A Micellar Multitasking Device: Sensing pH Windows and Gauging the Lipophilicity of Drugs with Fluorescent Signals

ARTICLE *in* CHEMISTRY - A EUROPEAN JOURNAL · DECEMBER 2009

Impact Factor: 5.73 · DOI: 10.1002/chem.200902427 · Source: PubMed

5 AUTHORS, INCLUDING:

Franck Denat
University of Burgundy
128 PUBLICATIONS **1,370** CITATIONS
SEE PROFILE

Y.A. Diaz Fernandez
University of Liverpool
45 PUBLICATIONS **809** CITATIONS
SEE PROFILE

Luca Pasotti
University of Pavia
15 PUBLICATIONS **325** CITATIONS
SEE PROFILE

Sok Nicolas
UMR PAM Procédés Alimentaires et Microbi…
10 PUBLICATIONS **80** CITATIONS
SEE PROFILE

DOI: 10.1002/chem.200902427

A Micellar Multitasking Device: Sensing pH Windows and Gauging the Lipophilicity of Drugs with Fluorescent Signals

Franck Denat,[b] Yuri Antonio Diaz-Fernandez,[a] Luca Pasotti,[a] Nicolas Sok,[b] and Piersandro Pallavicini*[a]

Abstract: A multitasking fluorescent device can be obtained by forming micelles of Triton X-100, containing a lipophilic macrocyclic Cu^{2+} complex and the coordinating fluorophore Coumarin 343 (C343), which features a COOH moiety. At low pH the two micellised components do not interact, and the fluorescence of Courmarin 343 (C343) is intense. At intermediate pH, C343 is deprotonated and coordinates to the Cu^{2+} centre in its apical position, with fluorescence quenching. At higher pH the deprotonated C343 is displaced from Cu^{2+} by the formation of an OH^- complex, and the fluorescence is revived. This allows the system to carry out its first task as it behaves as an "on–off–on" fluorescent sensor for pH windows. The "off" part of the window ranges from pH 6 to 8. In this interval, in which the carboxylate form of C343 is apically coordinated to the Cu^{2+} complex inside micelles, the device carries out its second task, that is, it behaves as a gauge for lipophilicity. For pHs between 6 and 8, molecules containing a COOH group are in their COO^- form and distribute between bulk water and micelles proportionally to their lipophilicity. Upon entering the micelle, their COO^- moiety competes for coordination with C343, displacing it from the Cu^{2+} centre, and this results in fluorescence revival, the intensity of which is also proportional to the lipophilicity of the examined molecule. We have chosen the physiological pH value (7.4) as the working pH, and we have examined the lipophilicity of fatty acids and of the widely used family of non-steroidal anti-inflammatory drugs (NSAIDs). The device successfully measures their lipophilicity, expressing it with an "off–on" type fluorescent signal, as demonstrated by the correlation of the fluorescence increase with the logarithmic water/octanol partition coefficient ($\log P$) and with the difference between the pK_a observed in micelles and that measured in water for NSAIDs.

Keywords: fluorescent probes · lipophilicity · micelles · pH windows · self-assembled devices

Introduction

Molecular fluorescent sensors generally monitor a single parameter, typically cation or anion concentration or bulk values such as pH.[1] Few examples have been proposed of molecular sensors capable of sensing multiple species[2] or of answering with multiple outputs.[3] The concept of a "lab-on-a-molecule" has been put forward for this kind of multitasking sensing molecules.[4] Supramolecular micellar sensors are particularly promising in this perspective, because they allow the facile self-assembling of multiple lipophilic molecular components inside a nanoscaled container, the micelle, in which interactions are dramatically promoted with respect to bulk water.[5] As an example of the potential of supramolecular micellar sensors, the complex "off–on–off" window response for fluorescent pH sensing[6] has been obtained by self-assembling in the same micelle three different molecular components.[7]

It has to be stressed that water-soluble, easy-to-prepare sensors for pH windows are intensely sought-after systems, because most biological processes proceed only within restricted pH ranges, and the cell metabolism of anomalous entities (e.g., tumours[8] or bacterial biofilms[9]) results in a change of the local pH from its normal, physiological value (usually pH 7.4). Noticeably, most of the few reported examples show an "off–on–off" response,[10] whereas the reversed

[a] Dr. Y. A. Diaz-Fernandez, Dr. L. Pasotti, Dr. P. Pallavicini
Dipartimenro di Chimica Generale
Università di Pavia, viale Taramelli 12
27100 Pavia (Italy)
Fax: (+39) 0382-528544
E-mail: piersandro.pallavicini@unipv.it

[b] Prof. Dr. F. Denat, Dr. N. Sok
Institut de Chimie Moléculaire de l'Université de Bourgogne
UMR CNRS 5260, Université de Bourgogne
9 avenue Alain Savary, 21078 Dijon (France)

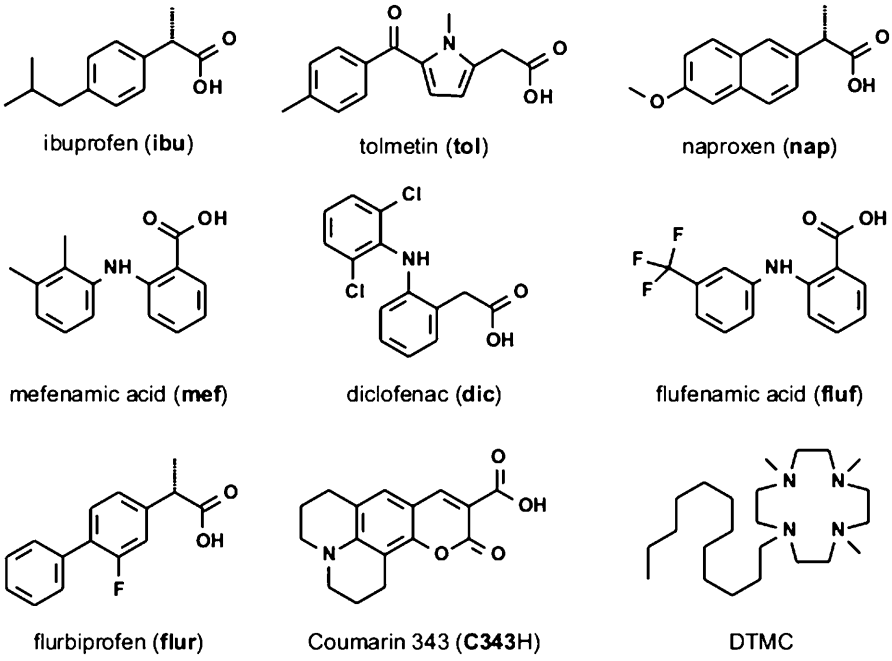
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.200902427.

one, that is, "on–off–on" response appears to be even more difficult to obtain.[11] On the other hand, the latter should be the most useful response type from the perspective of monitoring biotechnological processes or in imaging anomalous cell tissues because it would generate a fluorescence signal (instead of a less easy-to-read decrease) when the pH is drifting out of the normal, or chosen, interval.

Micelles act not just as mere containers because they have a hydrophobic core and a hydrophilic external layer, and some parameters display a centre-to-periphery gradient. In this regard, it must be mentioned that recently a multiplexing sensing system has been proposed for mapping proton concentration and micropolarity in micelles.[12] Moreover, the water–micelle interface mimics a cell membrane and it discriminates the penetration of external molecules on the basis of their lipophilicity.

We have recently described a micellar fluorescent gauge for fatty acids[13] that measures lipophilicity with a decrease in a fluorescent signal. The importance of such a gauge can be stressed by considering that the ability of a molecular drug to dissolve in physiological solutions and to cross natural lipid membranes, which is implicit in drug delivery and pharmacokinetics is a function of its lipophilicity.[14] Moreover, in the expanding area of polymeric micelles that are used as nanocarriers for pharmacological therapies[15] the same physicochemical property is a fundamental parameter in controlling the micelle compatibility with a drug and its load capacity. Lipophilicity also plays a major role in regulating the penetration of a liposome membrane in the strictly related field of liposomal drug delivery.[16] However, the most common parameter for evaluating the lipophilicity of a molecular drug is still its $\log P$ value, that is, its logarithmic partition coefficient. The parameter P is defined as the ratio of the concentration of a compound in the lipid phase to its concentration in the aqueous phase. In pharmaceutical sciences *n*-octanol is the standard lipid phase, and the $\log P$ determination relies on shake-flask or HPLC methods.[17] It should also be considered that many drugs contain acid or basic moieties and evidently have to be used under physiological conditions, that is, in the pH 6.8–7.4 interval, at which they can be ionised. In some cases, the use of $\log D_{7.4}$ has been proposed as an alternative to $\log P$; $\log D_{7.4}$ is the ratio of the concentration of the non-ionised compound in the lipid phase (*n*-octanol) to the concentration of all species in the aqueous phase at pH 7.4. A rough approximation is commonly employed to calculate $\log D_{7.4}$ from $\log P$: the ionised species are assumed to be not soluble in the lipid phase and to remain in water, and the relation $\log D_{7.4} = \log P + \log[1/(1+10^{7.4-pKa})]$ holds for monoprotic acids (the alternative exponent, $pK_a - 7.4$, is used for protonable monobases; pK_a refers to the acidic species in water).[18] Recently, instrumental methods (reversed-phase HPLC) for direct evaluation of $\log D_{7.4}$ have been proposed, in which retention times are proportional to $\log D_{7.4}$ and correlate with $\log P$.[19]

We now demonstrate that by choosing the molecular components appropriately, a multitasking micellar device can be obtained by self-assembling two molecular components in the same micellar container and by exploiting the properties of the container–water interface. The device is capable of carrying out both of the two described, different, nontrivial sensing activities, although of course not simultaneously: 1) it signals pH windows with the rare "well-like" ("on–off–on") fluorescent response and 2) it is capable of measuring the lipophilicity both of simple molecules (fatty acids) and of an important class of drugs (NSAIDs)[20] directly at the physiological pH value 7.4, by means of an easy-to-read switch-on ("off–on" response) of the fluorescent signal.



Results and Discussion

The components of the micellar multitasking device: NSAIDs all have aromatic rings in their structures and display intense absorptions in the 320–400 nm range (e.g., Figure 1, black dash-dot-dot line, **tol** absorption). In the perspective of working with this class of drugs, we chose Coumarin 343 as a fluorophore because it features a pH-dependent coordinating function, COOH, and it absorbs in the

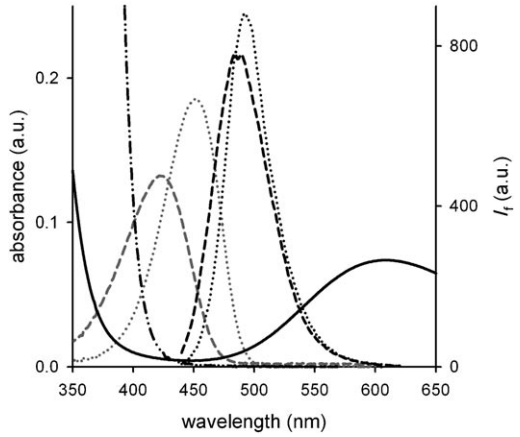


Figure 1. Absorption and emission spectra of relevant species in Triton X-100 solution. Black dash-dot-dot line: **tol** absorption (pH 7.4); black solid line: Cu^{2+} complex of DTMC (pH 7.4); grey dashed line: absorption of **C343**$^-$ (pH 7.4); grey dotted line: absorption of **C343**H (pH 3.2); black dashed line: emission of **C343**$^-$ (pH 7.4); black dotted line: of **C343**H (pH 3.2).

400–500 nm range, which does not interfere with NSAIDs absorption.

By using the non-ionic Triton X-100 (TX-100) surfactant at 0.01 M concentration, we carried out coupled spectrophotometric and pH-metric titrations on micellised Coumarin 343 (8×10^{-7} M concentration; the solution was also 0.05 M $NaNO_3$, as an ionic strength buffer). The carboxylic acid (**C343**H) and the carboxylate form (**C343**$^-$) display shifted absorption bands (Figure 1 grey dotted and dashed lines, respectively), with an isosbestic point at 430 nm. On exciting at 430 nm, the observed emission is also pH-dependent, as can be seen in Figure 1 (black dotted line = **C343**H, black dashed line = **C343**$^-$), with an isosbestic point at 484 nm. This is in agreement with that reported for **C343**H in bulk water in which $pK_a = 4.65$ was calculated.[21] By fitting absorbance versus pH data we found $pK_a = 5.68$ in TX-100, which is in agreement with the decreased tendency of micelle-included carboxylic acids to become deprotonated.[13] The pK_a of Coumarin 343 was also determined under different fluorophore/TX-100 ratios both by spectrophotometric and fluorimetric pH titrations, and values of 4.94 (5×10^{-4} M TX-100), 5.40 (3×10^{-3} M TX-100), 5.68 (1×10^{-2} M TX-100) and 5.76 (2×10^{-2} M TX-100) were obtained. The observed protonation and deprotonation constants of acid/base species in surfactant solutions change on variation of the micelle/species molar ratio if the species distributes between bulk water and micelles. In particular, at high micelle/species molar ratios, full inclusion is obtained, and the observed pK_a reaches its limiting value.[22] A plot of pK_a versus TX-100 concentration (Supporting Information Figure S2) clearly shows a plateau-like trend at M(TX-100) $\geq 1\times10^{-2}$ M; this indicates that at our working TX-100 concentration, Coumarin 343 is prevalently included in micelles with respect to bulk water.

To complete our micellar device, we used the Cu^{2+} complex of a *N*-dodecylated trimethylcyclen (DTMC). This is a complex with one available apical coordination site; in this position Cu^{2+} forms stable complexes with carboxylate ligands and is expected to quench the fluorescence of coordinated fluorophores, because it is a d^9 cation.[23] Its absorption band (pH 7.4) is also shown in Figure 1 (black solid line). It can be seen that both the NSAID and $[Cu(dtmc)]^{2+}$ spectra do not superimpose significantly to the **C343**H/**C343**$^-$ absorption (in particular to the isosbestic point, 430 nm) or emission.

We investigated spectrophotometrically the solution behaviour of the $[Cu(dtmc)]^{2+}$ complex in the 3–11 pH range in TX-100 micelles (complex 5×10^{-4} M; TX-100, 0.01 M; $NaNO_3$, 0.05 M). In the acidic side of the range (pH 3) the complex does not demetallate, that is, it is kinetically inert, as expected for macrocyclic complexes, at least on the timescale of the experiment (<3% absorbance variation in 60 min). For $3<pH<7$ we found $\lambda_{max}=605$ nm ($\varepsilon=260$ M^{-1}cm^{-1}). We also observed a blue shift in the absorption band at pH > 7.5, with λ_{max} changing to 609 nm ($\varepsilon=275$ M^{-1}cm^{-1}; see also the Supporting Information Figure S9). This is consistent with a square pyramidal coordination of Cu^{2+} that is completed by an apical water molecule that deprotonates to give an apically coordinated hydroxide complex, as summarised by Equation (1).[24]

$$[Cu(dtmc)(H_2O)]^{2+} \rightleftharpoons [Cu(dtmc)(OH)]^{+} + H^{+} \quad (1)$$

By means of potentiometric titrations we found $pK_a = 7.41(\pm0.05)$ for the coordinated water molecule. When the same experiments were carried out in water, the pK_a is 7.65($\pm$0.04). The similarity of the two values is not surprising due to the positioning of the Cu–cyclen charged moiety in the more hydrated external layer of the micelle.[25] As a control, the prevalent distribution of the copper complex inside micelles with respect to bulk water was checked by ultrafiltration. In an ultrafiltration experiment, the micelles and their content are rejected from the membrane and remain in the retentate phase, whereas small molecules and electrolytes cross the membrane. When a species is distributed only in part inside the micelles and the ultrafiltration is carried out on a micellar solution, it is expected that the species is found in the permeate at a concentration approximately equal to that it has in the bulk (i.e., outside micelles) of the micellar solution.[26] In our case, a negligible quantity of complex was found in the permeate. Starting from 50 mL of 1.5×10^{-4} M $[Cu(dtmc)]^{2+}$ at pH 7.4, after ultrafiltration we obtained 25 mL of permeate and 25 mL retentate with concentrations of 1.81×10^{-5} M and 2.61×10^{-4} M respectively.

Task 1—sensing pH window with an "on–off–on" response: We assembled the multitask sensing system by co-micellising Coumarin 343 and $[Cu(dtmc)]^{2+}$ in TX-100 micelles. We used 1.5×10^{-4} M copper complex, 8×10^{-7} M Coumarin 343, 0.01 M TX-100 and 0.05 M $NaNO_3$. The chosen surfactant has a cmc of 2×10^{-4} (cmc = critical micelle concentration).[27] Assuming that the aggregation number is 140,[28] each micelle contains an average of about two copper complexes

and ≈0.01 Coumarin 343 molecules. The low fluorophore concentration has been chosen both to minimise the probability of having two fluorophores per micelle (thus ruling out excimer emissions) and to avoid instrumental saturation when measuring emission spectra. Although it is known that added salts in bulk water and the addition of lipophilic species solubilised in the micellar core might modify the dimensions of micelles made of non-ionic surfactants,[29] this concentration of added Cu^{2+} complex and $NaNO_3$ salt is not expected to exert significant effects. TX-100 micelles are nanodimensioned oblate ellipsoids with $r_a = 2.8$ nm and $r_b = 5.9$ nm;[13] in our previous work we have shown this to be not significantly modified by the addition of comparable concentrations (2×10^{-4} M in 0.01 M TX-100) of the Zn^{2+} complex of the DTMC ligand and by 0.05 M $NaNO_3$.[13]

Addition of excess acid and titration with standard base allowed us to collect coupled fluorescence spectra and pH data. We read emission at 485 nm (isosbestic point in the absence of the Cu^{2+} complex) and plotted the percent fluorescence intensity (%I_f) values versus pH (100 % I_f is assigned to **C343**H emission at pH 2.3). The profile is shown in Figure 2 (top; triangles). The data show a well-shaped (or reverse dumb-bell) profile, that is, a window response of the "on–off–on" type. At low pH values coumarin is in the **C343**H form and not coordinated to Cu^{2+}: fluorescence is on (Figure 2, A). At intermediate pH values **C343**$^-$ is formed, which interacts with the copper complex in its apical position by substituting the coordinated water molecule. The $[Cu(dtmc)(\mathbf{C343})]^+$ complex is formed according to Equation (2), and fluorescence goes off (Figure 2, B). When the pH is raised to basic values, the $[Cu(dtmc)(OH)]^+$ complex is formed. OH^- displaces **C343**$^-$ from the Cu^{2+} centre according to Equation (3), and I_f goes on again (Figure 2, C).[30] By using the pK_a of **C343**H and of $[Cu(dtmc)(H_2O)]^{2+}$ we were able to fit I_f versus pH experimental data, to obtain $\log K = 4.8(\pm0.1)$ for Equation (2).

$$[Cu(dtmc)(H_2O)]^{2+} + \mathbf{C343}^- \rightleftharpoons [Cu(dtmc)(\mathbf{C343})]^+ + H_2O \quad (2)$$

$$[Cu(dtmc)(\mathbf{C343})]^+ + OH^- \rightleftharpoons [Cu(dtmc)(OH)]^+ + \mathbf{C343}^- \quad (3)$$

The distribution diagram of the species containing Coumarin 343 is drawn in Figure 2 (top, solid lines). A perfect superimposition of the % I_f versus pH profile is observed in correspondence of the formation of $[Cu(dtmc)(\mathbf{C343})]^+$. For comparison % I_f versus pH is also shown (Figure 2 top, circles) for **C343**H/**C343**$^-$ in TX-100 in the absence of the Cu^{2+} complex. We should stress that the physiological pH range (6.8–7.4) corresponds to the bottom of the fluorescence well and that this is the perfect condition for controlling an anomalous drifting from this interval, as it would be signalled by an easy-to-visualise fluorescence reviving.

Task 2—gauging lipophilicity: In an even larger pH interval (6–8), the micellar device also carries out its second function, that is, it behaves as a lipophilicity gauge. As already mentioned, 6.8–7.4 is considered to be the physiological pH interval, and 7.4 is the "physiological pH value" par excellence. In some cases lipophilicity is evaluated in pharmacology by measuring the water/*n*-octanol distribution of a given molecule at this pH,[31] so it has been chosen as our working value. What we expected is illustrated in Figure 3: at pH 7.4, CH_3-$(CH_2)_n$COOH and NSAIDs are 100 % in their carboxylate form (see also Table S1 in the Supporting Information) and like any $RCOO^-$ molecule, they can distribute between bulk water and the micellar containers proportionally to their lipophilicity. If poorly lipophilic, they will not enter the micellar space, leaving I_f unvaried (Figure 3, top). On the other hand, when sufficiently lipophilic, by enter-

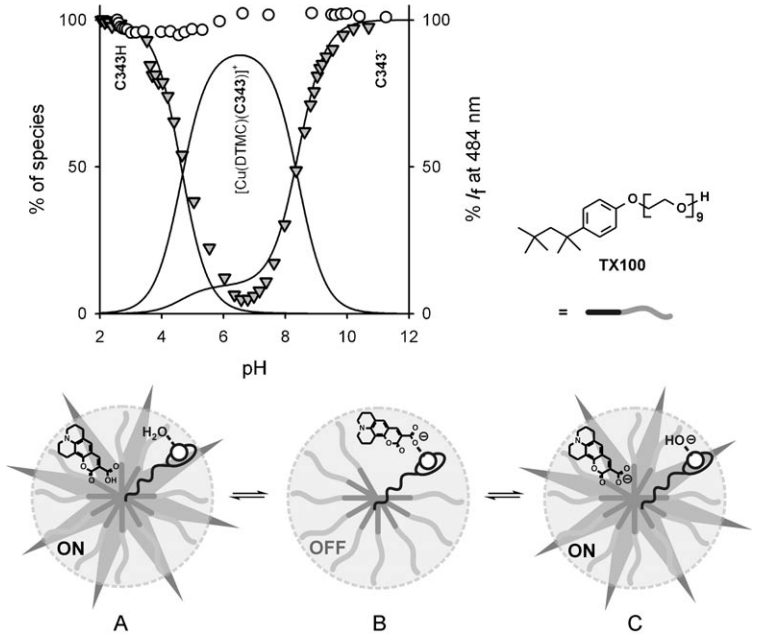


Figure 2. Top: Solid lines: distribution of the species containing Coumarin 343, as a function of pH, expressed as % concentration with respect to the total Coumarin concentration (**C343**$^-$ + **C343**H), calculated for 1.5×10^{-4} M copper complex, and 8×10^{-7} M Coumarin 343; white circles: % emission (484 nm) of Coumarine 343 versus pH in TX-100 micelles; grey triangles: same, in the presence of the Cu^{2+} complex of DTMC. Bottom: working scheme of the micellar device behaving as "on–off–on" sensor for pH windows; the pH value increases on moving from form A to form B and C; form B is at >80 % for pH 6–8, as can be read in the top part of the figure.

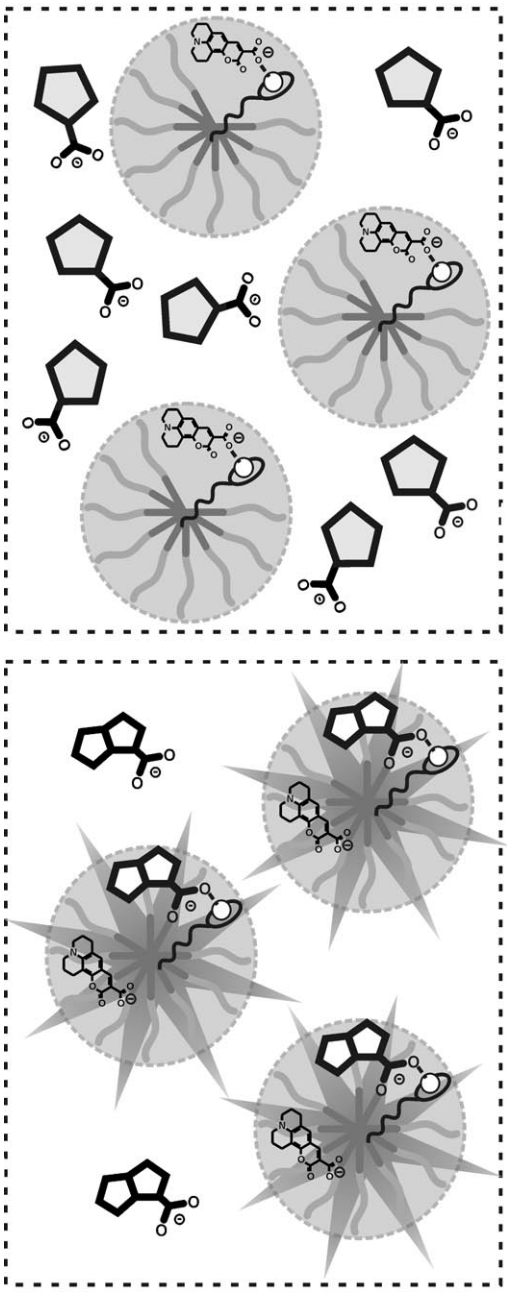


Figure 3. Rationale behind the use of our micellar device as a fluorescent gauge for lipophilicity. Top: poorly lipophilic molecules remain in water; they do not compete with **C343**$^-$ for the coordination to Cu^{2+} and leave the fluorescence unvaried (i.e., "off"). Bottom: more lipophilic molecules distribute between water and micelles, displacing **C343**$^-$ from Cu^{2+} when entering the micellar core, with fluorescence reviving. The intensity of fluorescence increase is proportional to the molecule's lipophilicity.

ing the micellar space they will compete with **C343**$^-$ for apical coordination to Cu^{2+}; this promotes the displacement of **C343**$^-$ and its fluorescence reviving (Figure 3, bottom). The intensity of the fluorescence reviving, ΔI_f, is thus directly proportional to the lipophilicity of $RCOO^-$. As a control, we first measured the lipophilicity of the $CH_3(CH_2)_nCOOH$ series ($n=0$–16) by addition of excess of the chosen fatty acid to the sensing system buffered at pH 7.4 (HEPES buffer, 0.05 M); we found that I_f increases with increasing acid concentration. The entity of the increase is a function of the chain length (Figure S5 in the Supporting Information). At a given acid concentration (we choose 5×10^{-4} M) $\Delta I_f\%$ can be calculated by assigning $I_f=100$ to the starting situation (no acid added). The $\Delta I_f\%$ values were plotted as a function of n (i.e., chain length) to obtain a profile that was in perfect agreement with what we have already reported for the lipophilicity of fatty acids[13] (see Supporting Information, Figure S6). In brief, for $0<n<3$ $\Delta I_f\%=0$ because the chain is too short, and carboxylates are so poorly lipophilic that they remain 100% in bulk water. For $4<n<9$, $\Delta I_f\%$ increases with n, as the fatty acids carboxylates distribute between bulk water and micelles. For $n\geq10$, $\Delta I_f\%$ reaches a limiting value: all $CH_3(CH_2)_nCOO^-$ molecules are lipophilic enough to distribute fully inside micelles.[32]

We then applied our sensing system to the NSAIDs series, by adding to the buffered solution of the micellar sensor (pH 7.4, 0.05 M Hepes) up to a fivefold excess of the chosen molecule. The $I_f\%$ versus NSAID molar concentration profiles are displayed in Figure 4 (top). The $I_f\%$ variation observed with three reference fatty acids, $CH_3(CH_2)_5COOH$, $CH_3(CH_2)_7COOH$ and $CH_3(CH_2)_9COOH$ is also reported in the Figure for comparison. For NSAIDs the fluorescence increase can be defined at any concentration as $\Delta I_f\%$ by assigning $I_f=100$ to the value read with no added drugs. In the case of NSAIDs there is no evident structural feature like the chain length in the fatty acids series to be put in relation with the observed $\Delta I_f\%$. However, the reliability of $\Delta I_f\%$ values as a direct evaluation of NSAIDs lipophilicity can be demonstrated by comparing $\Delta I_f\%$ with two series of values. One is the $\log P$ of NSAIDs, that is, the water/*n*-octanol partition coefficient, the values of which can be collected from literature.[33] The $\Delta I_f\%$ versus $\log P$ plot in Figure 4 (bottom, grey triangles) displays a sharp correlation. The second series of values is the ΔpK_a values of the carboxylic acid function of NSAIDs, that is, the difference between the pK_a determined in micelle and the pK_a obtained in water. It has been shown[13] that for fatty acids, ΔpK_a is directly proportional to the degree of inclusion in TX-100 micelles (an effect connected to the well-known modification of local acid–base properties due to the poor solvation inside micelles of non-ionic surfactants[34]). We determined the dissociation constants of NSAIDs in TX-100 by means of potentiometric titrations (solutions were 0.05 M in $NaNO_3$, 0.01 M in Triton X-100 and 0.001 M with the chosen NSAID), and then we calculated the ΔpK_a by using the pK_a values that were determined in water, which were taken from the literature.[35] Also in this case, Figure 4 (bottom, white triangles) shows a sharp correlation between $\Delta I_f\%$ and ΔpK_a . The ΔpK_a values found by us for NSAIDs are in the 1.0–1.6 range (see Supporting Information) that is the same range that was found for medium-chain fatty acids (e.g., octaonic–decanoic) by comparing data obtained in TX-100[13] and those reported in water.[31] It has been shown that addition of salts to TX-100 solutions increases the hydration of the

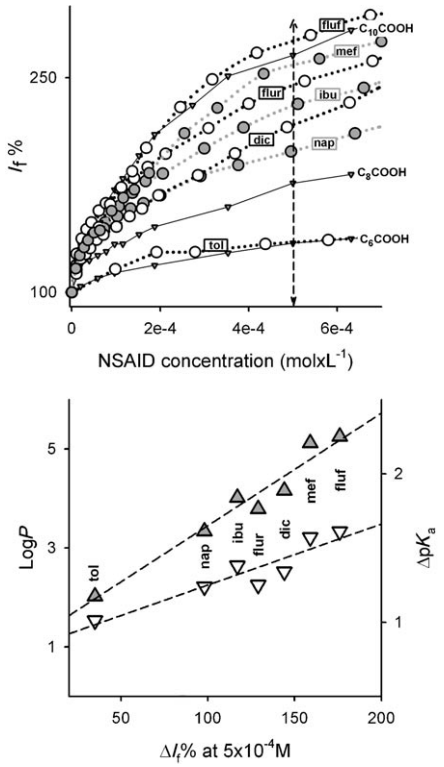


Figure 4. Top: I_f percentual variation at 484 nm as a function of the concentration of added NSAID (grey and white circles and lines). The NSAID to which is referred each profile is indicated on the plot. The I_f% variation of three fatty acids (small grey triangles with black lines) is also reported for comparison. The dashed arrow indicate the chosen concentration at which I_f% values were taken to calculated the ΔI_f% values used to draw the bottom part of the figure. Bottom: grey triangles: log P values (left axis) and ΔI_f% values for NSAIDs; the dashed line is the linear regression ($r^2 = 0.96$). Log P values are taken from ref. [33a] (**tol**, **ibu**, **dic**) and ref [33b] (**nap**, **flur**, **mef**, **fluf**). White triangles: ΔpK_a and ΔI_f% for NSAIDs ($r^2 = 0.86$, dashed line = linear regression). The pertinent NSAID is indicated on the plot.

polyethyleneoxide layer of the micelles.[36] In our case, and in the already-examined case of fatty acids,[13] the significant but not dramatic difference of the pK_a values between TX-100 and water might be due to the increased hydration expected with the 0.05 M $NaNO_3$ concentration that was used in the titration experiments.

Conclusions

In summary, we have demonstrated that our micellar multicomponent molecular system is able to carry out multiple tasks, that is, to signal with a fluorescence reviving if the pH is drifting out of a sharp window (that includes the physiological pH interval) and, inside this window, to measure with a fluorescence "off–on" modality the lipophilicity of carboxylate-containing molecules, including the widespread drug family of NSAIDs. It can be envisaged that this micellar device can be modified by changing both the metal complex and the fluorophore to be tailored for measuring the lipophilicity of other families of drugs or biomedically interesting molecules, and/or to shift the position of the "off" window on a different interval of the pH axis. Moreover, it can be foreseen that changing the container to polymeric-type micelles, as used in drug delivery, would allow this approach to be used for in vivo pH monitoring and for the evaluation of the micellar load capacity by means of a simple fluorescent signal. Studies are currently carried out in our laboratories in these directions.

Experimental Section

Materials: Triton X-100 (TX-100; *tert*-octylphenoxypoly(oxyethylene glycol) with an average of 9–10 oxyethylene units) was purchased from Caledon (average molecular weight: MW = 647). Fatty acids, NSAIDS, anhydrous $Cu(CF_2SO_3)_2$, Coumarin 343 were purchased from Sigma–Aldrich and used as received. DTMC was prepared as already described.[13] The $[Cu(dtmc)]^{2+}$ complex was obtained as a solution: DTMC (150 mg, 0.392 mmol) was dissolved in *tert*-butanol (10 mL) in a 25 mL flask, to which $Cu(CF_3SO_3)_2$ (141 mg, 0.392 mmol) was added. The violet solution was refluxed for 90 min, after which the product was obtained as a waxy violet solid after solvent removal with a rotary evaporator. The complex was redissolved in pure *tert*-butanol (10.0 mL) to obtain a 3.92×10^{-2} M solution that was used as such for preparing diluted solutions in H_2O. ESI-MS: m/z: 222, 223 [$M^{2+}/2$].

Potentiometric titrations for the determination of the pK_a of NSAIDs in micellar solution: A 0.001 M solution (25 mL) of the chosen acid was prepared in a aqueous solution of both 0.05 M $NaNO_3$ and 0.01 M Triton X-100. The solution was placed in the cell of the automatic titrating system, thermostatted at 25 °C and kept under a N_2 atmosphere. Excess acid (100 μL of 1.00 M standard HNO_3) was added, and the acidic solution was titrated automatically with portions (20 μL) of 0.200 M standard NaOH. The results were obtained as E (potential in mV at the glass electrode) versus volume of added base (in mL) and the protonation constants were calculated with the Hyperquad package.[37] Before titrations, the cell $E°$ values for the hydrogen glass electrode were determined by using the Gran method.[38] The same conditions were used to determine the pK_a of deprotonation of the water molecule coordinated to $[Cu(dtmc)]^{2+}$, except that the complex was 5×10^{-4} M in 0.01 M Triton X-100 or in water (in both cases solutions were also 0.05 M in $NaNO_3$).

Coupled fluorimetric–pHmetric titrations: a) On–off–on emission behaviour: an aqueous solution (20 mL) was prepared containing 0.05 M $NaNO_3$, 0.01 M TX-100, 1.5×10^{-4} M copper complex and 8×10^{-7} M Coumarin 343. The solution was placed in a thermostatted cell at 25 °C and kept under a N_2 atmosphere. A standard solution of 1.00 M HNO_3 (100 μL) was added, and the acidic solution was titrated with portions of standard 0.100 M NaOH (20 μL), whilst the pH was monitored by using a double-electrode pH meter. After each addition of base, the pH of the solution was allowed to stabilise, then a portion (3 mL) was withdrawn and placed in a quartz cell to record the emission fluorescence spectrum, after which the sample was returned to the bulk solution. b) Control experiment on Coumarin 343: the same conditions were used, except that the $[Cu(dtmc)]^{2+}$ complex was not added.

Competition titrations: Aqueous solutions (0.01–0.005 M) of the chosen fatty acid or NSAID and 0.01 M Triton X-100 were prepared, and the pH was adjusted to 7.4 by the addition of standard NaOH. This solution was used to titrate an aqueous solution (10 mL) containing HEPES (0.05 M, pH 7.4), Triton X-100 (0.01 M), $[Cu(dtmc)]^{2+}$ (1.5×10^{-4} M, by addition of 230 μL of the stock solution in H_2O) and Coumarin 343 (8×10^{-7} M). The solutions were thermostatted at 25 °C and kept under a N_2 atmosphere. The chosen acid or NSAID was added in portions of 20–100 μL, up to a total concentration of 2×10^{-3} M (fatty acids and NSAIDs). After each addition, a portion (3 mL) of the titrated solution was transferred to a quartz cuvette to measure the fluorescence emission spectrum, after which it was returned the bulk titrated solution.

Acknowledgements

Financial support: Università Italo-Francese and Egide (Galileo/Galilée grant), MIUR, Università di Pavia and Regione Lombardia (Reglom16), CNRS, the French Ministry for Research, and the Regional Council of Burgundy. N.S. acknowledges the French Ministery for Research for Ph.D. grants.

[1] a) T. Gunnlaugsson, M. Glynn, G. M. Tocci, P. E. Kruger, F. M. Pfeffer, *Coord. Chem. Rev.* **2006**, *250*, 3094–3117; b) L. Fabbrizzi, M. Licchelli, P. Pallavicini, *Acc. Chem. Res.* **1999**, *32*, 846–853; c) 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.* **1997**, *97*, 1515–1566; d) A. W. Czarnik, *Acc. Chem. Res.* **1994**, *27*, 302–308.

[2] M. Schmittel, H.-W. Lin, *Angew. Chem.* **2007**, *119*, 911–914; *Angew. Chem. Int. Ed.* **2007**, *46*, 893–896.

[3] T. Suzuki, K. Ohta, T. Nehira, H. Higuchi, E. Ohta, H. Kawai, K. Fujiwara, *Tetrahedron Lett.* **2008**, *49*, 772–776.

[4] D. C. Magri, G. J. Brown, G. D. McClean, A. P. de Silva, *J. Am. Chem. Soc.* **2006**, *128*, 4950–4951.

[5] P. Pallavicini, Y.-A. Diaz-Fernandez, L. Pasotti, *Coord. Chem. Rev.* **2009**, *253*, 2226–2240.

[6] A. P. de Silva, H. Q. N. Gunaratne, C. P. McCoy, *Chem. Commun. (Cambridge)* **1996**, 2399–2400.

[7] Y.-A Diaz-Fernandez, F. Foti, C. Mangano, P. Pallavicini, A. Perez-Gramatges, S. Rodriguez-Calvo, *Chem. Eur. J.* **2006**, *12*, 921–930.

[8] D. C. Drummond, M. Zignani, J.-C. Leroux, *Prog. Lipid Res.* **2000**, *39*, 409–460.

[9] P. R. Murray, M. A. Pfaller, K. S. Rosenthal, *Medical Microbiology*, 5th ed., Elsevier, Amsterdam, **2005**.

[10] a) S. A. de Silva, A. Zavaleta, D. E. Baron, O. Allam, E. V. Isidor, N. Kashimura, J. M. Precarpio, *Tetrahedron Lett.* **1997**, *38*, 2237; b) S. A. de Silva, B. Amorelli, D. C. Isidor, K. C. Loo, K. E. Crooker, Y. E. Pena, *Chem. Commun.* **2002**, 1360; c) L. Fabbrizzi, F. Gatti, P. Pallavicini, L. Parodi, *New J. Chem.* **1998**, *22*, 1403; d) T. Gunnlaugsson, *Tetrahedron Lett.* **2001**, *42*, 8901.

[11] a) P. Pallavicini, V. Amendola, C. Massera, E. Mundum, A. Taglietti, *Chem. Commun.* **2002**, 2452; b) V. Amendola, C. Mangano, P. Pallavicini, *Dalton Trans.* **2004**, 2850.

[12] S. Uchiyama, K. Iwai, A. P. de Silva, *Angew. Chem.* **2008**, *120*, 4745–4747; *Angew. Chem. Int. Ed.* **2008**, *47*, 4667–4669.

[13] G. Chirico, M. Collini, L. D′Alfonso, F. Denat, Y. A. Diaz-Fernandez, L. Pasotti, Y. Rousselin, N. Sok, P. Pallavicini, *ChemPhysChem* **2008**, *9*, 1729–1737.

[14] H. Van de Waterbeemd, D. A. Smith, B. C. Jones, *Comput. Aided Mol. Des.* **2001**, *15*, 273–286.

[15] a) V. P. Torchilin, *Pharm. Res.* **2006**, *24*, 1–16; b) P. P. Constantinides, M. V. Chaubal , R. Shorr, *Adv. Drug Delivery Rev.* **2008**, *60*, 757–767; c) G. A. Husseini, W. P. Pitt, *Adv. Drug Delivery Rev.* **2008**, *60*, 1137–1152; d) V. P. Torchilin, *Eur. J. Pharm. Biopharm.* **2009**, *71*, 431–444.

[16] a) D. Peer, J. M. Karp, S. Hong, O. C. Farok Hzad, R. Margalit, R. Langer, *Nat. Nanotechnol.* **2007**, *2*, 751–760; b) T. X. Xiang, B. D. Anderson, *Adv. Drug Delivery Rev.* **2006**, *58*, 1357–1378.

[17] a) J. Sangster, *Octanol–Water Partition Coefficients: Fundamentals and Physical Chemistry*, Wiley, New York, **1997**; b) F. Lombardo, M. Y. Shalaeva, K. A. Tupper, F. Gao, M. H. Abraham, *J. Med. Chem.* **2000**, *43*, 2922–2928.

[18] R. A. Scherrer, S. M. Howard, *J. Med. Chem.* **1977**, *20*, 53–58.

[19] F. Lombardo, M. Y. Shalaeva, K. A. Tupper, F. Gao, *J. Med. Chem.* **2001**, *44*, 2490–2497.

[20] NSAID = Non-steroidal anti-inflammatory drug; the most common representative of this class of massively used drugs is Ibuprofen.

[21] R. E. Riter, E. P. Undiks, N. E. Levinger, *J. Am. Chem. Soc.* **1998**, *120*, 6062–6067.

[22] a) F. Grieser, C. J. Drummond, *J. Phys. Chem.* **1988**, *92*, 5580; b) C. J. Drummond, F. Grieser, T. W. Healy, *J. Chem. Soc., Faraday Trans.* **1989**, *85*, 551; c) N. O. Mchedlov-Petrossyan, A. V. Plichko and A. S. Shumaker, *Chem. Phys. Rep.* **1996**, *15*, 1661; d) G. P. Gorbenko, N. O. Mchedlov-Petrossyan, T. A. Chernaya, *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 2117.

[23] V. Amendola, L. Fabbrizzi, F. Foti, M. Licchelli, C. Mangano, P. Pallavicini, A. Poggi, D. Sacchi, A. Taglietti, *Coord. Chem. Rev.* **2006**, *250*, 273–299.

[24] M. C. Styka, R. C. Smierciak, E. L. Blinn, R. E. DeSimone, J. V. Passariello, *Inorg. Chem.* **1978**, *17*, 82–86.

[25] Y. Diaz Fernandez, A. Perez Gramatges, V. Amendola, F. Foti, C. Mangano, P. Pallavicini, S. Patroni, *Chem. Commun. (Cambridge)* **2004**, 1650–1651.

[26] a) S. D. Christian, G. A. Smith, E. E. Tucker, *Langmuir* **1985**, *1*, 564–567; b) F. Denat, Y. A. Diaz-Fernandez, P. Pallavicini, L. Pasotti, Y. Rousselin, N. Sok, *Dalton Trans.* **2009**, 6751–6758.

[27] R. J. Jobson, E. A. Dennis, *J. Phys. Chem.* **1977**, *81*, 1075–1078.

[28] M. Wolszczak, J. Miller, *J. Photochem. Photobiol. A* **2002**, *147*, 45–54.

[29] J. Robson, E. A. Dennis, *Acc. Chem. Res.* **1983**, *16*, 251–258.

[30] Control experiments were run at pH 3 and 11, by adding up to 10^{-3} M of the copper complex to micellised Coumarine 343. In both cases we observed a slight I_f decrease (<15%), indicating a very inefficient intramicellar quenching by the Cu complex in the absence of a Cu^{2+}–$C343^{-}$ coordinative interaction.

[31] J. Sangster, *J. Phys. Chem. Ref. Data* **1989**, *18*, 1111–1229.

[32] Correlation of ΔI_f with $\log P$ data was also tried for fatty acids, based on $\log P$ collected from free-access database, containing literature-retrieved data. We used SRC PhysProp Database, http://www.syrres.com/what-we-do/product.aspx?id=133, accessed August 2009. Linear correlation between $\log P$ and ΔI_f is good, see Figure S7 in the Supporting Information, except for short-chain fatty acids ($n<4$). This is not surprising, as experimental $\log P$ determination might be difficult for poorly lipophilic substances, see A. Pyka, K. Bober, *J. Am. Oil Chem. Soc.* **2006**, *83*, 747–752. The latter paper reports also experimentally calculated R_M factors, that displays an even better linear correlation with our ΔI_f values, see Figure S8. R_M is the hydrophobicity index, and $R_M = \log[(1/R_F)-1]$, with R_F = ratio of the distance covered by the centre of the spot (of the examined fatty acid) to the distance simultaneously covered by the mobile-phase front in a RP-HPTLC experiment (methanol/water 95:5 as the mobile phase).

[33] a) L. Perioli, V. Ambrogi, C. Bernardini, G. Grandolini, M. Ricci, S. Giovagnoli, C. Rossi, *Eur. J. Med. Chem.* **2004**, *39*, 715–727; b) F. Barbato, M. I. La Rotonda, F. Quaglia, *J. Pharm. Sci.* **1997**, *86*, 225–229.

[34] P. Pallavicini, L. Pasotti, S. Patroni, *Dalton Trans.* **2007**, 5670.

[35] **tol** ($pK_a = 3.50$), **nap** (4.15), **mef** (4.20), **fluf** (3.90): ref. [25b]; **ibu** (4.40): G. Pitarresi, P. Pierro, G. Giammona, F. Iemma, R. Muzzalupo, N. Picci, *Biomaterials* **2004**, *25*, 4333–4343; **flur** (4.20): G. S. Oladiran, H. K. Batchelor, *Chem. Eng. Res. Des.* **2007**, *85*, 1039–1043; **dic** (4.0): P. van der Bijl, A. D. van Eyk, H. I. Seifart, I. Viljoen, M. Jooste, *J. Appl. Res.* **2003**, *3*, 505–511.

[36] G. D. J. Phillies, J. E. Yambert, *Langmuir* **1996**, *12*, 3431–3436.

[37] P. Gans, A. Sabatini, A. Vacca, *Talanta* **1996**, *43*, 1739–1753.

[38] G. Gran, *Analyst* **1952**, *77*, 661–671.

Received: September 2, 2009
Published online: December 8, 2009

