

NJC

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

Aggregation-induced emission enhancement and living cell imaging of novel diarylanthracene conjugated dyes

Chao Xia, Ying Qian*

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

In this work, two novel diarylanthracene conjugated dyes, CzAn and BCzAn, which contain carbazolyl substituted triarylamine groups, were synthesized by Ullmann reaction and characterized by ^1H NMR, ^{13}C NMR, and HRMS-MALDI-TOF. Both of the diarylanthracene conjugated dyes CzAn and BCzAn demonstrated aggregation-induced emission enhancement (AIEE) properties. CzAn and BCzAn emitted brightly in solid states with maximum emission wavelengths at 576 and 601 nm. BCzAn emitted weakly in THF solutions with fluorescence quantum yield of 8%, became strong emitters in the aggregated states with the fluorescence peak intensity increased in THF-water mixtures and DMF-glycerol mixtures rather than in pure solvents. Nanoparticles (Nps) were prepared from CzAn and BCzAn by hydrophobic interaction with bovine serum albumin (BSA). The diameters of the AIEE dye based BSA nanoparticles were characterized by transmission electron microscopy (TEM) and their photophysical properties were also measured. These BSA nanoparticles showed high water dispersibility, stable uniform morphology (100-200 nm), strong fluorescence, low cytotoxicity and excellent biocompatibility, making them good optical for living HeLa cells imaging.

Introduction

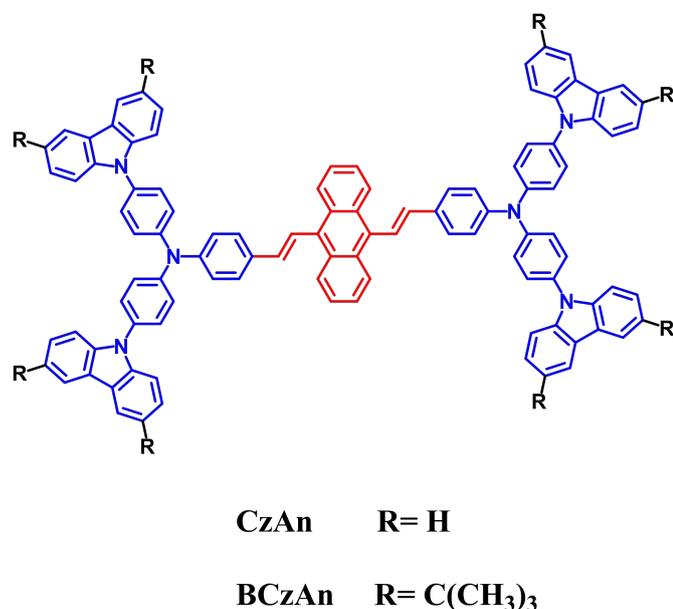
The design and synthesis of high-efficiency organic conjugated molecules with aggregation-induced emission enhancement (AIEE) properties have attracted considerable attention¹. Since the first report by Tang², a great number of AIEE-active dyes have been synthesized and researched³. The AIEE-active dyes have the general feature that they exhibit low or no fluorescence when freely dissolved in solvent. However, upon addition of a solvent which reduce their solubility, the fluorescence intensity drastically increases as a result of the aggregate formation⁴. Restricted intramolecular rotation (RIR) of molecules in aggregated state has been suggested as the main cause of the AIEE phenomenon⁵. Diarylanthracene derivatives are an important class of AIEE-active compounds with high fluorescence quantum yields and photostability⁶. A great number of diarylanthracene derivatives have been synthesized and considered to be candidates for fluorescence sensors⁷ and electroluminescent devices⁸. A series of AIEE-active pyridine-end-capped diarylanthracene derivatives which possess good two-photon absorption properties were reported by our group⁹.

Although diarylanthracene and other AIEE-active dyes have many advantages aforementioned, many of them and dyes

only having aggregation-induced emission (AIE) properties can not be introduced into biological applications such as cell imaging directly because of poor water-solubility and poor biocompatibility¹⁰. Researchers come up with some methods to overcome these disadvantages, such as modifying AIE molecules with ions to improve their solubility in cell media. However, the electric charges of the highly concentrated ionized dyes may affect intracellular physiology and even kill the cells¹¹. Our group has reported a series of AIE dye doped silica nanoparticles with high water-dispersity and excellent photophysical properties, but they have poor degradability in biological tissues¹². Combining AIEE/AIE dye with amphiphilic polymer matrix to form AIEE/AIE dye based nanoparticles can provide us with a valuable method to overcome the challenging issue¹³. The frequently-used polymer matrix included chitosan¹⁴, poly(DL-lactide-co-glycolide) (PLGA)¹⁵, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-(polyethylene glycol) (DSPE-PEG)¹⁶, etc. AIEE/AIE dye based fluorescent nanoparticles were extensively explored for biosensor, bioimaging and disease treatment because of their distinctive features such as hydrophilicity, size tuning, low cytotoxicity and biocompatibility¹⁷. Tang¹⁸ has reported AIE

dye based nanoparticles with photodynamic activity and could be successfully used in image-guided photodynamic therapy. Lu¹⁹ has reported folate-functionalized AIE dye based nanoparticles by co-encapsulating two dyes acting as donor-acceptor to achieve fluorescence resonance energy transfer (FRET) and showed specific targeting effect for cancer cells. Zhang²⁰ has developed a simple method by combining a AIE-active 9,10-distyrylanthracene and a non-ionic surfactant to achieve hydrophobic nanoparticles which showed novel AIE property, excellent biocompatibility and were applied in cell imaging.

Based on the discussions above, we can conclude that the synthesis of novel high-efficiency AIEE dyes and preparation of relevant nanoparticles for biological applications are still desirable. In this paper, two novel diarylanthracene conjugated dyes, CzAn and BCzAn, which contain carbazolyl substituted triarylamine groups, were synthesized by Ullmann reaction and characterized by ¹H NMR, ¹³C NMR, and HRMS-MALDI-TOF. Chemical structures of CzAn and BCzAn are given in Scheme 1. Fluorescence spectra of the diarylanthracene conjugated dyes in solid state and AIEE properties of them in mixed solvents were measured. Bovine serum albumin (BSA) is a commercially available hydrophilic polymer with great biocompatibility²¹. BSA nanoparticles based on AIEE dyes CzAn and BCzAn were successfully used in living HeLa cells imaging.



Scheme 1 Chemical structures of diarylanthracene conjugated dyes CzAn and BCzAn.

Experimental procedure

Materials and instrument

Solvents were purified and dried using standard protocols. The original compound An-BIPAS was synthesized according to the literature reported by our laboratory²². The intermediate 3,6-di-tert-butyl-9H-carbazole was synthesised according to the literature²³ and identified by ¹H NMR. All other chemical reagents were obtained commercially and were used as received without further purification.

Chemical structures were identified by high resolution mass spectrometry (HRMS) and nuclear magnetic resonance (NMR). HRMS were recorded on an Ultraflex MALDI-TOF mass spectro-photometer. ¹H NMR measurements were determined with a Bruker 300 MHz spectrometer, with TMS (tetramethyl silane) as the internal standard. ¹³C NMR measurements were determined with a Bruker 300 MHz spectrometer. UV-visible absorption spectra were measured on a Shimadzu UV-3600 spectrophotometer. Fluorescence spectra were recorded on an HORIBA FL-4 max spectrometer. The melting points were measured on a Microscopic Melting Point Meter X-4. Transmission electron microscopy (TEM) images were recorded on a Tecnai G20 microscope operated at 200 kV, the TEM specimens were made by placing a drop of the nanoparticles suspension on a carbon-coated copper grid. The cell imaging equipment used in our experiment were Upright Fluorescence Microscope (Imager A1) provided by ZEISS, Germany.

Synthesis

Preparation of An-BIPAS. The synthetic route of An-BIPAS was in accordance with the known literature procedure reported by our group²².

Preparation of CzAn. A mixture of An-BIPAS (0.50 g, 0.41 mmol), carbazole (0.33 g, 1.97 mmol), 1,10-phenanthroline (0.12 g, 0.67 mmol), CuI (0.07 g, 0.37 mmol), K₂CO₃ (0.25 g, 1.81 mmol) in 15 mL of DMF was stirred at room temperature for 1 h. And then the mixture was stirred and heated to 160 °C for 72 h under a nitrogen atmosphere. After cooling, it was poured into 100 mL of water. The precipitate was filtered and dried. The crude product was purified by column chromatography on silica gel (petroleum ether/CH₂Cl₂) to afford the pure solid CzAn. Yield 60%. m.p. > 300 °C. ¹H NMR (C₆D₆, 300 MHz) δ: 8.65~8.68 (m, 4H), 8.22 (d, 8H, *J* = 7.65 Hz), 7.95 (d, 2H, *J* = 16.41 Hz), 7.57 (dd, 12H, *J* = 8.10 Hz), 7.46~7.51 (m, 12H), 7.39~7.41 (m, 16H), 7.30~7.36 (m, 12H), 6.00 (d, 2H, *J* = 16.44 Hz); IR (KBr) ν: 3028, 1634, 1595, 1582, 1506, 1468, 1326, 1289, 971 cm⁻¹; ¹³C NMR (C₆D₆, 300 MHz) δ 147.82, 147.16, 141.96, 141.81, 137.50, 133.87, 133.73, 133.41, 130.78, 127.35, 126.70, 126.59, 126.09, 125.82, 125.74, 125.61, 125.32, 125.05, 124.43, 124.30, 121.21, 121.10, 121.04, 120.85, 120.73, 110.47, 110.35. HRMS-MALDI-TOF calcd for C₁₀₂H₆₉N₆ [M+H]⁺ C₁₀₂H₆₉N₆ 1377.5578, found 1377.5582.

Preparation of BCzAn. A mixture of An-BIPAS (0.50 g, 0.41 mmol), 3,6-bis(tert-butyl)carbazole (0.55 g, 1.97 mmol), 1,10-

phenanthroline (0.12 g, 0.67 mmol), CuI (0.07 g, 0.37 mmol), K_2CO_3 (0.25 g, 1.81 mmol) in 15 mL of DMF was stirred at room temperature for 1 h. And then the mixture was stirred and heated to 160 °C for 72 h under a nitrogen atmosphere. After cooling, it was poured into 100 mL of water. The precipitate was filtered and dried. The crude product was purified by column chromatography on silica gel (petroleum ether/ CH_2Cl_2) to afford the pure solid BCzAn. Yield 53%. m.p. >300 °C; 1H NMR ($CDCl_3$, 300 MHz) δ : 8.50~8.53 (m, 4H), 8.23 (s, 8H), 8.01 (d, 2H, $J=16.38$ Hz), 7.78 (d, 4H, $J=8.25$ Hz), 7.61 (d, 12H, $J=8.64$ Hz), 7.55 (s, 8H), 7.52 (d, 12H, $J=6.30$ Hz), 7.43~7.49 (m, 8H), 7.05 (d, 2H, $J=16.32$ Hz), 1.55 (s, 72H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ : 149.92, 148.71, 145.45, 141.98, 139.43, 135.74, 132.27, 130.35, 129.18, 127.76, 127.17, 126.66, 126.17, 125.83, 118.89, 111.85, 37.38, 34.68; HRMS-MALDI-TOF calcd for $C_{134}H_{133}N_6$ $[M+H]^+$ 1826.0586 found 1826.0592.

Preparation of AIEE dye based BSA Nps. BSA (0.1300 g) was dissolved in deionized water (50 mL) and a solution of THF (80 mL) containing 0.0013 g CzAn/BCzAn was added dropwise under sonication. Then 100 μ L glutaraldehyde solution (25 %) was added and the mixture was stirred for 4 h at room temperature. The THF was removed by rotary evaporation and stored at 5 °C for later use.

Cell culture and cell imaging. HeLa cells (human cancer cell lines) were grown in Dulbecco minimum essential media (DMEM) with 10% fetal bovine serum (FBS), 1% penicillin, and 1% amphotericin B. Before the treatment, the cell were plated in 24-well plates and incubated for 24 h. Then BSA nanoparticles were added at a concentration equal to 1.4 μ g \cdot L $^{-1}$ of pure dyes and incubated for 4 hours at 37 °C with 5% CO_2 . Then the cells were washed twice with PBS and imaged with a Fluorescence Microscopy.

Cytotoxicity of AIEE dye based BSA Nps. The cytotoxicity of the BSA Nps against HeLa cells was assessed by MTT assay. HeLa cells were seeded in 96-well microplates at a density of 10^5 cells mL^{-1} and cultured at 37 °C with 5% CO_2 for 24 h. BSA Nps were added into 96-well plate. The concentrations of BSA Nps were 0.7, 1.4, 2.8 μ g \cdot L $^{-1}$. After 24 h and 48 h, 10 μ L of MTT solution (5 mg \cdot mL $^{-1}$ in PBS) was added into each sample well. After 4 h, DMSO (100 μ L) was then added and the plate was lightly shaken for 10 min. The absorbance was measured at 570 nm. Every experiment was performed three times.

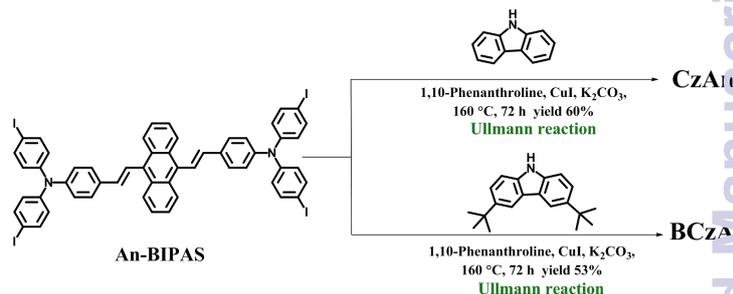
Results and discussion

Design and synthesis of the diarylanthracene conjugated dyes CzAn and BCzAn

The Synthesis routes of the targeted diarylanthracene conjugated dyes CzAn and BCzAn were shown in Scheme 2. The original compound An-BIPAS was synthesized according

to the literature reported by our group. Final compounds CzAn and BCzAn were synthesized by Ullmann reaction with connecting carbazole and 3,6-di-tert-butyl-9H-carbazole to An-BIPAS at the sites of iodine atoms in 60% and 53% yield.

The final compounds were confirmed by magnetic resonance (1H NMR and ^{13}C NMR) and high resolution mass spectrometry (HRMS-MALDI-TOF). The data were found to be in good agreement with the proposed structures.



Scheme 2 Synthesis of target diarylanthracene conjugated compounds CzAn and BCzAn.

Fluorescence of the diarylanthracene conjugated dyes in solid states

The normalized fluorescence spectra of the two diarylanthracene conjugated dyes in solid states was showed in Fig. 1. The maximum emission wavelengths of them were 576 nm and 601 nm, respectively. Obvious bathochromic shift was observed when tert-butyl was added to the carbazole. As shown in the insets of Fig. 1, under illumination with a 365 nm UV lamp, CzAn and BCzAn in solid states showed bright emission from yellow to orange. As shown in Fig. 2, the CIE chromaticity coordinate values of CzAn and BCzAn were calculated to be (0.47, 0.46) and (0.58, 0.43).

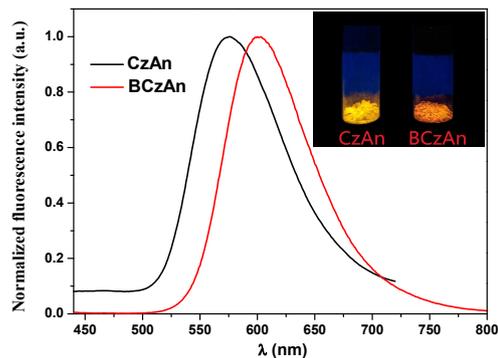


Fig. 1 The normalized fluorescence spectra of CzAn and BCzAn in solid state excited at 400 nm; the insets are photographs of CzAn and BCzAn in solid state recorded under 365 nm UV irradiation.

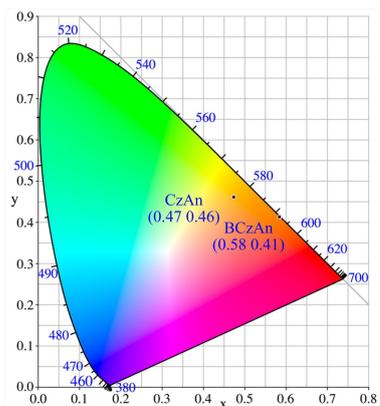


Fig. 2 The CIE chromaticity coordinate of CzAn and BCzAn.

Aggregation-induced emission enhancement of the diarylanthracene conjugated dyes in mixed solvents

CzAn and BCzAn are soluble in common organic solvents, such as THF, DMF, dichloromethane and chloroform. However, water is a poor solvent. Considering that the good and poor solvents should be miscible and the potential biological application will be subsequently investigated, we used anhydrous THF as the good solvent and water as the poor solvent to investigate the aggregation optical properties of compounds. Adding water to a THF solution of the two dyes will cause the well-dissolved molecules to aggregate. The optical photographs of BCzAn in THF-water mixtures with same concentration under illumination with a 365 nm UV lamp were showed in Fig. 3. The solution exhibited very yellow emission and an obvious enhancement of fluorescence intensity was observed when water fraction increased. This phenomenon was consistent with that described by the aggregation-induced emission enhancement (AIEE) concept.

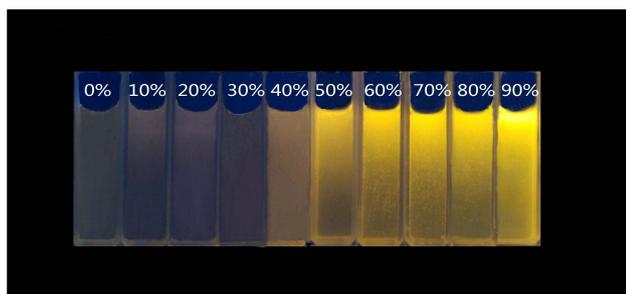


Fig. 3 Optical photographs recorded under 365 nm UV irradiation for and BCzAn in THF-H₂O mixtures with various volume fractions of water.

In order to further determine the AIEE properties of the diarylanthracene conjugated dyes, fluorescence spectra of them in THF with different water fractions were measured. Fluorescence spectra of BCzAn in THF-water mixtures was shown in Fig. 4a (Fluorescence spectra of CzAn in THF-water mixtures was provided in ESI, Fig. S8). The excitation wavelength was kept at 425 nm and the concentration was kept at 1×10^{-5} mol·L⁻¹. In pure THF, BCzAn exhibited very low fluorescence and fluorescence quantum yield was only 0.08.

When the volume fraction of water (f_w) was < 40%, the fluorescence intensity of BCzAn increased slowly. With further addition of water ($f_w > 40%$), the fluorescence intensity increased dramatically. When $f_w = 50%$, the fluorescence quantum yield (Φ_f) was up to 0.16 (Φ_f was measured by using quinine in 0.5 N H₂SO₄ as a standard²⁴). When $f_w = 90%$, the fluorescence intensity reached the maximum and the peak value was found to get 25-fold of that in pure THF. The Φ_f of BCzAn also reached the maximum value of 0.41 (Fig.4b). Adding water to a THF solution will cause the well-dissolved molecules to aggregate. The results showed that the compound molecules might started to aggregate when the volume fraction of water reached 40%. The maximum emission wavelength of the compound was at 560 nm approximately and changed weakly in different water-THF mixtures.

Therefore, we can generally rationalize that the diarylanthracene conjugated dyes were characterized as AIEE-active.

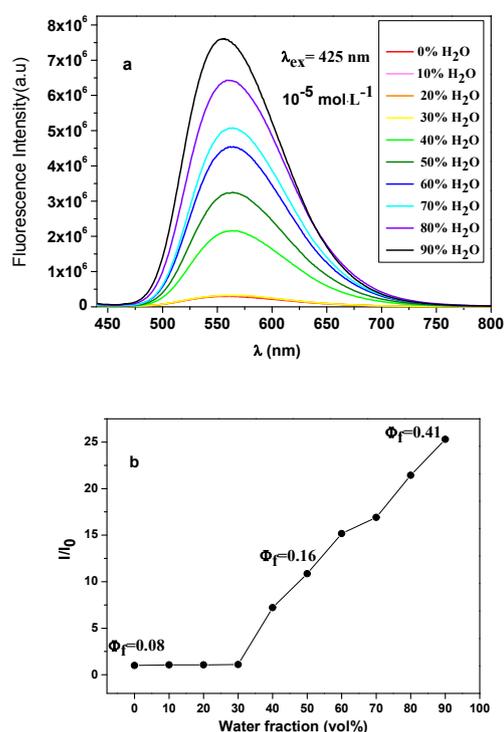


Fig. 4 Fluorescence spectra of BCzAn (10^{-5} mol·L⁻¹) in THF with different water fractions excited at 425nm (a); fluorescence peak intensities of BCzAn in THF (10^{-5} mol·L⁻¹) with different water fractions (I/I_0 , I_0 = fluorescence intensity in pure THF solution, $\lambda_{ex}=425$ nm, Φ_f represents fluorescence quantum yield) (b).

To get insight into the possible mechanism of AIEE, DMF-glycerol mixtures were selected. Glycerol is a common viscous solvent. Blending glycerol with DMF at different ratios can afford solvent mixtures with different viscosities²⁵. In pure DMF, the rotation of peripheral carbazolyl through C-N single bond might consume the excited state energy through the nonradiative process and the chromophores emitted weakly (Fig. 5). Fluorescence spectra of BCzAn in DMF-glycerol

mixtures was shown in Fig. 6a (Fluorescence spectra of CzAn in DMF-glycerol mixtures was provided in ESI, Fig. S11). The excitation wavelength was kept at 425 nm and the concentration was kept at $1 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$. The fluorescence intensity of BCzAn was increased by adding glycerol into its DMF solutions. When the volume fraction of glycerol (f_g) was $< 30\%$, the intensity increases were due to a pure viscosity effect. When f_g reached over 30% , the increases were due to the effects of both viscosity and aggregation. In mixtures with high content of glycerol ($f_g=60\%$), the fluorescence intensity reached the maximum and peak value was found to get 17-fold of that in pure DMF (Fig. 6b). The increasing solvent viscosity of the mixed solvents can slow down intramolecular rotations, which blocks the nonradiative decay path and activates the radiative decay, hence changing the chromophores highly emissive. The viscosity effects can give us the general conclusion that the formation of aggregates restricts the intramolecular rotations (RIR) might be the cause of enhancement of fluorescence intensity.

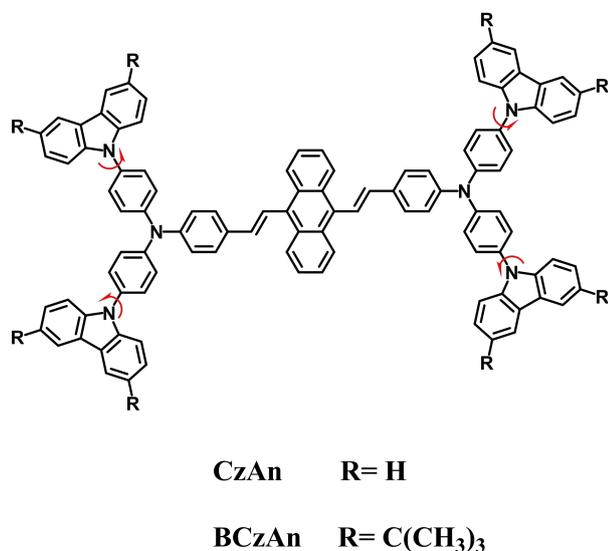


Fig. 5 The rotation of peripheral carbazolyl through C-N single bond.

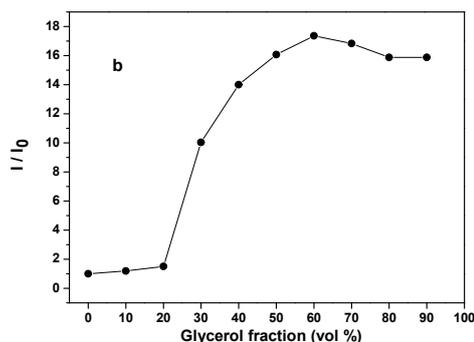
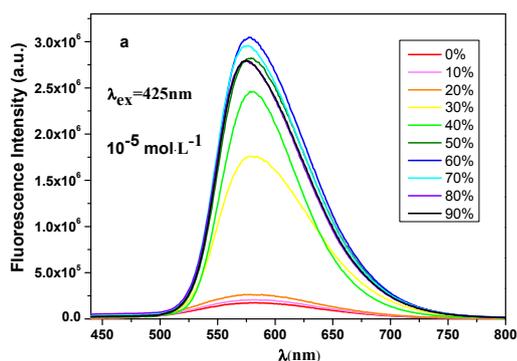


Fig. 6 Fluorescence spectra of BCzAn at $10^{-5} \text{ mol} \cdot \text{L}^{-1}$ in DMF with different glycerol fractions ($\lambda_{\text{ex}}=425 \text{ nm}$) (a); fluorescence peak intensities of BCzAn in DMF ($10^{-5} \text{ mol} \cdot \text{L}^{-1}$) with different glycerol fractions (I/I_0 , I_0 = fluorescence intensity in pure DMF solution, $\lambda_{\text{ex}}=425 \text{ nm}$) (b).

The detailed photophysical properties of CzAn and BCzAn in solid states and in different mixed solvents were summarized in Table 1.

Table 1 Photophysical properties of CzAn and BCzAn in solid state, in pure THF, in mixed THF and water, in pure DMF, in mixed DMF and glycerol.

	Solid	THF		$f_w=90\%$ THF-water		DMF	$f_g=60\%$ DMF-glycerol
	λ_{em} nm	λ_{abs} nm	λ_{em} nm	λ_{abs} nm	λ_{em} nm	λ_{em} nm	λ_{em} nm
CzAn	576	424	540 / Φ_f	430 / Φ_f	541 / Φ_f	571	577
BCzAn	601	420	564 / Φ_f	430 / Φ_f	559 / Φ_f	583	577

λ_{abs} UV-vis maximum absorption wavelength; λ_{em} maximum emission wavelength; f_w water fraction of THF-water mixtures; f_g glycerol fraction of DMF-glycerol mixtures; Φ_f fluorescence quantum yield.

BSA nanoparticles based on AIEE dyes CzAn, BCzAn and cell imaging

From above discussions, it can be concluded that the diarylanthracene conjugated dyes CzAn and BCzAn have excellent AIEE properties. However, their biological applications may be limited because they have poor solubility in water. Nevertheless, bovine serum albumin (BSA) is a commercially available hydrophilic polymer with great biocompatibility. Therefore we used BSA as matrix to prepare AIEE dye based nanoparticles (NPs), CzAn-BSA NPs and BCzAn-BSA NPs, and explore their biological applications.

The procedure for preparation of the AIEE dye based BSA Nps is illustrated in Fig. 7. With the THF solution of CzAn/BCzAn gradually added into aqueous solution of BSA, the CzAn/BCzAn molecules gradually aggregate and entangle

with the BSA chains. The AIEE dye based BSA Nps form instantly upon sonication. The BSA matrix was knitted together when glutaraldehyde (GA), a common cross-linker, was added. After the reaction finished, the THF is removed and the mixture returned to aqueous system²⁶.

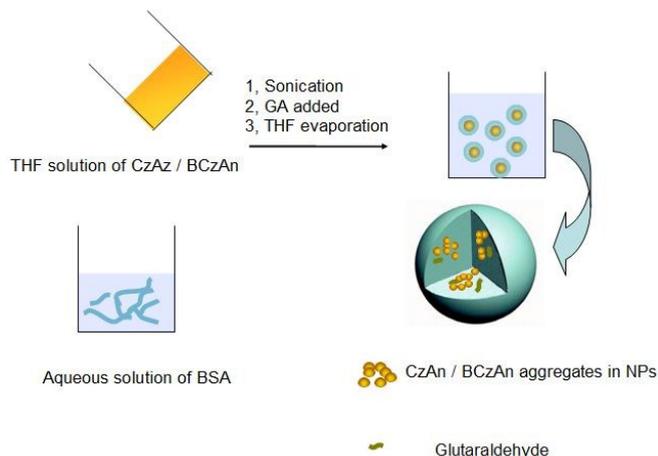


Fig 7 The preparation process and model structure of the AIEE dye based BSA nanoparticles.

The UV-vis absorption and fluorescence spectra of AIEE dye based BSA Nps in aqueous solution were investigated to assess their photophysical properties. As seen in Fig. 8, the maximum absorption of CzAn-BSA and BCzAn-BSA Nps in aqueous solution were located at 425, 427 nm and maximum emission wavelength of them were located at 545, 557 nm, which were similar with the maximum absorption and emission wavelength of pure AIEE dyes in THF-water mixtures with high water content. Meanwhile, the quantum yield of BSA NPs is 0.18 and 0.26, respectively. So it can be concluded that the BSA NPs inherit the excellent photophysical properties of AIEE dyes, CzAn and BCzAn.

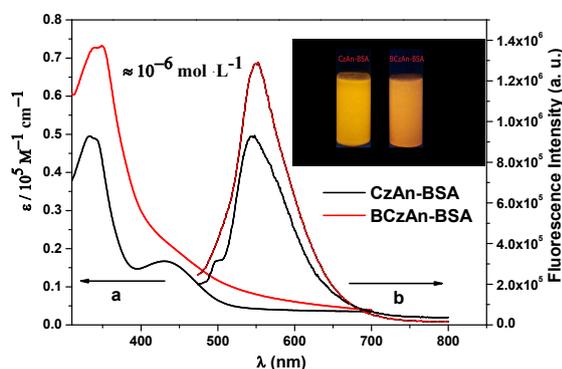


Fig. 8 UV-vis absorbance spectra (a) and fluorescence spectra (b) of AIEE dye based BSA Nps in aqueous solution; the inserts are optical photographs of Nps in aqueous solution recorded under 365 nm UV irradiation.

Transmission electron microscopy (TEM) images of the AIEE dye based BSA Nps are shown in Fig. 9. The images indicate that these NPs had a spherical shape and a smooth surface with an almost uniform size of around 150 nm.

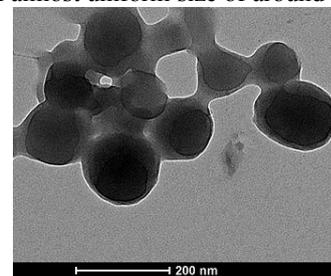


Fig. 9 TEM images of AIEE dye based BSA Nps.

The biocompatibilities of BSA Nps with HeLa cells were also investigated using MTT assay. Fig. 10 shows the Cell viability of HeLa cells after incubation with BCzAn-BSA Nps at concentrations of 0.7, 1.4, 2.8 μg·L⁻¹ for 24 and 48 h, respectively. The results show that no obvious cell viability decrease was found and further confirm that such BSA Nps are biocompatible with cells and promising for biomedical applications.

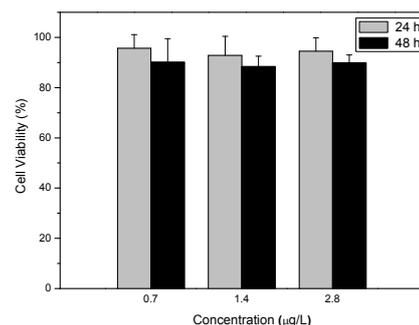


Fig. 10 Cell viability of HeLa cells after incubation with BCzAn-BSA Nps at different concentrations for 24 and 48 h.

To exploit the potential biological applications of AIEE dye based BSA Nps, the cell imaging of AIEE dye based BSA Nps using upright fluorescence microscope was examined. Fluorescence imaging picture of HeLa cells after incubation with BAnCz-BSA Nps was shown in Fig. 11 as an example, bright green fluorescence were observed when cells were incubated with 1.4 μg·L⁻¹ of nanoparticles for 4 h. The fluorescence covered the cells very well and BSA Nps showed an enhanced intracellular uptake and a high fluorescence contrast. These data indicate the good photostability of the AIEE dye based BSA Nps in a biological environment, which meets the requirements for bioimaging applications.

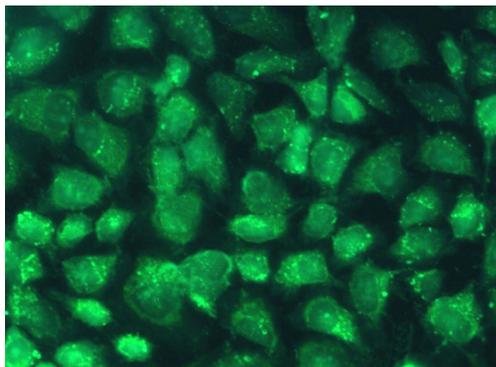


Fig. 11 Fluorescence imaging picture of HeLa cells after incubation with BAnCz-BSA Nps.

Conclusions

In the present work, two novel diarylanthracene conjugated dyes containing carbazolyl substituted triarylamine groups, CzAn and BCzAn, were synthesized and demonstrated aggregation-induced emission enhancement (AIEE) properties. CzAn and BCzAn emitted brightly in solid state with maximum emission wavelength at 576 and 601 nm. BAnCz emitted weakly in THF solutions with low fluorescence quantum yield of 0.08, became strong emitters in the aggregated states. The fluorescence intensity and fluorescence quantum yield increased dramatically in mixtures with high content of water compared in with that in pure THF. The viscosity effects showed that the formation of aggregates restricts the intramolecular rotations (RIR) might be the cause of enhancement of fluorescence intensity. Moreover, AIEE dye based BSA nanoparticles were prepared to explore the potential biological applications. These nanoparticles were demonstrated stable uniform morphology, strong fluorescence, low cytotoxicity properties and were successfully used in HeLa cells imaging. Therefore, we believe that these BSA nanoparticles based on AIEE-active diarylanthracene conjugated dyes CzAn, BCzAn are excellent fluorescence probes and will have a high potential for biological applications.

Acknowledgements

We thank the financial support from the National Natural Science Foundation of China (Grant numbers: 61178057). We thank Prof. S. Q. Liu, Dr H. N. Li for their kind help with the cells culture and the MTT assay.

Notes and references

*School of Chemistry and Chemical Engineering, Southeast University, Nanjing, 211189, China. E-mail: yingqian@seu.edu.cn.

1 Y. N. Hong, J.W. Y. Lam and B. Z. Tang, *Chem. Commun.*, 2009, 4332.
 2 J. D. Luo, Z. L. Xie, J. W. Y. Lam, L. Cheng, H. Y. Chen, C. F. Qiu, H. S. Kwok, X. W. Zhan, Y. Q. Liu, D. B. Zhu and B. Z. Tang, *Chem. Commun.*, 2001, 1740.

- 3 (a) B. K. An, S. K. Kwon, S. D. Jung, S. Y. Park, *J. Am. Chem. Soc.*, 2002, **124**, 14410; (b) H. Li, Z. Chi, B. Xu, X. Zhang, X. Li, S. Liu, Y. Zhang and J. Xu, *J. Mater. Chem.*, 2011, **21**, 3760; (c) Y. Zhang, J. Sun, G. Bian, Y. Chen, M. Ouyang, B. Hu and C. Zhang, *Photochem. Photobiol. Sci.*, 2012, **11**, 1414; (d) L. Yang, J. Ye, L. Xu, X. Yang, W. Gong, Y. Lin and G. Ning, *RSC Adv.*, 2012, **2**, 11529; (e) M. Baglan, S. Ozturk, B. Guer, K. Meral, U. Bozkaya, O.A. Bozdemir and S. Atilgan, *RSC Advances*, 2013, **3**, 15866; (f) Y. Wang, G. Lai, Z. Li, Y. Ma, Y. Shen and C. Wang, *Tetrahedron.*, 2015, **71**, 2761; (g) S. Sharma, C.P. Pradeep and A. Dhir, *New J. Chem.*, 2015, **39**, 1822.
- 4 Y. Hong, J.W.Y. Lam and B.Z. Tang, *Chem. Soc. Rev.*, 2011, **40**, 5361.
- 5 (a) S. Kamino, Y. Horio, S. Komeda, K. Minoura, H. Ichikawa, J. Horigome, A. Tatsumi, S. Kaji, T. Yamaguchi, Y. Usami, S. Hirota, S. Enomoto and Y. Fujita, *Chem. Commun.*, 2010, **46**, 9013; (b) X. Feng, B. Tong, J. Shen, J. Shi, T. Han, L. Chen, J. Zhi, P. Lu, Y. Ma and Y. Dong, *J. Phys. Chem. B*, 2010, **114**, 16731; (c) S. Kaur, A. Gupta, V. Bhalla and M. Kumar, *J. Mater. Chem. C.*, 2014, **2**, 7356.
- 6 B. Xu, J. Zhang, H. Fang, S. Ma, Q. Chen, H. Sun, C. Im and W. Tian, *Polym. Chem.*, 2014, **5**, 479.
- 7 (a) H. Li, X. Zhang, Z. Chi, B. Xu, W. Zhou, S. Liu, Y. Zhang and J. Xu, *Org. Lett.*, 2011, **13**, 556; (b) X. Zhang, Z. Chi, J. Zhang, H. Li, B. Xu, X. Li, S. Liu, Y. Zhang and J. Xu, *J. Phys. Chem. B.*, 2011, **115**, 7606 (c) X. Zhang, Z. Chi, X. Zhou, S. Liu, Y. Zhang and J. Xu, *J. Phys. Chem. C*, 2012, **116**, 23629; (d) Y. Dong, B. Xu, J. Zhang, X. Tan, L. Wang, J. Chen, H. Lv, S. Wen, B. Li, L. Ye, B. Zou and W. Tian, *Angew. Chem., Int. Ed.*, 2012, **51**, 10782.
- 8 (a) Y. C. Chang, S. C. Yeh, Y. H. Chen, C. T. Chen, R. H. Lee and R. J. Jeng, *Dyes. Pigm.*, 2013, **99**, 577; (b) C. Chitpakdee, S. Namuangruk, P. Khongpracha, S. Jungsuttiwong, R. Tarsang, T. Sudyoasuk and V. Promarak, *Spectrochim. Acta, Part A*, 2014, **125**, 36.
- 9 C. Guan and Y. Qian, *Chin. J. Org. Chem.*, 2014, **34**, 537.
- 10 (a) X. Zhang, X. Zhang, S. Wang, M. Liu, L. Tao and Y. Wei, *Nanoscale*, 2013, **5**, 147; (b) H. Lu, X. Zhao, W. Tian, Q. Wang and J. Shi, *RSC Adv.*, 2014, **4**, 18460
- 11 Y. Yu, C. Feng, Y. Hong, J. Liu, S. Chen, K. M. Ng, K. Q. Luo and B. Z. Tang, *Adv. Mater.*, 2011, **23**, 3298.
- 12 Y. Jin and Y. Qian, *New J. Chem.*, 2015, **39**, 2872.
- 13 (a) D. Ding, K. Li, B. Liu and B. Z. Tang, *Acc. Chem. Res.*, 2013, **46**, 2441; (b) X. Zhang, X. Zhang, L. Tao, Z. Chi, J. Xu and Y. Wei, *J. Mater. Chem. B*, 2014, **2**, 4398; (c) K. Li and B. Liu, *Chem. Soc. Rev.*, 2014, **43**, 6570; (d) X. Zhang, K. Wang, M. Liu, X. Zhang, L. Tao, Y. Chen and Y. Wei, *Nanoscale*, 2015, **7**, 11486.
- 14 (a) Z. Wang, S. Chen, J. W. Y. Lam, W. Qin, R. T. K. Kwok, N. Xie, Q. Hu and B. Z. Tang, *J. Am. Chem. Soc.*, 2013, **135**, 8238; (b) X. Zhang, X. Zhang, B. Yang, M. Liu, W. Liu, Y. Chen and Y. Wei, *Polym. Chem.*, 2013, **4**, 4317.
- 15 J. Geng, K. Li, W. Qin, L. Ma, G. G. Gurzadyan, B. Z. Tang and B. Liu, *Small*, 2013, **9**, 2012.
- 16 (a) K. Li, Y. Jiang, D. Ding, X. Zhang, Y. Liu, J. Hua, S. S. Feng and B. Liu, *Chem. Commun.*, 2011, **47**, 7323; (b) K. Li, W. Qin, D. Ding, N. Tomczak, J. Geng, R. Liu, J. Liu, X. Zhang, H. Liu, B. Liu and B. Z. Tang, *Sci. Rep.*, 2013, **3**, 1150; (c) W. Qin, K. Li, G. Feng, M. Li, Z. Yang, B. Liu and B. Z. Tang, *Adv. Funct. Mater.*, 2014, **24**, 635; (d) D. Ding, C. C. Goh, G. Feng, Z. Zhao, J. Liu, R. Liu, N. Tomczak, J. Geng, B. Z. Tang, L. G. Ng and B. Liu, *Adv. Mater.*, 2013, **25**, 6083.

- 17 (a) X. Zhang, X. Zhang, B. Yang, Y. Zhang and Y. Wei, *ACS Appl. Mater. Interfaces.*, 2014, **6**, 3600; (b) M. Liu, X. Zhang, B. Yang, F. Deng, Z. Huang, Y. Yang, Z. Li, X. Zhang and Y. Wei, *RSC Adv.*, 2014, **4**, 35137; (c) J. Geng, Z. Zhu, W. Qin, L. Ma, Y. Hu, G.G. Gurzadyan, B.Z. Tang and B. Liu, *Nanoscale*, 2014, **6**, 939.
- 18 Y. Yuan, G. Feng, W. Qin, B.Z. Tang and B. Liu, *Chem. Commun.*, 2014, **50**, 8757.
- 19 H. Lu, X. Zhao, W. Tian, Q. Wang and J. Shi, *RSC Adv.*, 2014, **4**, 18460.
- 20 X. Zhang, X. Zhang, S. Wang, M. Liu, L. Tao and Y. Wei, *Nanoscale*, 2013, **5**, 147.
- 21 M. Chen, M. Yin, *Prog. Polym. Sci.*, 2014, **39**, 365.
- 22 B. Wang and Y. Qian, *New J. Chem.*, 2013, **37**, 1402.
- 23 J. Li, T. Zhang, Y. Liang and R. Yang, *Adv. Funct. Mater.*, 2013, **23**, 619.
- 24 A. M. Brouwe, *Pure Appl. Chem.*, 2011, **83**, 2213.
- 25 Q. Zeng, Z. Li, Y. Dong, C.a. Di, A. Qin, Y. Hong, L. Ji, Z. Zhu, C. K. W. Jim, G. Yu, Q. Li, Z. Li, Y. Liu, J. Qin and B. Z. Tang, *Chem. Commun.*, 2007, 70.
- 26 W. Qin, D. Ding, J. Liu, W.Z. Yuan, Y. Hu, B. Liu and B.Z. Tang, *Adv. Funct. Mater.*, 2012, **22**, 771.

Diarylanthracene conjugated dyes with good aggregation-induced emission enhancement (AIEE) properties were synthesized and their BSA nanoparticles were successfully used in HeLa cells imaging.

