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The Molecular Design of Fluorescent Sensors for Ionic Analytes

Luigi Fabbrizzi,[1] Maurizio Licchelli,[1] Luisa Parodi,[1] Antonio Poggi,[1] and Angelo Taglietti[1]

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Molecular fluorescent sensors can be synthesized by covalently linking a photoactive fragment (e.g., anthracene) to a receptor subunit displaying affinity toward the envisaged substrate. The electron transfer process is the privileged signal transduction mechanism: redox active substrates (e.g., transition metals) typically release/uptake an electron to/from the proximate photoexcited fluorophore, the recognition being signaled through fluorescence quenching; redox inactive substrates (d^0 and d^{10} metals, H^+) deactivate an existing quenching relay (e.g., a tertiary nitrogen atom close to the fluorophore) and their recognition is signaled through fluorescence enhancement. Anionic substrates can be conveniently recognized on the basis of the metal–ligand interaction: polyamine receptors containing the photophysically inactive Zn^{II} ion bind the carboxylate group. In the case of amino acids, NH_3^+–CH(**R**)–COO^-, selectivity is improved when the receptor platform bears additional groups capable to interact specifically with the **R** substituent. If **R** is capable of transferring an electron to the nearby photoexcited fluorophore, the recognition is signaled through fluorescence quenching.

KEY WORDS: Fluorosensors; electron transfer; anthracene; metal ions; amino acids.

LINKING A RECEPTOR AND A FLUOROPHORE TO BUILD A MOLECULAR SENSOR

The interaction of a light-emitting molecule with a given substrate in solution may cause the change of the intensity of its emission (either enhancement or quenching), a process which, in principle, can be used for analytical purposes. A classical example of fluorescence quenching refers to the interaction of anthracene (An) with *N,N*-dimethylaniline (DMA), in an ethanolic solution [1]. On successive additions of DMA, the intensity of anthracene emission bands progressively decreases.

The Stern–Volmer plot, i.e., the plot of I_0/I (intensity of the maximum emission, at 400 nm; I_0 intensity before DMA addition; I, intensity after each addition) vs DMA molar concentration, as shown in Fig. 1, is linear, indicating that a *dynamic* quenching mechanism is operative. Quenching is due to the transfer of an electron from the donor molecule DMA to the photoexcited fluorophore An*. The occurrence of a DMA-to-An* electron transfer (eT) process is accounted for on a thermodynamic basis. In particular, the associated ΔG°_{eT} value can be calculated from the combination of pertinent photophysical and electrochemical quantities, through the thermodynamic cycle reported in Fig. 2. Specifically, the spectroscopic energy $E^{0\text{-}0}$ is obtained from the frequency of the emission band at 386 nm, whereas the electrode potentials $E^\circ(An/An^-)$ and $E^\circ(DMA^+/DMA)$ can be obtained from cyclic voltammetry experiments (e is the electron charge). The distinctly negative ΔG°_{eT} value ($= -E^{0\text{-}0} - eE^\circ(An/An^-) + eE^\circ(DMA^+/DMA) = -0.4\ eV$) guarantees the occurrence of the DMA-to-An* eT process.

The procedure is simple, the Stern–Volmer plot is beautifully linear, and the explanation of the quenching

[1] Dipartimento di Chimica Generale, Università di Pavia, via Taramelli 12, I-27100 Pavia, Italy.

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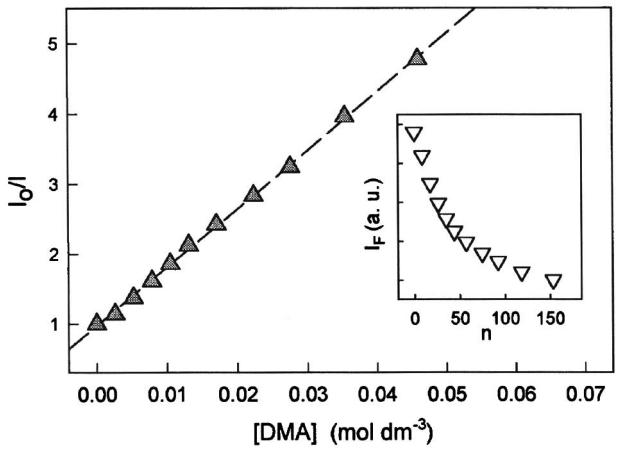
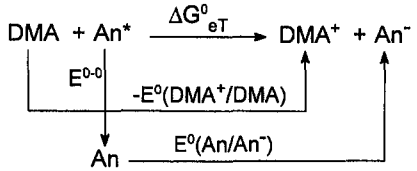


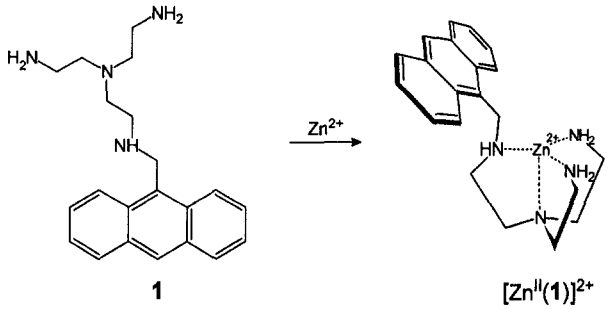
Fig. 1. Stern–Volmer plot for the titration of anthracene (An) with *N,N*-dimethylaniline (DMA). The concentration of An in the ethanolic solution is 3×10^{-5} mol dm^{-3}. Inset: The fluorescence intensity is plotted vs n = (mol of DMA)/(mol of An): a 150-fold excess of DMA is required to quench An fluorescence.



$$\Delta G^0_{eT} = -[E^{0\text{-}0} - eE^0(DMA^+/DMA) + eE^0(An/An^-)] = -0.4 \text{ eV}$$

Fig. 2. Thermodynamic cycle for the calculation of the free energy change ΔG°_{eT} associated with the DMA-to-An* photoinduced electron transfer process. $E^{0\text{-}0}$ is the spectroscopic energy of anthracene, E° are standard reduction potentials for both the An/An$^-$ couple and the DMA$^+$/DMA couple, and e is the electron charge.

mechanism is convincing. In fact, an experiment like this can be proposed to students as an exercise in a course of photochemistry. But, definitely, it cannot be suggested to an analytical chemist as a way to determine DMA in solution. The inset in Fig. 1 indicates that almost-complete quenching of the fluorophore ($I_F \leq 5\%$) is achieved after the addition of 150 equiv. On the contrary, a spectrofluorimetric titration experiment would require that quenching of fluorescence occurs at a 1:1 fluorophore/analyte ratio (the equivalent point). This is not the case for the experiment described in Figs. 1 and 2, in which An and DMA molecules interact very weakly and only a small fraction of them forms an {An*–DMA} pair lasting long enough to allow the eT process responsible for fluorescence quenching to take place. In order to design an analytically successful titration experiment, one should engineer the An and DMA fragments in such a way that they interact in a permanent way, thus allowing the occurrence of an easy and efficient eT process. In the following, we describe an experiment which profits from the metal–ligand interaction. In particular, DMA is functionalized with a $-COO^-$ group in the 4-position (to give 4-*N,N*-dimethylaminebenzoate, DMA–COO^-), whereas the anthracene fragment is appended to one of the terminal amine groups of the tripodal tetraamine *tren,* to give **1** (trenAn) [2].



1 $[Zn^{II}(\mathbf{1})]^{2+}$

Then, on addition of 1 equiv of Zn^{II}, the trenAn molecule organizes itself around the metal center: a five-coordinate metal complex is formed, in which the amine groups occupy four coordination sites of a trigonal bipyramidal coordination polyhedron. One of the axial positions is left vacant: it can be temporarily occupied by a solvent molecule or by an anion coming from the solution. Zinc(II) tetraamine systems display a special affinity toward the $-COO^-$ group. This leads to a situation favorable for a spectrofluorimetric titration experiment (Fig. 3).

The $[Zn^{II}(\mathbf{1})]^{2+}$ complex, in an ethanolic solution, shows the typical emission spectrum of anthracene. On addition of DMA–COO^-, the fluorescence intensity, I_F, decreases to reach complete quenching ($I_F \leq 5\%$) with the addition of 3 equiv. Very interestingly, the I_F vs. equiv. profile indicates the formation of a 1:1 adduct, $[Zn^{II}(\mathbf{1})(DMA\text{–}COO)]^+$, whose formation constant, as calculated through a nonlinear least-squares procedure, is 5.45 log units. The efficiency of the quenching process is due to the fact that coordination of the aromatic carboxylate to the Zn^{II} center brings the $-N(CH_3)_2$ donor group in front of the fluorophore, allowing the occurrence of a fast and efficient eT mechanism, as illustrated in Fig. 4.

The system $[Zn^{II}(\mathbf{1})]^{2+}$ can be considered as the prototype of a fluorescent sensor for aromatic carboxylates: it possesses (i) a subunit (the Zn^{II}–tren fragment) capable of interacting with the analyte and (ii) a subunit (the anthracene moiety) which signals the occurrence of the recognition process. The system works because the analyte itself activates the signaling mechanism, by trans-

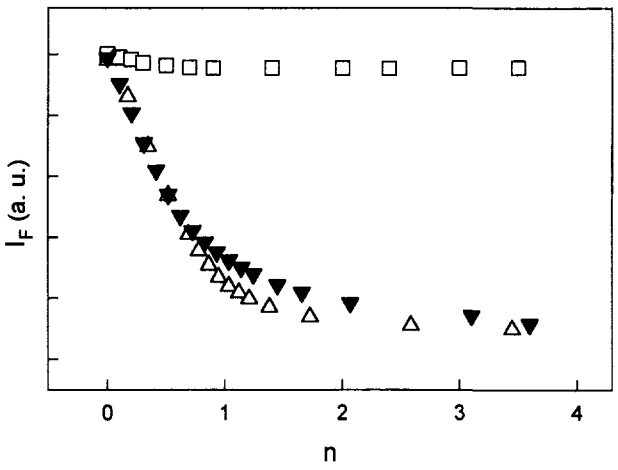


Fig. 3. Titration of a methanolic solution of the $[Zn^{II}(\mathbf{1})]^{2+}$ receptor with 4-substituted benzoates: 4-*N*,*N*-dimethylaminobenzoate (open triangles) and 4-nitrobenzoate (filled triangles) quench the fluoresence of the anthracene subunit of **1** via an eT mechanism. In both cases, a stable $[Zn^{II}(\mathbf{1})(X)]^{+}$ adduct is formed. Plain benzoate forms a stable adduct too but does not quench fluorescence.

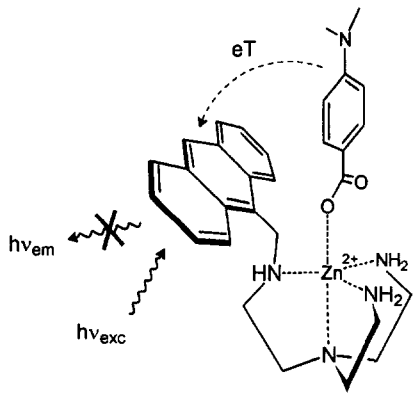


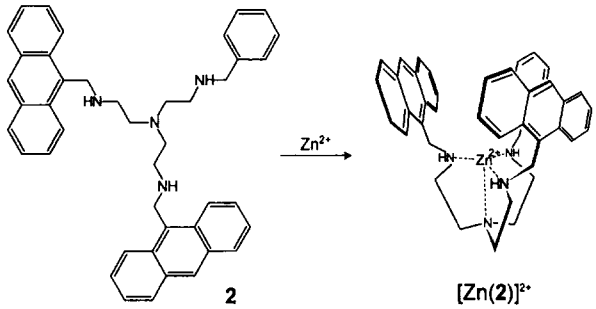
Fig. 4. Coordination to the Zn^{II} center places the dimethylaniline subunit in a favorable position to transfer an electron to the facing photoexcited anthracene fragment. This quenches fluorescence.

ferring an electron to the proximate photoexcited fluorophore. Noticeably, a similar behavior (formation of a 1:1 adduct $[Zn^{II}(\mathbf{1})(X)]^{+}$, monitored through fluorescence quenching) is observed with 4-nitrobenzoate anion: in this case the fluorescence quenching mechanism is provided by an eT process from the photoexcited fluorophore to the coordinated benzoate, which displays electron acceptor tendencies ($\Delta G^{\circ}_{eT} = -1.0$ eV). Notice that plain benzoate anion binds the Zn^{II} center but does not modify at all the emission of the anthracene subunit.

The examples discussed above help to illustrate what we define as the two-component approach to the design of a molecular fluorescent sensor: in particular, one should link covalently a receptor subunit capable of interacting with the envisaged substrate to a fluorescent fragment [3]. Sensor efficiency requires that (i) the receptor–analyte interaction is very selective, possibly specific, and (ii) a mechanism exists that drastically changes the emission of the fluorophore, following the recognition process. As far as point (ii) is concerned, the eT mechanism appears especially convenient and versatile, as we try to demonstrate with examples involving varying analytes: amino acids, metal ions, and protons.

A FLUORESCENT SENSOR FOR AMINO ACIDS BEARING AROMATIC SUBSTITUENTS

Since the Zn^{II} center displays affinity toward the carboxylate group, one could suggest the utilization of the two-component system $[Zn^{II}(\mathbf{1})]^{2+}$ as a receptor, and possibly as a fluorescent sensor, for any substrate bearing a $-COO^{-}$ fragment, in particular amino acids. In this connection $[Zn^{II}(\mathbf{1})]^{2+}$ in an ethanol/water mixture (4:1, v/v), buffered to pH ≥7 (the lowest pH value at which the Zn^{II} complex is completely formed), gives poorly stable 1:1 adducts with natural amino acids: the log*K* values are in any case <2, independent of the nature of the amino acid. The rather low affinity is to be ascribed to the electrostatic repulsion between the metal center and the ammonium group of the amino acid. In order to increase the adduct stability, one should engineer the receptor portion of the system by inserting further binding sites. To obtain selectivity, these groups should display specific affinity toward the investigated amino acid.



A successful example from our laboratory, not yet published, refers to system **2,** in which the tren framework has been armed with two antracenyl and one benzyl substituents [3]. The corresponding Zn^{II} complex, $[Zn^{II}(\mathbf{2})]^{2+}$, displays the typical anthracene emission and no spectral evidence exists for the formation of an excimer. $[Zn^{II}(\mathbf{2})]^{2+}$ shows a high affinity toward those natural amino acids that bear aromatic substituents:

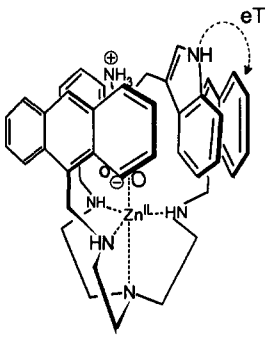


Fig. 5. Multipoint molecular recognition of tryptophane (trp) by the $[Zn^{II}(\mathbf{2})]^{2+}$ system. The $-COO^-$ group of the amino acid is coordinated to the metal center, whereas its aromatic moiety is involved in a π-stacking interaction with the anthracene substituents on the tetramine. Recognition is signaled through fluorescence quenching, due to an electron transfer process from the donor indole moiety of trp to a nearby photoexcited anthracene fragment.

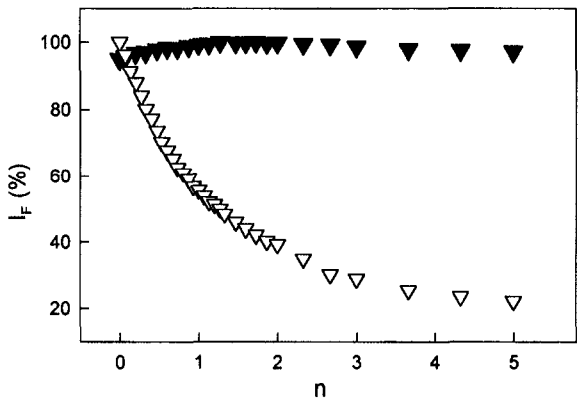


Fig. 6. Titration of a methanolic solution of the $[Zn^{II}(\mathbf{2})]^{2+}$ receptor with tryptophane (trp) and phenylalanine (phe). Both amino acids form stable 1:1 adducts, but only trp is able to quench the fluorescence of the nearby photoexcited An fragment (open triangles). Phe does not exhibit electron donor tendencies and cannot modify the emission of the facing An subunit (filled triangles).

phenylalanine (phe) and tryptophane (trp). The log$K$ values associated with the formation of the $[Zn^{II}(\mathbf{2})(phe)]^{2+}$ and $[Zn^{II}(\mathbf{2})(trp)]^{2+}$ adducts, in a 4:1 ethanol/water mixture, buffered to pH = 6.8, are 4.48 and 4.21, respectively (calculated through spectrophotometric titration experiments). These values are distinctly higher than those observed for all the other amino acids (e.g., glycine, log$K$ = 3.06).

The rather high solution stability of $[Zn^{II}(\mathbf{2})(phe)]^{2+}$ and $[Zn^{II}(\mathbf{2})(trp)]^{2+}$ adducts is due to the existence of π-stacking interactions between the aromatic part of the Zn^{II}-bound amino acid and one of the facing polyaromatic substituents of the tren framework. The binding situation in $[Zn^{II}(\mathbf{2})(trp)]^{2+}$ is sketched in Fig. 5.

Let us now consider the utilization of $[Zn^{II}(\mathbf{2})]^{2+}$ as a fluorescent sensor. Titration of an aqueous ethanolic solution of $[Zn^{II}(\mathbf{2})]^{2+}$ with trp induces fluorescence quenching. The titration profile, shown in Fig. 6, corresponds to the formation of a 1:1 adduct, whose log$K$ value is coincident with that obtained from the spectrophotometric titration. On the contrary, titration with the other amino acid bearing an aromatic substituent, phe, does not alter the anthracene emission of $[Zn^{II}(\mathbf{2})]^{2+}$. The signaling mechanism operating in the $[Zn^{II}(\mathbf{2})(trp)]^{2+}$ adduct is brought by the analyte itself. The indole substituent of trp possesses electron donor tendencies and fluorescence quenching is due to a "through-space" eT process from the secondary amine nitrogen atom of the trp subunit to the facing photoexcited An fragment. Such a mechanism cannot be provided by the other recognized amino acid, phe, whose substituent (a phenyl group) does not exhibit electron donor properties.

The selective behavior of the $[Zn^{II}(\mathbf{2})]^{2+}$ receptor derives from its capability to establish two distinct interactions with the $NH_3^+CH(R)COO^-$ substrate: (i) the Zn^{II}–COO^- metal–ligand interaction and (ii) the π-stacking interaction between R and aromatic substituents present on the receptor framework. The example above agrees well with the general rule of molecular recognition: the higher the number of the interaction points between receptor and substrate, the higher the selectivity [5].

eT FLUOROSENSORS FOR REDOX ACTIVE METAL IONS

The two-component approach outlined in the previous sections can be easily transferred to the design of fluorosensors for metal ions. In this case, the fluorescent fragment has to be linked to a receptor subunit displaying affinity toward the envisaged metal (a multidentate ligand, of either cyclic or noncyclic nature). If we still intend to profit from an eT-based signal transduction mechanism, the metal ion itself, when bound to the receptor moiety, must be able to release/uptake one electron to/from the proximate photoexcited fluorophore. This is a commonplace situation for transition metal ions, which typically display a rich one-electron redox activity. Electron transfer tendencies can be exalted and addressed through the coordination by the ligand acting as a receptor.

Let us consider the fluorescent sensing of 3d divalent metal ions (Fig. 7). In system **3**, an anthracene

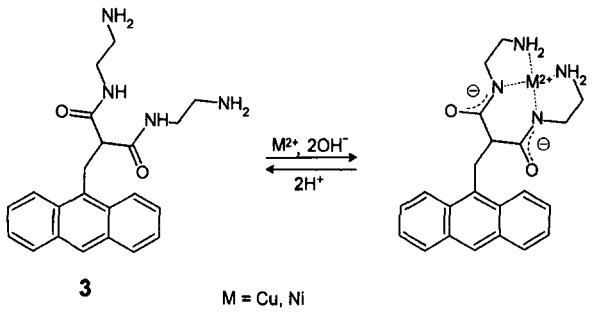


Fig. 7. Recognition and sensing of transition metals. At a given pH, the diamide–diamine receptor subunit of system **3** chelates the divalent metal center and undergoes simultaneous deprotonation of the amide groups. The endothermic effect associated with the latter process is more than compensated only by metals late in the 3d series (Cu^{II} and Ni^{II}) but not by earlier metals (Mn^{II}, Fe^{II}, Co^{II}), which do not profit enough from ligand field effects. Thus, the receptor recognizes the position of metal centers in the Periodic Table.

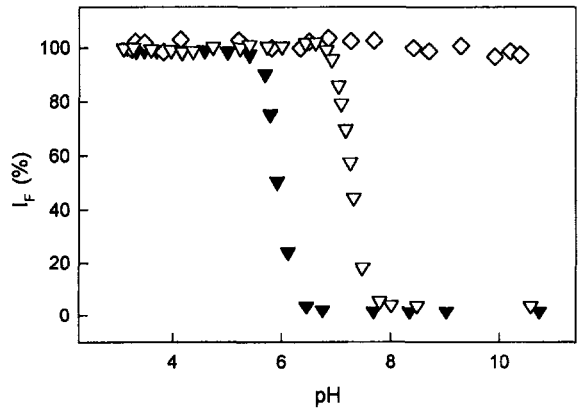


Fig. 8. Spectrofluorimetric titration with a standard base of (i) a solution containing **3** plus excess acid (diamonds); (ii) a solution containing **3**, 1 equiv of Cu^{II}, plus excess acid (filled triangles); and (iii) a solution containing **3**, 1 equiv of Ni^{II}, plus excess acid (open triangles). Titration in the presence of any other 3d metal (Mn^{II}, Fe^{II}, Co^{II}, or Zn^{II}) does not alter anthracene emission (same profile as in i). Medium: MeCN/water, 4:1 (v/v).

fragment has been linked through a $-CH_2-$ group to a quadridentate ligand containing two amide groups and two terminal primary amine groups [6]. In the presence of a divalent metal ion M^{II} (M = Cu, Ni), the two amide groups deprotonate and the quadridentate ligand chelates the metal center, according to a square stereochemistry. The complexation process in an MeCN/water solution (4:1, v/v) can be followed spectrofluorimetrically. In particular, the cuvette contains **3**, 1 equiv of Cu^{II} plus excess acid, and standard NaOH is added as a titrating agent.

In an acidic solution, the uncomplexed system **3** displays the full anthracene emission (see the I_F vs pH profile in Fig. 8). At pH=5.5, I_F starts decreasing according to a symmetric sigmoidal profile, and at pH = 6.5 it has a value lower than 5%. Fluorescence quenching is associated with the complexation of the Cu^{II} ion by the diamide–diamine chelating subunit, which involves the simultaneous deprotonation of the two amide groups, and is due to the transfer of an electron from the metal center to the photoexcited anthracene fragment. The Cu^{II}-to-An* eT process is characterized by a distinctly negative free energy change value ($\Delta G^{\circ}_{eT} = -0.5$ eV). It must be observed that the square coordination by two deprotonated amide and two amine groups favors the access to unusually high oxidation states of the chelated metal and, in particular, makes the otherwise arduous Cu^{II}-to-Cu^{III} process especially easy ($E^{\circ}_{Cu^{III}/Cu^{II}}$ = 0.6 V vs NHE, in MeCN).

A similar behavior is observed if Cu^{II} is replaced by Ni^{II} in the cuvette, but the I_F vs pH profile is displaced to higher pH values (see Fig. 8): this simply reflects the higher stability of the Cu^{II}–receptor complex with respect to Ni^{II}. Also, in this case, the quenching of anthracene fluorescence is due to a metal-to-fluorophore eT mechanism. In particular, the ΔG°_{eT} value associated with the Ni^{II}–to-An* process is quite negative (−0.35 eV).

If the titration is carried out in the presence of other divalent 3d metal ions (Mn^{II}, Fe^{II}, Co^{II}), the anthracene emission is maintained over the entire pH range. The reason is simple: the complexation involves the deprotonation of the amide groups, an especially endothermic process. The endothermic effect is more than compensated for only by metals late in the 3d series (Cu^{II} and Ni^{II}), and not by earlier metals, which do not profit enough from ligand field effects.

Thus, each component of sensor **3** does an excellent job: the receptor efficiently discriminates metal centers, in particular, recognizing their position in the Periodic Table. The fluorophore signals the recognition by switching off its emission. Further discrimination between Cu^{II} and Ni^{II} can be operated by taking into account the different stability of the metal–receptor complexes with respect to pH. The two separated I_F vs pH profiles in Fig. 8 indicate that at pH = 6.5 the Cu^{II} is completely chelated by the receptor, whereas the Ni^{II} complex is not formed yet.

This situation suggested the following titration experiment: a solution of **3** was adjusted to pH = 7 with 2,6-lutidine buffer and a standard solution of Ni^{II} was added. No modification of anthracene emission was observed even after the addition of 3 equiv (see Fig. 9). Then the same solution was titrated with Cu^{II}: I_F was

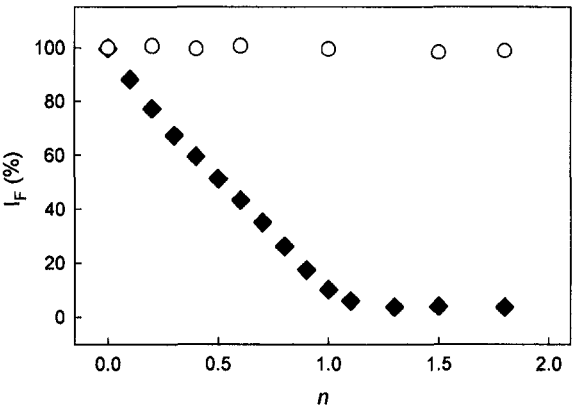


Fig. 9. Discrimination of Cu^{II} and Ni^{II} by fluorosensor **3**. A solution of **3** is adjusted to pH = 7 and titrated with a standard solution of Ni^{II} (circles). The same solution is then titrated with Cu^{II}. n = (mol of M^{II})/(mol of **3**).

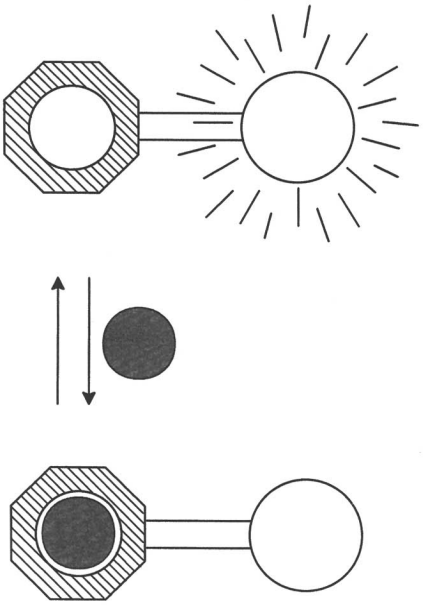


Fig. 10. The prototype of a fluorescent sensor for redox active substrates. Before recognition the fluorescence is ON; after, it is OFF.

observed to decrease linearly and full fluorescence quenching was obtained after the addition of 1 equiv of Cu^{II}.

eT FLUOROSENSORS FOR REDOX INACTIVE METAL IONS

The design of a two-component fluorosensor for a transition metal, as described in the preceding section,

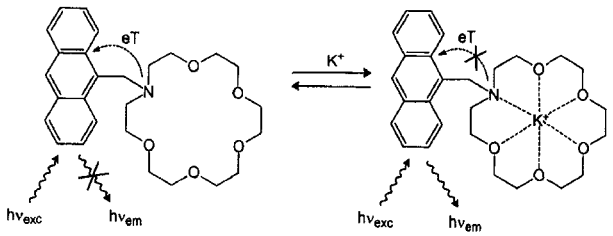


Fig. 11. Fluorosensing of the K^+ ion. Before metal complexation, the fluorescence of the An fragment is quenched by the nearby tertiary amine group (eT from the nonbonding electron pair). On complexation the lone pair is involved in the metal–ligand interaction, the eT process is prevented and fluorescence is restored.

is straightforward: simply connect by a spacer a fluorescent fragment to a suitable receptor. The 3d metal itself, which has an intrinsic tendency to exchange electrons, when bound to the receptor, will quench the emission of the proximate fluorophore through an eT mechanism, thus signaling the occurrence of the recognition process. In some cases, the quenching process may be due to an energy transfer (ET) mechanism of the Dexter type (or double electron exchange) [7]. Transition metals can be involved in the latter mechanism, as they possess empty or half-filled levels (the d orbitals) available for the circular exchange of electrons with the photoexcited fluorophore. Whatever mechanism operates, a d block metal will quench the fluorescent subunit of a two-component sensor, provided that it is not too far away. On these bases, a class of ON–OFF fluorosensors for transition metals can be generated (metal free sensor, fluorescence ON; metal bound sensor, fluorescence OFF) [8]. This situation is illustrated schematically in Fig. 10.

There exist metal ions which do not have any redox tendencies: they are metals having either a d^0 (alkali and alkaline-earth cations, Al^{III}) or a d^{10} electronic configuration (e.g., Zn^{II}, Ga^{III}). These metals cannot quench via an eT mechanism the fluorescence of the light-emitting subunit of any two-component system of the type illustrated in Fig. 10. Indeed, their fluorosensing requires the design of a more elaborate molecular system.

A successful approach is well illustrated with the design of a fluorosensor for the K^+ ion (Fig. 11). The molecular system **4** results from the linking, through a $-CH_2-$ group, of the classical anthracene fluorophore and of an 18-membered crown ligand [9]. The chosen receptor is very similar to the well-known cyclic hexaether 18-crown-6: simply, one ether oxygen atom has been replaced by an amine nitrogen atom. The presence of the nitrogen atom in the ring does not vary much the

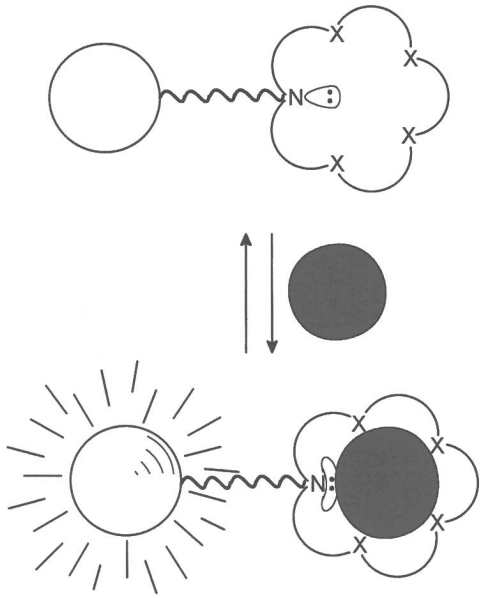


Fig. 12. The prototype of a fluorescent sensor for redox inactive substrates. The tertiary amine nitrogen atom present in the receptor subunit acts as a switch. Before recognition, it keeps fluorescence OFF. When "pressed" by the substrate, it is deactivated and fluorescence is ON.

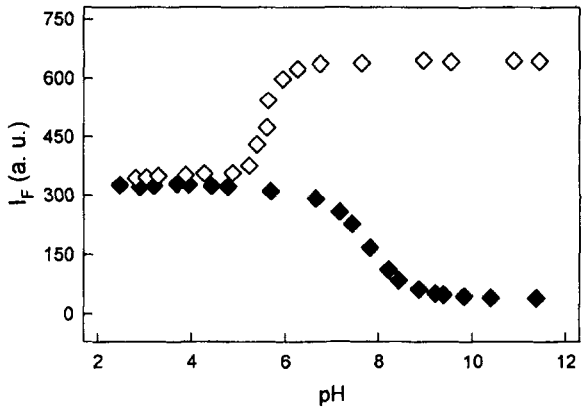
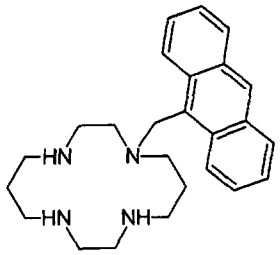


Fig. 13. Spectrofluorimetric titration with a standard base of (i) a solution containing **5** plus excess acid (filled symbols) or (ii) a solution containing **5**, 1 equiv of Zn^{II}, plus excess acid (open symbols). At pH $\geq$ 9 the fluorescence of solution i is fully quenched, whereas full emission is observed for solution ii. This indicates that **5** should behave as an efficient OFF–ON fluorosensor of Zn^{II} for any pH $\geq$ 9.

coordinating tendencies toward the K^+ ion (whose selective complexation derives from the favorable matching of the metal radius and the radius of the macrocyclic cavity) and, very interestingly, provides an efficient mechanism for recognition signaling. In fact, the tertiary amine nitrogen atom possesses reducing tendencies and, in the absence of K^+, quenches the nearby fluorophore through an N-to-An* eT process (ΔG°_{eT} = − 0.41 eV). When the metal ion enters the crown, the lone pair of the nitrogen atom becomes involved in coordination and is no longer available for the eT mechanism. Thus, the fluorescence is restored. System **4** is therefore a fluorosensor of the OFF–ON type: no emission in the absence of the analyte, recognition signaled through a sharp fluorescence revival. Its behavior fits well the metaphor of the bulb–switch–finger. The tertiary nitrogen atom in **4** is the switch, which is operated by the K^+ ion (the finger). Pressure by the finger (metal complexation) switches on the light bulb (anthracene emission activated). The prototype of an OFF–ON fluorosensor is sketched in Fig. 12.

Systems of the type illustrated in Fig. 12 have to be used in order to fluorosense analytes unable to promote either eT or ET mechanisms [10]. For instance, in the case of Zn^{II}, one can utilize a system analogous to **4**, in which the NO_5 crown subunit is replaced by a ligand more suitable for a d^{10} metal. In particular, in system **5** the receptor subunit is the 14-membered tetramine macrocycle cyclam, which exibits the highest affinity toward 3d divalent cations. The task of one of the amine donor atoms, the tertiary one, is to switch on–off the emission of the nearby anthracene fragment, according to whether or not the metal center is bound by the cyclam subunit.



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H^+ ions compete for amine nitrogen atoms of the receptor subunit of **5**. Thus, when investigating the sensing behavior of **5**, the pH of the solution has to be controlled.

Figure 13 displays the variation of the fluorescence intensity of **5** in an MeCN/water solution (4:1, v/v), over the 2–12 pH range, *in the absence of metal,* as obtained through a spectrofluorimetric titration experiment. In a strongly acidic solution, **5** is partially, not fully fluorescent: this is due to the fact that, due to repulsive electrostatic effects present in the cyclic molecule, even at pH = 2 not all the nitrogen atoms are protonated. In particular, the unprotonated amine group(s) is (are) close

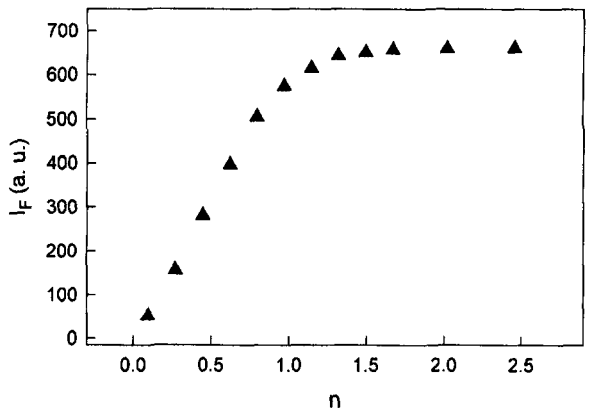


Fig. 14. Spectrofluorimetric titration of a solution of **5**, adjusted to pH = 12, with a standard solution of Zn^{II} (circles). n = (mol of Zn^{II})/(mol of **5**).

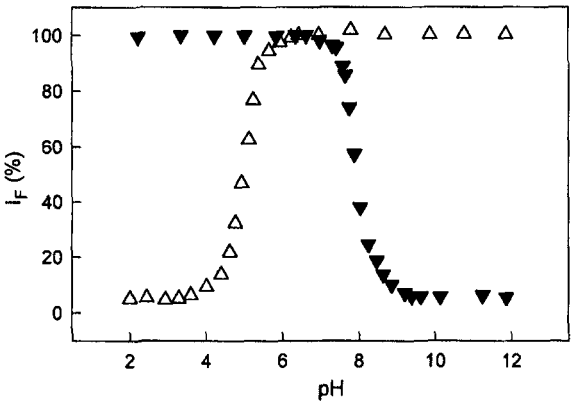


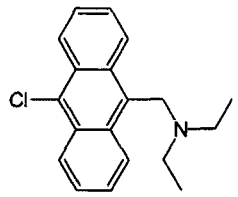
Fig. 15. pH dependence of the fluorescence intensity of anthrylamine **6** (filled symbols, ON–OFF behavior) and of the anthracene–pyridine derivative **7** (open symbols, OFF–ON behavior). Combination of a fluorescent fragment, of an amine subunit, and of a pyridine subunit in the same molecular system should give rise to an OFF–ON–OFF pH fluorosensor (a pH window indicator). A three-component system of this type, **8**, has recently been designed by de Silva [11].

enough to the An fragment to undergo an N-to-An* eT process which competes with the radiative decay of the excited fluorophore. At pH > 6 a more crucial ammonium group begins to deprotonate (probably the tertiary one closest to the An subunit), and a more efficient eT mechanism operates. I_F decreases until full quenching is achieved at pH ≥ 10. On the other hand, the I_F vs pH profile obtained in the presence of 1 equiv of Zn^{II} (open symbols in Fig. 13) superimposes that obtained in the absence of metal until pH = 5. At this pH, I_F increases to reach a plateau value at pH = 6. Fluorescence enhancement is associated to the complexation of Zn^{II} by the tetramine receptor. In particular, the Zn^{II}–N interaction interrupts any N-to-An* eT mechanism from both the nearest tertiary nitrogen atom and the secondary amine groups of the cyclam ring. At pH ≥ 6, the Zn^{II}–receptor complex is completely formed and I_F reaches the maximum value (full anthracene emission). Since at pH values >9, in the absence of Zn^{II}, the fluorescence of **5** is almost completely quenched (<5%), any pH value >9 is a correct value for the utilization of **5** as an OFF–ON sensor for Zn^{II}, in a spectrofluorimetric titration experiment.

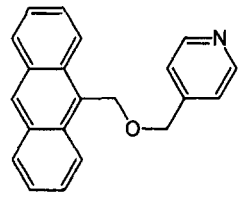
Figure 14 shows the OFF–ON profile obtained by titrating with Zn^{II} an aqueous MeCN solution of **5**, buffered to pH = 12. In particular, a sharp equivalent point is perceived, with complete revival of the fluorescent emission.

pH FLUORESCENT SENSORS

It has been outlined in the previous section that H^+, like K^+ and Zn^{2+}, can activate–deactivate a tertiary amine switch linked to an anthracene fragment. This opens the way to pH sensing by two-component fluorescent systems. A simple, yet efficient pH sensor is represented by the anthrylamine base **6.**



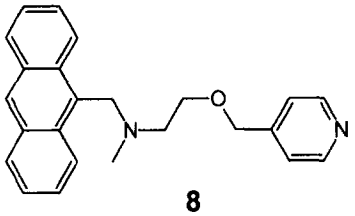
6



7

Figure 15 shows the sigmoidal dependence of I_F of **6** upon pH (filled symbols). At pH ≤7, the tertiary amine group is protonated and the An fragment displays full emission; at pH ≥ 9, the proton is released and fluorescence is quenched. The pH at which I_F is reduced to 50% corresponds to pK_A (= 7.9). System **6** is nothing more than an acid–base indicator, like the well-known fluoresceine (but fluoresceine is a one-piece system, you cannot discern the receptor subunit and the fluorescent fragment). Like any other indicator, **6** can be employed to visualize the equivalent point in an acid–base titration experiment. It cannot be used for the direct determination of pH: if the solution emits light, one can state that pH is ≤pK_A − 1 and that pH is ≥pK_A + 1, if no anthracene emission is observed.

Another acid–base couple which can be utilized as an eT switch of fluorescence is represented by the pyridinium/pyridine (pyH^+/py) system. In fact, the pyH^+ group shows definite eT tendencies (being prone to the reduction to the pyH· radical, $E°(pyH^+/pyH·) = -1.25$ V vs SCE). On the other hand, unlike the amine group, the pyridine nitrogen atom does not show any reducing tendencies. On these bases, it happens that in the two-component anthracene–pyridine system **7,** the protonated form has its fluorescence quenched, due to the occurrence of an An*-to-pyH^+ eT process ($\Delta G°_{eT} = -0.89$ eV). On deprotonation, the redox tendencies are completely lost and the fluorescence is fully restored. This generates an OFF–ON sigmoidal I_F vs pH profile centered at pH 5 (see open symbols in Fig. 15), opposite to the ON–OFF profile observed for system **6.** Examination of the curves displayed in Fig. 15 suggests that a system in which the fluorophore is linked to both a tertiary amine and a pyridine group should give rise to an I_F vs pH bell-shaped profile.



8

Actually, this is observed with the three-component system **8** [11]. System **8** does something more than acid–base indicators usually do. In fact, light emission does not simply indicate that pH is higher (or lower) than pK_A. It states that pH is comprised within a known window: pK_A(pyridine) - pK_A(amine). The position and width of the window can be varied at will, by choosing fragments of appropriate acidity.

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