Coordination Chemistry Reviews 253 (2009) 2226–2240

Contents lists available at ScienceDirect

Coordination Chemistry Reviews

journal homepage: www.elsevier.com/locate/ccr

Review

Micelles as nanosized containers for the self-assembly of multicomponent fluorescent sensors

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Contents

ARTICLE INFO

Article history:
Received 6 October 2008
Accepted 14 November 2008
Available online 25 November 2008

Keywords:
Fluorescence
Micelles
Sensors
Molecular devices
Self-assembly

ABSTRACT

Micelles can be used as containers to confine different lipophilic molecules in the same nanometric volume. When two or more molecules are contained in the same micelle their local concentration is dramatically increased, they are much less solvated than in bulk water and their mobility is still allowed as if they were included in an organic solvent nanodroplet. As a consequence, intramicellar dynamic interactions are strongly promoted and new overall functions may be expressed by the combination of the properties of each molecular component, obtaining a nanosized supramolecular device. In this paper, we will review the micellar systems in which the promotion of metal-ligand coordination between lipophilic fluorescent ligands and lipophilic complexes, and the co-micellization of lipophilic fluorophores with lipophilic ligands and bases allow the micellar self-assembling of supramolecular fluorescent sensors for cations, anions, pH-windows, and for some chemical–physical properties of molecules.

1. Introduction

The most useful property of micelles is the ability of including lipophilic molecules in their hydrophobic core. Although they had little idea of what an amphiphilic molecule or a micelle was, humans exploited this ability since the discovery of soap, which seems to date back to 2800 BC [1]. The development of surfactant science in the 20th century brought the rationalization of the solubilization processes of hydrophobic molecules in micellar aqueous solutions [2]. Beside the obvious improvement of the detergents industry, more subtle chemical processes, like those involving catalysis, took great advantage of the rational development of this feature [3]. In this paper, a more recent approach to the exploitation of micellar inclusion is reviewed. This approach takes into account the controlled inclusion in micelles of two or more molecular species, with the aim of obtaining self-assembled multicomponent devices capable of working as fluorescent sensors.

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doi:10.1016/j.ccr.2008.11.010

1.1. Micelles as nanosized containers for self-assembled molecular devices

Classical micelles in water are built up from small monomeric amphiphilic molecules, featuring a polar head and a lipophilic tail, that self-assemble into supramolecular structures. These have an internal hydrophobic core and an external hydrophilic surface (or palisade layer), described by the polar heads of the amphiphilic monomers. Their shape is quite commonly spherical, less frequently ellipsoidal (Fig. 1) but also rod-like shapes or extended sheet structures are possible, depending on many factors such as the surfactant type and structure, and on the concentration of organic additives (e.g. alcohols and co-surfactants) and electrolytes. The bulk aqueous solutions obtained are microheterogeneous, featuring lipophilic domains of nanometric dimensions (the micellar cores) homogeneously dispersed into water. A lipophilic molecule may dissolve in an aqueous micellar solution even if it is not soluble in pure water. This happens thanks to the molecule inclusion in the micellar core, where it is subject to a different set of conditions with respect to bulk water: (i) hydration is very low if not negligible [4]; (ii) similarly, the concentration of H^+ and of ions is very low (especially when micelles of non-ionic surfactant are considered) [5]; (iii) viscosity is in many cases comparable to that of an organic solvent droplet [6]; (iv) although inclusion is a dynamic process, i.e. included molecules continuously exchange with water, residence times in micelles may be quite long (hundreds of nanoseconds) [7].

The shape and the dimension of micelles have been studied both with calculations based on theoretical models [8,9] and with a plethora of experimental techniques which in most cases are based on the determination of the micellar hydrodynamic radius by light scattering [10–15]. Traditional micelles are made of an average number (Aggregation Number, AN) of surfactant monomers that generally ranges from 50 to 200, depending on the surfactant monomer structure and properties. Although sometimes a dependence of the micellar shape and size is also found as a function of the surfactant concentration, in most cases micelles have a defined AN for a large surfactant concentration range, obviously higher than CMC (critical micelle concentration, i.e. the molar concentration of the monomer at which micelles begin to form, at a given temperature) and have a defined shape. When spherical, the radius of a micelle is not too different from the length of a surfactant monomer in its full extension, which in most cases is in the 1–3 nm range. As an example, cetylpyridinium chloride (CPC, **1**) micelles are spheres with a hydrodynamic radius of 2.6 nm [16]. As a matter of fact, micelles have nanometric dimensions. If we consider them as containers, we should think that each included molecule is confined inside a nanovolume, this bringing a huge increase of the molecular local concentration. Using the example of CPC micelles, a sphere of 2.6 nm radius has a volume of 73.6 nm^3. A single molecule confined in such a volume can be considered as 1.66×10^{-24} mol dissolved in a 7.36×10^{-23} dm^3 volume. This sums up to a local (inside micelle) concentration as high as 2.2×10^{-2} M. Although this calculation is just a rough approximation, it is useful for understanding that if a lipophilic molecule is added to a bulk aqueous micellar solution even at micromolar or lower concentration, its local (i.e. inside micelle) concentration jumps up several orders of magnitude, thanks to micellar inclusion.

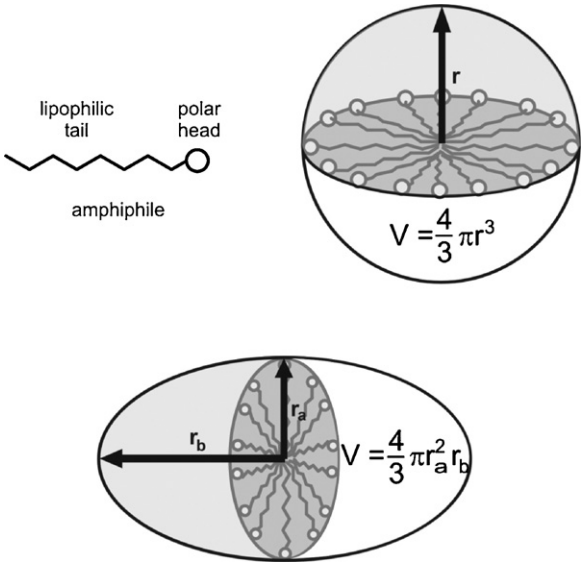


Fig. 1. Schematic representation of a surfactant monomer and of the micelles formed by it, both with a spherical and an oblate ellipsoid shape. Formula for volume calculation is taken from classical geometry.

These considerations bring advantages if we think of two or more different molecules included into the same micelle. They will "feel each other" as if their concentration is dramatically increased, they will be allowed to establish dynamic processes while residing in the micelle, and their reciprocal interactions (e.g. coordinative, donor–acceptor, hydrogen bonding) will be strongly promoted by poor solvation. This is an ideal situation to obtain a nanosized supramolecular device [17,18], as the promoted intramicellar dynamic interactions between the included molecules may allow the micelle and its content to express a new function, that is not the mere sum of the functions brought by each micelle-included molecular component. Moreover, a device of this kind is obtained with a straightforward self-assembling process. Molecular components bringing different functions are gathered in the micellar container without the need of any particular programming [19] during their synthesis, except (in some cases) lipophilization with suitable fragments. What is needed is just to mix them in the correct molar ratio with respect to the surfactant. Finally, being microheterogeneous, the macroscopic response of a micellar solution (e.g. its fluorescence intensity or its absorbance) will depend on the overall analytical concentration of the molecular components.

1.2. Effects of micellar inclusion on acid/base properties

As a consequence of the low hydration and low ion concentration in the hydrophobic core, the acid/base (and coordination) properties of the micelle-included molecules may significantly vary with respect to bulk water. A micelle-included base, B, will display a lower tendency to be protonated with respect to a solution in bulk water, as local proton concentration is lowered and BH^+ is poorly stabilized by solvation. As an example, triethylamine has a logarithmic protonation constant of 10.68 in water [20] while dodecyldimethylamine (**2**) displays a log K of protonation as low as 7.84 when included in micelles formed by the non ionic TritonX-100 surfactant (**3**) [21]. On the other hand, an acid of the AH type will show a decreased tendency to deprotonate when included in micelles, due to the poor stabilization of A^-: the 4.72 pK_a value of acetic acid in water [22] may be compared with the 6.7 pK_a value of dodecanoic acid found in a micellar solution of the non-ionic C12E8 surfactant (**4**) [23]. Interestingly, a certain degree of control on the pK_a and log K values of micelle-included species may be exerted acting on the electrostatic effect played by the overall charge on the micellar surface: as an example, dodecanoic acid has a pK_a of 7.6 in sodium dodecyl sulphate micelles (SDS, anionic surfactant, **5**) while its pK_a is 5.7–6.2 in dodecyl trimethyl ammonium bromide micelles (DTAB, cationic surfactant, **6**) [23].

The measurement of a log K or pK_a value of a molecule in a micellar solution is a matter of carrying out a potentiometric titration and fitting the potential vs. added base data as in traditional experiments carried out in water. In the micellar solution,

however, a macroscopic glass electrode is used to investigate a microheterogeneous system and the read data are space-averaged potential values. What is calculated is not the intrinsic acidity or basicity constant of the examined molecule but an *observed* value, that is influenced by the distribution of the molecule between the micellar container and bulk water. Considering the same moiety in the same micellar solution, dramatically different log *K* values can be observed for acid/base equilibria depending on the overall lipophilicity of the molecule to which the moiety belongs. Considering, e.g. the protonation of an *n*-alkyl dimethyl amine, short chains would result in a preferential solubilization in bulk water (high observed log *K*), long chains in a preferential solubilization inside micelles (low observed log *K*) and intermediate length chains in a distribution between water and micelles (intermediate observed log *K*). In this latter case, the observed log *K* values are also strongly dependent on the molar ratio between the examined molecule and the chosen surfactant: increasing the surfactant concentration means increasing micellar concentration and shifting the distribution of the molecule inside the micelles. At sufficiently high surfactant concentrations, limiting observed acid/base constants are obtained, as the molecule considered is at 100% included in micelles [24–28]. Even when an acid or basic molecule is 100% distributed inside a micelle, the observed acid/base constants depend on the position of the acid or base moiety inside the micelle, which in turn depends on the structure of the molecular backbone [29]. As an example, for an amino group, the deeper its position inside the micellar core, the lower its protonation constant.

For the aim of this review, the macroscopic behaviour of a micellar solution is exactly regulated by the values of the pertinent observed constants (acidity, basicity, complexation) of the micelle-included molecules. These values will allow an operator to foresee, e.g. the fluorescence intensity of a bulk solution of a micellar fluorescent pH sensor at any pH value, provided that the concentration of the surfactant and of the included molecules are the same as in the determination of the observed values. It is thus evident that for any practical application the lipophilicity and the structure of a molecular component may be useful parameters to modify in order to fine-tuning the micellar devices properties.

1.3. Micellar inclusion of two species

Papers describing experiments in which two different molecules are included and interact in the same micelle have appeared in the literature since the early seventies and can be considered classics of surfactant chemistry [30]. These papers exploited the micellar inclusion of an hydrophobic fluorophore and an hydrophobic quencher, and their interactions in the flash photolysis timescale, to investigate the diffusion rates of the fluorophore inside the micelle [31], the specific adsorption of counter ions in the Stern layer [31–33] or to model the luminescence decay of hydrophobic molecules solubilized in micelles [34]. Moreover, static and dynamic observation of intramicellar interactions between a fluorophore and a quencher has become a common method for the determination of the micelle AN [35–41]. It has been only with the end of the nineties that variations in fluorescence taking place thanks to intramicellar interactions of included molecules have been recognized as useful in the attainment of supramolecular devices, namely fluorescent sensors for cations, pH windows and anions.

1.4. Non-micellar multicomponent fluorescent sensors

Sensing chemical species in solution using fluorescence has attracted a lot of attention, due to the intense nature of the fluorescent signal, to the easiness of its visual or instrumental reading, to the plethora of fluorophores available from organic and coordination chemistry and to the deep influence played by chemical species on a fluorophore's emission properties. A chronology of this branch of chemistry should begin with fluorophores that feature interaction sites for the chosen substrate that are directly included in their molecular structure. Systems of this kind include, e.g. polyaromatic molecules with protonable atoms in their backbone, that may work as fluorescent pH sensors [42]. However selectivity, tunability and application flexibility are limited when using molecules of this kind. A more sophisticated class of fluorescent sensors was developed with the concepts of supramolecular chemistry, capable of selectively sensing cations in solution. The first papers appeared

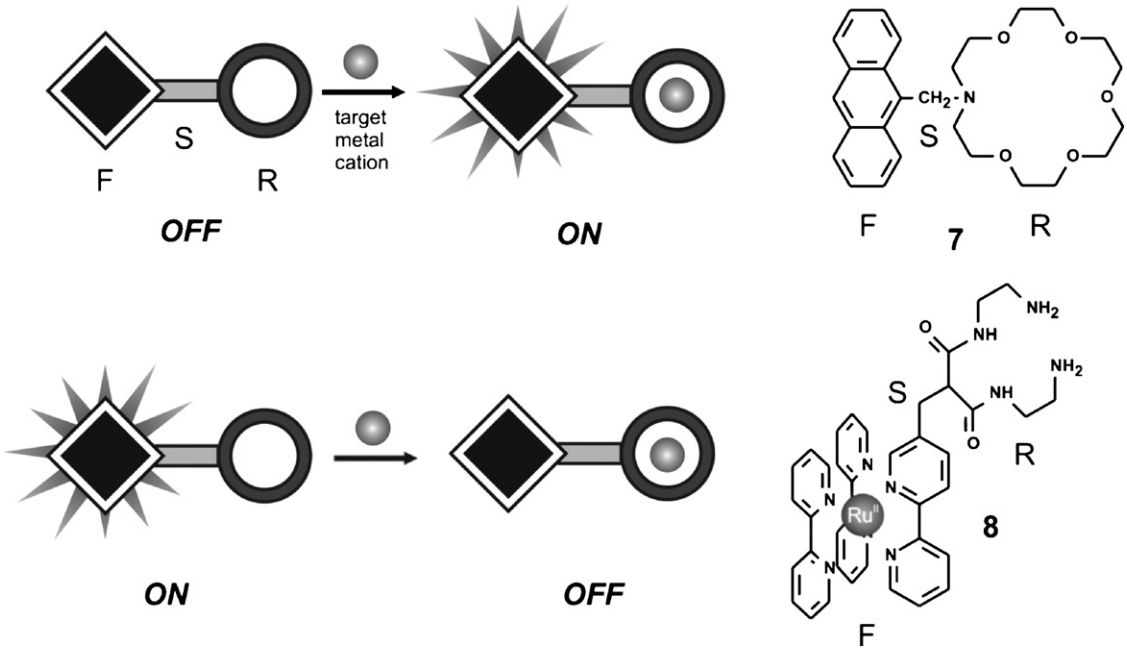


Fig. 2. Working scheme and examples for an OFF–ON (**7**) and an ON–OFF (**8**) covalent fluorescent sensor for cations of the FSR type. The F, S and R tags in the molecular formula indicate the fluorophore, the spacer and the receptor components, respectively. Molecule **7** is a selective fluorescent sensor for K^+, molecule **8** for Cu^{2+}. Complexation of Cu^{2+} in the binding unit of **8** takes place with a pH-dependent equilibrium (vide infra and Fig. 7).

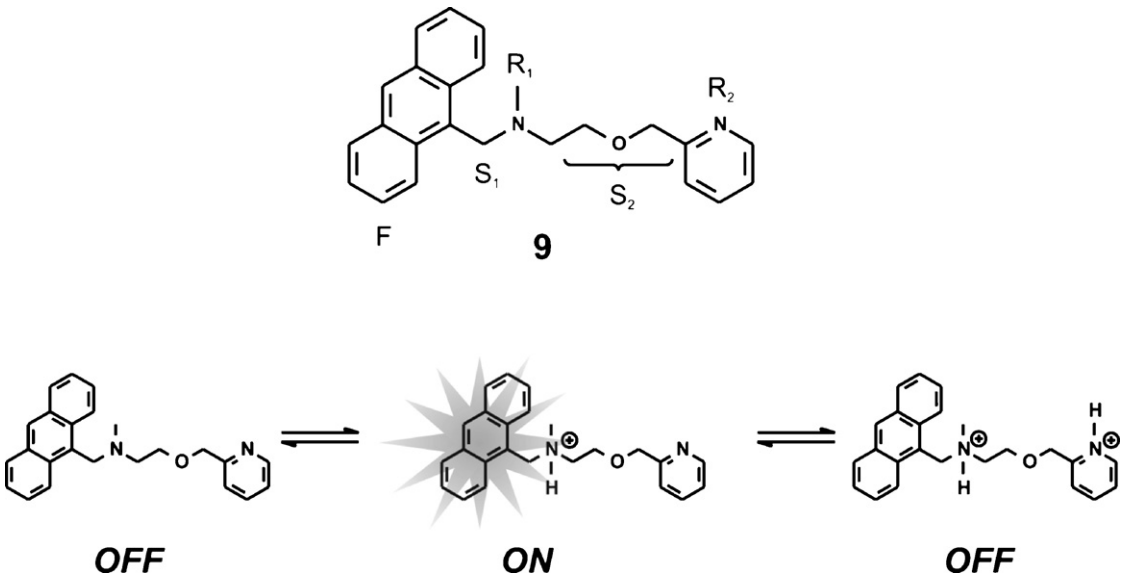


Fig. 3. Working scheme for an OFF–ON–OFF fluorescent sensor for pH windows. The F, R_n and S_n tags in the molecular formula indicate the fluorophore and the two spacer and receptor units. Starting from acidic pH, the switching from OFF to ON takes place at pH values around the pK_a of the pyridinium moiety (3.9). On further increasing pH, the switching back from ON to OFF takes place at pH values around the pK_a of the ammonium moiety (7.9). The example is taken from ref. [50].

between 1985 and 1988 and dealt with the sensing of H^+ [43], alkali-metal cations [44–45] and Zn^{2+} [46] These examples were based on a multi-component covalent approach, later called FSR (fluorophore–spacer–receptor), in which a fluorophore and a receptor molecular component, capable of acting also as a quencher in the absence of the target species, were covalently linked by a spacer (see Fig. 2; the example of molecule **7** is taken from Ref. [43]). These covalent multicomponent molecular devices thus behaved as sensors of the "OFF–ON" type for the target cation. Later, examples in which a fluorophore was covalently linked to a receptor component capable of acting as a quencher only in the presence of the target cation were also introduced, leading to the FSR "ON–OFF" sensors [47–49] (Fig. 2; the example of molecule **8** is taken from Ref. [49]). Moreover, sophisticated fluorescent pH-sensors were developed in the 1990s with an extension of this approach, named pH-window sensors [50,51]. These are capable of signaling if the pH of a solution is inside or outside a given range. Two receptor fragments (two different bases), R_1 and R_2, are covalently connected by two spacers to the same fluorophore. R_1 has a lower protonation constant than R_2 and the first quenches fluorescence when it is protonated, while the latter quenches fluorescence when it is in its neutral form. An OFF–ON–OFF response with increasing pH is thus obtained, as in the example of molecule **9**, Fig. 3 [50].

The FSR or "covalent" approach to fluorescent sensors has flourished and a huge number of papers have been published and continue to be published both in the field of cation sensing [52–57] and, more recently, of anion sensing [58–61]. In the latter case, an approach exploiting a different concept has also been elaborated, the so-called "chemosensing ensemble" approach [62,63]. This requires that a fluorophore is non-covalently bound to a receptor, with its fluorescence quenched by the latter. Addition of a target substrate capable of strongly binding the receptor results in the displacement of the fluorophore and fluorescence reviving, as sketched in Fig. 4. In most cases, the fluorophore-receptor and substrate–receptor interactions are of the coordinative type, as in the examples **10** (receptor), **11** (fluorophore) and **12** (substrate) [62] in Fig. 4. It is obvious that FSR sensors require some (or a lot) of organic synthesis for the construction of their covalent structures. Several synthetic steps may also be required for the sensors based on the chemosensing ensemble approach, e.g. for the synthesis of the receptor that in many cases is a coordinatively unsaturated metal complex based on a polydentate ligand [64,65]. Some fully supramolecular fluorescent sensors have also been described more recently [66], based on the self-assembling of metallacyclic structures containing luminescent metal complexes, capable of a variation of the emission intensity when molecular species [67–69], inorganic anions [70] or metal cations [71] are included in the metallacycle hole. However, as the metallacycles which are involved do not have trivial architectures and are based on the formation of kinetically inert metal complexes, multistep complicated synthetic procedures may be required with this approach.

2. Micellar self-assembled fluorescent sensors

2.1. Sensing transition and heavy metal cations

The idea behind micellar self-assembled sensors for transition and heavy metal cations is to combine the chemistry of ON–OFF FSR fluorescent sensors and the use of intramicellar fluorescence quenching, possibly taking advantage of the promotion of intramicellar interactions brought by inclusion of molecular components in micelles. Given a target metal cation to be sensed in aqueous solution, a suitable, selective ligand can be derived from classical coordination chemistry, or even better from the chemistry of FSR sensors. The ligand has to be lipophilic or to be lipophilized (e.g. with a long *n*-alkyl chain), so that in a micellar solution it is preferentially included in micelles. Moreover, it should not be capable of quenching fluorescence, at least in the pH range in which it should be used to bind the metal cation. A hydrophobic fluorescent molecule also has to be chosen and added to the micellar solution containing the lipophilic ligand. If the receptor component of an FSR sensor has been chosen as the ligand, the most suitable fluorophore could be the same coupled to it in the FSR molecule. Playing on the concentrations of both components and of the surfactant concentration, a significant population of micelles containing both the lipophilic fluorophore and the lipophilic ligand is found. Fluorescence will be ON, see Fig. 5. Addition of the target metal cation will result in its complexation by the lipophilic ligand, inside the micellar container, Fig. 5. Intramicellar photo-electron transfer (PeT) or energy transfer (ET) mechanisms between the excited fluorophore and the metal complex will result in fluorescence decrease (OFF state), allowing one to signal the cation.

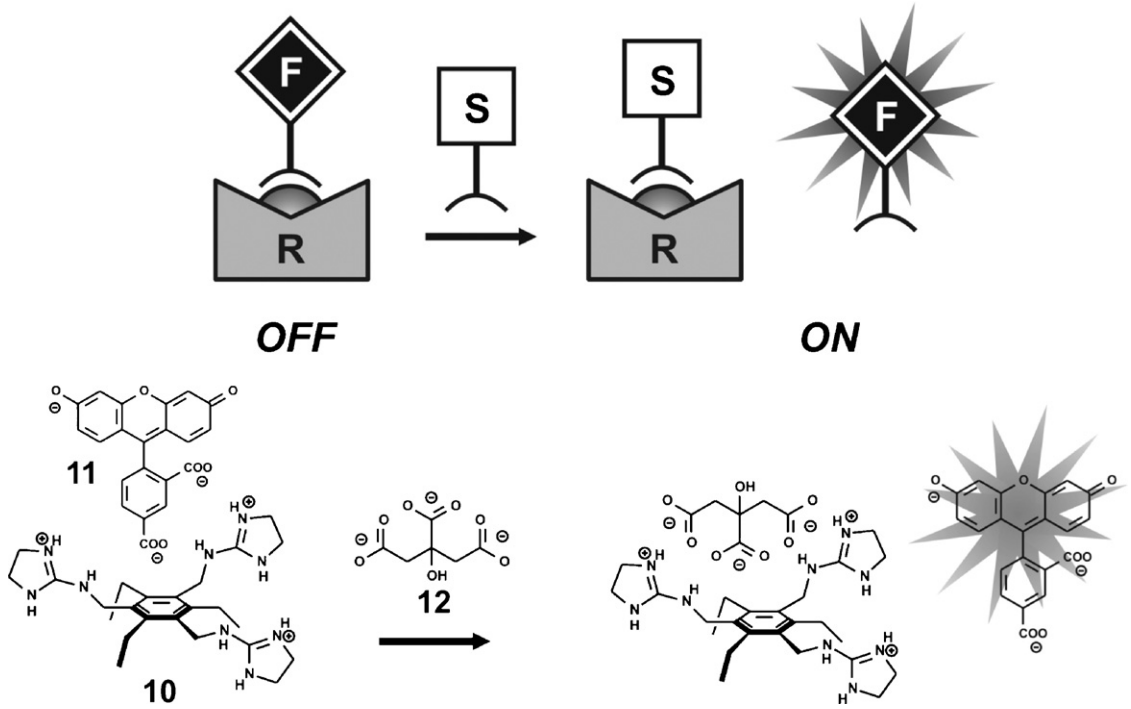


Fig. 4. Working scheme for the chemosensing ensemble approach. Molecule **10** is the receptor, molecule **11** the fluorophore and molecule **12** the substrate (the example is taken from Ref. 62b).

The first example was proposed by Tecilla, Tonellato et al. in 1999 [72]. The target cation to be sensed was Cu^{2+}, the chosen ligand was the glycylclycine dipeptide **13**, capable of selectively coordinating Cu^{2+} (at least in a defined pH range) forming a neutral chelate complex by deprotonation of the amido and carboxylic acid fragments, as sketched in Fig. 6 [73,74]. The authors appended a *n*-decyl chain to lipophilize the ligand (**14**), that was included in cetyltrimethylammonium bromide (CTAB, **15**) micelles together with the 8-anilino-1-naphthalenesulfonic acid (**16**), a commercial fluorophore that did not need any further functionalization. The resulting aqueous micellar solution was intensely fluorescent. Addition of Cu^{2+} at pH 7 resulted in its complexation by the lipophilized glycylglycine ligand and in fluorescence quenching. The sensor selectivity for Cu^{2+} was demonstrated over Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} (only Fe^{2+} showed a certain degree of interference). One of the advantages of the micellar self-assembling approach was indicated by the authors, who demonstrated that the same ligand could be used to sense Cu^{2+} in a set of different containers (i.e. micelles of different surfactants) and coupled to different fluorophores. The surfactants used were Brij 35 (**17**), Triton X-100 (**3**) and DMMAPS (3-(*N*,*N*-dimethylmyristylammonio)propanesulfonate, **18**) while the fluorophores were 9-anthracene carboxylic acid, dansylamide and 1-naphthylphosphate. Later, the same authors also proposed a simplified version of a micelle-based self-assembled sensor for Cu^{2+}, in which the dipeptided GlyLys and GlyGlu were functionalized with long *n*-alkyl chain [75]. These molecules were actually amphiphilic, at least in well defined pH ranges, as the *n*-alkyl-GlyLys feature a primary amine that can be protonated while GlyGlu has an additional carboxylic acid function that does not participate in Cu^{2+} coordina-

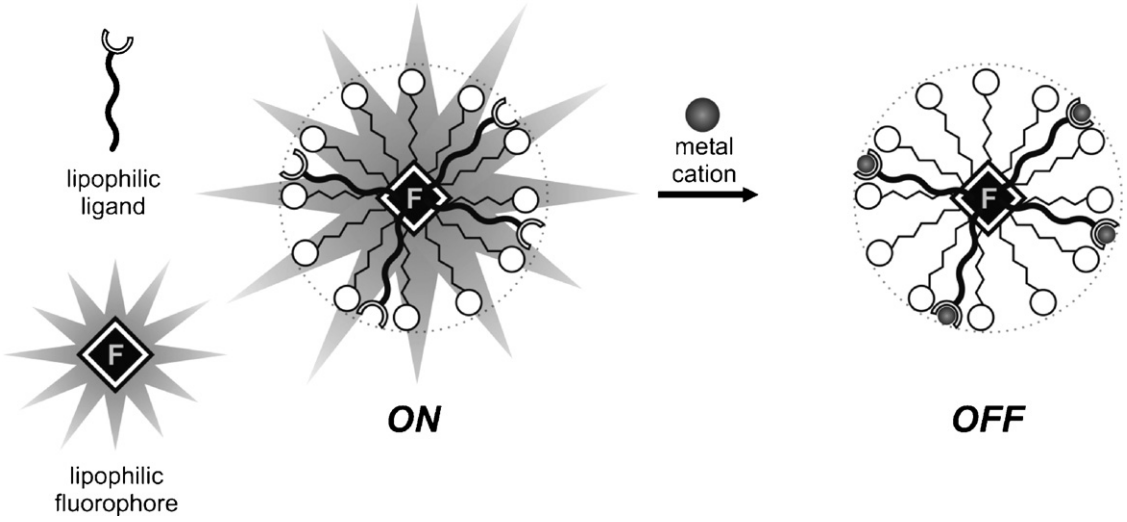


Fig. 5. Working scheme for the self-assembled ON–OFF fluorescent micellar sensors for metal cations.

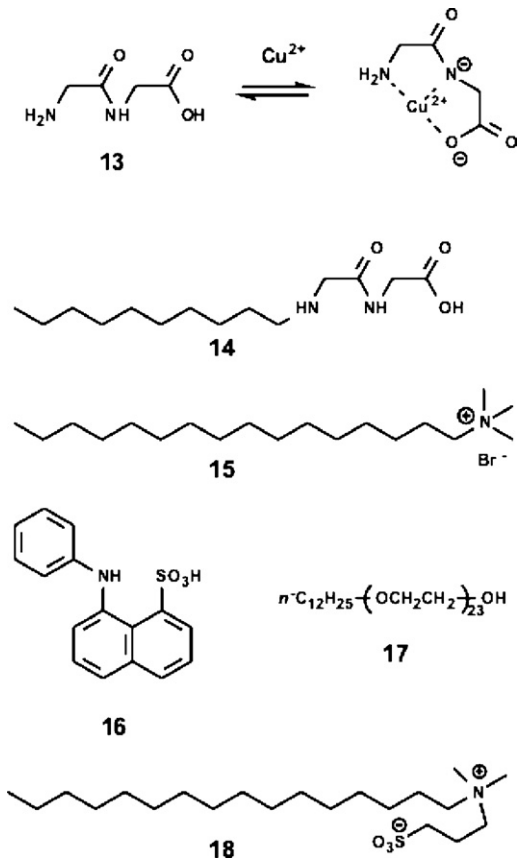


Fig. 6. The complexation equilibrium involving molecule **13** is pH-dependent: binding of Cu^{2+} takes place with the release of the proton of the amide NH group and of the proton of the carboxylic acid group.

tion and can deprotonate. These molecules behaved as surfactants, forming micelles without the need of adding any co-surfactant. Micellar inclusion of hydrophobic commercial fluorophores like 8-anilino-naphthalensulfonic acid or Rhodamine 6G allowed one to signal, by fluorescence quenching, the copper(II) cations, that were complexed by the polar heads of the surfactants with an equilibrium similar to that described in Fig. 6.

Our contribution to this area started in 2004, with an ON–OFF fluorescent sensor for Cu^{2+} [76]. We used the well-known non-ionic surfactant Triton X-100 (**3**) to obtain the micellar containers. The fluorophore was plain pyrene (**19**), a strongly hydrophobic and intensely fluorescent commercial molecule. We used the 1,4,8,11-tetraaza-5,7-dione fragment (also known as dioxo-2,3,2) as the ligand. Dioxo-2,3,2 (**20**) is a classical ligand from co ordination chemistry. This undergoes the pH dependent equilibrium displayed in Fig. 7, forming a very stable, neutral complex with Cu^{2+} at pH >6. Noticeably, dioxo-2,3,2 has been previously used by some of us as the receptor in the first FSR ON–OFF sensor for Cu^{2+} [47], **21**, a molecule that was capable of working only in an organic-enriched 8:2 dioxane/water solvent mixture, with a methylene unit as the spacer and anthracene as the fluorophore. To use it in a micellar sensor, dioxo-2,3,2 was lipophilized by appending an *n*-dodecyl chain to the maleimidic carbon atom, obtaining **22**. Determination of the protonation and complexation constants of micelle-included **22** allowed one to fully describe the system with a distribution diagram (Fig. 8a). The constants observed are interestingly similar to the constants determined for plain dioxo-2,3,2 and Cu^{2+} in water [77], suggesting that the diamino-diamido fragment of **22** is located near to the palisade layer of Triton X-100 micelles, where the polyethyleneoxide chains allow a high degree of hydration. When both **22** and pyrene are added to Triton X-100 micelles, a sigmoidal fluorescence intensity (I_f) profile is obtained with pH only in the presence of Cu^{2+} (Fig. 8a, black circles) while no variation is observed when Cu^{2+} is absent (white triangles). I_f vs. pH profile superimposition to the distribution diagram in Fig. 8a, sharply visualizes that complexation of Cu^{2+} by **22** inside the micelles is responsible for the fluorescence quenching. Addition of Cu^{2+} to a micellar solution of pyrene plus **22** at pH >7 results in fluorescence quenching, with the system acting as a self-assembled fluorescent ON–OFF sensor for Cu^{2+}, as sketched in Fig. 9. Dioxo-2,3,2 ligands may incorporate only Cu^{2+} and Ni^{2+} with the equilibrium described in Fig. 7, so they are intrinsically selective for these two cations [47,48]. The Ni^{2+} cation is also effectively complexed by **22** in Triton X-100 micelles, with pyrene fluorescence quenching, Fig. 8b, black triangles. However, the lower affinity of Ni^{2+} with respect to Cu^{2+} for the deprotonated dioxo-2,3,2 framework shifts the descending part of the I_f vs. pH sigmoid to a higher pH values. Selectivity for Cu^{2+} over Ni^{2+} may be obtained by selecting the working pH interval (e.g. 7.0 < pH < 7.5).

The versatility of the micellar self-assembled approach was also stressed by us. Later, we used the same container (Triton X-100 micelles) and the same fluorophore (pyrene) to obtain both an ON–OFF and an OFF–ON fluorescent sensor for Hg^{2+}, by changing the lipophilized ligand [78]. From classical coordination chemistry we used the [16]aneNS4 macrocycle, **23**, as a suitable, selective lig-

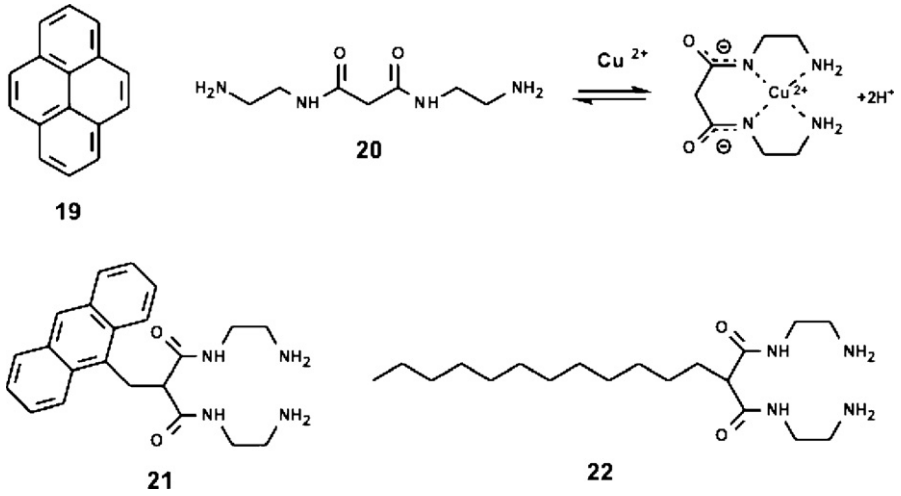


Fig. 7. The complexation equilibrium involving molecule **20** is pH-dependent: binding of Cu^{2+} (or Ni^{2+}) takes place with the release of the two protons of the amide NH groups. The complexes obtained are neutral and square planar.

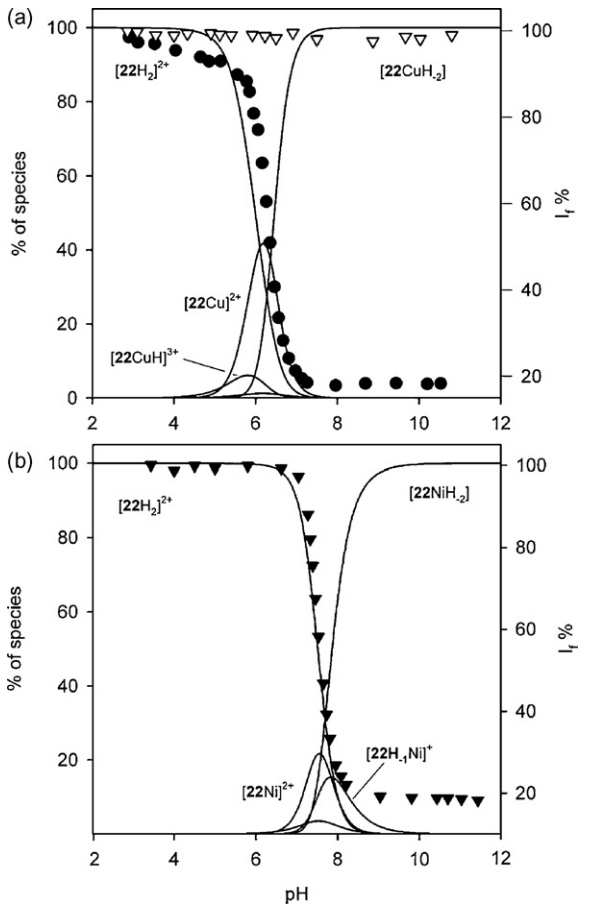


Fig. 8. (a) Distribution diagram for the system **22**/Cu^{2+} (both 0.001 M) in micellar solution of Triton X-100 (0.01 M). Solid lines indicate the percent concentration of each species relative to the total ligand concentration. The pertinent species for each profile are indicated in the figure. White triangles show the percentage emission intensity of micelle-included pyrene (at 393 nm) as a function of pH, with no added Cu^{2+}. Black circles report the percentage emission when Cu^{2+} is added (1:1 molar ratio with respect to **22**); (b) Distribution diagram for the system **22**/Ni^{2+} (both 0.001 M) in micellar solution of Triton X-100 (0.01 M). Black triangles report the percentage emission of pyrene as a function of pH, with Ni^{2+} in 1:1 molar ratio with respect to **22**. Figures have been adapted from Ref. [76].

and for Hg^{2+}. Functionalization with an *n*-dodecyl chain gave the highly lipophilic ligand **26**. This is included in Triton X-100 micelles in the 2–12 pH range with the amino group both in its protonated or neutral form. Potentiometric studies on the ligand alone in micelle and on the ligand plus Hg^{2+} allowed one to determine the protonation and complexation constants of the system, and to draw the distribution diagram described by Fig. 10a (solid lines). The percentage of protonated ligand when no metal is added is also reported as a dashed line. Due to the inclusion in micelles and deep positioning in the micellar core of the NR_3 fragment, the protonation log *K* is as low as 3.81. This can be compared to the 7.0 log *K* value determined in water (no surfactant added) for the non-lipophilized methyl-substituted macrocycle **24**. The I_f vs. pH profiles in the absence and in the presence of Hg^{2+} are also reported in Fig. 10**a** (white triangles and grey triangles, respectively). In the absence of mercury, the pyrene fluorescence is regulated by the protonation of the tertiary amine of **26**, with a typical sigmoidal ON–OFF profile. On the other hand, Hg^{2+} complexation is effective in the full pH range, with consequent fluorescence quenching. Addition of Hg^{2+} to Triton X-100 micelles containing **26** at pH $\leq$4 results in fluorescence quenching due to the heavy-metal effect played by Hg^{2+} on the pyrene fluorophore. The system works as an ON–OFF sensor for Hg^{2+}, (Fig. 11a). The sensor is selective for Hg^{2+} (no interference was observed with Ca^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}), although Ag^{+} is a serious competitor for coordination by **26**. Determination of complexation constants, however, showed a preference larger than one order of magnitude in the complexation of Hg^{2+} over Ag^{+}. Hg^{2+} quantities lower than 1 ppm may be easily sensed by this device.

Using the same components we can transform the fluorescent sensor from an ON–OFF to an OFF–ON type, by playing on the receptor lipophilicity [78]. A shorter substituent on the [16]aneNS4 macrocycle, *n*-butyl (**25**), results in a different distribution of species for the ligand/Hg^{2+} system in micelles (Fig. 10b). The logarithmic protonation constant of **25** is 5.86, intermediate between that of **24** and **26**, see dashed line in Fig. 10b. When no Hg^{2+} is added I_f follows sharply the protonation state of the tertiary amino group of **26** (white triangles in Fig. 10b). With mercury added, the $[Hg(\mathbf{25})]^{2+}$ complex is predominant for $6 < pH < 10$ but it is located outside the micelles. This is due to its +2 charge and to the shortened alkyl chain, i.e. the complex is too hydrophilic to remain inside the Triton X-100 micelles. As a consequence, between pH 7 and 9 addition of Hg^{2+} to micelles containing pyrene and **25** results in complexation and expulsion of $[Hg(\mathbf{25})]^{2+}$ with fluorescence recovery, as can be seen observing the I_f vs. pH profile in Fig. 10b, grey triangles. The working scheme of the OFF–ON sensor is sketched in Fig. 11b.

Using chains of different lengths produced similar responses when *n*-propyl substitution was chosen. Shorter chains or longer chains did not yield effective systems. In the former case because both the free ligand and its complex are both distributed mainly outside the micelles (obtaining an ON–ON fluorescence response),

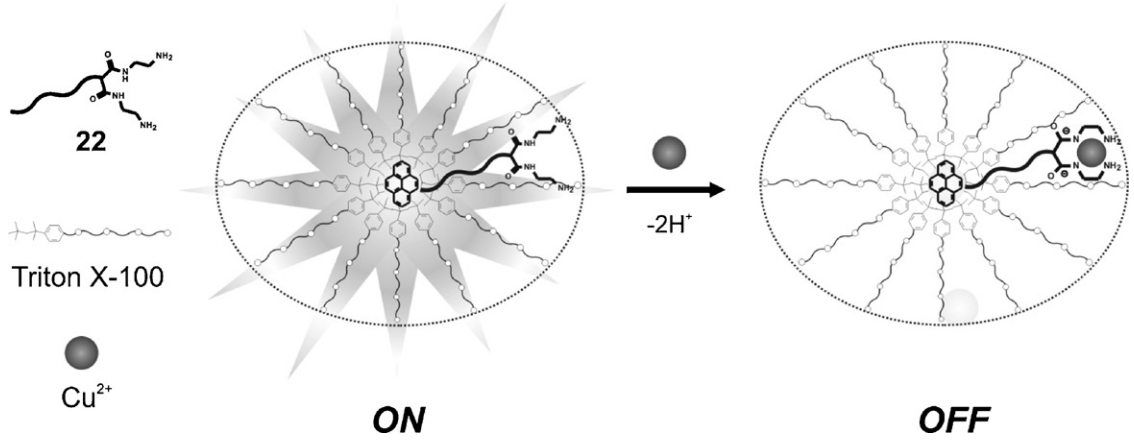


Fig. 9. Sketch of the working scheme of the micellar system made of pyrene and **22** as an ON–OFF fluorescent sensor for Cu^{2+}.

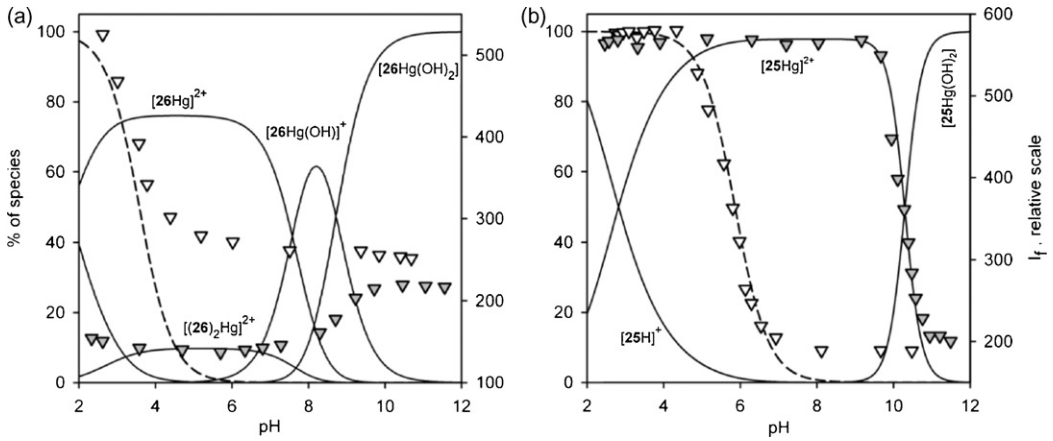


Fig. 10. (a) Distribution diagram for the system **26**/Hg^{2+} (both 0.001 M) in a micellar solution of Triton X-100 (0.01 M). Solid lines indicate the percent concentration of each species relative to the total ligand concentration. The pertinent species for each profile are indicated in the figure. White triangles show the percentage emission intensity of micelle-included pyrene (at 393 nm) as a function of pH, when no Hg^{2+} is added. Grey triangles describe the percentage emission when Hg^{2+} is present in 1:1 molar ratio with **26**); (b) Same, for ligand **25**. Figures are adapted from Ref. [78].

in the latter case because both are distributed prevalently inside the micelle (obtaining an OFF–OFF response).

2.2. Sensing pH windows

We also obtained fluorescent sensors for pH windows by including multiple components in micelles. Reasoning on the few systems described in the literature (e.g. **9** in Fig. 3) we decided to use the same quenching groups but as separate lipophilic components and to self-assemble them in micelles. We used the Triton X-100 micelles as containers. The components were pyrene as the fluorophore, a lipophilized pyridine (**27**) and one tertiary amine (**28** or **29**). An OFF–ON–OFF response is obtained on moving along the pH axis. Fig. 12a (black triangles) reports I_f vs. pH profile for the example of **27** with **28** and pyrene as components [21]. The inflection points on the ascending and descending sides of the dumb bell shaped I_f vs. pH profile of the figure correspond to the protonation log*K* values of the pyridine and amine fragments, 3.73 and 7.84 respectively, as indicated by the solid lines describing the distribution diagram. The working scheme of the system is sketched in Fig. 12b. Similar results were obtained by covalently linking both a pyridine and a tertiary amine moiety in the same lipophilic molecule (**30**–**33**). These bi-bases are included in micelles with pyrene, to give dumb bell shaped I_f vs. pH responses that shift along the pH window and feature a larger or more narrow "ON" state as a function of the observed protonation log*K* values. These,

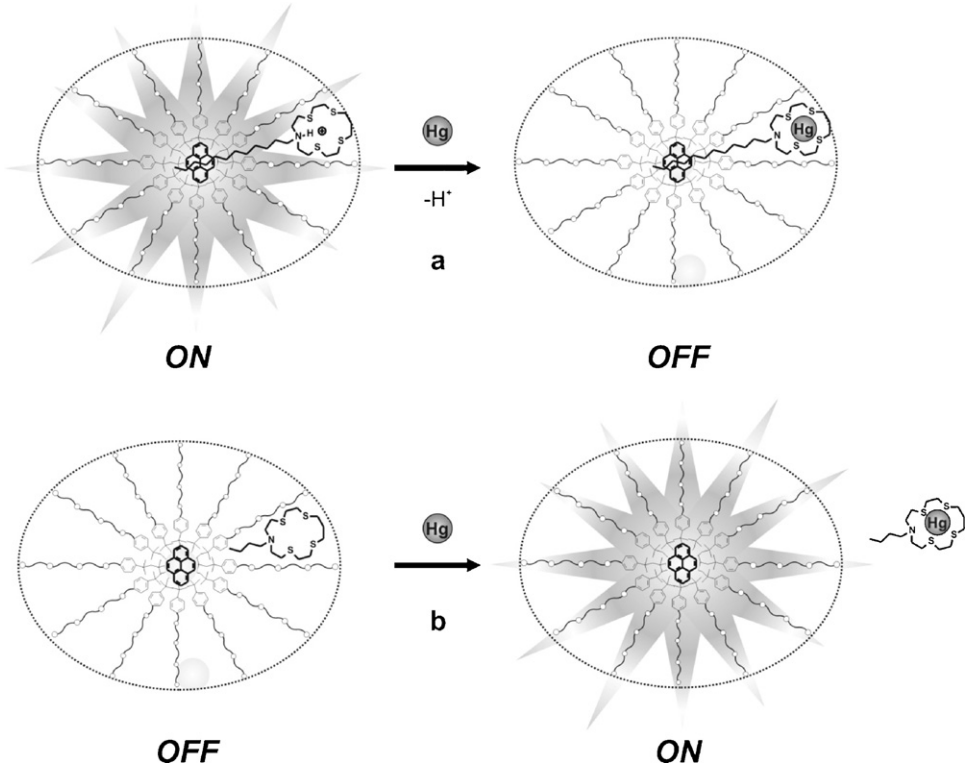


Fig. 11. (a) Working scheme for the micellar system made of pyrene and **26**, as an ON–OFF fluorescent sensor for Hg^{2+} (pH must be <4 as the tertiary amine of **26** must be protonated before Hg^{2+} addition); (b) working scheme for the micellar system made of pyrene and **25**, as an OFF–ON fluorescent sensor for Hg^{2+} (pH must be in the 6–10 interval, where **25** is micelle-included and not protonated in the absence of Hg^{2+}).

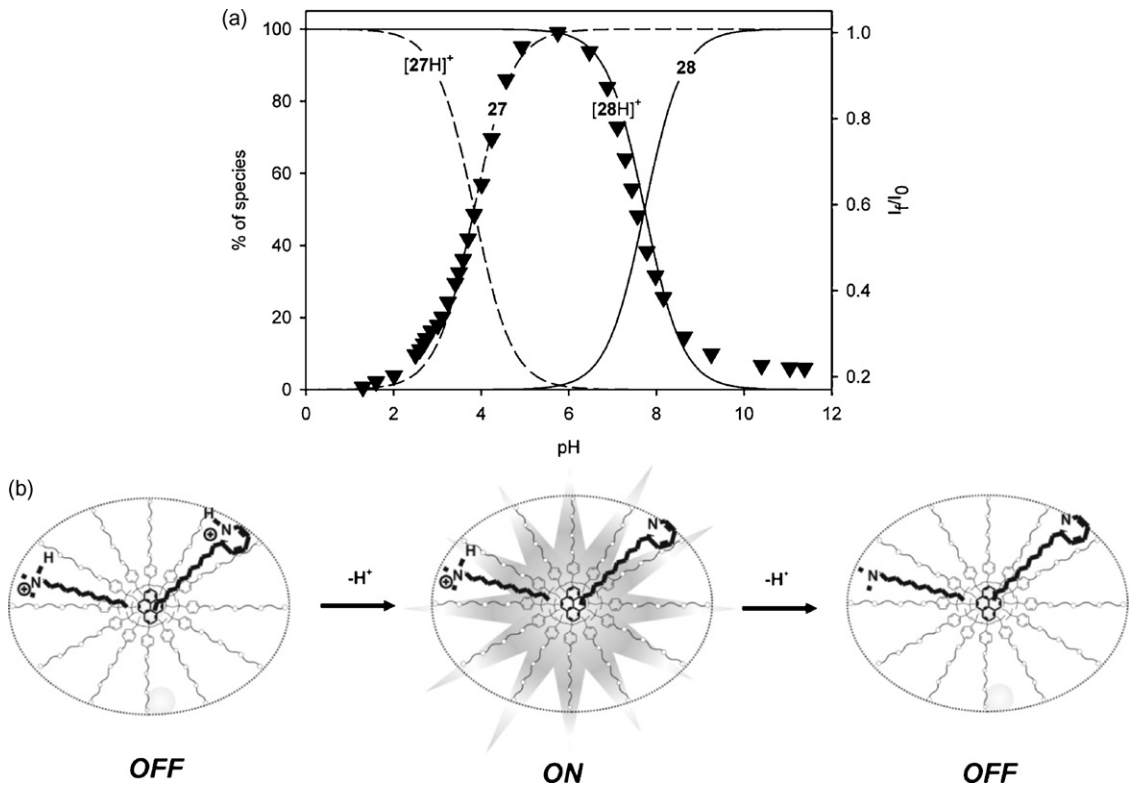


Fig. 12. (a) Distribution diagram for the system made by micelle-included bases **27** and **28** (both 5×10^{-4} M) plus pyrene in Triton X-100 (0.01 M). The species pertinent to each profile are reported in the figure. Dashed and solid lines refer to species containing **27** and **28**, respectively. Black triangles report the I_f/I_0 emission intensity of pyrene, as a function of pH (figure adapted from Ref. [21]); (b) working scheme of the system as an OFF–ON–OFF fluorescent sensor for pH windows. Transitions between OFF and ON, and ON and OFF, are found on moving along the pH axis when crossing the pK_a of the pyridinium group of **27** (3.73) and of the ammonium group of **28** (7.84).

in turn, depend on the molecular structure. With these bi-bases all the pyridine log *K* values are shifted to very low values due to the vicinity of a protonated amino group and to the poor solvation inside micelles (e.g. log *K* for the pyridine fragment is 2.13 in **31**), while more subtle structural and electronic effects finely tune the protonation constants of the amino groups. Lipophilicity and positioning inside the micelle also play an important role, as the very lipophilic molecule **32** has observed log *K* values of 4.58 (NR_3 fragment) and 1.8 (pyridine fragment), to be compared with those of the less lipophilic **30** (6.27 and 2.13 for NR_3 and pyridine, respectively). The tunability and flexibility in application of this system can be appreciated from Fig. 13. The horizontal segments represent the ideal half-height width of the OFF–ON–OFF fluorescence response, i.e. their limiting values are the observed protonation log *K*s of the bases responsible of the quenching processes.

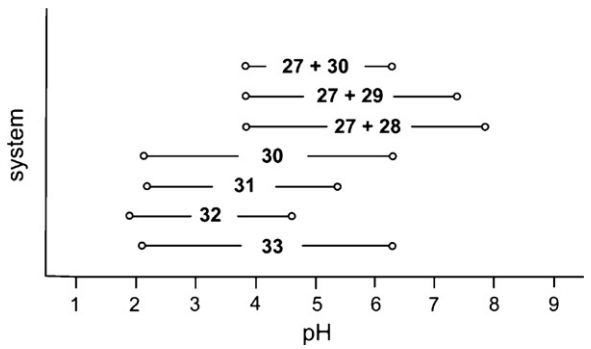


Fig. 13. Horizontal segments represent the ideal half-height width of the I_f vs. pH curves of each system. Their limiting values on the pH scale correspond to the pK_a values of the protonated base moieties responsible of the quenching processes.

Fine regulation can also be achieved by playing on the overall charge of the micellar container, thus influencing the observed log *K* values of the bases included in the micelle without the need of changing their molecular backbone. We have used molecules **27** and **28** plus pyrene and included them in co-micelles of Triton X-100 and SDS (**5**, from 0 to 1 molar fraction) [79]. On increasing the SDS molar fraction the increased negative charge on the surface of the micellar containers pushes the observed log *K*s towards higher values, due to the favourable electrostatic effect exerted on protonated species. As a consequence, the ON portion of the I_f vs. pH profile shifts progressively towards higher pH values.

2.3. Sensing anions

Niikura and Anslyn published in 2003 an interesting example of a fluorescent sensor of the chemosensing ensemble type, that takes advantage of micellar inclusion and of the consequent poor solvation of the molecular components [80]. The molecular receptor **34** and the fluorescent 5-carboxy fluorescein molecule (**35**) form a stable chemosensing ensemble in methanol, capable of efficiently signal the inositol triphosphate anion (**36**). **34** plus **35** in methanol interact and promote the stabilization of the yellow fluorescent open form of the latter, due to the favourable charge pairing interactions. In the same solvent, addition of inositol triphosphate is signaled, because it results in the preferential interaction between **34** and **36**, with the release in the solvent of **35**, that once free, prefers its cyclized, colorless and non-fluorescent form. The affinity of **36** for **34** is strongly reduced in water, due to solvation, and moreover the closed form of **35** is not stabilized by water, so the inositol triphosphate cannot be signaled. However, the same system works well in water plus Triton X-100 solution, due to inclusion

in the micellar containers where the affinity of **36** for **34** is promoted by low solvation and by local concentration increase effects. Moreover, when **36** displaces 5-carboxy fluorescein from **34**, **35** remains inside the micelles, where its closed form is more stable than the open one, due to the poorly polar environment. As a consequence, a very efficient ON–OFF sensor for inositol triphosphate is obtained in water plus Triton X-100 micelles. Interestingly, the authors report that the use of a non-ionic surfactant is exclusive, as ionic surfactants as SDS (**5**) and CTAB (**14**) does not induce the formation of the colorless, non fluorescent form of **35**.

3. Working on receptor's lipophilicity and structure to improve sensing efficiency in micellar self-assembled fluorescent sensors for Cu^{2+}

It has been another goal of ours to understand the features that regulate the efficiency of fluorescent self-assembled micellar sensors for Cu^{2+}. For a sensor, the percentage residual fluorescence, I_{RES}, may be defined as in Eq. (1)

$$I_{RES} = \frac{I_f}{I_0} \times 100 \quad (1)$$

where I_f and I_0 are the fluorescence intensity of the sensing system when all the receptors are loaded with the target substrate and when no substrate is added (at the working conditions), respectively. Moreover I_0, the fluorescence of the free sensor in the working conditions, may be lower than the maximum fluorescence intensity available from the fluorophore/micelle/ligand(s) assembly, that may be higher under different conditions (typically pH). Accordingly, I_0 is the "exploitable" fluorescence of the sensor. The lower I_{RES} and the higher is the exploitable fluorescence, the more efficient is a sensor. For any kind of fluorescent ON–OFF sensors and for micellar self-assembled fluorescent in particular, I_{RES} maybe significantly >0, see Fig. 14a. The Cu^{2+} sensor made of pyrene and **22** contained in Triton X-100 micelles displayed a I_{RES} = 18%[R]. Explanation of such high I_{RES} value has been put forward by us, investigating the sensing system by means of time resolved and steady-state fluorescence experiments [81]. Under the working concentration conditions, a ratio of 10 molecules of **22** per Triton X-100 micelle was used, which does not affect the properties of the container micelles [81]. Pyrene was used in such a low concentration to obtain a ratio of 0.01 molecules per micelle. Under these conditions each micelle containing 1 pyrene would also contain 10 ligand molecules (and 10 quenching Cu^{2+} complexes when the ligands are all saturated by the metal cation). Fluorescence kinetics in micellar solutions can be described by the expression proposed by Infelta et al. [34] successively modified by Tachiya [82], to fit the observed intensity decay after a pulse at $t = 0$, as a function of time and of the k_1, k_2 and k_3 constants.

$$\ln\left(\frac{I(t)}{I(0)}\right) = -k_1 t - k_2(1 - e^{-k_3 t}) \quad (2)$$

In Eq. (2) constants k_1 and k_2 are functions of τ_0 (pyrene lifetime in the absence of quencher), τ_q (effective fluorophore-quencher collision time in micelle), τ_- (residence time of the quencher in micelle) and <*q*> (average number of quencher per micelle), while k_3 is a function of τ_q and τ_-. Values of 268 ns (τ_0), 118 ns (τq) and 665 ns (τ_-) were found by us for our system, with <*q*> = 10, sharply indicating that the quenching process in intramicellar. An expression for steady state fluorescence can be written [83] using these calculated parameters, obtaining I_{RES} as a function of <*q*>, Fig. 14b. With <*q*> = 10 an I_{RES} value as low as 5% should be expected. The observed I_{RES} value of 18% corresponds to an "effective" <*q*> value of 4. This means that only 4 out of 10 molecules of the micelle-included Cu^{2+}-complexed ligands are capable of effectively quenching the pyrene excited state. TritonX-100 micelles have an oblate ellipsoid shape [8] that drives compartmentalization and preferential accommodation of ligand **22** (and of its Cu^{2+} complex) in the flattest region of the micelle. It is the preferential accommodation of the complex **22**/Cu^{2+} in a certain region of the micellar container that reduces the number of available quenchers, resulting in a higher steady state fluorescence response [84].

Variation in the total number and type of carbon atoms appended to the dioxo-2,3,2 fragment can help to control the I_{RES} values [85]. Molecules **37**–**40** have been used in the same concentration and pH conditions of **22** with pyrene in Triton X-100 micelles. With fully Cu^{2+}-complexed ligands I_{RES} values were found to be 18% for **37**, 8% for **38** and 38% for **39**, while the Cu^{2+} complex of **40** gave only a poor response, with I_{RES} = 87%. This latter result was attributed to the poor lipophilicity of the Cu^{2+} complex of **40** that is distributed only for a small percentage inside the micellar containers. The different I_{RES} values found with the Cu^{2+} complexes of **37**–**40** correlate with the pK_a of the ammonium moieties of their amino groups. The pK_a values in turn may be put in relation with the positioning inside the micellar core of each molecule: the highest the lipophilicity, the deeper is the positioning and the lower is the observed basicity (higher pK_a). Changing the structure with respect to **22** changes also the exploitable fluorescence. For **22** in the absence of Cu^{2+} I_f is constant (within 2%) all over the pH range, due to the poor quenching ability of primary amines. Ligands **37**–**40** have secondary amines that are able to partially quench pyrene fluorescence in micelles. Their "exploitable" fluorescence may be evaluated as the percentage fluorescence intensity, with respect to

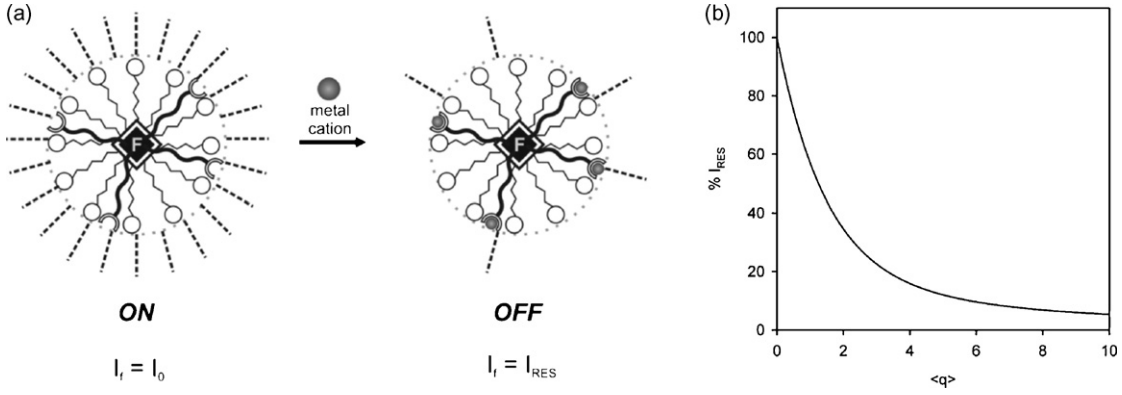


Fig. 14. (a) Pictorial representation of the residual fluorescence intensity item: when all the receptors included in the micellar container bind the substrate, a residual emission may remain; (b) calculated residual percentage I_{RES} as a function of number of quenchers included in Triton X-100 micelles.

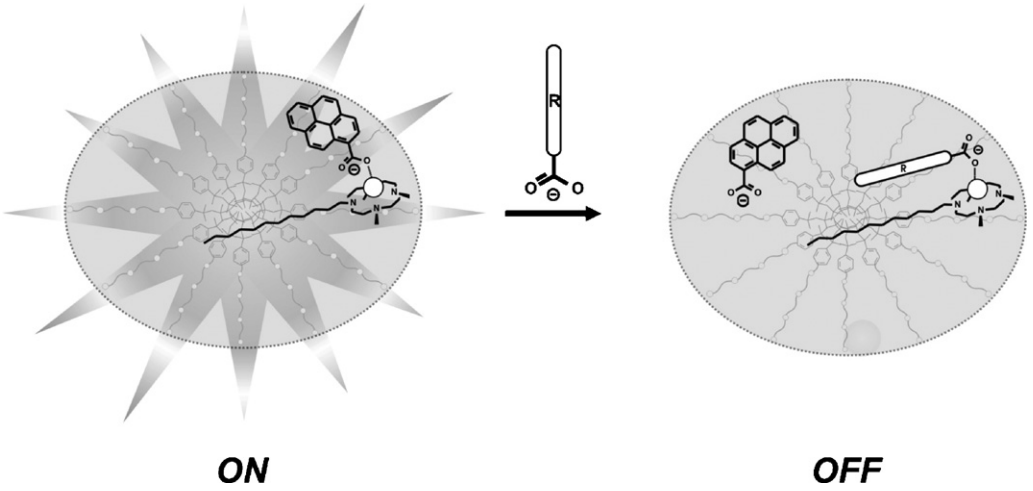


Fig. 15. Working scheme for the lipophilicity sensor made of micelle-included molecular components **48** and **49**. They interact exclusively inside micelles to form **50** (ON state). A competing carboxylate that is sufficiently lipophilic to enter the micellar container will displace **48** from the apical position of Zn^{2+}, with a huge fluorescence decrease (OFF state).

that of micellar pyrene alone, at the pH value at which Cu^{2+} is fully complexed. Exploitable fluorescence values of 96%, 87% and 86% are found for **37**, **38** and **39**, respectively.

The effect of the variation of the container type and shape on I_{RES} has also been examined by us, changing the surfactant and leaving unchanged both the fluorophore (pyrene) and the ligand (**22**) [86]. We have considered micelles made of the series of surfactants Triton X-100 (**3**), CxEy (**41**–**43**), PONPE n (**44**–**46**), Tween20 (**47**). In each case time-resolved experiments were carried out to individuate the kinetic parameters of the quenching processes and a model was proposed for calculating the expected I_{RES} % on the basis of the surfactant and of the Cu^{2+}/**22** complex (i.e. quencher) concentrations. Steady state fluorescence was also measured to obtain experimental I_{RES} values. Polarity and microviscosity inside the micelles were also calculated using respectively the I_1/I_3 ratio of the vibrational peaks of plain pyrene [87–89] and the ratio of monomer/excimer emission of 1,3-dipyrenyl-propane [86]. No correlation was found for I_{RES} neither with the polarity, nor the microviscosity nor the size of the micellar containers. On the other hand, the shape of the micelles seems to play an important role. In micelles with an oblate ellipsoid shape, where compartmentalization of included species is expected, the calculated I_{RES} is significantly lower than experimental I_{RES}. In micelles with a spherical shape, like those made of CxEy surfactants, the symmetry of the container rules out compartmentalization and all the included Cu^{2+} complexes can participate in the quenching processes. Accordingly, calculated and experimental I_{RES} are identical. An I_{RES} % as low as 10.8 was found in the OleylE_{20} (**43**) surfactant micelles.

4. A step forward: micellar sensors for lipophilicity

We recently proposed an evolution of the micellar self-assembled fluorescent sensors, capable of reporting about a complex molecular property, i.e. lipophilicity [90]. In this case, besides fully exploiting the local concentration increase and the promotion of interactions exerted by the nanosize and by the organic-like properties of the micellar core, we also exploited the capability of the micellar containers to discriminate the entrance of molecular species on the basis of their lipophilicity. Using Triton X-100 as surfactant (**3**), 1-pyrenecarboxylic acid (**48**) and the Zn^{2+} complex of the lipophilic N-dodecyl, *N′*,*N″*,*N‴*-trimethyl Cyclen ligand (**49**) were assembled in micelles. While **48** and **49** do not interact in water, when they are included in the micellar containers the $-COO^-/Zn^{2+}$ apical coordination is dramatically promoted, and an observed log*K* value of 5.6 can be measured for the formation of complex **50**. The complex exists in micelles in the 5–8 pH range, with the fluorescence of the pyrene molecule boosted by 600% due to the reduced wobbling dynamics experienced when coordinated to Zn^{2+} inside the micellar containers. This is the ON state for sensing molecules with a competing group, in particular $-COO^-$, that can displace **48** from the Zn^{2+} apical position, releasing it inside the micelle with a huge I_f decrease (OFF state, see Fig. 15). Due to the intrinsic selectivity of a micelle towards the lipophilic features of a molecule, the entity of the fluorescence decrease is proportional to the ability of the competing molecule to enter the micellar containers, i.e. to the competing molecule's lipophilicity. The sensor has been applied to the fatty acid series $CH_3(CH_2)_nCOOH$ (n = 0–16), obtaining the I_f vs. fatty acid concentration profiles of Fig. 16a. The differences in the response are a function of the number of carbon atoms as demonstrated by Fig. 16b, illustrating the I_f% values at a concentration of $CH_3(CH_2)_nCOOH$ ~50 times larger than that of **48**. In turn, the chain length of a fatty acid is proportional to its lipophilicity, as is well known from the literature [91]. This is also demonstrated by the observed log*K* values measured for the same series of fatty acids in Triton X-100 micelles, which reproduce the same sigmoidal profile of Fig. 16b as a function of carbon atom number.

5. Using micelles as containers to improve the efficiency of F–S–R and chemosensing ensemble sensors

Recently, a bunch of papers has appeared, in which sensors from "classical" F–S–R chemistry are used in aqueous micellar solution, taking advantage either of the poor solvation inside the micellar core or of the favourable electrostatic effect played by the charged heads of the ionic surfactants in the palisade layer. These systems are not properly a multicomponent self-assembly, as usually one single molecular component is included in the micellar container, but the continuity with the systems reviewed in sections 2–4 is evident and they have to be included in this review.

Selective sensing of alkali and alkaline earth cations has been achieved using monoaza cryptands **51** and **52** featuring pyrene as the fluorophore. Complexation of the metal cations inside the

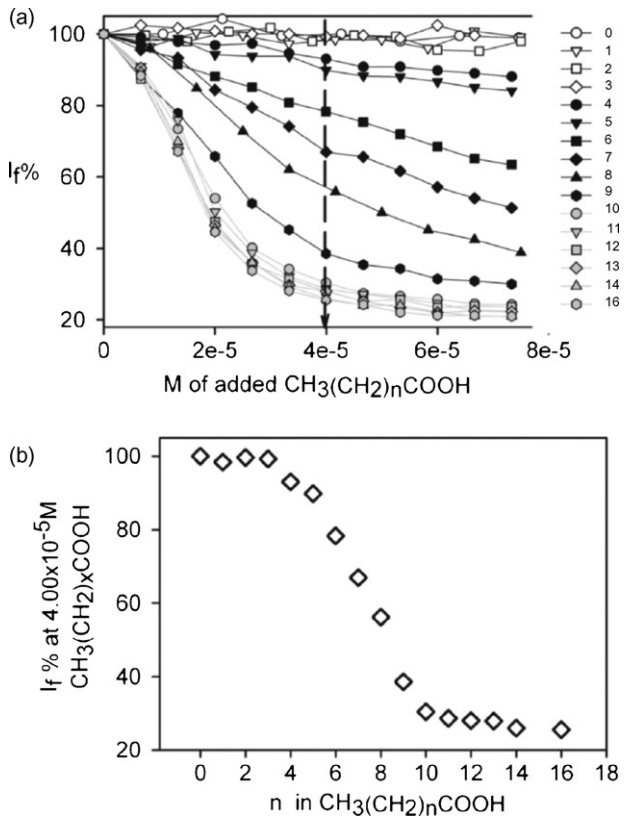


Fig. 16. (a) I_f vs. molar concentration of added $CH_3(CH_2)_nCOOH$ for the micellar system made of **48** + **49** + pyrene. The correspondence between the graphical symbols and the *n* value of the pertinent species is on the right of the figure. The dashed arrow indicates the concentration value at which I_f data are taken to build (b); (b) Percentage fluorescence for the micellar **48** + **49** + pyrene system at 4.00×10^{-5} M concentration of added $CH_3(CH_2)_nCOOH$, as a function of *n*. Figures are adapted from Ref. [90].

cryptand cavity would prevent the PeT quenching mechanism that involves the lone pair on the tertiary amine moiety, resulting in an OFF–ON behaviour of the sensor. However, sensitivity is negligible in pure water, due to the competition of water for cation (and ligand) solvation, which results in a weak or nil complexation in the cryptand cavity. In the presence of micelles of non-ionic surfactants (Tween-60 and Triton X-100 for **51** and **52**, respectively) the ligands are reasonably distributed mainly inside the micelles, where complexation is promoted by poor solvation. In micellar medium, dramatic I_f revivals are observed when adding K^+ (**51**) and Ba^{2+} (**52**) as a consequence of the huge increase of the observed complexation constants. Potassium and Barium are selectively signaled among alkali and alkaline earth cations [92,93].

More micellar sensors for alkali metal ions have also been reported, based on the water-insoluble spirobenzopyrane-azacrown ethers conjugates **52**–**55**. This series of molecules dissolves well in micellar solutions of the anionic surfactant tetramethylammoniumdodecylsulfate (TMADS). Also these systems may work as OFF–ON fluorescent sensors, as the lone pair of the tertiary amine nitrogen participates in the complexation of an alkali metal cation inside the azacrown cavity, this preventing fluorescence quenching due to PeT to the excited state of the spirobenzopyrane fluorophore. However, inclusion in a negatively charged micelle dramatically increases the observed basicity of the NR_3 moiety, so that its full deprotonation and full fluorescence revival in the absence of added metal is obtained only at pH 13. Working at this pH, addition of alkali metal cations results in complexation in the micelle-included azacrown ethers, promoted also by the overall negative charge of the micellar container. I_f increase is observed, with some selectivity dictated by the macrocyclic cavity-ion radius match. The most effective selectivity is found for ligand **53** and Li^+, over the Na^+, K^+ and Cs^+ competitors [94].

Fluorescent sensors for Hg^{2+} have also been proposed, that take advantage of the micellar inclusion of a single molecule that contains both the receptor and the fluorophore. Molecules **56**–**58** feature a rod-shaped hydrophobic 2-phenylbenzoxazole fluorophore and a hydrophilic tetraamide Hg^{2+}-ion receptor. The response to Hg^{2+} binding is of the ON–OFF type. Inclusion in SDS (**5**) micelles substantially increases the tetraamide affinity for the Hg^{2+} cation and also seems to improve the ligands selectivity for this cation. Moreover, **56**–**58** are intramolecular charge-transfer fluorescent species, i.e. they are environment-sensitive and inclusion in micelles advantageously changes some of their properties. For example, emission intensities in the absence of Hg^{2+} are increased and the ON–OFF response of **57** is transformed into a self-calibrated two-wavelength ratiometric signal [95].

Another brilliant example of the exploitation of the particular features brought by inclusion in the micellar containers is a foldamer-based sensor for Hg^{2+} [96]. The authors chose a long foldamer molecule made of six cholate and two methionine units, bearing a covalently linked dansyl fluorescent group at one end. This molecule dissolves and folds into an helical structure in nonpolar solvents, while it is insoluble in water. When folded, it is preorganized as a bidentate ligand that strongly and selectively binds Hg^{2+}. Aqueous micellar solutions of Triton X-100, cetyltrimethylammonium bromide (CTAB) and SDS solubilize well the foldamer, with fluorescence increase of the dansyl unit, as expected by the inclusion of the latter in a poorly polar environment [97]. Inside the micelles the foldamer is preorganized in the conformation capable of efficiently bind Hg^{2+}. However, the fluorescence response to Hg^{2+} is poor in the cationic micelles of CTAB, as the overall positive charge of the container rejects the mercury cations. Very efficient quenching is observed in Triton X-100 and, even better, in the anionic micelles of SDS.

Exclusive Zn^{2+} sensing by means of the FSR molecule 1-pyrenylmethyl-bis(2-picolyl) amine (**59**) is observed in micellar solution. Interestingly **59** aggregates in water, due to pyrene–pyrene π-stacking promoted by hydrophobic interactions, and this makes its bis(2-picolyl)amine binding unit unavailable for any metal cation, Zn^{2+} included. The use of a micellar solution of the ionic SDS and CTAB, or of the non-ionic Tween20 surfactants results in the micellar inclusion and disaggregation of the pyrene units (as can be noted from the disappearance of the pyrene–pyrene excimer emission band). This makes the bis-picolylamine tridentate unit available for coordination, and addition of Zn^{2+} is followed by fluorescence revival (fluorescence is otherwise quenched by NR_3 by PeT). More intense effects are observed in Tween20 and SDS micelles, while the electrostatic repulsion effect exerted by CTAB depresses coordination of the metal cation and I_f increase [98].

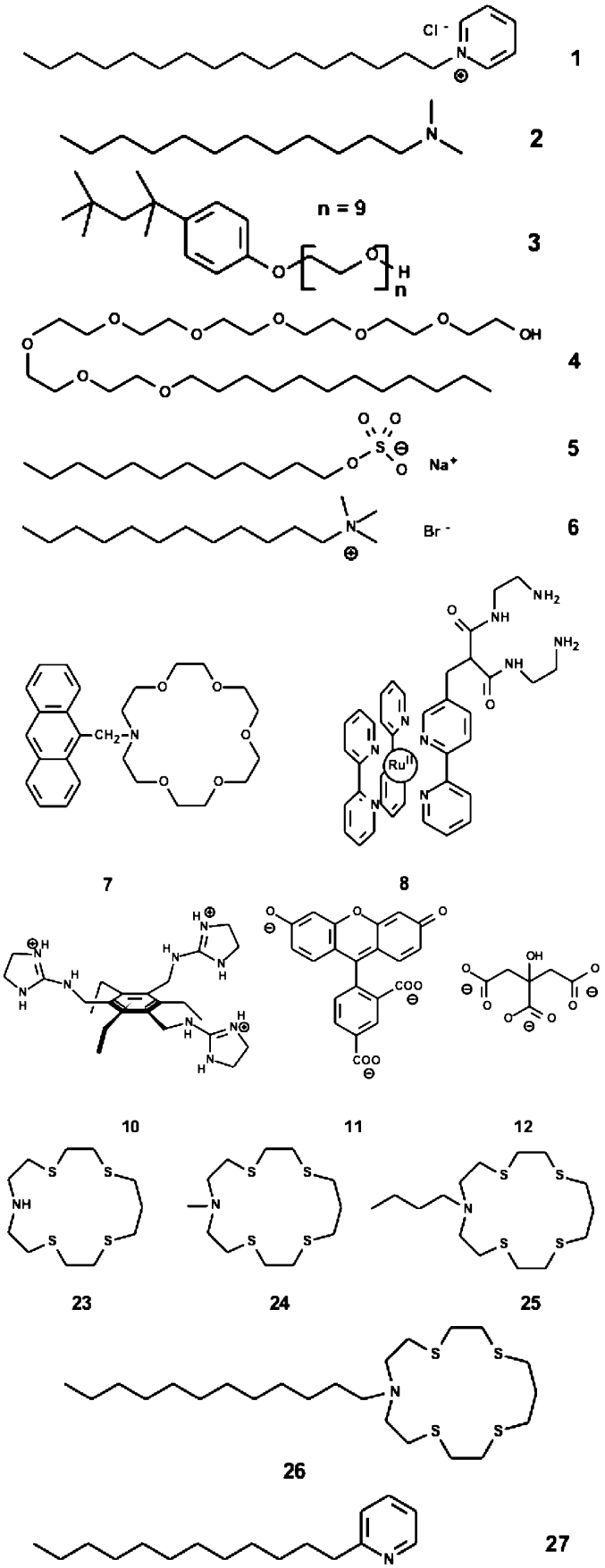
Sensing of Cu^{2+} with an indoloquinolizine molecule (**60**) has been studied in SDS as a function of the surfactant concentration [99,100]. Inclusion of the fluorophore in micelles results in an increase of I_f and quantum yield. The fluorophore does not feature a specific interaction site for Cu^{2+}, so the I_0/I_f response follows the Stern–Volmer equation with the concentration of Cu^{2+} that acts as the quencher. However, in the presence of surfactant a clear magnification of the sensor response is observed, due to the K_{SV} increase. This is in turn due to the favourable electrostatic effect played by the SDS micelles surface, which hugely increases the local concentration of the Cu^{2+} cations.

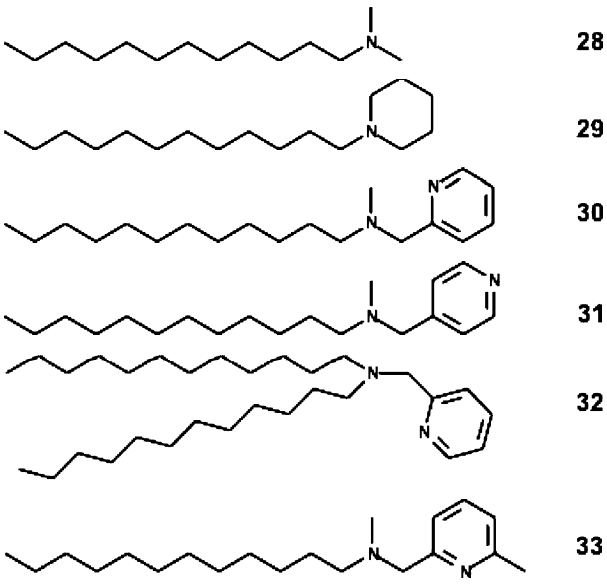
Finally, a smart example of the use of local cation concentration increase in micelles of anionic surfactants has been reported, leading to a micellar AND logic gate [101]. In this case, the lipophilic molecule **61** is dissolved in a micellar aqueous solution. **61** contains one fluorophore, anthracene, and two quenchers, i.e. the tertiary amino group and the dimethoxybenzene group (embedded in a crown ether backbone). Both are strong electron donors capable of giving PeT. When the tertiary amine is protonated and the crown ether coordinates Na^+ the quenching mechanisms are not active and the anthracene fluorescence is recovered. The state of the system (fluorescence) steps from 0 to 1 (quenched/recovered) only in the presence of two distinct inputs (H^+ and Na^+), making **61** to behave as an AND molecular logic gate. However, **61** does not react with the Na^+ input in water, due to the poor coordinating ability of the crown ether unit in comparison to solvation. Micelles of cationic (cetyltrimethylammonium chloride) or non-ionic (Triton X-100) surfactants include **61**, but they are not able to locally concentrate Na^+ and no response is observed in I_f with Na^+. When the anionic TMADS (tetramethylammonium dodecylsulfate) is used as the surfactant, the local concentration of Na^+ in micelles is sufficiently high to obtain a two order of magnitude increase of the observed complexation log K in the crown ether unit (stepping from <0.5 to 1.9). This allows one to recover fluorescence when both the amino group is protonated and Na^+ is added and to obtain the desired AND logic response.

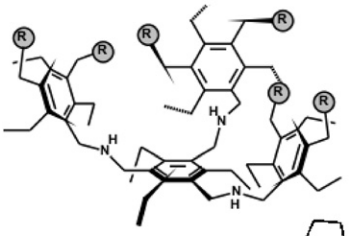
6. Conclusions

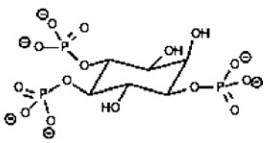
We have shown that micelles can be advantageously used as nanosized containers for the self-assembling of fluorescent sensors for cations, anions, bulk properties such as pH and molecular properties such as lipophilicity. This type of device is characterized by high versatility, as the components (or the container) may be changed almost at will, without the need for particularly complicated synthetic effort, e.g. transforming a fluorescent sensor for Cu^{2+} into a sensor for Hg^{2+} by simply changing the binding unit appended to a long *n*-alkyl chain. Moreover, the properties of a micellar device can be fine-tuned by changing simple structural features of the molecular components included, i.e. acting on their lipophilicity and structure to obtain a different degree of micellar inclusion or a different position inside the micelle. This allows one to regulate the type of response of the sensor (e.g. changing an ON–OFF into an OFF–ON sensor) or its working pH range, or even its signaling efficiency. It has been shown also that acting on the container (particularly on the charges of the surfactant molecules) may bring useful variations in the device properties. A huge range of new developments are possible starting from these results. First, most of the chemistry developed for FSR sensors can be adapted to micellar sensors with many advantages in terms of synthetic simplification, money saving and easiness of use in aqueous solutions (many FSR systems are bound to work only in organic or water-organic solvents). Moreover, we foresee important developments in the use of these kind of sensors for the measurements of molecular properties useful, e.g. in drug design and drug delivery, such as the acid/base behaviour and the stability in a membrane-like environment, and the lipophilicity of pharmaceutically interesting molecules. Studies in these fields are currently going on in our laboratories. Finally, the in-vivo use of this kind of sensor may be envisaged, e.g. for visual signaling if a process is taking place inside the desired pH window. However, this kind of use may be prevented by the unavoidable presence of monodispersed surfactant monomers in a concentration as high as cmc. Accordingly, we are now moving also in the use of micelles made of biologically compatible block-copolymers as an alternative to traditional surfactants for the self-assembling of multicomponent nanosized sensors and devices.

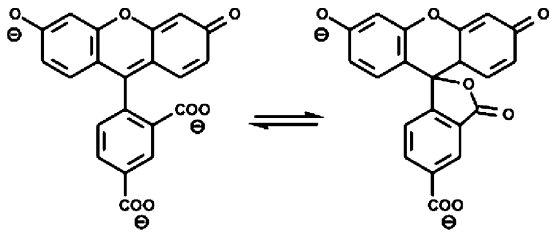
Appendix A. Formula

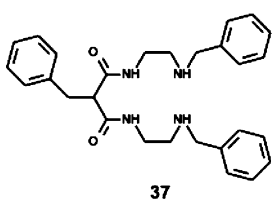


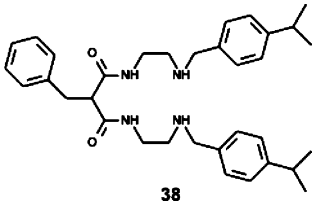


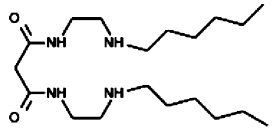


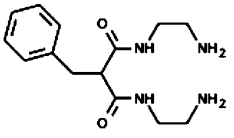


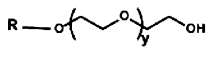








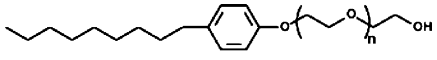




41 R = *n*-dodecyl, y = 25

42 R = cetyl, y = 23

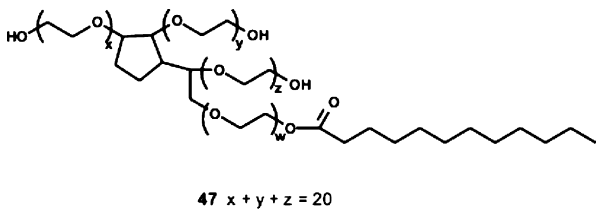
43 R = oleyl, y = 20



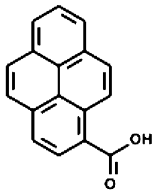
44 n = 15

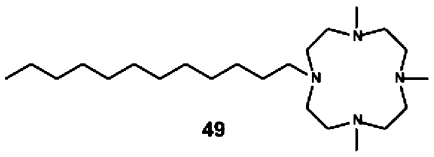
45 n = 18

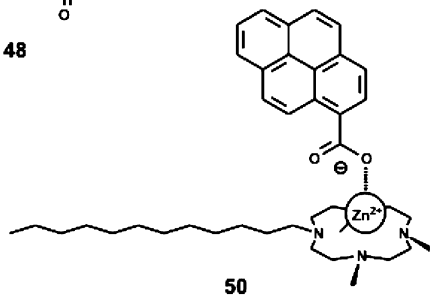
46 n = 20

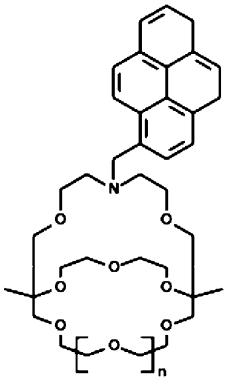


47 x + y + z = 20



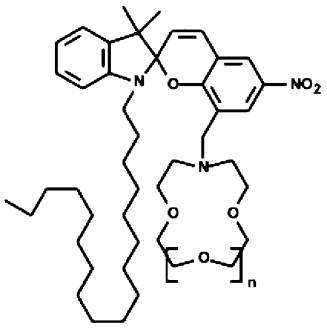






51 n = 0

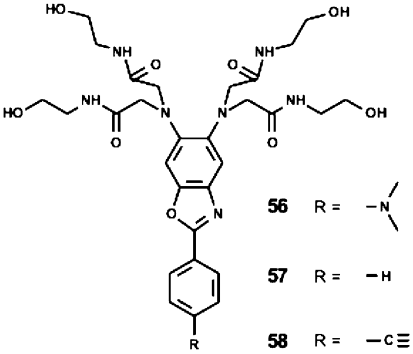
52 n = 1



53 n = 1

54 n = 2

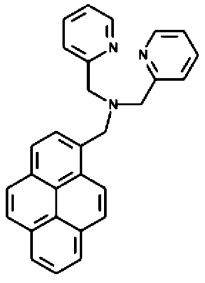
55 n = 3

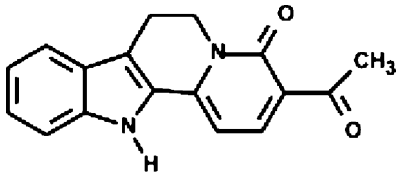


56 R = $-N$

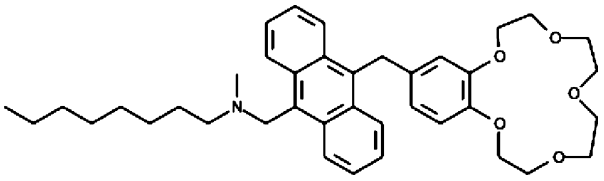
57 R = $-H$

58 R = $-C\equiv N$





60



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References

[1] J.A. Hunt, Pharm J. 263 (1999) 985.
[2] (a) T.F. Tadros, Surfactants, Academic, London, 1984;
(b) M.J. Rosen, Surfactant and Interfacial Phenomena, 3rd ed., Wiley Interscience, New York, 2004;
(c) D. Myers, Surfactant Science and Technology, 3rd ed., Wiley Interscience, New York, 2005;
(d) R.J. Farn, Chemistry and Technology of Surfactants, Blackwell Publishing, Oxford, 2006.
[3] (a) M. Gratzel, Kinetics and Catalysis in Microheterogeneous systems, Marcel Dekker, New York, 1991;
(b) M.N. Kahn, Micellar Catalysis, Taylor & Francis, Boca Raton, 2006.
[4] C. Tanford, The Hydrophobic Effect: Formation of Micelles and Biological Membranes, 2nd ed., Wiley Interscience, New York, 1980.
[5] V.-R. Hanke, W. Knoche, E. Dutkiewicz, J. Chem. Soc., Faraday Trans. 1 (83) (1987) 2847.
[6] R. Zana, J. Phys. Chem. B 103 (1999) 9117.
[7] M. Tachiya, Chem. Phys. Lett. 69 (1980) 605.
[8] R.J. Robson, E.A. Dennis, J. Phys. Chem. 81 (1977) 1075.
[9] P.H. Nelson, G.C. Rutledge, T.A. Hatton, J. Chem. Phys. 107 (1997) 10777.
[10] G.D.J. Phillies, J. Stott, S.Z.J. Ren, J. Phys. Chem. 97 (1993) 11563.
[11] K. Streletzky, G.D.J. Phillies, Langmuir 11 (1995) 42.
[12] W. Brown, R. Rymden, J. van Stam, M. Almgren, Svensk, J. Phys. Chem. 93 (1989) 2512.
[13] G.B. Dutt, Langmuir 21 (2005) 10391.
[14] L. Kong, M. Cao, M. Hai, J. Chem. Eng. Data 52 (2007) 721.
[15] E. Freitosa, W. Brown, Langmuir 14 (1998) 4460.
[16] K.A. Johnson, G.B. Westermann-Clark, D.O. Shah, Langmuir 5 (1989) 932.
[17] V. Balzani, P. Ceroni, B. Ferrer, Pure Appl. Chem. 76 (2004) 1887.
[18] V. Balzani, A. Credi, M. Venturi, Phys. World 17 (2004) 39.
[19] J.M. Lehn, Angew. Chem., Int. Ed. Engl. 29 (1990) 1304.
[20] V. Frenna, N. Vivona, G. Consiglio, D. Spinelli, J. Chem. Soc. Perkin Trans. 2 (1985) 1865.
[21] Y. Diaz-Fernandez, F. Foti, C. Mangano, P. Pallavicini, S. Patroni, A. Perez-Gramatges, S. Rodriguez-Calvo, Chem. Eur. J. 12 (2006) 921.
[22] IUPAC Stability Constant Database (SC-Database), Academic Software, UK.
[23] F.L.B. da Silva, D. Bogren, O. Söderman, T. Åkesson, B. Jönsson, J. Phys. Chem. B 106 (2002) 3515.
[24] F. Grieser, C.J. Drummond, J. Phys. Chem. 92 (1988) 5580.
[25] C.J. Drummond, F. Grieser, T.W. Healy, J. Chem. Soc., Faraday Trans. 85 (1989) 551.
[26] N.O. Mchedlov-Petrossyan, A.V. Plichko, A.S. Shumaker, Chem. Phys. Reports 15 (1996) 1661.
[27] G.P. Gorbenko, N.O. Mchedlov-Petrossyan, T.A. Chernaya, J. Chem. Soc., Faraday Trans. 94 (1998) 2117.
[28] N.O. Mchedlov-Petrossyan, N.A. Vodolazkaya, A.O. Doroshenko, J. Fluoresc. 13 (2003) 235.
[29] R.A. Bissell, A.J. Bryan, A.P. de Silva, C.P. McCoy, J. Chem. Soc., Chem. Commun. (1994) 405.
[30] J.K. Thomas, Chem. Rev. 80 (1980) 283.
[31] M. Grätzel, J.K. Thomas, J. Am. Chem. Soc. 95 (1973) 6885.
[32] R.R. Hautala, N.J. Turro, Mol. Photochem. 4 (1972) 545.
[33] L.K. Patterson, E. Vieil, J. Phys. Chem. 77 (1973) 1191.
[34] P.P. Infelta, M. Grätzel, J.K. Thomas, J. Phys. Chem. 78 (1974) 190.
[35] J. Lang, J. Phys. Chem. 94 (1990) 3734.
[36] P.J. Tummino, A. Gafni, Biophys. J. 64 (1993) 1580.
[37] S. Reekemans, D. Bernik, M. Gehlen, J. Vanstam, M. Vanderauweraer, F.C. Deschryver, Langmuir 9 (1993) 2289.
[38] R. Nagarajan, Langmuir 10 (1994) 2028.
[39] A. Jover, F. Meijide, E. Rodríguez Núñez, J. Vázquez Tato, M. Mosquera, Langmuir 13 (1997) 161.
[40] O. Vorobyova, W. Lau, M.A. Winnik, Langmuir 17 (2001) 1357.
[41] C.A. Silverio, L.T. Okano, Colloids Surf. B 38 (2004) 41.
[42] B.L. Van Duuren, Chem. Rev. 63 (1963) 325.
[43] A.P. de Silva, R.A.D. Dayasiri Rupasinghe, J. Chem. Soc. Chem. Commun. (1985) 1669.
[44] J.P. Konopelski, F. Kotzyba-Hibert, J.-M. Lehn, J.-P. Desvergne, F. Fage, A. Castellan, H. Bouas-Laurent, J. Chem. Soc. Chem. Commun. (1985) 433.
[45] A.P. de Silva, S.A. de Silva, J. Chem. Soc. Chem. Commun. (1986) 1709.
[46] M. Huston, K. Haider, A.W. Czarnik, J. Am. Chem. Soc. 110 (1988) 4460.
[47] L. Fabbrizzi, M. Licchelli, P. Pallavicini, A. Perotti, D. Sacchi, Angew. Chem. Int. Ed. 33 (1994) 1975.
[48] L. Fabbrizzi, M. Licchelli, P. Pallavicini, A. Perotti, A. Taglietti, D. Sacchi, Chem. Eur. J. 2 (1996) 75.
[49] F. Bolletta, I. Costa, L. Fabbrizzi, M. Licchelli, M. Montalti, P. Pallavicini, L. Prodi, N. Zaccheroni, J. Chem. Soc., Dalton Trans. (1999) 1381.
[50] A.P. de Silva, H.Q.N. Gunaratne, C.P. McCoy, Chem. Commun. (1996) 2399.
[51] S.A. de Silva, A. Zavaleta, D.E. Baron, O. Allam, E.V. Isidor, N. Kashimura, J.M. Percarpio, Tetrahedron Lett. 38 (1997) 2237.
[52] B. Valeur, I. Leray, Coord. Chem. Rev. 205 (2000) 3.
[53] A.G. Bryan, A.P. de Silva, S.A. de Silva, R. Rupasinghe, K. Sandanayake, Biosensors 4 (1989) 169.
[54] A.P. de Silva, H.Q.N. Gunaratne, T. Gunnlaugsson, A.J.M. Huxley, C.P. McCoy, J.T. Rademacher, T.E. Rice, Chem. Rev. 97 (1997) 1515.
[55] L. Fabbrizzi, M. Licchelli, P. Pallavicini, Acc. Chem. Res. 32 (1999) 846.
[56] L. Fabbrizzi, M. Licchelli, P. Pallavicini, D. Sacchi, A. Taglietti, Analyst 121 (1996) 1763.
[57] A.W. Czarnik, Acc. Chem. Res. 27 (1994) 302.
[58] T. Gunnlaugsson, M. Glynn, G.M. Tocci, P.E. Kruger, F.M. Pfeffer, Coord. Chem. Rev. 250 (2006) 3094.
[59] R. Martinez-Manez, F. Sancenon, J. Fluoresc. 15 (2005) 267.
[60] P.D. Beer, P.A. Gale, Chem. Angew, Int. Ed. Engl. 40 (2001) 486.
[61] L. Fabbrizzi, I. Faravelli, G. Francese, M. Licchelli, A. Perotti, A. Taglietti, Chem. Commun. (1998) 971.
[62] (a) B.T. Nguyen, E.V. Anslyn, Coord. Chem. Rev. 250 (2006) 3118;
(b) A. Metzeger, E.V. Anslyn, Angew. Chem. Int. Ed. Engl. 37 (1998) 649.
[63] S.L. Wiskur, H. Ait-Haddou, J.J. Lavigne, E.V. Aslyn, Acc. Chem. Res. 34 (2001) 963.
[64] L. Fabbrizzi, F. Foti, A. Taglietti, Org. Lett. 7 (2006) 2603.
[65] V. Amendola, M. Bonizzoni, D. Esteban-Gomez, L. Fabbrizzi, M. Licchelli, F. Sancenon, A. Taglietti, Coord. Chem. Rev. 250 (2006) 1451.
[66] A. Kumar, S.-S. Sun, A.J. Lees, Coord. Chem. Rev. 252 (2008) 922.
[67] S.-S. Sun, A.J. Lees, J. Am. Chem. Soc. 122 (2000) 8596.
[68] P. Thanasekaran, R.-T. Liao, B. Manimaran, Y.-H. Liu, P.-T. Chou, S. Rajagopal, K.-L. Lu, J. Phys. Chem. A 110 (2006) 10683.
[69] R. Lin, J.H.K. Yip, K. Zhang, L.L. Koh, K.-Y. Wong, K.P. Ho, J. Am. Chem. Soc. 126 (2004) 15852.
[70] R.V. Slone, D.I. Yoon, R.M. Calhoun, J.T. Hupp, J. Am. Chem. Soc. 117 (1995) 11813.
[71] M. Sathiyendiran, R.-T. Liao, P. Thanasekaran, T.-T. Luo, N.S. Venkatramana, G.-H. Lee, S.-M. Peng, K.-L. Ku, Inorg. Chem. 45 (2006) 10052.
[72] P. Grandini, F. Mancini, P. Tecilla, P. Scrimin, U. Tonellato, Angew. Chem. Int. Ed. 38 (1999) 3061.
[73] W.L. Koltun, R.H. Roth, F.R.N. Gurd, J. Biol. Chem. 238 (1963) 124.
[74] W.L. Koltun, M. Fried, F.R.N. Gurd, J. Am. Chem. Soc. 82 (1960) 233.
[75] M. Berton, F. Mancin, G. Stocchero, P. Tecilla, U. Tonellato, Langmuir 17 (2001) 7521.
[76] Y. Diaz-Fernandez, A. Perez-Gramatges, V. Amendola, F. Foti, C. Mangano, P. Pallavicini, S. Patroni, Chem. Commun. (2004) 1650.
[77] G. Desantis, L. Fabbrizzi, A.M. Manotti Lanfredi, P. Pallavicini, A. Perotti, F. Ugozzoli, M. Zema, Inorg. Chem. 34 (1995) 4529.
[78] P. Pallavicini, Y.A. Diaz-Fernandez, F. Foti, C. Mangano, S. Patroni, Chem. Eur. J. 13 (2007) 178.
[79] P. Pallavicini, Y.A. Diaz-Fernandez, L. Pasotti, in preparation.
[80] K. Niikura, E.V. Anslyn, J. Org. Chem. 68 (2003) 10156.
[81] Y. Diaz-Fernàndez, A. Pérez-Gramatges, S. Rodrìguez-Calvo, C. Mangano, P. Pallavicini, Chem. Phys. Lett. 398 (2004) 245.
[82] M. Tachiya, J. Chem. Phys. 76 (1982) 340.
[83] M.H. Gehlen, M. Van de Auweraer, S. Reekmans, M.G. Neumann, F.C. De Schryver, J. Phys. Chem. 95 (1991) 5684.
[84] Y. Díaz-Fernández, A. Pérez-Gramatges, S. Rodríguez-Calvo, C. Mangano, P. Pallavicini, Chem. Phys. Lett. 398 (2004) 245.
[85] P. Pallavicini, L. Pasotti, S. Patroni, Dalton Trans. (2007) 5670.
[86] Y. Díaz-Fernández, S. Rodríguez-Calvo, A. Pérez-Gramatges, P. Pallavicini, S. Patroni, C. Mangano, J. Colloid Interf. Sci. 313 (2007) 638.
[87] K. Kalyanasundaram, J.K. Thomas, J. Am. Chem. Soc. 99 (1977) 2039.
[88] N.J. Turro, P.L. Kuo, Langmuir 1 (1985) 170.
[89] G. Komaromy-Hiller, N. Calkins, R. Von Wandruszka, Langmuir 12 (1996) 916.
[90] G. Chirico, M. Collini, L. D'Alfonso, F. Denat, Y.A. Diaz-Fernandez, L. Pasotti, Y. Rousselin, N. Sok, P. Pallavicini, ChemPhysChem 9 (2008) 1729.
[91] J. Sangster, J. Phys. Chem. Ref. Data 18 (1989) 1111.
[92] Y. Nakahara, T. Kida, Y. Nakatsuji, M. Akashi, Chem. Commun. (2004) 224.
[93] Y. Nakahara, T. Kida, Y. Nakatsuji, M. Akashi, Org. Biomol. Chem. 3 (2005) 1787.
[94] H. Sakamoto, T. Yamamura, K. Takumi, K. Kimura, J. Phys. Chem. 20 (2007) 900.
[95] J. Wang, X. Qian, J. Qian, Y. Xu, Chem. Eur. J. 13 (2007) 7543.
[96] Y. Zhao, Z. Zhong, Org. Lett. 8 (2006) 4715.
[97] Y.-H. Li, L.-M. Chan, L. Tyer, R.-T. Moody, C.M. Himel, D.M. Hercules, J. Am. Chem. Soc. 97 (1975) 3118.
[98] S. Bhattacharya, A. Gulyani, Chem. Commun. (2003) 1158.
[99] P. Das, A. Mallick, D. Sarkar, N. Chattopadhyay, J. Colloid. Interf. Sci. 320 (2008) 9.
[100] A. Mallick, M.C. Mandal, B. Haldar, A. Chakrabarty, P. Das, N. Chattopadhyay, J. Am. Chem. Soc. 128 (2006) 3126.
[101] S. Uchiyama, G.D. McClean, K. Iwai, A.P. de Silva, J. Am. Chem. Soc. 127 (2005) 8920.