Investigation on photophysical properties of a substituted 3*H*-indole-modified β-cyclodextrin
II. Efficient photoinduced energy transfer with naphthalene and its derivatives

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Abstract

Photoinduced energy transfer is observed in the supramolecular assemblies of mono-6-deoxy-(2-[(*p*-amino) phenyl]-3,3-dimethyl-5-carboxyl-3*H*-indole)-β-CD (compound A) with naphthalene and its derivatives, which are stabilized via hydrophobic interactions in aqueous solutions. According to Förster theory, the critical transfer radius R_0 was calculated. It was found that the energy transfer efficiency from naphthalene and its derivatives to compound A is very high and is increased with increasing their inclusion percents in the β-CD cavity of compound A. Energy transfer efficiencies for 2-methoxynaphthalene and 2-naphthol are not much different, but larger than that of naphthalene at the same molar ratio of donor to acceptor. But, when the molar ratio reaching 1:10, the difference in the energy transfer efficiency for the three donors is negligible.
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1. Introduction

As photoinduced energy transfer (PET) plays a key role in photosynthesis [1], it has been frequently applied to probe biology and estimate chromophore separation and structure on the nanometer scale [2]. It provides information about distances on the order of 10–100 Å and is thus suitable for investigating spatial relationships of interest in biochemistry [3]. Large amounts of research on covalently linked chromophore arrays for exploring long-range energy transfer and electron transfer phenomena have been documented [4], and the research concerning non-covalently linked chromophore arrays has also been described [5]. Generally speaking, the energy transfer mechanism in the non-covalent systems is more complicated than that in the covalent systems. Non-covalent interactions including hydrogen bonding, π-stacking, metal-ligand coordination and hydrophobic interactions often help assemble energy transfer donors and acceptors [6–10]. To further understand and mimic

the energy transfer mechanism in biological systems where hydrophobic interactions are usually dominant, some research groups designed and synthesized artificial systems of photoinduced electron transfer in which the donor and acceptor were held together via hydrophobic interactions [11,12]. Ueno and co-workers investigated the quenching mechanism of the fluorescence of naphthalene by the combination process of Förster energy transfer and electron transfer to the trinitrophenyl units [13].

Cyclodextrin, containing 6 (α-CD), 7 (β-CD), or 8 (γ-CD) D-glucose units, is one of the most popular host molecules to construct various supramolecular assemblies. A wide variety of cyclodextrin derivatives have been designed and synthesized for their application on enzyme mimics and as supramolecular receptors and chiral selectors in separation science and technology, pharmaceutical chemistry, food technology and analytical chemistry [14]. Very recently, we have synthesized a new compound, mono-6-deoxy-(2-[(*p*-amino)phenyl]-3,3-dimethyl-5-carboxyl-3*H*-indole)-β-CD (compound A) [15,16]. Both experimental and computational analyses show that the substituted 3*H*-indole moiety of compound A adopts rim-covering conformation in

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aqueous solution [16]. It undergoes novel recognition mechanism, in contrast to the conventional CD-based chemosensors that exhibit self-inclusion conformation [16]. The substituted 3*H*-indole moiety of compound A located at the rim of the β-CD cavity is surrounded by water molecules both inside and outside of the cavity. Upon addition of guests, some water molecules inside the cavity are excluded leading to the substituted 3*H*-indole moiety transferring from a hydrophilic microenvironment to a less hydrophilic one and thus an increase in its fluorescence intensity [16]. In this article, we will report that a photoinduced energy transfer phenomenon occurs from naphthalene and its derivatives to compound A, which can afford deeper insight into the mechanism of energy transfer in the non-covalent systems. Naphthalene and its derivatives are included in the β-CD cavity of compound A through hydrophobic interaction. In these supramolecular assemblies, the energy transfer from naphthalene and its derivatives (donors) to the substituted 3*H*-indole moiety of compound A (acceptor) is very efficient.

2. Experimental

2.1. Materials

β-CD (Beijing Shuanghuan, China) was recrystallized three times from tridistilled water. Naphthalene, 2-naphthol and 2-methoxynaphthene were purified by sublimation at reduced pressure. All other chemical reagents were of analytical grade.

2.2. Instrumentations

Absorption spectra were recorded on UV-3010 (Hitachi) spectrophotometer using 1 cm quartz cells. Steady-state fluorescence measurements were performed on F-4500 (Hitachi) spectrofluorimeter. The excitation and emission bandpasses were 10 and 5 nm, respectively. Each solution was excited near its maximum absorption wavelength using 1 cm quartz cells. Fluorescence lifetime measurements were made on a multiplexed time-correlated single-photon counting fluorimeter FLS920 (EDINBURGH). The fluorescence lifetime was determined from data on the fluorescence transient waveform of the material to be tested and the lamp waveform data using the least-squares iterative deconvolution method [17]. Three thousand counts were collected for each sample.

2.3. Methods

Tridistilled water and fresh sample solutions were used throughout the experiments. The pH value of solutions, fixed at 9.5, was adjusted by adding NaOH and no buffers were used [18]. Stock solutions of naphthalene and its derivatives were prepared in methanol. The fluorescence spectra for the mixture of the donor naphthalene with the acceptor compound A and for the acceptor compound A only were recorded under same conditions. The latter has been subtracted from the former to give accurate spectral information and the net change in the fluorescence intensities for both donor and acceptor. The influence

of dissolved oxygen on the systems in the present work was investigated and it was found to be typically less than 5%. Thus, all samples for the measurements were not deoxygenated and measured directly in the air.

3. Results and discussion

The interaction between compound A and naphthalene in aqueous solution has been studied with absorption and steady-state fluorescence spectra. As can be seen from Fig. 1, there is much overlap between the emission spectrum of naphthalene and the absorption spectrum of compound A. This indicates that the occurrence of the photoinduced energy transfer from naphthalene (donor) to the substituted 3*H*-indole moiety of compound A (acceptor) is possible [19].

The fluorescence spectra for the mixture of naphthalene at fixed concentration and compound A at various concentrations are shown in Fig. 2. Considering the fact that the fluorescence
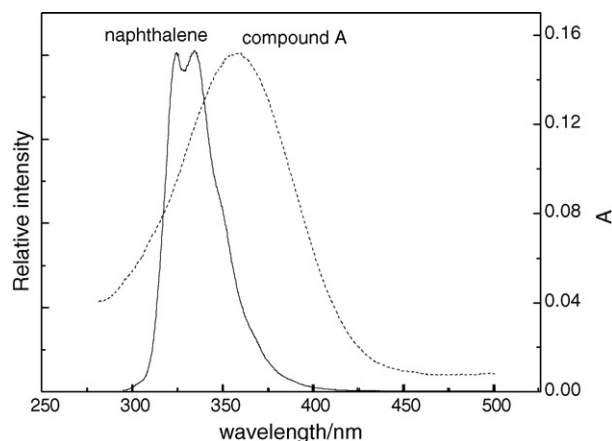

Fig. 1. Fluorescence emission of naphthalene (10 μM) and absorption of compound A (10 μM) in aqueous solution.

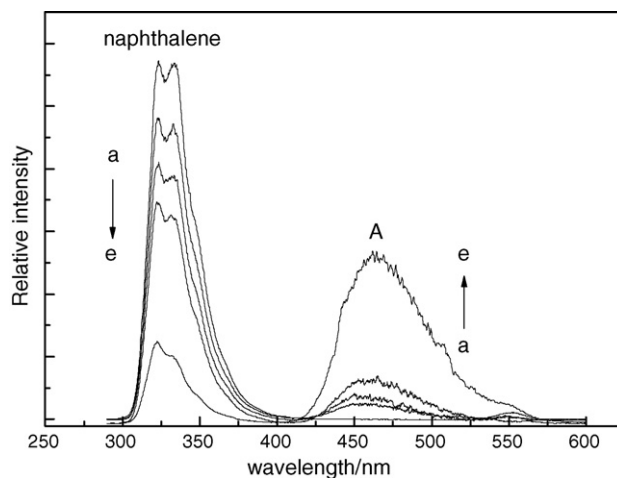
Fig. 2. Fluorescence spectra of naphthalene (10 μM) in the presence of compound A at various concentrations (from (a) to (e): 0, 10, 20, 30 and 100 μM). The excitation wavelength = 275 nm. Note that the spectrum of the compound A only has been subtracted from the fluorescence spectra of the mixture of donor naphthalene and acceptor compound A, which gives net changes of fluorescence intensities for both donor and acceptor.

Table 1
Förster energy transfer parameters, inclusion association constants of donor–acceptor pair and the inclusion percents of donor in the β-CD cavity of compound A

Donor	R_0 (Å)	K_1 ($\times 10^3$ M^{-1})	Molar ratio (donor:acceptor)	Inclusion percent of donor (%)	E_{ET}	R (Å)
Naphthalene	20.8	7.2 ± 0.9	1:1	6	0.10	30.0
			1:2	11	0.23	25.3
			1:10	40	0.80	16.5
2-Naphthol	26.6	11.1 ± 0.6	1:1	9	0.27	31.0
			1:2	16	0.41	28.4
			1:10	51	0.83	20.4
2-Methoxy naphthalene	33.4	12.4 ± 0.1	1:1	10	0.22	40.9
			1:2	18	0.38	36.3
			1:10	54	0.84	25.3

spectra for both donor and acceptor are net ones, we can see that the PET phenomenon does take place. Since compound A adopts the rim-covering conformation, energy transfer can undergo in two routes, one is the dynamic quenching between compound A and the free excited naphthalene molecule and the other is the static quenching between compound A and the naphthalene molecule inside the β-CD cavity of compound A. To assess the capability of compound A including naphthalene, we carried out the non-linear regression (NLR) analysis [16] with the intensity at a fixed wavelength ($\lambda = 333$ nm) near the maximum emission of naphthalene and found that the fit based on the 1:1 (guest:host) model converged well with good correlation coefficient (see Fig. 3). The value of the association constant of the 1:1 inclusion complex K_1 was estimated to be $(7.23 \pm 0.90) \times 10^3$ M^{-1}, from which the inclusion percent of naphthalene by compound A can be calculated based on the concentrations of guest and host (see Table 1).

For steady-state fluorescence, the Stern–Volmer relationship has been derived previously for a system with dynamic and static quenching components and especially, for small concentration of quencher the equation is given by [20]:

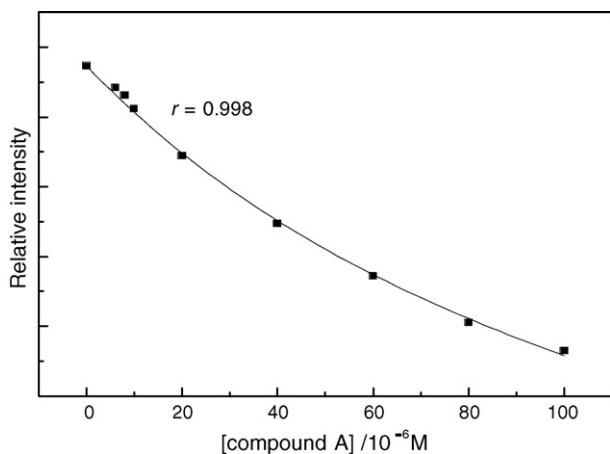$$\frac{I}{I_q} = 1 + (k_b + \tau_0 k_q)[Q] \qquad (1)$$

Fig. 3. Non-linear regression (NLR) result of the fluorescence intensity of naphthalene (1 μM) at the fixed wavelength ($\lambda = 333$ nm) against various concentrations of compound A.
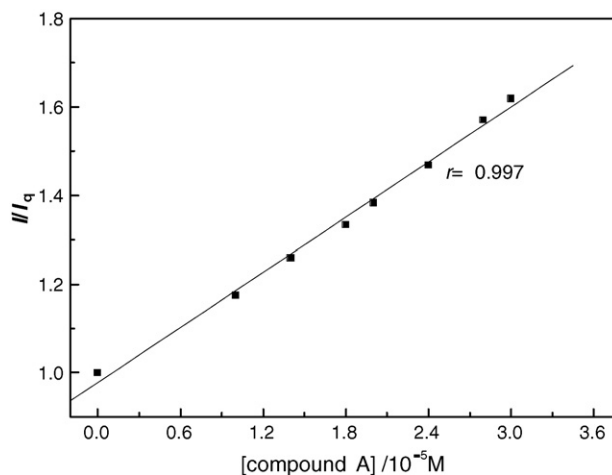
Fig. 4. Stern–Volmer plot for the quenching of naphthalene (10 μM) by compound A in aqueous solutions.

where I and I_q are the fluorescence intensities in the absence and presence of quencher (Q), respectively, k_b the supramolecular binding constant, τ_0 the lifetime of the fluorophore in the absence of quencher, and k_q is the bimolecular quenching constant. When there is only dynamic quenching, the Stern–Volmer relationship can be described as

$$\frac{I}{I_q} = 1 + \tau_0 k_q[Q] \qquad (2)$$

If there were only dynamic quenching in the studied system, the k_q value calculated from the slope $\tau_0 k_q$ (see Fig. 4) would be $(5.9 \pm 0.2) \times 10^{11}$ M^{-1} s^{-1}, which is listed in Table 2. This value is much higher than the value of the diffusion rate constant

Table 2
Apparent Stern–Volmer constants (K_{sv})[a], and hypothetical fluorescence quenching constants (K_q) [b] of donors

Donor	K_{sv} ($\times 10^4$ M^{-1})	T_0 (ns)	K_q ($\times 10^{11}$ M^{-1} s^{-1})
Naphthalene	2.07 ± 0.06	35.3	5.9 ± 0.2
2-Naphthol	3.61 ± 0.16	5.1	70.8 ± 3.1
2-Methoxy naphthalene	3.28 ± 0.16	10.0	32.8 ± 1.6

[a] K_{sv} is the slope of the plot of I/I_q vs. concentration of compound A.
[b] $K_q = K_{sv}/\tau_0$.

[21], i.e.:

$$k_{\text{diff}} = \frac{8kT}{3\eta} = 6.6 \times 10^9 \, \text{M}^{-1} \, \text{s}^{-1} \tag{3}$$

This leads us to conclude that molecular dynamic diffusion is not the unique mechanism for the quenching studied herein. According to the literature, the bimolecular quenching concerning the singlet state of an organic molecule is mostly attributed to the long-range Förster energy transfer [22], or electron transfer [23].

In the time-resolved fluorescence measurements, the lifetime of naphthalene in pure water was estimated to be 35.3 ns. For we measured the sample directly in the air, the possible weak quenching of O_2 [19] made the value slightly lower than 39.0 ns reported in the literature [24]. For the same reason, the lifetime of naphthalene in the aqueous solution of β-CD (10 mM) was estimated to be 56.3 ns, also slightly lower than 60.9 ns reported in the literature [24]. To our knowledge, the 1:1 (guest:host) inclusion complex was formed between naphthalene of low concentration and β-CD in aqueous solution, whereas the 2:2 inclusion complex was formed at high concentration of naphthalene [24,25]. Thus, naphthalene in the hydrophobic cavity of β-CD can be well shielded from water molecules. This can reduce the intersystem crossing rate of naphthalene to some extent and results in a much longer lifetime [24]. To the mixture of naphthalene and compound A at the molar ratio of 1:2 (donor:acceptor) that was excited at 275 nm, the fluorescence decay curves at emission wavelengths of 333, 365, 470 nm were monitored, respectively (see Fig. 5). At 333 nm that was near the maximum emission of naphthalene and the minimum emission of compound A, the fluorescence decay was fitted by a single-exponential curve with a lifetime of 34.38 ns. We ascribed it to the lifetime of naphthalene. As we mentioned above, dynamic and static quenching coexist in the mixture. At the molar ratio 1:2 of donor to acceptor (see Table 1), 11% of naphthalene molecules included in the β-CD cavity of compound A undergo static
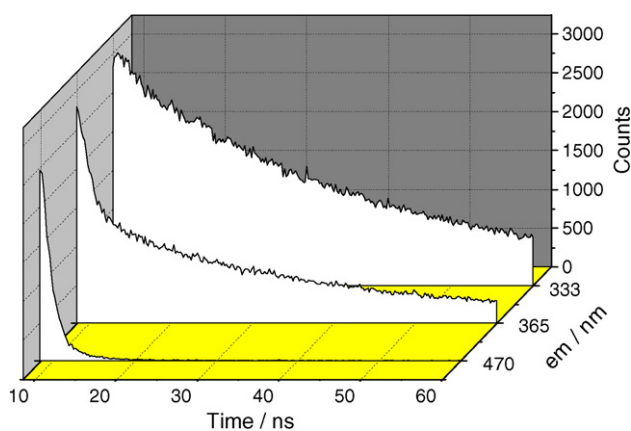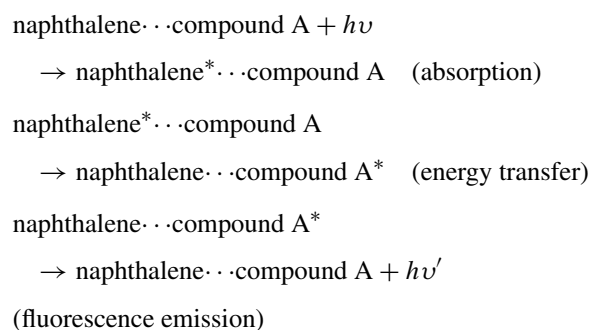

Fig. 5. Time-resolved fluorescence of the mixture of naphthalene (10 μM) and compound A (20 μM). The excitation wavelength was 275 nm, while the emission wavelengths were 333, 365 and 470 nm, respectively. B_i is a pre-exponential factor representing the fractional contribution to the time-resolved decay of the component with a lifetime τ_i, $I(t) = \sum_i B_i \, e^{-t/\tau_i}$.

quenching with the substituted 3H-indole moiety. The inclusion process increases the lifetime of naphthalene, while the static quenching process decreases it. A compensation of these two processes makes the lifetime of naphthalene in the presence of compound A similar to that in pure water. To mimic the effect of dynamic quenching between the substituted 3H-indole moiety of compound A and the naphthalene outside the β-CD cavity, we measured the lifetime of naphthalene in the presence of the substituted 3H-indole instead of compound A at the same molar ratio of 1:2. The fluorescence decay monitored at 333 nm was also fitted by a single-exponential curve with a lifetime of 34.42 ns (figures not shown). This seems to indicate that the dynamic quenching does not affect obviously the lifetime of naphthalene in the system we studied. This is the reason that the fluorescence decay at 333 nm was fitted by a single-exponential curve. As shown in Fig. 5, with increasing emission wavelength to 365 nm, the fluorescence decay was fitted by a dual-exponential curve with a long lifetime of 32.1 ns ($B_1 = 86.92\%$) and a short lifetime of 1.37 ns ($B_2 = 13.08\%$). The former was ascribed to the lifetime of naphthalene and the latter to that of compound A. At the wavelength of 470 nm that was near the maximum emission of compound A and the minimum emission of naphthalene, the fluorescence decay was also fitted by a dual-exponential curve with a long lifetime of 9.48 ns ($B_1 = 4.4\%$) and a short lifetime of 0.93 ns ($B_2 = 95.6\%$). We ascribed these two lifetimes to the two components of compound A. The longer one corresponds to the component that participated in the energy transfer process, and the shorter one that similar to the value of compound A in pure water corresponds to the free molecules without energy transfer. Obviously, the fact that the long lifetime of donor is weakened and the lifetime of acceptor is appeared with increasing the emission wavelength strongly supports the occurrence of energy transfer. Thus, in this system the energy transfer can proceed as the following steps [26]:

naphthalene···compound A + $h\upsilon$

   → naphthalene*···compound A   (absorption)

naphthalene*···compound A

   → naphthalene···compound A*   (energy transfer)

naphthalene···compound A*

   → naphthalene···compound A + $h\upsilon'$

(fluorescence emission)

where a naphthalene molecule in the ground state absorbs light and converts to its first excited single state naphthalene*, then the rapid Förster energy transfer occurs to the nearby substituted 3H-indole moiety of compound A. According to the Förster theory [27], the efficiency of energy transfer is given by

$$E_{\text{ET}} = 1 - \frac{I_{\text{DA}}}{I_{\text{D}}} = \frac{R_0^6}{R_0^6 + R^6} \tag{4}$$

where I_{DA} and I_{D} are the donor intensities in the presence and absence of acceptor, R the distance between the donor and accep-

tor, and R_0 is the Förster or critical transfer distance at which the energy transfer rate is equal to the decay rate. R_0 being a function of the spectral properties of a donor–acceptor pair can be represented as [27]:

$$R_0^6 = \frac{(9000 \ln 10)\kappa^2 \phi_D}{128\pi^5 n^4 N} \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 \, d(\lambda)$$
$$= 8.79 \times 10^{-5} (\kappa^2 n^{-4} \phi_D J(\lambda)) \tag{5}$$

where κ^2 is the orientation factor related to the geometry of the donor–acceptor dipoles and for random orientation as in fluid solution $\kappa^2 = 2/3$, n the refractive index of the medium, ϕ_D the quantum yield of the donor in the absence of acceptor, N the Avogadro number, $F_D(\lambda)$ the spectral distribution of corrected donor emission, and $\varepsilon_A(\lambda)$ is the extinction coefficient of the acceptor [28]. For this naphthalene-compound A pair, the spectral overlap integral $J(\lambda)$ was calculated to be $1.514 \times 10^{14} \, \mathrm{M}^{-1} \, \mathrm{cm}^{-1} \, (\mathrm{nm})^4$ for the emission spectrum of naphthalene and the absorption spectrum of compound A. Thus, the value of R_0 was estimated to be 20.8 Å, which is in the range of 20–60 Å for a typical donor–acceptor pair [29]. Based on Eq. (4) and Fig. 2, we know that the energy transfer efficiency is strongly dependent on donor–acceptor distance and can calculate the molecular distance between naphthalene and the substituted 3H-indole moiety of compound A at their different molar ratios. The results listed in Table 1 show that the energy transfer efficiency is increased with increasing the molar ratio of donor to acceptor, while the donor–acceptor distance is decreased gradually. In the case of all naphthalene molecules included in the β-CD cavity of compound A, the donor–acceptor molecular distance within the supramolecular assembly would be not larger than 7.9 Å, the height of β-CD cavity, since compound A adopts rim-covering conformation [16]. On the contrary, Table 1 shows that the distance is actually much larger than 7.9 Å. Thus, it is believed that the static quenching is not the unique mechanism responsible for the energy transfer either.

On the basis of above results, we can now conclude that the dynamic and static quenching mechanisms work simultaneously. Obviously, the R value is the average of those for the included donor–acceptor pair (R_1) and for the free donor–acceptor pair (R_2). Provided that the R_1 value is smaller than the R_2 value, one can infer that the more inclusion complexes between naphthalene and compound A formed, the smaller the R value will be. The estimated inclusion percents of naphthalene and the corresponding R values as shown in Table 1 strongly support our supposition.

To obtain more details about this energy transfer mechanism, we also studied the interactions of compound A with two derivatives of naphthalene, i.e., 2-naphthol and 2-methoxynaphthalene. Like naphthalene, they also exhibit considerable overlaps between their fluorescence emission spectra and the absorption spectrum of compound A (figures not shown), which are the necessary conditions for the Förster energy transfer to occur. All the corresponding results obtained for 2-naphthol and 2-methoxynaphthalene are complied in Tables 1 and 2. The results suggest that such supramolecular assemblies of 2-naphthol and 2-methoxynaphthalene included in the β-CD cav-

ity of compound A also make the energy transfer very efficient, which is similar to the situation of naphthalene. Furthermore, when comparing the results of the above three donors, one can find that the order of the association constant (K_1) is 2-methoxynaphthalene > 2-naphthol > naphthalene, and so is the inclusion percent at the same molar ratio of donor to acceptor. One can also find that the energy transfer efficiencies for 2-methoxynaphthalene and 2-naphthol are not much different considering experimental errors, while they are obviously larger than that of naphthalene at the same molar ratio of donor to acceptor. But, when the molar ratio reaching 1:10, the difference in the energy transfer efficiency for the three donors is negligible.

4. Conclusion

In the present study, steady-state and time-resolved fluorescence measurements show that the photoinduced energy transfer occurs from naphthalene and its derivatives (donors) to the substituted 3H-indole moiety of compound A (acceptor). Notably, naphthalene and its derivatives can form inclusion complexes with compound A via hydrophobic interactions. According to Förster theory, the critical transfer radius R_0 was calculated. The energy transfer efficiencies of the three donors increase with the increasing of their inclusion percents, which suggests that such supramolecular assemblies of naphthalene and its two derivatives included in the β-CD cavity of compound A make the energy transfer very efficient.

Acknowledgements

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