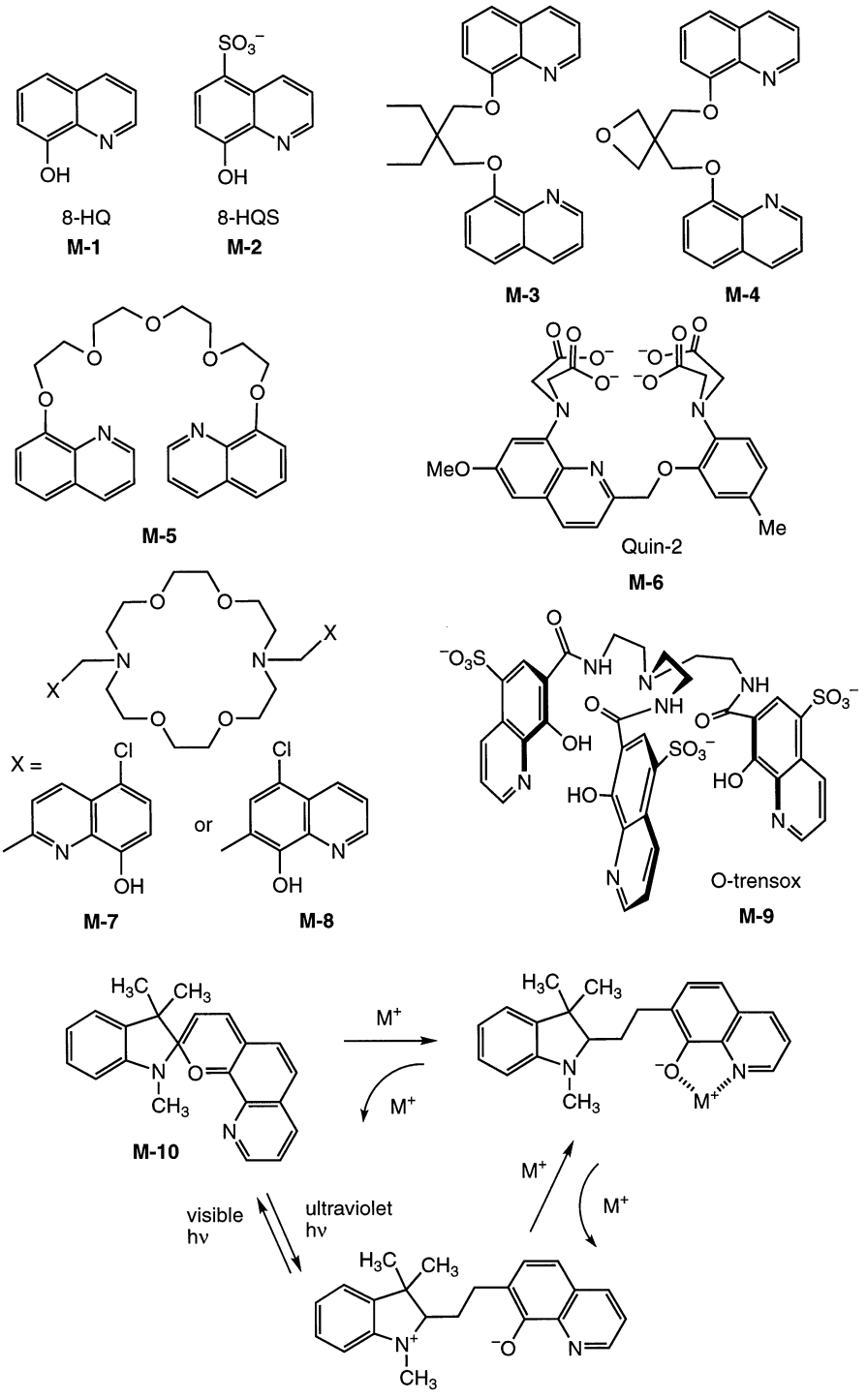


Fig. 25. Oxyquinoline-based chelators, podands and coronands.

authors. On the other hand, the fluorescence intensity of **M-8** [104] was shown to increase by a factor of 1000 in the presence of Mg^{2+} (in a mixture methanol/water, 1:1, v/v) whereas other alkaline-earth ions have no effect.

Another interesting example is the tripodand O-Trensox (**M-9**) in which three 8-HQS moieties are covalently linked. This compound was designed to achieve efficient complexation of both Fe^{II} and Fe^{III}; it turned out to be indeed an outstanding siderophore. A strong fluorogenic effect has been observed with the same cations as for 8-HQS, in particular with Al^{III} (Bardez et al., to be published).

Finally, two original photoisomerizable fluoroionophores based on quinolinospiropyranindoline have been designed [105]. One of them, **M-10**, is shown in Fig. 25. These systems operate by either photochemically- or chemically-induced reversible formation of merocyanine-oxyquinoline metal ion complexes.

5.2. Further calixarene-based fluorescent sensors

In calixarene-based compound **M-11** [106] (Fig. 26) bearing four anthracene moieties on the lower rim, some changes in fluorescence intensity were observed on binding of alkali metal ions but no excimer emission was detected. Quenching of the fluorescence by Na^+ may arise from interaction of four anthracene residues brought in closer proximity to one another; enhancement of fluorescence by K^+ is difficult to explain.

A fluorescent sensor for Na^+ has been developed using calix[4]arene tetraester linked with diaminopyridine **M-12** [107]. Complexation of Na^+ in the cavity induces a break of the intramolecular hydrogen bonding evolving with the diaminopyridine moieties and consequently reorientation to diamine pyridine to an external flavin was found to be possible. This effect results in fluorescence quenching.

An original fluoroionophore consisting of a conjugated poly(phenylene bithiophene), **M-13** [108] linked with calixarene was designed and tested with various cations. An outstanding selectivity towards Na^+ was observed, whereas Li^+, K^+ or Ca^{2+} induce negligible effects. The blue shift of the emission spectrum upon sodium binding appeared to be much more pronounced than with a monomeric model compound and no emissive point was observed. This interesting behavior may be due to migration of excitation energy to regions of the polymer which do not have bound Na^+ and can relax to lower energy conformations.

5.3. Semaphorene

It is appropriate to end this review on signaling fluorescent sensors with a semaphore-shaped compound. **M-14** [109] (Fig. 27), called semaphorene, belongs to the anthraceno-cryptand family (see **PET-5**) but it exhibits quite different photophysical features because the nitrogen lone-pairs can approach essentially the less reactive 1,4 positions, the fluorescence quantum yield being high in all solvents. Interestingly, the dipole moment in the excited state in much larger than in the ground state ($\Delta\mu \approx 10$ Debye). The changes in UV and fluorescence spectra upon

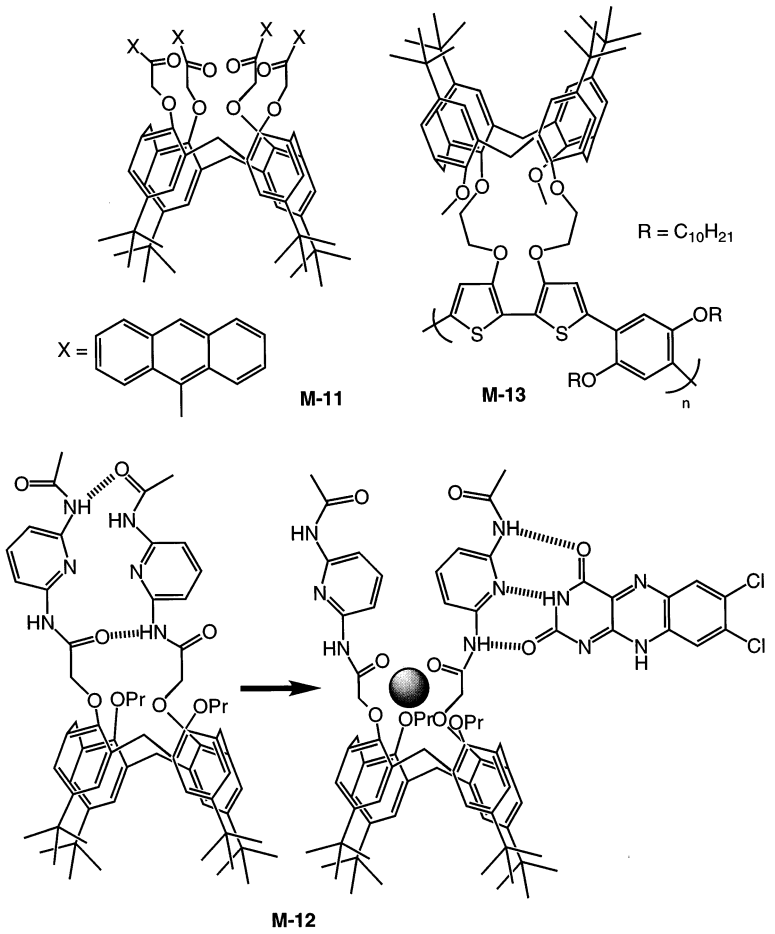
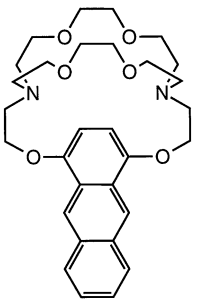


Fig. 26. Calixarene-based sensors.



M-14

Fig. 27. Structure of semaphorene.

cation binding can be explained by the relief of the interaction between the nitrogen lone-pairs and anthracene. In particular, a blue shift of the fluorescence spectrum was observed; the largest shift resulting from protonation of the nitrogen atoms supports this interpretation. Therefore, the strongest effects are expected from cations that have a high affinity for the nitrogens. In particular, the soft cations Ag^+ and Tl^+ lead to very stable complexes in methanol in accordance with their affinity for the nitrogen lone-pairs and π clouds that coordinate the cation. It should be noted that semaphorene is very sensitive to the presence of protons.

6. Concluding remarks

The present review illustrates the immense variety of fluoroionophores that have been designed for cation recognition. Emphasis was put on the understanding of cation-induced photophysical changes which should help the user and the designer of this kind of sensor.

A distinct advantage of PET sensors is the very large change in fluorescence intensity usually observed upon cation binding, so that the expression 'off–on' and 'on–off' fluorescent sensors is often employed. Another characteristic is the absence of shift of the fluorescence or excitation spectra which precludes the possibility of intensity-ratio measurements at two wavelengths. Furthermore, PET often arises from a tertiary amine whose pH sensitivity may affect the response to cations. Nevertheless, pH sensitivity around neutral pH can be avoided by working with substituted anilines (whose pK is ca. 5.5) instead of aliphatic amines.

In PCT sensors, the changes in fluorescence quantum yield on cation complexation are generally not very large as compared to those observed with PET sensors. Nevertheless, exceptions can be found (see **PCT-8** and **PCT-33**). However, the absorption and fluorescence spectra are shifted upon cation binding so that an appropriate choice of the excitation and observation wavelengths often allows one to observe quite large changes in fluorescence intensity. Moreover, ratiometric measurements are possible: the ratio of the fluorescence intensities at two appropriate emission or excitation wavelengths provides a measure of the cation concentration which is independent of the probe concentration (provided that the ion is in excess) and is insensitive to the intensity of incident light, scattering, inner-filter effects and photobleaching. Ratiometric measurements are also possible with excimer-based sensors.

It should be emphasized that progress is still to be made regarding the selectivity towards a given cation in a specific range of concentration which can be very different according to the application. In this respect, the choice of the recognition moiety is of major importance but it is important to note that the fluorophore itself often participates in the complexation and thus plays a role in the selectivity. Cryptands are known to be very selective of alkali ions but they often contain a tertiary amine which is pH sensitive. Calixarenes with appropriate appended groups including fluorophores turn out to be a most promising class of fluoroionophores because they have great potential in terms of molecular design. Several examples of outstanding selectivity have already been reported.

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