Rhenium *fac*-tricarbonyl bisimine complexes: luminescence modulation by hydrophobically driven intramolecular interactions

Michael P. Coogan,* Vanesa Fernández-Moreira, Jacob B. Hess, Simon J. A. Pope* and Craig Williams

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A triplet metal-to-ligand charge transfer emitting cationic Re^{I} complex (**1c**) functionalised with a C_{12} alkyl chain possesses unique solvent-dependent photophysical properties. In acetonitrile solution the luminescence properties of **1c** are typical of related *fac*-$\{Re(CO)_3(diimine)L\}^+$ species with emission at 555 nm (τ = 135 ns, Φ_{em} = 1.7%) whereas in water, emission was blue-shifted to 523 nm with an increase in luminescence lifetime (688 ns) and quantum yield (9.2%). These unusual properties are attributed to a dynamic intramolecular mechanism involving fold-back of the alkyl chain onto or around the coordinated 2,2′-bipyridine ligand, thus shielding the excited state from the surrounding water solvent. Comparison of **1c** with Re^{I} complexes either lacking a chain or incorporating varying chain lengths (C_8 and C_{16}) showed these properties to be unique to **1c**. The intramolecular fold-back conformation was shown to be highly temperature dependent between 278 and 318 K, with elevated temperatures resulting in far less effective shielding. These unique photophysical properties can therefore be exploited in aqueous environments through interaction with lipophilic entities such as liposomes or biomolecules such as human serum albumin, which both result in a reverted red-shifted emission for **1c** at 552–555 nm.

Introduction

The application of phosphorescent metal complexes as lumophores in cell imaging is an emerging area, with a number of recent papers demonstrating the applicability of coordination complexes of rhenium,[1–3] ruthenium[4] and iridium[5] in confocal fluorescence microscopy. These complexes emit from a triplet metal-to-ligand charge transfer (^{3}MLCT) excited state, which is accessed *via* intersystem crossing from the initially formed singlet excited state, mediated by spin–orbit coupling which is efficient in heavy metal complexes.[6] As a result of our investigations into the design and syntheses of luminescent, multifunctional transition metal compounds as biologically useful fluorochromes for cell imaging applications, it has become clear that bisimine derivatives of the *fac*-$\{Re^{I}(CO)_3\}$ core possess ideal photophysical attributes for fluorescence microscopy, allowing visible wavelength excitation and detection. Recently it has become apparent[1,2] that in addition to these advantageous photophysical properties, such complexes show good biocompatibility, being simultaneously stable under physiological conditions, of low toxicity and possessing high membrane permeability. The combined photophysical attributes and biocompatibility recommend such complexes to those interested in the design and application of imaging agents and reporter devices. The chemical literature contains many reports of metal-based systems which are designed to report on their environment,[7] with their photophysical properties being modulated by the presence of certain analytes;[8] however, as yet few exploit emission lifetime[9] and even fewer have been applied in physiological conditions or in real cellular environments.

While fluorophores can display solvatochromism with respect to their emission maxima, the effects are rarely of such a magnitude that they enable well-resolved images highlighting *e.g.* lipophilic *versus* hydrophilic zones, but rather it is common to use separate membrane (lipophilic) and cytoplasm (hydrophilic) staining dyes with markedly different fluorescent properties.[10] Green fluorescent proteins are widely used in cellular imaging since expression in various organelles allows differentiation on the basis of fluorescence lifetime imaging microscopy[11] although the luminescence lifetimes tend to be $<$5 ns. At the same time there is a popular area of research concerned with the phenomena of changes in photophysical properties upon inclusion, complexation, or other intermolecular interactions. Particularly well examined in this area is the inclusion of small lumophores into cyclodextrins, with the sometimes dramatic changes in photophysical properties ensuing as the microenvironment of the lumophore alters.[12]

The incorporation into the core structure of the parent rhenium complexes of long hydrophobic aliphatic chains should endow these complexes with membrane permeability (important for cellular uptake and for entry to organelles), and with a sufficiently long alkyl chain it may be expected that the complex will itself localise in membranes. Such a lipid environment, of higher viscosity and lower polarity than water, would be expected to shield the solvent-dependent properties of the ^{3}MLCT excited state, which tends to be localised on the bisimine ligand, from the aqueous, physiological

School of Chemistry, Main Building, Cardiff University, Cardiff, Cymru/Wales, UK CF10 3AT. E-mail: popesj@cardiff.ac.uk. E-mail: cooganmp@cf.ac.uk; Fax: +44 (0)292 087 4030; Tel: +44 (0)292 087 4066

medium and thus modulate the emission properties of the fluorochrome. Thus, measurement of the luminescence lifetime of such species in a variety of media may provide an environmentally sensitive probe for use in imaging experiments.

Results and discussion

Synthesis

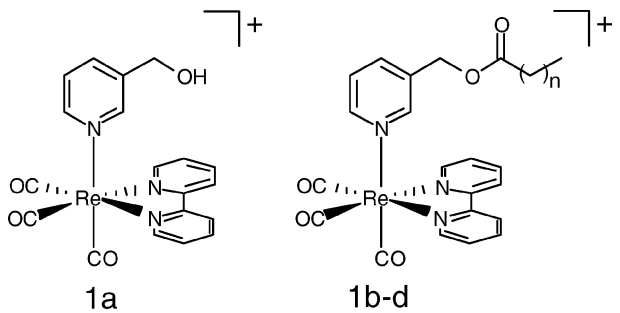
Complexes (Scheme 1) of the form *fac*-{$Re(CO)_3$(2,2′-bipyridine)L}$^+$ in which L is a highly lipophilic ester derivative of 3-hydroxymethylpyridine, Py-3-CH_2O_2CR' (R′ = octyl, myristyl, steryl), were synthesised as reported in the literature.[1] This involved initial formation of rhenium tricarbonyl bipyridyl chloride, then activation of the chloride (either exchange to the triflate, or *via* halide abstraction to the acetonitrile complex) allowing final displacement with the substituted pyridine, L.

Photophysical studies

The electronic spectra of the complexes showed the typical characteristics associated with bisimine derivatives of Re^I.[13] Ligand centred bands associated with $\pi \rightarrow \pi^*$ transitions of the coordinated ligands were generally observed at higher energies (<330 nm), whilst the broad metal-to-ligand charge transfer (^{1}MLCT) bands (formally Re(dπ)→bisimine(π^*)) for complexes **1a–d** appeared around 340–370 nm.

Having assessed the UV-Vis absorption properties of the complexes our attention turned to a photophysical investigation, initially in acetonitrile. For compounds **1a–d** direct irradiation of the ^{1}MLCT bands using an excitation wavelength of 400 nm resulted in a broad, structureless visible emission centred *ca.* 555 nm. Such features are typical of cationic bisimine Re^I complexes where the emission is frequently assigned as ^{3}MLCT in origin. Luminescence lifetime measurements on **1a–d**, in aerated acetonitrile solution using a pulsed excitation source at 355 nm and a detection wavelength of 600 nm, each showed a single exponential decay (indicative of one excited state environment) in the 110–140 ns domain. Again, such an observation is entirely consistent with a ^{3}MLCT assignment of complexes of the generic type *fac*-{$Re(CO)_3$(diimine)L}$^+$.[13]

In aqueous solution **1a**, the hydroxymethylpyridine species, possesses an emission wavelength similar to that in acetonitrile, the luminescence lifetime of which is 117 ns (λ_{ex} = 355 nm). Moving to the extended chain species, however, revealed some interesting changes in photophysical properties in aqueous solution compared to acetonitrile. Whereas **1b** (eight carbon chain) has photophysical attributes which mimic those of **1a**, doubling the length of the chain (**1c**) caused profound changes in both the steady state and the emission lifetime characteristics in aqueous solutions, whereas in organic solvents these species were, essentially, identical. **1c** possesses an emission maximum at 523 nm, approximately a 1000 cm^{-1} blue-shift when compared to **1a** or **1b**, suggesting that the ^{3}MLCT excited state is being *destabilised* in water, raising the π^* energy level and thus the dπ–π^* separation, and thus reflected in the emission wavelength. A simple solvation model would have predicted precisely the opposite effect, since it is commonly observed that more polar solvents, such as water (dielectric constants: water 80; acetonitrile 37), stabilise dipolar CT excited states and thus cause red-shifts in emission maxima when compared to less polar media.[10d] Quantum yield measurements show that moving to water as solvent increased Φ_{em} of **1c** by over five-fold (1.7% in aerated acetonitrile; 9.2% in aerated water with associated errors of ±15%). The lifetime analysis (λ_{ex} = 355 nm, λ_{em} = 600 nm) of **1c** revealed that the data fitted well to a bi-exponential decay profile with τ = 176 and 688 ns (Fig. 1), the relative weightings of which give predominance to the longer component (Table 1). From the measured parameters (luminescence lifetimes and quantum yields) the rates of radiative (k_r) and non-radiative (k_{nr}) decay can be estimated. In MeCN, k_r and k_{nr} for **1c** are 1.26×10^5 s^{-1} and 7.28×10^6 s^{-1}, respectively, whereas in water (utilising τ = 688 ns) these values are 1.33×10^5 s^{-1} and 1.31×10^6 s^{-1}, thus suggesting that it is the non-radiative decay rate that increases with decreasing emission energy, in accordance with the energy gap law.[10d,14] In comparison, the data for **1a** (and **1b**) suggest very little photophysical change



Scheme 1 The cationic rhenium complexes **1a** and **1b**, **1c**, **1d** (*n* = 6, 12, 16).

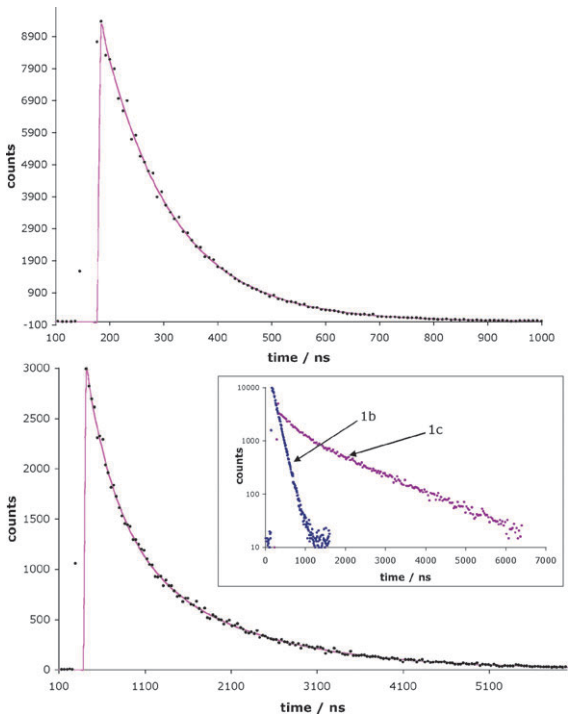


Fig. 1 Fitted luminescence decay profiles for **1b** (top) and **1c** (bottom) recorded in aerated water (λ_{ex} = 355 nm, λ_{em} = 600 nm). Inset: direct comparison of **1b** and **1c** emission lifetimes.

Table 1 Photophysical data of the complexes, **1a–d**

Complex	λ/nm[ad]	λ/nm[bd]	λ/nm[cd]	τ/ns[ae]	τ/ns[be]	τ/ns[ce]
1a	552	553	—	121	117	—
1b	552	554	—	134	129	—
1c	523	555	517	176 (21%), 688 (79%)	135	887
1d	552	556	—	119 (64%), 412 (36%)	130	—

[a] Aerated water. [b] Aerated acetonitrile. [c] Aerated hexane. [d] λ_{ex} = 400 nm. [e] λ_{ex} = 355 nm, λ_{em} = 600 nm.

when altering the solvent from acetonitrile to water even though the latter could be expected to be a more efficient vibrational quencher than acetonitrile. Thus, both the unexpected blue-shift of emission maximum and the presence of an anomalously long-lived excited state for **1c** in water suggest a more complex effect at play than that explained by a simple solvation model.

Studies in liposomes

Given the goal of identifying photophysical properties characteristic of local environment, which would allow localisation in membranes to be determined, preliminary studies were undertaken using liposomes.[15] Liposomes are spherical vesicles formed from the self-assembly of membrane lipids into curved bilayers upon hydration. They initially form as complex mixtures of large, multilamellar vesicles, but can be converted to small, unilamellar vesicles (SUVs) by a variety of techniques, including extrusion through nanometre pore membranes. SUVs have been widely applied as models for cell membranes in permeability and diffusion studies as they can be constituted to provide mimics for a variety of membrane structures by varying lipid and cholesterol ratios and extrusion technique. Thus, as a model for the behaviour of these rhenium complexes in the presence of cell membranes, SUVs provide an attractively comparative system. Complex **1c** was thus incorporated into egg phosphatidyl choline (PC) at a 0.1% molar ratio (deliberately low in order not to disrupt the membranes) by co-dissolution in chloroform followed by thorough mixing and evaporation. This modified lipid was then used to prepare SUVs by standard hydration and extrusion techniques. The formation of vesicles with this modified lipid was confirmed (on a separate sample) by encapsulation–quenching experiments with calcein, according to the literature.[15] The steady-state emission spectrum of this preparation revealed an emission maximum of 552 nm. Although this unexpectedly contrasts with **1c** (λ_{em} = 523 nm), it is comparable to the chain-less species, **1a**, in water. Emission lifetime analysis of the 552 nm band revealed τ = 94 ns, which, again, is far more comparable to **1a** (τ = 117 ns) than the extended lifetimes of **1c** in water alone.

Taken together, the trio of observations—(i) complex **1a**, with no alkyl chain, displays emission lifetime and maximum in water similar to MeCN solvent; (ii) complex **1c**, with a C_{12} chain, displays an anomalously long lifetime and blue-shifted emission maximum in water; (iii) complex **1c** in aqueous media in the presence of lipid membranes reverts to typical MeCN behaviour—can be interpreted to imply that in water the C_{12} chain is responsible for the altered photophysical properties, but that in the presence of MeCN solvent or lipophilic zones (which interact with this chain) the effect is disrupted. One hypothesis for the effects of alkyl chains on the properties of appended molecules in aqueous solvents is referred to as chain-wrapping or fold-back;[16a] that is a conformation in which the alkyl chain is driven by hydrophobic forces to wrap around the molecule and in some way alter its behaviour (typically by protection from the solvent environment). In this case it is possible to attribute the enhanced photophysical attributes of **1c** in water to an intramolecular process involving fold-back of the aliphatic chain onto, or around, the bipyridine ligand. Both the blue-shift in λ_{em} (523 nm) and the dramatically extended emission lifetime (688 ns) suggest that the excited state is shielded from solvent quenching effects. The minor, shorter lifetime component of 176 ns is assigned to a form of the complex where fold-back does not predominate and thus the bipyridine is not as effectively shielded. It is reasonable to assume that the conformational re-arrangement is a dynamic process, with two or more preferred major conformations, likely to be chain-wrapping around bipyridine and self-wrapping of the chain, and that these have been resolved on the timescale of the lifetime decay measurements (*i.e.* a microsecond). The fold-back form of the complex would be expected to possess a family of conformations each giving subtly different degrees of excited state shielding, but which cannot be deconvoluted from one another. These observations also suggest that it is the lipophilic chain of **1c** that predominantly interacts with the liposomal membrane (rather than the coordinated bipyridine), thus exposing the Re-bisimine moiety to the solvent.

An alternative explanation for the drastically altered photophysical properties of **1c** in water could be proposed by invoking micelle formation. However, one would expect that such micelles would be structured with the lipophilic chains internalised and the charged complexes (head-groups) protruding into bulk water. By analogy with the liposome results, such an observation would not be expected to result in enhanced photophysical attributes. Additionally, as a precaution, we worked within concentration ranges that would not encourage micelle formation for these chain lengths ($<10^{-5}$ M).

The hypothesis that the blue-shift in emission wavelength and the extension of lifetime were due to intramolecular lipophilic interactions was further investigated by obtaining a solution of **1c** in hexane. Following excitation of the ^{1}MLCT band a broad structureless feature with λ_{em} 517 nm was observed (Fig. 2). The luminescence lifetime analysis for this band revealed a single component with τ = 887 ns. In hexane the aliphatic tail should be well solvated and therefore not involved in intramolecular fold-back processes. However, the non-polar hexane environment should destabilise the dipolar ^{3}MLCT excited state and cause a blue-shift in emission maximum when compared to acetonitrile, for example. Comparing the results in water and hexane clearly demonstrates that the pyridine-appended aliphatic chain of **1c** interacts very strongly with the bipyridine ligand and effectively shields the excited state from water by creating a microenvironment which mimics that of hexane. The inefficient quenching (and thus long lifetime) in hexane is explicable in terms of the non-polar nature of the solvent, preventing efficient dipolar

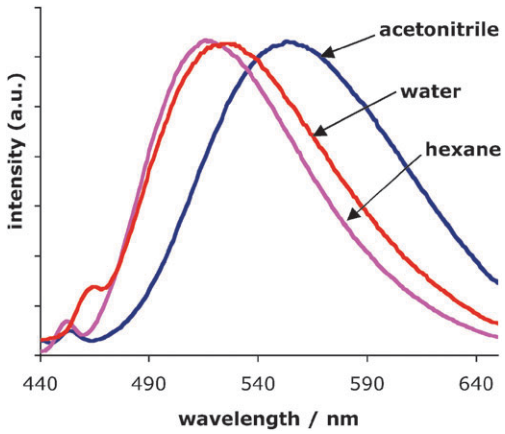


Fig. 2 Normalised steady-state spectra for **1c** in various aerated solvents (λ_{ex} = 400 nm).

coupling of excited state and solvent, important to vibrational quenching processes and thus k_{nr}. Since the emission lifetime (887 ns) in hexane can be thought to represent the hydrophobic extreme, the properties of **1c** in water can be interpreted in terms of a localised hydrophobic and hexane-like microenvironment at the bipyridine unit.

Bipyridine shielding *via* inclusion complexation with β-cyclodextrin complexes of Re^{I} chelates has been reported by DeGraff *et al.*, wherein changes in the luminescence lifetimes of the species were rationalised in terms of the solvent–bipyridine interaction.[17] Hydrophobically driven intramolecular interactions have also been exploited in reactions where the rate of cleavage of an ester is dramatically increased by the incorporation of a nucleophilic unit in the terminal position of an appended alkyl chain.[16] That this effect is only observed in aqueous solvent implies that when solvated the chain is free to occupy multiple extended conformations, and hence the nucleophilic substituent is remote from the ester, while in water, hydrophobic forces induce chain-wrapping which brings the nucleophilic group into proximity with the ester, accelerating its cleavage.

Interestingly, further extension of the chain to eighteen carbon units (**1d**) did not enhance the observed properties. The emission wavelength for **1d** was observed at 552 nm and thus did not replicate the blue-shift noted for **1c**. However, the lifetime analysis did reveal two components of 119 and 412 ns, although in this case the shorter component predominated (Table 1) suggesting that **1c** represents the 'ideal' chain length for intramolecular fold-back. It may be that the longer chain length of **1d** promotes inter- as opposed to intramolecular interactions by overcoming electrostatic repulsions of the cationic rhenium units, or, that the longer chain makes a simple folding involving alkyl chain–alkyl chain interactions preferred over a wrapping which involves alkyl chain–bipyridine interactions. In a related study, Lakowicz and co-workers have reported enhanced emission intensity and emission lifetime in water-soluble Re^{I} complexes incorporating hydrophobic diphosphine ligands. However, they attributed these characteristics to shielding of the ^{3}MLCT excited state *via* aggregation of the complexes in water.[18] Such behaviour does not explain our observations since the enhanced emissive properties are only observed for **1c**.

Interaction with human serum albumin

The hypothesis that the unusual photophysical properties of **1c** are explicable in terms of a hydrophobically driven interaction between the bisimine unit and the myristyl chain, which is deactivated in the presence of lipophilic solvents or lipophilic zones in the medium (*e.g.* liposome membrane), suggests that a range of other species should be capable of interacting with the myristyl chain to prevent this effect. Not only could demonstration of this effect provide further evidence in support of the hypothesis, but in addition the expected change of the photophysics could provide a prototype sensor for lipophilic binding sites in *e.g.* biomolecules. Human serum albumin (HSA) is an abundant plasma protein which is responsible for the transport of many lipophilic species (both endogenous and drugs).[19] The overall binding behaviour of HSA is complex, but the primary cargo are long chain fatty acids. These (as the unesterified acids) bind in hydrophobic pockets or tunnels,[20] with binding reinforced by charge–charge interactions from protonated amines in the side chains of lysine or arginine residues.[21] There is evidence that the primary binding sites for long chain acids are tunnels, while others may be pockets, but in either case a hydrophobic pocket or tunnel could provide zones which could stabilise the extended chain form of **1c**. Thus, if the hypothesis concerning the interaction of the myristyl chain with the bipyridine ring is correct, titration of HSA into an aqueous solution of **1c** should result in a switch from the photophysical properties observed in water to something akin to those observed in acetonitrile solvent, as the chain interacts with HSA binding sites, exposing the bipyridine to water. Steady-state measurements showed that upon addition of a solution of HSA to an aqueous solution of **1c**, the photophysical properties were indeed modulated showing a red-shift of *ca.* 30 nm to 555 nm together with a significant loss of integrated intensity. As these new properties closely match those that have been interpreted as having the bipyridine ligand exposed to solvent, these observations imply that the aliphatic chain is no longer intramolecularly associated with the bipyridine unit. This finding strongly supports the above hypothesis concerning chain-wrapping.

Variable-temperature luminescence studies

As a final investigation of the putative chain-wrapping phenomenon, variable-temperature luminescence spectra were recorded (Fig. 3). It was reasoned that a hydrophobically driven chain-wrapping event would be sensitive to temperature as it involves an ordering of the molecule in terms of loss of rotational freedom in the myristyl chain, which would be disfavoured at higher temperatures. Thus, at elevated temperatures it is to be expected that the spontaneous chain-wrapping which is observed in aqueous solution at ambient temperature would be reversed, with a concomitant reversion to the photophysical properties associated with an exposed bipyridine unit. Steady-state spectra were recorded in 5 K increments between 318 and 278 K and showed profound changes. Upon warming the λ_{max} of the ^{3}MLCT emission reduced in intensity and steadily red-shifted to *ca.* 550 nm at 318 K, suggesting that the average bipyridine environment of

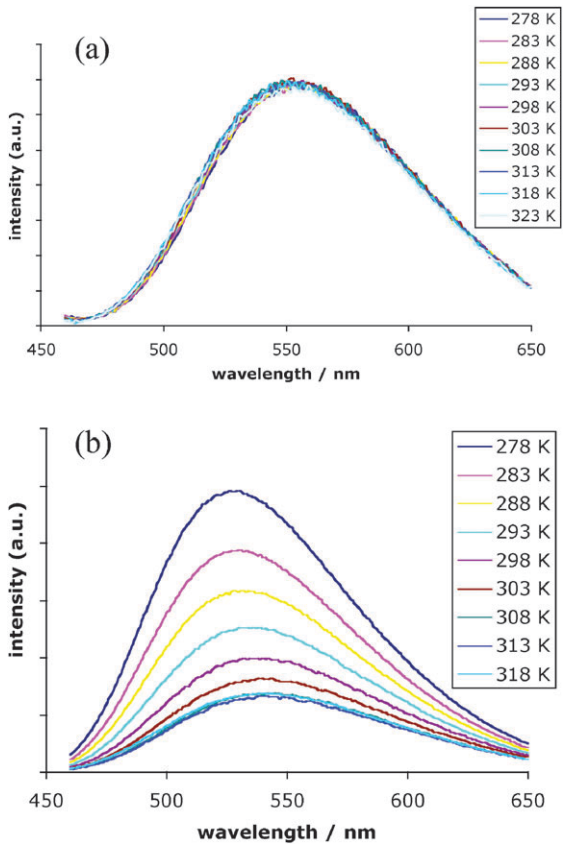


Fig. 3 Variable-temperature steady-state spectra for **1c** (λ_{ex} = 380 nm). (a) Recorded in MeCN. (b) Recorded in H_2O at 278, 283, 288, 293, 298, 303, 308, 313 and 318 K (from top to bottom).

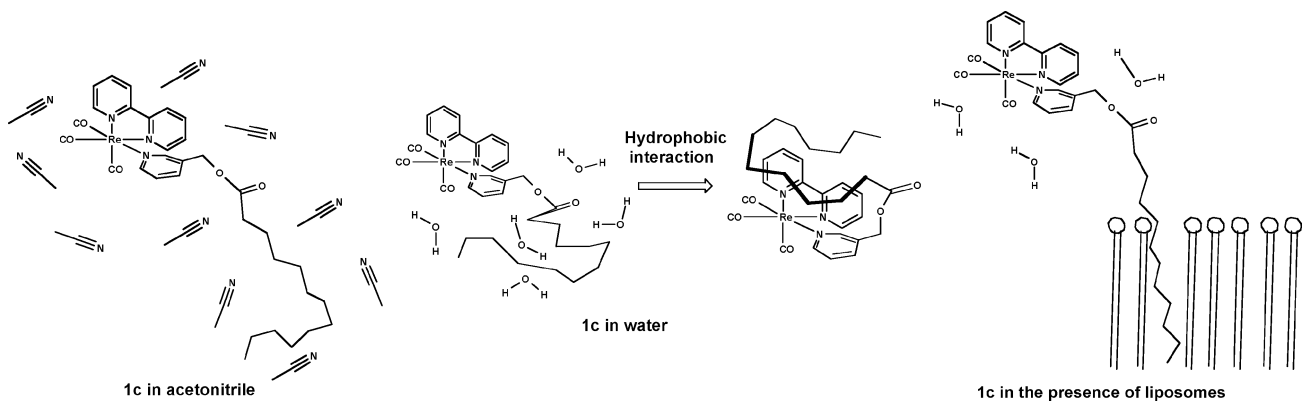
1c becomes progressively more solvated by the aqueous medium. Reducing the temperature of the sample back to 278 K induced the opposite effect: a blue-shift and increased intensity of λ_{max} indicative of a reversal of this process (and eliminating the possibility that thermal decomposition accounted for the changes). Such profound changes within a relatively narrow temperature range suggest that an entropic contribution to the system probably dominates, but that at room temperature (293 K) or below, hydrophobically driven intramolecular (alkyl chain–bipyridine) interactions are sufficiently strong to make the chain-wrapped conformer the dominant form of **1c**. An identical series of steady-state measurements were conducted on **1c** in acetonitrile (Fig. 3) and showed very little temperature dependence, the emission maximum remaining comparatively unaltered in terms of wavelength and intensity within this temperature range.

Conclusions

In this paper we have discussed the unique photophysical behaviour of a *fac*-tricarbonyl rhenium complex incorporating a 2,2′-bipyridine and an axial pyridyl ligand functionalised with a myristyl ester group. In acetonitrile solution the luminescence properties of the complex are typical and easily interpreted. In aqueous solution the emission properties are altered, resulting in a high energy blue-shift in emission maximum, an increase in quantum yield and a dramatic extension of luminescence lifetime. These photophysical changes are attributed to a hydrophobically driven, intramolecular fold-back of the myristyl chain, which results in partial shielding of the bipyridine unit from the surrounding solvent environment (Scheme 2). The extent of intramolecular 'solvation' was temperature dependent; elevated temperatures (up to 318 K) resulted in poorer shielding. Inclusion in the predominantly aqueous system of lipophilic entities (HSA, liposomes) reversed this effect, both providing evidence for the proposed explanation and suggestive of possible extension to environmentally sensitive probe applications. The fold-back conformation appeared to be extremely sensitive to alkyl chain length. Shortening the chain (octyl) demonstrated no evidence of fold-back, whilst lengthening (styryl) revealed a less effective shielding effect; fortuitously the myristyl chain appears to represent an optimum length for allowing intramolecular fold-back to occur in these systems.

Experimental

Room temperature steady-state emission spectra were obtained using aerated solutions and a JobinYvon-Horiba Fluorolog spectrometer fitted with a JY TBX picosecond photodetection module. Aerated solution samples for luminescence lifetime decays were irradiated using a pulsed Continuum Minilite Nd:YAG configured for 355 nm output and emission detected at 600 nm using a Hamamatsu R5509-73



Scheme 2 Cartoon representing the proposed conformational variations of **1c**.

detector (cooled to −80 °C using a C9940 housing). Data sets were obtained using the JY-Horiba FluorHub single photon counting module and lifetimes determined using the provided decay analysis software package, v6.1. Aqueous solutions were obtained by dissolving the complex in the minimum of acetonitrile and diluting with water. Quantum yield measurements on **1c** were obtained with aerated solutions, using $\{Ru(bpy)_3\}(PF_6)_2$ (recrystallised from acetone–water mixtures at least three times) in aerated acetonitrile as a standard (Φ_{em} = 0.016)[22] and corrected for the refractive indices of the solvents used in the measurement. Variable-temperature steady-state spectra were obtained utilising a Peltier sample cooler in conjunction with a Wavelength Electronics temperature controller, model LFI-3751.

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