Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 5055

www.rsc.org/obc

PAPER

Non-covalent interactions of coumarin dyes with cucurbit[7]uril macrocycle: modulation of ICT to TICT state conversion†

Nilotpal Barooah, Jyotirmayee Mohanty, Haridas Pal and Achikanath C. Bhasikuttan*

Received 13th January 2012, Accepted 10th May 2012
DOI: 10.1039/c2ob25759a

Non-covalent interaction of coumarin laser dyes, namely coumarin-1 (C1), coumarin-481 (C481) and coumarin-6H (C6H), with a versatile macrocyclic host molecule cucurbit[7]uril (CB7), has been investigated in aqueous solution using photophysical methods. Steady-state and time-resolved fluorescence studies illustrate significant enhancements/modifications in the fluorescence yields, lifetimes and spectral features of C1, C481 and C6H on interaction with CB7, and are assigned to 1 : 1 complex formation between the dyes and the CB7 host. The complex formation is mainly driven by charge–dipole interaction, as evident from the binding constant values ($K \sim 10^4$–10^5 M^{-1}). The large changes in the excited state behaviour of C1 and C481 as compared to C6H in the presence of CB7 indicate that CB7 binds C1 and C481 through the encapsulation of the 7-*N*,*N*′-diethylamino group of the dyes and the structural rigidity imposed by this interaction dramatically alters the excited state properties of the dyes by preventing the conversion of their emissive intramolecular charge transfer (ICT) state to the non-radiative twisted intramolecular charge transfer (TICT) state. The present results direct towards the probable supramolecular approach using water soluble macrocyclic CB7, in the development of aqueous dye laser systems in the blue-green region.

Introduction

Engineering of intrinsic molecular properties for desired applications through supramolecular self-assembly has attracted many investigations in recent years.[1–5] Host–guest complexes serve as examples of the simplest yet interesting dynamic self-assembled systems. The phenomenon of inclusion of a guest by a complementary host molecule provides the unique opportunity to tune the appropriate molecular properties of the guest in a desired way through stimuli responsive, and reversible non-covalent interactions.[1,2,6,7] In this direction host–guest assemblies involving macrocycles such as cyclodextrins, calixarenes and more recently cucurbiturils have been the subject of numerous studies.[2,3,8] Especially the complexes of a water soluble synthetic receptor namely cucurbit[*n*]urils (CB*n*, *n* = 5–10, Chart 1)[1,9,10] with various organic compounds/dyes have been studied for their probable applications in fluorescence sensing, model drug delivery systems, novel functional materials and optoelectronics, *etc.*[1,11–16] CB*n*, the highly symmetrical cyclic congeners obtained from the acid catalysed condensation of glycoluril with formaldehyde, possess a hydrophobic cavity with two polarizable carbonyl laced portals.[1,6,9,10] Due to their unique structural features cucubiturils are capable of imparting a combination of ion-dipole and hydrophobic interactions toward the guests leading to the formation of strong host–guest complexes with cationic and neutral organic dyes, organometallic compounds, inorganic complexes and even with protein residues.[1,2,17–19] Most interestingly, the efficient inclusion of fluorogenic dyes in the hydrophobic and rigid macrocyclic cavity of CB*n* often dramatically alters the excited state

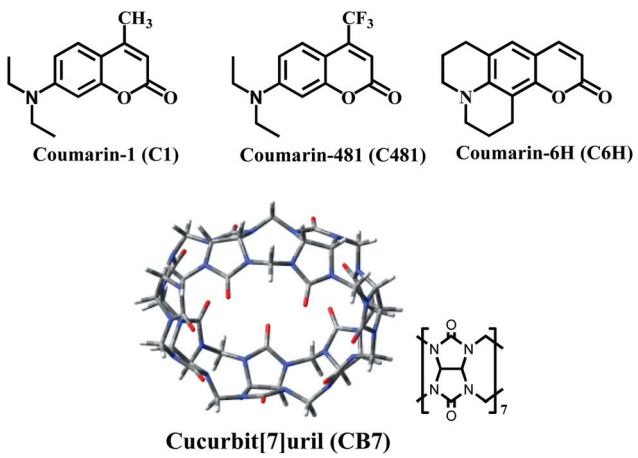

Chart 1 Chemical structures of the coumarin guests and the CB7 host.

Radiation & Photochemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai, India. E-mail: bkac@barc.gov.in; Fax: +91 22 2550 5151

†Electronic supplementary information (ESI) available: Additional figures and discussions. See DOI: 10.1039/c2ob25759a

dynamics of the guest dyes leading to novel optical responses.[20–23] In recent years, studies on various laser dyes embedded inside CB*n*, particularly with cucurbit[7]uril (CB7), have demonstrated promising optical behaviour supporting the development of aqueous dye lasers in the visible region.[24,25] In this perspective, the interactions of well known laser dyes in the blue–green region such as coumarin dyes,[26–28] with cucurbiturils is of current research interest.[17,29,30]

Coumarin dyes with a 7-alkylamino substituent are known to display unique optical properties such as very high quantum yield in non-polar solvents, solvent polarity dependent Stoke's shift and large excited state dipole moment, *etc*.[31–35] On the other hand the observation of a very low quantum yield of these coumarin derivatives in high polarity solvents has been reported to be caused by the conversion of their fluorescent intramolecular charge transfer (ICT) state to the non-fluorescent twisted intramolecular charge transfer (TICT) state.[32] The mechanism of such radiationless decay in 7-alkylamino coumarins is interpreted in terms of a charge stabilizing influence by substituents, solvent or other microenvironments of the dye, which tends to affect the barrier for torsional motion leading to the TICT state. Such control on the charge distribution in the coumarins and hence their physico-chemical properties have been widely studied in homogeneous and heterogeneous media of different solvent characteristics and microenvironments.[33] Some of the coumarin derivatives are also projected as environment sensitive solvatochromic fluorophores suitable for *in vivo* imaging of cells in living organisms.[36] It is expected that the coumarins will be ideal fluorescent guests to probe the nature and binding capacity of various host molecules, as both polarity/polarizability and the constriction due to the host binding will greatly affect the formation of the TICT state,[37,38] which would result in significant changes in the dye fluorescence. Very recently we have established an efficient host-assisted guest protonation mechanism in coumarin 6 (C6) dye and demonstrated that CB7 interaction at the coumarin part of the C6 greatly influences the stereo-electronics at the benzothiazole part, leading to a considerable change in its protolytic as well as photophysical characteristics.[39] The remarkable supramolecular pK_a shift achieved in the CB7–C6 host–guest system, which was more than 5.2 units, was established as tunable for the controlled dissociation of the protonated/deprotonated complex by a simple stimulus such as a thermal or metal ion responsive mechanism.[39]

Having understood the important roles of the ICT/TICT states in the 7-alkylamino coumarins in dictating the excited state dynamics and their overall molecular characteristics, the remarkable modulation of the same reported on cucurbituril encapsulation is highly promising for improving their applicability. With this perspective, herein, we report the effect of macrocyclic encapsulation on the radiative yield, ICT to TICT conversion and water solubility of 7-dialkylamino substituted coumarin derivatives namely, coumarin-1 (C1) and coumarin-481 (C481) (Chart 1) by the synthetic receptor molecule, CB7, in aqueous media. The results are further compared with that of CB7 bound coumarin-6H (C6H) dye (Chart 1), where unlike the previous dyes, the dialkylamino group is substituted by a bridged julolidinyl structure that does not allow TICT formation. It is observed that the inclusion of these coumarin dyes by CB7 has tremendous effects on the excited state relaxation dynamics of the dyes

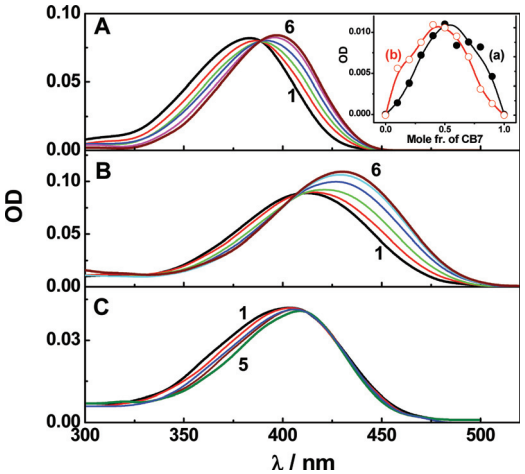

Fig. 1 Absorption spectra of aqueous solution of the coumarin dyes with CB7. (A) C1 (4.0 μM) with [CB7]/μM: 0.0 (1); 3.7 (2); 7.5 (3); 12.5 (4), 25.0 (5); 82 (6). (B) C481 (4.7 μM) with [CB7]/μM: 0 (1); 5 (2); 12 (3); 25 (4); 50 (5); 262 (6). (C) C6H (~2.2 μM) with [CB7/μM: (1) 0, (2) 17.5, (3) 37.5, (4) 50 and (5) 200. Inset A: Absorbance changes in C1 (a) and C481 (b) with continuous variation of the mole fraction of CB7 (Job plot) to evaluate the complex stoichiometry.

in aqueous media. The water solubility of these dyes in the presence of CB7 is found to be substantially high and in combination with the enhanced fluorescence yield accessible in the presence of CB7, makes the cucurbituril complexed dyes suitable candidates for aqueous dye laser systems in the blue–green region.

Results and discussion

Absorption spectral characteristics of C1, C481 and C6H in the presence of cucurbit[7]uril

Fig. 1 shows the characteristic absorption profile of C1, C481 and C6H in aqueous solution exhibiting maxima at ~383 nm, ~410 nm and ~403 nm, respectively.[35–38] The interaction of the macrocyclic host CB7 with C1, C481 and C6H was followed by the changes in the absorption profile of the guests, which are shown in Fig. 1. Incremental addition of CB7 to the C1 solution (~4 μM) resulted in ~10 nm bathochromic shift along with an isosbestic point at ~388 nm (Fig. 1A) and attained saturation at ~50 μM concentration of CB7. Whereas the absorption profile of C481 (~4.7 μM) with CB7 displayed broader and hyperchromic spectral changes with ~20 nm bathochromic shift with a clear isosbestic point at ~407 nm (Fig. 1B) and required a higher CB7 concentration (~200 μM) for saturation. In the case of C6H (~2.2 μM), the absorption spectra became slightly narrower with a small red shift of ~7 nm and an isosbestic point at ~410 nm, as shown in Fig. 1C. This required ~200 μM CB7 for saturation in the spectral changes. The distinct changes in the absorption profile of the guest dyes with the addition of CB7 clearly demonstrate the strong interaction of CB7 with the coumarin derivatives in aqueous solution and are indicative of the host–guest complex formation in these systems. It should be mentioned here that it has been verified that the trace amount of acidity prevalent in the synthesized CB7 samples does not cause the above spectral changes.

Steady-state fluorescence characteristics of C1, C481 and C6H in the presence of CB7

The emission characteristics of 7-*N*,*N*′-disubstituted amino coumarin derivatives, such as C1, C481 and C6H, are the subject of numerous investigations owing to their rich and intriguing photophysics in various homogeneous and heterogeneous media.[31–37] The emission yields of these laser dyes and their other optical properties such as strong solvent polarity dependent Stoke's shifts, large change in the dipole moments on photo-excitation *etc*., critically depend on the substituent at the amine functionality.[31–36] C1 and C481 display low quantum yields in aqueous media (Φ_f C1 = 0.05, Φ_f C481 = 0.01).[35] To explain the observed emission behaviour of these dyes in polar solvents Jones *et al*. proposed the idea of an planar emissive intramolecular charge transfer (ICT) excited state of the dyes capable of converting to a non-fluorescent twisted intramolecular charge transfer (TICT) state through the rotation of the *N*,*N*′-dialkyl moiety.[35] Based on similar studies from our group, it is proposed that the conversion of planar ICT state to non-planar TICT state introduces a fast non-radiative deexcitation channel for the excited state of these dyes and quite interestingly, the activation energy for this new deexcitation channel is found to increase gradually with the increasing solvent polarity parameter (Δf).[32,33] It is suggested that the large difference in the geometry for the excited TICT state and the ground state causes the probability for the TICT to ground state radiative transition to be extremely negligible, and de-excitation of the TICT state to ground state thus occurs mainly by the non-radiative transition.[32,33] In the present study the quantum yields of the studied coumarin dyes in aqueous solution are found to be in excellent agreement with those reported earlier.[35] The fluorescence emission profiles of free C1 and C481 along with the observed changes with the gradual addition of CB7 are shown in Fig. 2. In the case of C1 (~4 μM), addition of CB7 (~80 μM) resulted in an overall enhancement of fluorescence yield by a factor of 17 but, without any notable shift in the emission maxima at 467 nm (Fig. 2A). In a similar manner, in presence of CB7 (~200 μM), C481 (~4.7 μM) displayed an enhanced emission yield by ~16-fold with a slight blue shift in emission maxima (523 nm) by ~5 nm (Fig. 2B).

The accompanying changes in the photophysical behaviour of fluorophores through complexation to CBs are quite generally interpreted in terms of the rigid confinement of the guest inside the host and the hydrophobic environment inside CB cavity.[39–41] These two cooperative effects induce dramatic enhancement in the emission yield by retarding the non-radiative de-excitation mechanisms and reasonable blue shift in the emission maxima of the encapsulated guest due to lower polarity inside the CB*n* cavity. In the present case, while the significant enhancement in the emission intensity for C1 and C481 in aqueous medium in the presence of CB7 clearly demonstrates the interaction of the guest dyes with the receptor CB7, no significant blue shift in the emission maxima of the dyes was observed. To explain this rather unusual spectral behaviour, we anticipate that in case of both the coumarin derivatives, the core coumarin chromophore resides outside the CB7 cavity and hence exposed to the solvent medium and the binding of the dyes with CB7 actually occurs through the encapsulation of their 7-*N*,*N*′-diethylamino group.

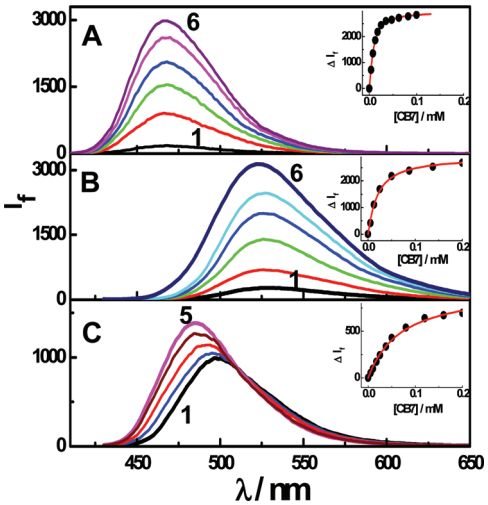

Fig. 2 Fluorescence spectra of aqueous solution of the coumarin dyes with CB7. (A) C1 (4.0 μM) with [CB7]/μM: 0.0 (1); 3.7 (2); 7.5 (3); 12.5 (4), 25.0 (5); 82 (6). (B) C481 (4.7 μM) with [CB7]/μM: 0 (1); 5 (2); 12 (3); 25 (4); 50 (5); 262 (6). (C) C6H (~2.2 μM) with [CB7/μM: (1) 0, (2) 17.5, (3) 37.5, (4) 50 and (5) 200. Insets show the fluorescence titration curves for the respective dyes with CB7.

Complexation with CB7 in this fashion imposes restriction on the torsional motion of *N*,*N*′-diethylamino substituent and prevents the conversion of emissive ICT to non-radiative TICT, which in turn results in the enhancement in the emission yield. Confirmation of this mechanism was evident from the relatively nominal change observed in C6H, in which the *N*,*N*′-diethylamino substituent is rigidized by the julolidinyl structure. As presented in Fig. 2C, even in the absence of CB7, the dye exhibited strong emission at 498 nm (Φ_f C6H = 0.58) and the presence of CB7 did not bring out any large changes as observed in the other two dyes. The fluorescence quantum yield (Φ_f) of CB7·C1, CB7·C481 and CB7·C6H complexes in aqueous solution at the saturation concentration of CB7 were determined and found to be 0.85, 0.16 and 0.73, respectively, by taking the parent dyes in ethanol solution as standards.[35] It is expected that the remarkable changes in the excited state relaxation mechanism will be well reflected in the excited state decay dynamics of the guest dye systems. Therefore, fluorescence decays for the parent systems were measured using both time-correlated single photon counting (TCSPC) and fluorescence up-conversion techniques and the results are discussed below.

Effect of complexation on the fluorescence lifetime of the coumarin dyes

Fig. 3 presents the fluorescence decay traces recorded for the coumarin dyes at their respective emission maxima using the TCSPC setup, both in the absence and presence of CB7. As discussed earlier, due to the involvement of efficient ICT to TICT transformation in the excited state in polar solvents like water, C1 and C481 undergo very fast non-radiative decay and hence exhibit unusually fast excited state decay profiles. As shown in Fig. 3A, B and Table 1, for uncomplexed (free) C1 and C481 dyes, the major component of the double exponential decays

provided the reasonably short lifetime values as ~320 ps (83%) and ~210 ps (86%), respectively for the two dyes. Both the dyes, however, having a minor contribution from a very fast decay component (<50 ps), which is apparently shorter than the time resolution of the TCSPC setup used in this study. However, in the presence of CB7, the decay traces were found to fit fairly well with a double exponential decay function having slower decay constants and the decays gradually became slower on increasing the host concentration. For the C1 dye, the initial decay constants at lower CB7 concentration (~ 280 ps, 14% and ~5 ns, 86%) slowly transformed almost in to a single component of 5.1 ns (97%) having negligible contribution from the faster component. For the C481 dye, the initial ~190 ps (22%) and ~1.95 ns decay constants became ~210 ps (5%) and ~2 ns (95%) components in the presence of >100 μM of CB7. It is clear from the present results (Table 1) that the two lifetime components in both the cases apparently remain in a similar range but there is a steady increase in the amplitude contributions for the slower components (τ_2) with a gradual increase in the concentration of CB7. At this moment we would like to attribute the fast component to the free C1/C481 dye in water and the longer component to the CB7·C1 or CB7·C481 host–guest complex, where the faster relaxation routes for the dyes have been restricted due to host binding. Here again, the decay traces recorded for C6H dye, both in the absence and presence of CB7 (Fig. 3C), display only a single long lifetime component, which increased from 5.3 ns in the absence of CB7 to 6.7 ns in the presence of CB7, obviously due to the absence of any free *N*,*N*′-diethylamino group in the C6H dye. Using the estimated average lifetime and the fluorescence quantum yield values, we evaluated the changes in the radiative and non-radiative decay rates of the excited states of these dyes on interaction with CB7 host and the values are provided in Table 1. As expected, the decreasing trend of overall non-radiative decay rate (k_{nr}) with increase in CB7 concentration reflects the restriction on the rotation of 7-*N*,*N*′-diethylamino group of C1 and C481 due to its encapsulation inside the CB7 cavity which in turn reduces the excited state ICT to TICT conversion process. As expected due to the absence of ICT to TICT process, the reduction in the K_{nr} value is only nominal in the case of C6H dye in the presence of CB7 (Table 1).

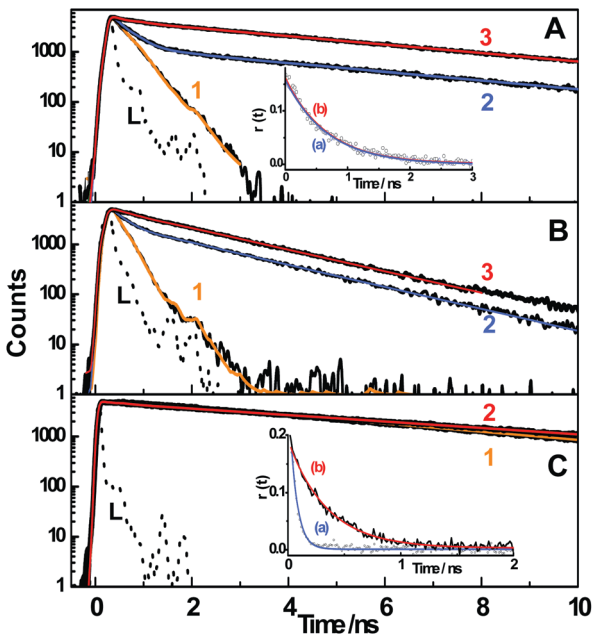

Fig. 3 Fluorescence decay traces of C1 (~4 μM) (A), C481(~4.7 μM) (B) and C6H (~2.2 μM) (C) at their respective emission maxima, in the absence of CB7 host (1); in the presence of 5 μM (2) and ~100 μM (3) CB7. L represents the excitation lamp profile. The fitted (solid lines) values are given in Table 1. Inset A: Anisotropy decay traces of C1 (a) and C481 (b) in presence of CB7. Inset C: Anisotropy decay traces of C6H in the absence (a) and presence (b) of CB7.

Further, moving down to the early time domain, we investigated the effect of CB7 cavitization on the ultrafast fluorescence dynamics of the coumarin dyes using femtosecond fluorescence up-conversion measurements. Ultrafast processes in coumarin dyes have been of immense interest to photochemists in the past two decades and are well documented as originating due to processes like intramolecular torsional relaxation such as ICT to TICT conversion and/or dielectric solvent relaxation. To understand the effect of CB7 interaction, we recorded the time-resolved fluorescence decay traces for C1 and C481 dyes in water in the ultrafast time domain, both in the absence and presence of CB7 host. In these time scales, diffusional interactions are quite unlikely at the concentrations used here, and any effect on the decay dynamics due to the presence of the host molecules is expected to be due to the close contact of the host with the guest dyes. As shown in Fig. 4 in both the cases, the time resolved traces displayed a fast decay component in ~2–3 ps followed by a much slower component. A deconvolution analysis,

Table 1 Excited state decay time constants and other photophysical parameters evaluated for CB7-C1, CB7-C481 and CB7-C6H complexes at varying CB7 concentrations (also see Table S1 for other concentrations of CB7). The decays were analyzed by biexponential kinetics

	[CB7] (μM)	τ_1 (ns) a_1 (%)	τ_2 (ns) a_2 (%)	χ^2	Φ_f	$k_r \times 10^8$ s^{-1}	$k_{nr} \times 10^8$ s^{-1}
CB7-C1	0	0.028 (17)	0.32 (83)	1.04	0.051	1.9	35.1
	8	0.28 (14)	5.07 (86)	1.12	0.42	0.96	1.31
	35	0.26 (5)	5.09 (95)	1.07	0.78	1.61	0.45
	82	0.26 (3)	5.10 (97)	1.02	0.85	1.71	0.30
CB7-C481	0	0.053 (14)	0.21 (86)	1.04	0.012	0.64	52.5
	5	0.19 (22)	1.95 (78)	1.00	0.04	0.26	6.31
	25	0.21 (8)	1.95 (92)	1.12	0.11	0.61	4.97
	138	0.21 (5)	1.99 (95)	1.18	0.16	0.84	4.44
CB7-C6H	0	5.32 (100)	—	1.06	0.58	0.11	0.78
	25	5.78 (100)	—	1.15	0.62	0.11	0.65
	150	6.73 (100)	—	1.07	0.73	0.11	0.41

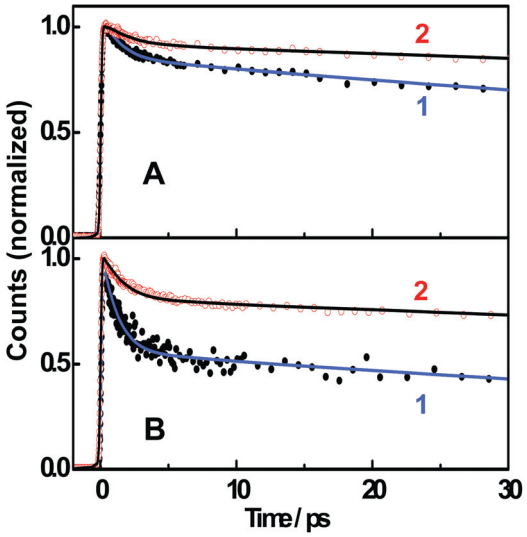

Fig. 4 The symbols represent the fluorescence decay traces of C1 (A) and C481 (B) generated from the fluorescence upconversion measurements, both in the absence (1) and presence (2) of CB7. The solid lines are the deconvoluted fitted traces having two exponential decays constants as discussed in the text.

considering the excitation pulse profile, provided the fast decay component as 1.2 ps & 1.0 ps and the slower decay time as 150 ps & 120 ps, respectively, for C1 and C481 in the absence of CB7. On forming an inclusion complex with CB7, we expect significant restriction in these fast processes due to the rigidization and the less polar microenvironment inside the CB7 cavity. This is what manifested from the decay traces recorded for the dyes in the presence of CB7 (Fig. 4). The deconvolution analysis provided the faster component as 1.6 ps & 1.4 ps and the slower component as 380 ps & 300 ps, respectively, for C1 and C481 in the presence of CB7.

It has been widely discussed that the early time dynamics in 7-aminocoumarins do involve the intramolecular bond twisting motions, thus transforming the fluorescent ICT states effectively to the non-fluorescent TICT states. Since the large polarization in the charge distribution caused by the ICT to TICT conversion is stabilized in polar solvents, the non-radiative relaxation dynamics of the ICT state is very prominent in polar solvents. Apart from the slight retardation in the faster (~1 ps) decay components, the slower (~120 ps & 150 ps) components are seen to be largely affected by the CB7 complexation. As the ICT to TICT conversion do have a contribution in the same time scale as that of the solvent relaxation process, much detailed experimentation, analysis and discussions are required to comment on the intricacies involved in separating these contributions and will be attempted in a separate study. In the context of the present discussion, as the fluorescence decay in the upconversion measurement becomes significantly slower in the presence of CB7, we assert that the fast ICT to TICT conversion in C1/C481 dyes do get affected by their complexation with CB7 host.

Time resolved fluorescence anisotropy studies

Time resolved fluorescence anisotropy studies can provide valuable information regarding the hydrodynamic molecular volume of the emitting species from which information on the complex formation can be predicted. Theoretically, the rotational correlation time, τ_r, for the fluorophore can be related to its rotational diffusion coefficient (D_r) and the viscosity (η) of the medium (water in the present case) and is given by the Stokes–Einstein relationship (eqn (1)):[42]

$$\tau_r = 1/(6D_r), \text{ where } D_r = \frac{RT}{6V\eta} \quad (1)$$

Here, V is the hydrodynamic molecular volume of the fluorophore and T is the absolute temperature. In the present case, it is expected that the complexation of the coumarin dyes with CB7 will be reflected as an increase in the rotational correlation time of the complexes compared to those of the free dyes. It should be mentioned that in the absence of CB7, the anisotropy decays for the studied coumarin dyes are found to be very fast and thus rotational correlation time for the free dyes could not be measured reliably by using our present TCSPC set-up, except for C6H. However, the anisotropy decay for C1, C481 and C6H in the presence of CB7 was found to be significantly slower, with the τ_r values as about 315 ± 50 ps, 340 ± 50 ps and 325 ± 50 ps respectively, clearly supporting the formation of host–guest complexes in these systems in aqueous solution, all with similar stoichiometries.

Determination of stoichiometry and binding constants

It is well known that the cucurbituril homologues, particularly CB7 and CB8, can accommodate one or two organic chromophoric dyes partially or fully depending on the relative size of the guest and the host cavity. Based on the earlier reports[17,40,41,43] and considering the cavity size of CB7, it is anticipated that the dyes used in the present study (Chart 1) would form host–guest complexes with 1 : 1 stoichiometry. At the same time, although it is impossible to accommodate two dye molecules inside one CB7 host, it does not necessarily exclude the possibility of formation of 2 : 1 (host–guest) stoichiometric complexes in the present systems. It was reported earlier that thioflavin T forms complexes with CB7 in both 1 : 1 and 2 : 1 stoichiometries.[21] In the present cases, the changes observed in the absorption profile of C1 and C481 with the gradual increase in the CB7 concentration, however, indicate a typical 1 : 1 host–guest complexation equilibria with clear single isosbestic point in the absorption spectra. Further, the Jobs plots were constructed by selecting a wavelength where the maximum changes were observed in the absorption spectra (at 400 nm for CB7·C1 system and at 430 nm for CB7·C481system) and are shown in the inset of Fig. 1A. In both the cases the maxima appear at ~0.5 mole fraction of the host CB7 which clearly indicate the 1 : 1 stoichiometry of the host–guest complexes of C1 and C481 with the CB7 host.

Establishing the 1 : 1 stoichiometry for the complexes, the binding constants (K_{eq}) were evaluated by the fluorescence titration method as described in method M1, ESI.† Following this and from the titration curves of emission intensity changes with CB7 concentration, the binding constants (K_{eq}) for the 1 : 1 complexes (CB7·C1, CB7·C481 and CB7·C6H) were obtained from their nonlinear fittings[40,44] (*cf.* insets of Fig. 2A–C) and are

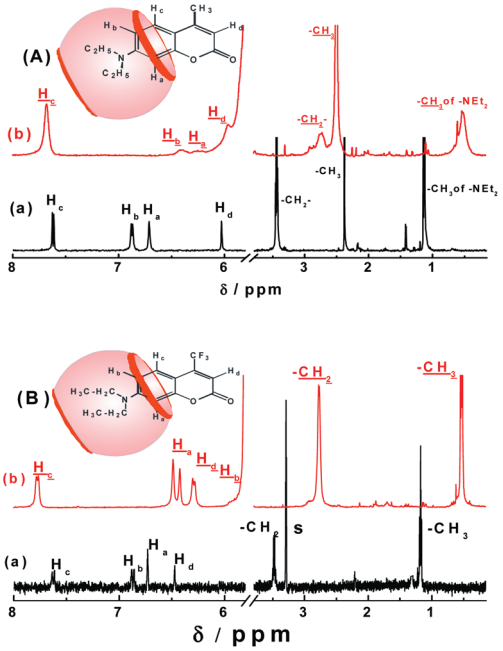

Fig. 5 ^{1}H NMR spectra (500 MHz) of ~100 μM C1 in D_2O (A) and ~50 μM C481 in MeOH-d_4 (B) in their free forms (a) and in presence of 1 mM CB7 in D_2O (b). The protons assigned to the complexed anilino aryl and ethyl groups are underscored and S indicates the signal from the residual solvent. Insets: Pictorial representation of the CB7 : C1 (A) and CB7 : C481 (B) complexes. CB7 and HOD resonances are not shown for clarity.

found to be (1.8 ± 0.1) × 10^5 M^{-1}, (6.0 ± 0.4) × 10^4 M^{-1} and (1.5 ± 0.1) × 10^4 M^{-1}, respectively. As indicated from the K_{eq} values, all the three dyes bind quite reasonably with CB7 but the binding for C1 is 3-times higher than that of C481 and one order higher than that of C6H dye. In the cases of C1 and C481, the binding mode is suggested to be governed by the combination of both charge–dipole and hydrophobic interactions inside the non-polar cavity of CB7 as the K_{eq} values are significantly large (>10^4 M^{-1}). With C6H, the julolidinyl group may hinder the formation of an inclusion complex, however, the strong hydrogen bonding interactions at the CB7 portal region causes the changes observed here possibly *via* exclusion complex formation. It should be mentioned that the interaction of the coumarin dyes with another versatile host molecule, β-cyclodextrin (β-CD), having a similar cavity dimension as CB7, showed binding constant values distinctly lower (K_{eq} of the order of 10^2 M^{-1})[30b,45] as compared to the CB7 complexes. Since β-CD lacks any strong polarizable group like the carbonyl portals in CB7, for βCD dye systems the interaction is mainly hydrophobic in nature and hence significantly weaker than in CB7 dye complexes.

^{1}H NMR studies on the CB7-Dye complexes

To get more insight into the binding modes in the present host–guest complexes, NMR measurements were carried out. Here, the approach of CB7 host from both the ends of the dyes is quite feasible and we investigated the shift in the proton resonances to understand the site of CB7 binding with the dyes. The ^{1}H NMR spectra recorded for C1 and C481 dyes in the absence and in the presence of CB7 are shown in Fig. 5. Encapsulation inside the hydrophobic cavity of CB7, in principle, renders an upfield shift of the proton resonances. At the same time, the protons residing in the vicinity of the negatively polarized carbonyl portals experience a strong deshielding effect and display a downfield shift with respect to their parent positions in ^{1}H-NMR spectra. In the present cases, the ^{1}H-NMR spectra of C1 in the presence of CB7 (1.3 equivalent) in D_2O displays clear upfield shifts of 0.7 ppm and 0.6 ppm for the $–CH_2$ and $–CH_3$ protons with respect to their original position at δ 3.44 (q, $J_{1,3}$ = 14 Hz) and δ 1.13 (t, J = 7 Hz), respectively, indicating that the $–NEt_2$ group is buried inside the hydrophobic cavity of CB7. On the other hand, the aromatic protons H_a (at δ 6.71, s) and H_b (at δ 6.87, d, J = 7Hz) show an upfield shift, appearing as broad unresolved signals in the range of δ 6.1–δ 6.5 in the presence of CB7. This allows us to propose the positioning of CB7 on C1 dye as represented in the inset of Fig. 5A. The effect of deshielding by the carbonyl portal of CB7 could not be observed for the H_c proton (at δ 7.61, d, J = 14 Hz) which instead, appears at 0.4 ppm downfield shifted and as a broad singlet. In this host–guest configuration, the resonance position for the H_d proton, which is away from the CB7, remains almost unaltered but partly overlapped with the CB7 signal. The 4-CH_3 group shows a small downfield shift of 0.1 ppm from its original resonance position at 2.39 ppm, probably due to its close proximity to the carbonyl portal of CB7 molecule in the host–guest complex.

Similarly, in the case of C481 in the presence of CB7, the $–CH_2$ and $–CH_3$ protons of the $–NEt_2$ group shows an upfield shift of 0.72 ppm and 0.65 ppm, respectively, with respect to their parent positions at δ 3.45 (q, $J_{1,3}$ = 10 Hz) and δ 1.13 (t, $J_{1,2}$ = 10 Hz). The aromatic protons H_a (at δ 6.73, s) and H_b (at δ 6.86, d, J = 10 Hz) show upfield shift of 0.24 ppm and 0.58 ppm, respectively, in the presence of CB7. The upfield shift of these protons clearly indicate that the $–NEt_2$ group and a part of the coumarin aromatic moiety resides inside the hydrophobic cavity of CB7, as represented in the inset of Fig. 5B. Encapsulation in this manner places the negatively polarized carbonyl portal of CB7 in close proximity to the H_c proton (at δ 7.76, d, J = 10 Hz). The deshielding effect of carbonyl portal of CB7 molecule results in the downfield shift of the H_c proton by approximately 0.2 ppm. Finally the H_d proton resonance, which would be away from the CB7 molecule in the host–guest complex remains unaltered. These observations clearly depict the interactions of C1 and C481 to CB7 leading to the complete encapsulation of 7-NEt_2 groups and a part of the aromatic coumarin moiety inside the hydrophobic cavity of CB7. Encapsulation in this manner would restrict any conformational change *via* the rotation of the 7-NEt_2 group and thus bring out the pronounced photophysical changes for the encapsulated dyes. However in case of C6H, inclusion of the bulky julolidinyl end is unlikely and the chemical shifts of the aromatic and aliphatic protons suggest that C6H forms an exclusion complex with CB7, mainly through the interaction at the portal region (see Note 1 and Fig. S2, ESI†).

Enhancement of solubility of the coumarin dyes in water in the presence of CB7

Most of the coumarin dyes are sparingly soluble in water. Enhancement of water solubility of coumarins has been reported

in aqueous micellar[46] and cyclodextrin solutions.[37,38,47] The solubility of C1, C481 and C6H increased significantly in aqueous solution in the presence of CB7. The concentration of the CB7 encapsulated dye was calculated from the absorbance of the solution (Fig. S1†) using the molar extinction coefficient of the complexed dye in water. The solubility of C1 increased by a factor of ~100, that of C481 increased by a factor of ~55 and C6H increased by a factor of ~20 in the presence of 0.5 mM of CB7. Since the solubility factor mainly depends on the binding constant values of the complexes as well as the concentration of host molecules, a larger solubility factor for C1 is observed as compared to C481 and C6H at a fixed CB7 concentration. Thus the encapsulation of the dyes into CB7 converts a poorly soluble (~2.5 μmol L^{-1}) hydrophobic compound into a more soluble (~0.25 mmol L^{-1}) inclusion complex. This is an important aspect of supramolecularly enhanced solubilization of sparingly soluble drugs for medicinal uses and that of chromophoric dyes for various photochemical uses, especially laser operation in water by increasing the concentration of laser dyes from μmol L^{-1} to mmol L^{-1} range as usually required for such applications.

Experimental section

Laser grade coumarin-1 (C1: 7-diethylamino-4-methylcoumarin; coumarin-481 (C481: 7-diethylamino-4-(trifluoromethyl) coumarin) and coumarin-6H (C6H: 2,3,5,6–1*H*,4*H*-tetrahydroquinolizino[9,9a,1-gh]coumarin) were obtained from Exciton Inc. and used as received. CB7 was synthesized and purified following the reported procedures in the literatures.[48–50] Purity of the CB7 was checked using ^{1}H-NMR spectroscopy. Nanopure water (conductivity less than 0.06 μS cm^{-1}), obtained from a Millipore Gradiant A10 system, was used to prepare the sample solutions. Chemical structures of the studied coumarin dyes and cucurbit [7]uril (CB7) host are shown in Chart 1. Absorption spectra were recorded with a Shimadzu 160 A UV-vis spectrophotometer (Tokyo, Japan). Steady-state fluorescence spectra were recorded using a Hitachi F-4500 spectrofluorimeter (Tokyo, Japan). ^{1}H NMR spectra were recorded on a Bruker Avance WB 500 MHz spectrometer at TIFR, Mumbai, India.

The time-resolved (TR) fluorescence measurements were carried out using a time-correlated-single-photon-counting (TCSPC) spectrometer (IBH, UK), described elsewhere.[40] In the present work, 374 nm (for C1 dye) and 445 nm (for C481 and C6H dyes) diode lasers (~100 ps, 1 MHz repetition rate) were used for sample excitation and a MCP PMT was used for fluorescence detection. The instrument response function (IRF) of the instrument was collected by replacing the sample cell with a light scatterer (suspension of TiO_2 particle in water) and the full width half maximum (FWHM) was ~100 ps. From the measured decay traces, the time constants were evaluated following a reconvolution procedure.[51,52] The fluorescence decays, $I(t)$ were in general analyzed using a multi-exponential function as

$$I(t) = \sum_i B_i \exp(-t/\tau_i) \quad (2)$$

where, B_i and τ_i are respectively the pre-exponential factor and the fluorescence lifetime for the ith component of the fluorescence decay.

Ultrafast fluorescence decays were measured using a femtosecond fluorescence up-conversion setup (FOG 100, CDP Inc., Russia) based on diode pumped solid state (DPSS) laser pumped 50 fs titanium sapphire laser (CDP Inc., Russia), described elsewhere.[53] In these measurements, samples were excited with the second harmonic light (~400 nm) of the laser and fluorescence from the samples was upconverted by mixing it with the fundamental light pulse (gate pulse; ~800 nm, horizontally polarized) of the laser in a 0.5 mm type I beta barium borate (BBO) crystal. All the measurements were carried out with magic angle polarization of the excitation pulse (using a Berek variable compensator) with respect to the gate pulse to avoid the interference of the rotational relaxation of the fluorophore on the measured fluorescence decays. Optical delay between the excitation and the gate pulses was varied using a delay rail (6.6 fs per step) in the path of the gate pulses. The upconverted signals were measured with a photon counter after passing through a proper bandpass filter and a double monochromator. In all these measurements the samples were taken in a rotating cell (0.4 mm path length) to have a better heat dissipation and thus to avoid any photodegradation of the dye. All measured emission decays were fitted with a multi-exponential function using the standard convolute and compare nonlinear least squares procedure.[51] The IRF was measured through the cross correlation of the excitation and the fundamental gate pulse and was found to have FWHM of ~200 fs.

Conclusions

Supramolecular interactions of cucurbituril macrocycle, CB7 with the coumarin dyes, namely, C1, C481 and C6H have been investigated in aqueous solution using ground state absorption and steady-state and time-resolved fluorescence measurements. In the presence of CB7, distinct differences have been observed in the photophysical characteristics of C1 and C481, suggesting the formation of host–guest complexes with 1 : 1 stoichiometry which are driven by the combination of charge–dipole and hydrophobic interactions between the host CB7 and the bound coumarin dyes. The macrocyclic host–guest interactions impart structural rigidity to the dyes bringing out restrictions on the torsional motion on their 7-*N*,*N*′-diethylamino groups in the excited state and thus modulating the excited state deactivation processes, namely, the conversion of the emissive ICT state to the non-radiative TICT state of the dyes in aqueous solutions. This reduction in the ICT to TICT conversion in turn results in the observed enhancement in the emission yield of both C1 and C481 dyes in aqueous solution. Notably, CB7 encapsulation enhances the solubility of the dyes quite significantly in aqueous solution. The enhanced fluorescence intensity and increased solubility of these dyes in water with small amount of CB7 additive (0.5 mM) make the present host–guest systems quite promising for the development of aqueous dye laser systems in the blue–green region and support its application in cell imaging. Further studies in this direction are being attempted.

Acknowledgements

The authors thank Dr T. Mukherjee, Director, Chemistry Group and Dr S. K. Sarkar, Head, RPCD, for their constant support during this work. N.B. acknowledges Mr A. K. Mora, RPCD,

BARC for his help in the fluorescence upconversion measurements.

Notes and references

1 A. C. Bhasikuttan, H. Pal and J. Mohanty, *Chem. Commun.*, 2011, **47**, 9959–9971.
2 R. N. Dsouza, U. Pischel and W. M. Nau, *Chem. Rev.*, 2011, **111**, 7941–7980.
3 Y. Chen and Y. Liu, *Chem. Soc. Rev.*, 2010, **39**, 495–505.
4 E. Arunkumar, C. C. Forbes and B. D. Smith, *Eur. J. Org. Chem.*, 2005, 4051–4059.
5 M. R. Craig, M. G. Hutchings, T. D. W. Claridge and H. L. Anderson, *Angew. Chem., Int. Ed.*, 2001, **40**, 1071–1074.
6 A. C. Bhasikuttan, S. Dutta Choudhury, H. Pal and J. Mohanty, *Isr. J. Chem.*, 2011, **51**, 634–645.
7 A. L. Koner and W. M. Nau, *Supramol. Chem.*, 2007, **19**, 55–66.
8 H. Bakirci and W. M. Nau, *Adv. Funct. Mater.*, 2006, **16**, 237–242.
9 J. Lagona, P. Mukhopadhyay, S. Chakrabarti and L. Isaacs, *Angew. Chem., Int. Ed.*, 2005, **44**, 4844–4870.
10 J. W. Lee, S. Samal, N. Selvapalam, H.-J. Kim and K. Kim, *Acc. Chem. Res.*, 2003, **36**, 621–630.
11 R. Wang, L. Yuan and D. H. Macartney, *Chem. Commun.*, 2005, 5867–5869.
12 M. Shaikh, J. Mohanty, A. C. Bhasikuttan, V. D. Uzunova, W. M. Nau and H. Pal, *Chem. Commun.*, 2008, 3681–3683.
13 J. Mohanty, A. C. Bhasikuttan, S. Dutta Choudhury and H. Pal, *J. Phys. Chem. B*, 2008, **112**, 10782–10785.
14 N. Saleh, A. L. Koner and W. M. Nau, *Angew. Chem., Int. Ed.*, 2008, **47**, 5398–5401.
15 M. J. Frampton and H. L. Anderson, *Angew. Chem., Int. Ed.*, 2007, **46**, 1028–1064.
16 Y. Xu, M. Guo, X. Li, A. Malkovskiy, C. Wesdemiotisa and Y. Pang, *Chem. Commun.*, 2011, **47**, 8883–8885.
17 W. M. Nau and J. Mohanty, *Int. J. Photoenergy*, 2005, **7**, 133–141.
18 (*a*) N. Jayaraj, M. V. S. N. Maddipatla, R. Prabhakar, S. Jockush, N. J. Turro and V. Ramamurthy, *J. Phys. Chem. B*, 2010, **114**, 14320–14328; (*b*) S. Yi and A. E. Kaifer, *J. Org. Chem.*, 2011, **76**, 10275–10278.
19 J. M. Chinai, A. B. Taylor, L. M. Ryno, N. D. Hargreaves, C. A. Morris, P. J. Hart and A. R. Urbach, *J. Am. Chem. Soc.*, 2011, **133**, 8810–8813.
20 S. Dutta Choudhury, J. Mohanty, H. Pal and A. C. Bhasikuttan, *J. Am. Chem. Soc.*, 2010, **132**, 1395–1401.
21 S. Dutta Choudhury, J. Mohanty, H. P. Upadhyaya, A. C. Bhasikuttan and H. Pal, *J. Phys. Chem. B*, 2009, **113**, 1891–1898.
22 J. Mohanty, S. Dutta Choudhury, H. P. Upadhyaya, A. C. Bhasikuttan and H. Pal, *Chem.–Eur. J.*, 2009, **15**, 5215–5219.
23 J. Mohanty, N. Thakur, S. Dutta Choudhury, N. Barooah, H. Pal and A. C. Bhasikuttan, *J. Phys. Chem. B*, 2012, **116**, 130–135.
24 J. Mohanty, K. Jagtap, A. K. Ray, W. M. Nau and H. Pal, *ChemPhysChem*, 2010, **11**, 3333–3338.
25 J. Mohanty, H. Pal, A. K. Ray, S. Kumar and W. M. Nau, *ChemPhysChem*, 2007, **8**, 54–56.
26 S. S. Anufrik and V. V. Tarkovsky, *J. Appl. Spectrosc.*, 2010, **77**, 640–647.
27 Y. Yang, J. Zou, H. Rong, G. D. Qian, Z. Y. Wang and M. Q. Wang, *Appl. Phys. B: Lasers Opt.*, 2007, **86**, 309–313.
28 R. J. Nedumpara, K. J. Thomas, V. K. Jayasree, C. P. Girijavallabhan, V. P. N. Nampoori and P. Radhakrishnan, *Appl. Opt.*, 2007, **46**, 4786–4792.
29 N. Barooah, B. C. Pemberton and J. Sivaguru, *Org. Lett.*, 2008, **10**, 3339–3342.
30 (*a*) B. C. Pemberton, N. Barooah, D. K. Srivatsava and J. Sivaguru, *Chem. Commun.*, 2010, **46**, 225–227; (*b*) B. D. Wagner, *Molecules*, 2009, **14**, 210–237.
31 A. V. Pinheiro, A. J. Parola, P. V. Baptista and J. C. Lima, *J. Phys. Chem. A*, 2010, **114**, 12795.
32 (*a*) A. Barik, M. Kumbhakar, S. Nath and H. Pal, *Chem. Phys.*, 2005, **315**, 277–285; (*b*) T. L. Arbeloa, F. L. Arbeloa, M. J. Tapia and I. L. Arbeloa, *J. Phys. Chem.*, 1993, **97**, 4704–4707; (*c*) P. Dahiya, M. Kumbhakar, T. Mukherjee and H. Pal, *Chem. Phys. Lett.*, 2005, **414**, 148–154.
33 (*a*) S. Corrent, P. Hahn, G. Pohlers, T. J. Connolly, J. C. Scaiano, V. Fornes and H. Garcia, *J. Phys. Chem. B*, 1998, **102**, 5852–5858; (*b*) N. Sarkar, A. Dutta, S. Das and K. Bhattacharyya, *J. Phys. Chem.*, 1996, **100**, 15483–15486.
34 P. Zhou, P. Song, J. Liu, K. Han and G. He, *Phys. Chem. Chem. Phys.*, 2009, **11**, 9440.
35 G. Jones II, W. R. Jackson, C. Choi and W. R. Bergmark, *J. Phys. Chem.*, 1985, **89**, 294–300.
36 G. Signore, R. Nifosi, L. Albertazzi, B. Storti and R. Bizzarri, *J. Am. Chem. Soc.*, 2010, **132**, 1276–1288.
37 A. Nag, T. Chakrabarty and K. Bhattacharyya, *J. Phys. Chem.*, 1990, **94**, 4203–4206.
38 W. R. Bergmark, A. Davis, C. York, A. Macintosh and G. Jones II, *J. Phys. Chem.*, 1990, **94**, 5020–5022.
39 N. Barooah, J. Mohanty, H. Pal and A. C. Bhasikuttan, *J. Phys. Chem. B*, 2012, **116**, 3683–3689.
40 (*a*) J. Mohanty, A. C. Bhasikuttan, W. M. Nau and H. Pal, *J. Phys. Chem. B*, 2006, **110**, 5132–5138; (*b*) M. Shaikh, J. Mohanty, P. K. Singh, W. M. Nau and H. Pal, *Photochem. Photobiol. Sci.*, 2008, **7**, 408–414.
41 J. Mohanty and W. M. Nau, *Angew. Chem., Int. Ed.*, 2005, **44**, 3750–3754.
42 *Molecular Fluorescence Principles and Applications*, ed. B. Valeur, Wiley-VCH, 2002.
43 M. Shaikh, S. Dutta Choudhury, J. Mohanty, A. C. Bhasikuttan, W. M. Nau and H. Pal, *Chem.–Eur. J.*, 2009, **15**, 12362–12370.
44 S. Dutta Choudhury, J. Mohanty, A. C. Bhasikuttan and H. Pal, *J. Phys. Chem. B*, 2010, **114**, 10717–10727.
45 B. D. Wagner, S. J. Fitzpatrick and G. J. McManus, *J. Inclusion Phenom. Macrocyclic Chem.*, 2003, **47**, 187–192.
46 G. Jones II, W. R. Jackson, S. Kanoktanaporn and W. R. Bergmark, *Photochem. Photobiol.*, 1985, **42**, 477.
47 G. Jones II and M. A. Rahman, *J. Phys. Chem.*, 1994, **98**, 13028–13037.
48 C. Marquez, F. Huang and W. M. Nau, *IEEE Trans. NanoBiosci.*, 2004, **3**, 39–45.
49 J. Kim, I. S. Jung, S. Y. Kim, E. Lee, J. K. Kang, S. Sakamoto, K. Yamaguchi and K. Kim, *J. Am. Chem. Soc.*, 2000, **122**, 540–541.
50 A. Day, A. P. Arnold, R. J. Blanch and B. Snushall, *J. Org. Chem.*, 2001, **66**, 8094–8100.
51 D. V. O'Connor and D. Phillips, *Time Correlated Single Photon Counting*, Academic Press, New York, 1984.
52 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer, New York, 2006.
53 N. Kandoth, S. Dutta Choudhury, J. Mohanty, A. C. Bhasikuttan and H. Pal, *J. Phys. Chem. B*, 2010, **114**, 2617–2626.

