One-step preparation of AIE-active dextran via formation of phenyl borate and their bioimaging application

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Abstract

The fabrication of luminescent polymeric nanoparticles with aggregation induced emission (AIE) properties has been extensively investigated in recent years for their distinct optical properties, biocompatibility and designability. Although a number of AIE-active nanoparticles have been fabricated previously, the AIE-active nanoprobe based on carbohydrate polymers have received only limited attention. In this work, we reported for the first time that AIE-active organic nanoparticles could be prepared via a facile one-step strategy. To fabricate these AIE-active nanoparticles, the AIE dye 9, 10-bis (divinylphenylboronic acid) anthracene (An-B(OH)₂) terminated with phenylboronic acid was synthesized using anthracene as core chemical structure. After mixing with the An-B(OH)₂ and dextran, the resultant products (An-Dex) can be obtained through formation of phenyl borate in aqueous solution, air atmosphere and under room temperature. The intermediates and An-Dex were characterized by a series of characterization techniques including ¹H nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy, transmission electron microscopy and fluorescence spectroscopy. To further explore the biomedical applications of these glycosylated AIE-active nanoparticles, cell viability as well as cell imaging performance of An-Dex nanoparticles was also examined. We demonstrated that An-Dex nanoparticles showed strong fluorescent intensity, great water dispersibility and excellent biocompatibility. More importantly, it is well known that phenyl borate is a dynamic bond, which can potentially respond to the pH and glucose. Therefore, the resultant AIE-active probes should be promising candidates for different biomedical applications such as biological imaging and controlled drug delivery.

Key words: carbohydrate polymers, aggregation induced emission, luminescent organic nanoparticles, responsive phenyl borate, biological imaging

1. Introduction

The development of luminescent nanoparticles for biomedical applications has attracted considerable research interest in the past few decades.[1, 2] These luminescent nanoparticles can be used for fabrication of multifunctional nanocomposites, which are potential applicable for biosensors, disease diagnostic and imaging guide treatment and nanotheranostics.[3-11] Among all of these existed luminescent nanoparticles, fluorescent organic nanoparticles (FNPs) based on dyes with aggregation induced emission (AIE) properties should be the most promising ones because of their outstanding optical properties.[12, 13] As compared with the fluorescent inorganic nanoparticles and FNPs based on conventional organic dyes, the FNPs based on AIE-active dyes possess desirable biocompatibility and biodegradability, well designability of chemical structure and optical properties and more importantly the obviously enhanced fluorescent intensity in their aggregated state.[14-18] The unique optical properties of AIE dyes make them very suitable for fabrication of luminescent nanoparticles for biomedical applications.[6, 19-21] Since the first report of AIE properties of silole by Tang et al in 2001, the AIE phenomenon has attracted more and more research attention and a large number of materials based on the AIE dyes have been prepared and explored for diverse applications.[22-29] Especially, the AIE-active polymeric nanoparticles from self assembly of amphiphilic AIE dyes contained copolymers have demonstrated to be more suitable candidates for biological imaging applications because their AIE properties can elegantly and effectively overcome the aggregation caused quenching effect (ACQ) of conventional organic dyes.[30] In recent years, a large number of AIE dyes with different core chemical structure and functional groups have been synthesized.[31] These AIE dyes can be utilized for fabrication of AIE-active polymeric nanoparticles through different strategies such as non-covalent self assembly, polymerization, formation of dynamic Schiff base, ring-opening reaction and supermolecular interaction.[32-39] Although great progress has been made in the fabrication and biomedical applications of AIE-active polymeric nanoparticles, the synthesis of amphiphilic AIE-active carbohydrate polymers has seldom been reported.[14, 40, 41]

Carbohydrate polymers are an important class of natural polymers that can be obtained from the animals, plants and microorganisms etc. The carbohydrate polymers are abundant in nature, renewable and can be currently or potentially applied in areas such as biomaterials, nanotechnology, drug delivery, food, textiles, tissue engineering and environmental remediation etc.[42-49] Among these

carbohydrate polymers, dextran is one of the dominated carbohydrate polymers, which have been extensively for biomedical applications due to its high water solubility, biocompatibility, biodegradability and low cost.[50, 51] However, to the best of our knowledge, only very few reports have demonstrated the preparation of AIE-active carbohydrate polymers through covalent linkage.[14, 40] And only very limited reports have reported the fabrication of AIE-active carbohydrate polymers through dynamic bonds or with responsive properties. It is well known that the diol group of carbohydrate polymers can form phenyl borate with phenylboronic acid (PBA), which can response to the stimuli such as pH and glucose. Therefore, the AIE-active carbohydrate polymers with responsive properties can thus be fabricated via formation of phenyl borate.[52]

In this contribution, a rather facile strategy was developed for formation of AIE-active dextran through mixing the AIE dye 9, 10-bis (divinylphenylboronic acid) anthracene (An-B(OH)₂) and dextran under rather mild experimental conditions. Detailed information for synthesis of phenylboronic acid-terminated AIE dye and preparation of AIE-active dextran was shown in **Scheme 1**. The resultant products (An-Dex) are tended to self assemble into AIE-active polymeric nanoparticles due to their amphiphilic properties. The resultant AIE-active polymeric nanoparticles were investigated by means of a number of characterization techniques. The biocompatibility as well as cell uptake behavior of An-Dex was further examined to evaluate their biological imaging potential. Considered the responsive properties, the resultant products An-Dex should also be utilized for controlled drug delivery applications.

Scheme 1 Schematic showing the preparation of An-Dex FNPs which relied on the formation of specific interaction between phenylboronic acid and dextran. (Tp: triethyl phosphate, 4-FPBa: 4-Formylphenylboronic acid)

2. Experiment

2.1 Materials and characterization

All chemical agents were of analytical grade and were used as received without any further purification. All aqueous solutions were prepared with doubly distilled water. All the chemicals such as 4-Formylphenylboronic acid (M_w : 149.94 Da, 98%), Anthracene (M_w : 178.3 Da, 98%), Trethyl phosphite (M_w : 166.16 Da, 98%), Dextran (M_w : 10000 Da, 98%) and Aluminum chloride hexahydrate (M_w : 241.43 Da, 97%) were purchased from Sinopharm Chemical reagents Co., Ltd (Shanghai, China). The synthetic polymers and materials were characterized by Fourier transform infrared spectroscopy (FT-IR) using KBr pellets. The molecular weight of An-B(OH)₂ and An-Dex was determined by mass spectroscopy. Triple TOF5600+ was operated in positive mode with an ion spray voltage at 2.3 kV. The Fourier transform infrared (FT-IR) spectra were supplied from Nicolet5700 (Thermo Nicolet corporation). ¹H nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance-400 spectrometer with D₂O and CDCl₃ as the solvents. Transmission electron microscopy (TEM) images were recorded on a Hitachi 7650B microscope operated at 80 kV. The TEM specimens were got by putting a drop of the nanoparticle ethanol suspension on a carbon-coated copper grid. The fluorescence data were obtained from fluorescence spectrophotometer (FSP, model: C11367-11), which purchased from Hamamatsu (Japanese). The X-ray photoelectron (XPS) spectra were performed on a VGESCALAB 220-IXL spectrometer using an Al K α X-ray source (1486.6 eV). The energy scale was internally calibrated by referencing to the binding energy (Eb) of the C1s peak of a carbon contaminant at 284.6 eV.

2.2 Synthesis of 9, 10-Bis (bromomethyl) anthracene

The Anthracene (5.7 g, 32 mmol), (CH₂O)_n (3.6 g), 33% HBr in acetic acid (45 mL), and a catalytic amount of AlCl₃ were stirred for 6 h at 50 °C. The resulting solid was washed with water and dried in vacuum one night. The residue product was recrystallized in toluene three times to obtained flaxen solid. Yield 6.7 g (64%). ¹H NMR (300 MHz, CDCl₃): δ 8.38 (m, 4H), δ 7.65 (m, 4H), δ 5.54 ppm (m, 4H).

2.3 Synthesis of 9, 10-bis (diethylphosphorylmethyl) anthracene

9, 10-Bis (bromomethyl) anthracene (1.2 g, 3.3 mmol) was added to triethyl

phosphate (10 mL), and the resulting solution was refluxed for 10 h. The solvent was removed in vacuum and the residue products were purified by a column chromatography on silica gel using ethyl acetate and CH₂Cl₂ as the eluent. Yield: 0.9 g (65%). ¹H NMR (300 MHz, CDCl₃): δ 8.38 (m, 4H), δ 7.6 (m, 4H), 4.25 (d, 4H), 3.8 (m, 8H), 1.06 ppm (t, 12H).

2.4 Synthesis of An-B(OH)₂

9, 10-bis (diethylphosphorylmethyl) anthracene (1.37 g, 3 mmol), 4-Formylphenylboronic acid (1.125 g, 7.5 mmol) and t-BuOK (896 mg, 8 mmol) in dry THF solution were stirred at 40 °C for 5 h. The THF solution was removed by rotary evaporation and the crude product was separated by column chromatography on silica gel using ethyl acetate /CH₂Cl₂ (1:3) as the eluent. Yield: 1.11g (48%). ¹H NMR (300 MHz, CDCl₃): δ 8.12 (m, 4H), δ 7.65 (m, 4H), δ 7.56 ppm (m, 4H), δ 6.92 ppm (s, 4H), δ 2.98 ppm (m, 4H).

2.5 The preparation of An-Dex

The glycosylated fluorescent nanoparticles with unique AIE feature were prepared via utilizing glucose-responsive property. 50 mg 9, 10-bis (divinylphenylboronic acid) anthracene (An-B(OH)₂) was dissolved in THF solution. Then adjust pH of resulting solution to 10 by adding potassium carbonate solution and stir for 10 min. Furthermore, Dextran (200 mg) in aqueous solution was charged via a gastight syringe and stir at room temperature for 30 min. Finally the An-Dex would be obtained by rotary evaporation.

2.6 Cell viability evaluation of An-Dex

HepG2 Cells were seeded in 96-well microplates at a density of 5×10^4 cells per mL in 160 μL of the respective media containing 10% fetal bovine serum (FBS). After 24 h of cell attachment, HepG2 cells were incubated with 10, 20, 40, 80 and 120 μg mL⁻¹ of An-Dex FNPs for 8 and 24 h. Then the cells were washed with PBS for three times to remove the uninternalized nanoparticles. After that, 10 μL of CCK-8 dye and 100 μL of Dulbecco's Modified Eagle's Medium (DMEM) cell culture media was added to each well and incubated for 2 h at 37 °C. Plates were then analyzed with a microplate reader (Victor III, Perkin-Elmer). Measurements of dye absorbance were carried out at 450 nm, with the reference wavelength at 620 nm. The values were proportional to the number of live cells. The percent reduction of WST was compared to the control (cells not exposed to nanoparticles), which represented 100% WST

reduction. Three replicate wells were used for each control and test concentrations per microplate, and the experiment was repeated three times. Cell survival was expressed as absorbance relative to that of untreated controls. Results are presented as mean $\pm$ standard deviation (SD).

2.7 Cell imaging application of An-Dex

HepG2 cells were facilely bred in DMEM, which was supplied with 10% heat-inactivated FBS, 2 mM glutamine, 100 U mL⁻¹ penicillin, and 100 μ g mL⁻¹ of streptomycin. Before proceeding experiment of cell imaging, the cell culture should be controlled at 37 °C in a similar incubation environment of 95% air and 5% CO₂ in culture medium. In order to maintain the exponential growth of the cells, the culture medium should be updated every three days. Before treatment, cells were seeded in a glass bottom dish with a density of 1×10^5 cells per dish. On the day of treatment, the cells were incubated with An-Dex FNPs at a final concentration of 20 μ g mL⁻¹ for 3 h at 37 °C. Afterward, the cells were washed three times with PBS to remove the An-Dex FNPs and then fixed with 4% paraformaldehyde for 10 min at room temperature. Cell images were obtained using a confocal laser scanning microscope (CLSM) Zesis 710 3-channel (Zesis, Germany) with the excitation wavelength of 458 nm.

3. Result and discussion

The successful formation of An-Dex FNPs with AIE-active dyes was confirmed by ¹H NMR and ¹³C NMR measurement. As shown in **Fig. 1**, the chemical shifts of hydroxyl group in An-B(OH)₂ were around 2.8 ppm, which could confirm that the AIE dye (An-B(OH)₂) has been prepared successfully. Compared dextran with An-Dex, it is significant that a series of new peaks appeared around 6.5-8.2 ppm, which come from the aromatic protons of An-B(OH)₂. Surprisingly, no signals of dextran were detected in the sample of An-Dex, which is mainly ascribed to the poor dispersibility of dextran in the CDCl₃. The successful synthesis of An-B(OH)₂ was also confirmed by ¹³C NMR spectrum (**Fig. S3**). However, we have not obtained valid information for the formation of An-Dex based on ¹³C NMR spectrum. It is possible due to the poor solubility of An-Dex in CDCl₃. Furthermore, the MS determination showed that the *Mw* of An-B(OH)₂ is 515.18 Da, which is well consistent with the

theoretical molecular weight based on the formula. In a word, based on the above results, we can conclude that successful preparation of An-Dex via one-step strategy for formation of phenyl borate.

Fig. 1 The ^1H NMR spectrum of the An-B(OH)_2 , An-Dex, dextran. The new chemical shift at 2.8 ppm could be ascribed to the $-\text{OH}$, suggesting that An-B(OH)_2 has been prepared. Furthermore, the chemical shift appeared between 7.5-8.2 ppm, demonstrating that successful reaction between An-B(OH)_2 and dextran. The solvents for ^1H NMR measurement are D_2O and CDCl_3 .

Due to the amphiphilic properties of An-Dex, these AIE-active copolymers are tended to self-assemble into nanomaterials in pure aqueous solution. Therefore, the size and morphology of the resultant An-Dex copolymers were characterized by TEM. As shown in **Fig. S5**, many spherical nanoparticles with diameters ranging from 200 to 300 nm could be clearly identified. The successful formation of nanoparticles implied that dextran and An-B(OH)_2 have formed the amphiphilic copolymers through simply mixing dextran and An-B(OH)_2 under room temperature and without inert gas protection. During the self assembly procedure, the hydrophobic component An-B(OH)_2 was encapsulated in the core of luminescent nanoparticles, while the hydrophilic dextran was coated on the surface of AIE dye aggregates. It could not only provide the water dispersibility of the obtained nanoparticles, but also endow the biological properties of dextran. The hydrodynamic size distribution of An-Dex in water was determined by dynamic laser scattering (DLS) analysis. Results showed that the size of An-Dex is 272.7 ± 25.8 nm with narrow polydispersity index (PDI =

0.072). It is well consistent the size from TEM characterization. The FT-IR spectra of dextran, An-Dex and An-B(OH)₂ were used to determine their chemical structural information and functional groups. As displayed in **Fig. 2**, it is obvious that the peak at 1000 cm⁻¹ could be observed in the samples of Dex and An-Dex, which were attributed to C-O bond of Dex. On the other hand, the IR absorption peak at 3500 cm⁻¹ was found in An-B(OH)₂ and Dex, which can be assigned to the stretching vibration of hydroxyl group. Furthermore, the asymmetric stretching vibration of C-H in the samples of An-B(OH)₂ and Dex can be found at 2840 and 2920 cm⁻¹. However, a series of peaks at 700 cm⁻¹ ascribed to the aromatic ring of An-B(OH)₂ were appeared in An-Dex and the peaks strength of hydroxyl group at 3500 cm⁻¹ decreased significantly in An-Dex, which could give us direct evidence that we have successfully prepared the An-Dex via formation of stimuli responsive phenyl borate.

Fig. 2 FT-IR spectra of An-B(OH)₂, dextran and An-Dex samples. It can be seen that the intensity of -OH was obviously decreased, indicated the successful formation of An-Dex.

XPS spectra were further recorded to confirm the successful synthesis of An-Dex FNPs. As shown in **Fig. 3**, it is obvious that there are the elements including B, C and O in An-Dex samples. Because the dextran is absent of element B, the emergence of B in the sample of An-Dex clearly indicated that An-B(OH)₂ has successfully incorporated into An-Dex through formation of phenyl borate. Then, the content of elements in An-Dex samples was calculated based on XPS analysis (**Table 1**). The An-Dex FNPs are composed with C atom (71.52%), O atom (26.53%) and a fraction of B atom (1.95%). As compared with the pure dextran, the percentage of O is

obviously decreased while the content of C is increased. More importantly, the percentage of B in the sample of An-Dex is also significantly lower than that of theoretical value (4.35%) of An-B(OH)₂. The above comparison evidenced that An-B(OH)₂ has incorporated into dextran through formation of dynamic bond between the phenylboronic acid and diol groups. On the other hand, we could also calculate that the molar ratio of glucose units of dextran to that of AIE dye is about 3 based on the content of C, O and B in An-Dex.

Fig. 3 XPS spectra of An-Dex FNPs: (A) C1s spectrum; (B) O1s spectrum; (C) B1s spectrum; and (D) XPS spectrum of An-Dex FNPs showing the presence of carbon, oxygen and boron.

Table 1 Element content (%) of An-Dex were based on the XPS analysis

	C	O	B
An-Dex	71.52%	26.53%	1.95%

The UV absorption spectrum of An-Dex FNPs in aqueous solution was shown in **Fig. S6**. It is obvious that an adsorption peak at 262 nm was appeared in UV absorption spectrum. The adsorption peak at 262 nm should be attributed to the $\pi \rightarrow \pi^*$ transition of C=C and aromatic rings. On the other hand, due to the light scattering of An-Dex, the UV absorbance was raised from 500-200 nm, indicating that the An-Dex have formed nanoparticles in pure aqueous solution. Furthermore, we could observe the logo “AB” as background in the inset of **Fig. S6**, which demonstrate that An-Dex FNPs possess great water dispersibility. Because An-Dex FNPs contained both hydrophobic AIE dye and hydrophilic carbohydrate polymers, the resultant

amphiphilic copolymers would self-assemble into core-shell nanomaterials. The hydrophobic AIE dye An-B(OH)₂ was embedded in the core, which was covered by the hydrophilic dextran. Therefore the final products are expected to emit bright luminescence due to the AIE properties of An-B(OH)₂ (inset of **Fig. 4**). The PL properties of An-Dex FNPs were characterized by fluorescent spectroscopy in detailed. As shown in **Fig. 4**, maximum emission wavelength of An-Dex FNPs was located at 529 nm, while their maximum excitation wavelength was located at 475 nm. The fluorescence quantum yield (QY) of An-Dex FNPs was determined using quinine sulfate as the reference dye. We demonstrated that the QY of An-Dex FNPs is as high as 28.9%. The photostability is another very important parameter for the biomedical application of An-Dex FNPs. The excellent photostability of AIE dyes based nanoprobe has demonstrated by Tang et al.[14] In their work, the AIE dye TPE was conjugated with chitosan through condensation reaction between the isothiocyanate of TPE and amino group of chitosan. As compared with the fluorescein based chitosan nanoparticles, the TPE-CS showed better photostability when these fluorescent nanomaterials were irradiated with UV lamp. The excellent photostability as well as strong luminescence intensity of AIE dyes based polymeric nanoprobe is very useful for biological imaging applications. In this work, the photostability of An-Dex was also evaluated using continuous UV lamp irradiation. As shown in **Fig. S7**, the fluorescent intensity showed only a few decrease after An-Dex water suspension was irradiated by UV lamp for 1 h. These results demonstrated that An-Dex possess good photostability. Taken together, we confirmed that the An-Dex FNPs possess good water dispersibility, high fluorescent QY, unique AIE properties and excellent photostability, implied that An-Dex FNPs are of great potential for biological imaging applications.

Fig. 4 Fluorescence excitation and emission spectra of An-Dex. The inset is the fluorescence image of An-Dex. The An-Dex water suspension was irradiated by UV

lamp with $\lambda = 365$ nm.

Cell viability is an important parameter to determine the biomedical applications potential for biomaterials. In this work, the acute cytotoxicity of An-Dex FNPs towards HepG2 cells was examined using CCK-8 assay.[53-57] As displayed in **Fig. 5**, we found that the cell viability values have not changed obviously after cells were incubated with different concentrations of An-Dex FNPs for 8 and 24 h. The cell viability values even greater than 100% and all of these values are greater than 90%. The excellent cytocompatibility of An-Dex FNPs is mainly ascribed to the good water dispersibility and biocompatibility of dextran. All of these above results suggested that An-Dex FNPs have high potential for biomedical applications.

Fig. 5 Cell viability of An-Dex FNPs. HepG2 Cells was incubated with 10-120 $\mu\text{g mL}^{-1}$ of An-Dex FNPs for 8 and 24 h.

Considered the strong fluorescence, high water dispersibility, excellent cytocompatibility as well as the responsiveness of phenyl borate, the cell uptake behavior of An-Dex FNPs was evaluated using CLSM to explore their potential biological imaging applications. As shown in **Fig. 6**, intense fluorescent signal was observed in the location of cells after they were incubated with 20 $\mu\text{g mL}^{-1}$ of An-Dex FNPs for 3 h. Meanwhile, many areas with weak fluorescence intensity were also found in the location of cells, which were surrounded by areas with strong fluorescent signal. These areas with relative weak or no fluorescence should be the location of cell nucleus. Therefore we could conclude that An-Dex FNPs can be internalized by cells and mainly located at cytoplasm. On the other hand, we could also found that the cells still adhered very well to the cell plates and kept their normal morphology (**Fig.**

6B), further implied that An-Dex FNPs possess good biocompatibility. Furthermore, the dosage used for cell imaging is only $20\ \mu\text{g mL}^{-1}$, which is much lower than the biocompatible dosage of An-Dex FNPs. The dosage can be further decreased if the surface of An-Dex FNPs was functionalized with targeting agents. Therefore, the An-Dex FNPs should be biocompatibility enough for biological imaging applications. It is well known that phenyl borate can response to both pH and glucose. Therefore, these AIE active FNPs can be potentially used for self report carriers for controlled delivery of biological active agents. Furthermore, the dynamic cross-linked FNPs could possibly overcome the CMC of traditional of noncross-linked AIE active FNPs. They could also display better properties for biomedical applications as compared with the stable cross-linked AIE active FNPs due to the responsiveness of phenyl borate.

Fig. 6 Cell imaging of An-Dex FNPs. Cells were incubated with $20\ \mu\text{g mL}^{-1}$ of An-Dex FNPs for 3 h. (A) fluorescent images, (B) bright field, (C) the overlap image of A and B, scale bar = $20\ \mu\text{m}$. The HepG2 cells were excited by a laser with wavelength of 458 nm.

4. Conclusion

In summary, An-Dex FNPs were prepared through a “one-step” strategy that just need mixing the phenylboronic acid-terminated AIE dye (An-B(OH)_2) and dextran under rather mild reaction conditions such as room temperature, air atmosphere, aqueous solution and without using metal catalysts. Results showed that the resultant An-Dex copolymers showed amphiphilic properties and can self assembly into luminescent nanoparticles with size about 250 nm. More importantly, these An-Dex FNPs emitted strong green-yellow fluorescence in pure aqueous solution due to their AIE properties. Furthermore, these An-Dex FNPs exhibited good water dispersibility and biocompatibility due to their surface was covered with the hydrophilic dextran. Cell uptake behavior demonstrated that these water dispersible luminescent nanoparticles can be readily internalized by cells through endocytosis and mainly distributed in the cytoplasm. Finally, to the best of our knowledge, this is the first report for the

preparation of AIE-active carbohydrate polymers based nanoprobes through formation of the dynamic phenyl borate. Therefore, An-Dex FNPs should be promising candidates for various biomedical applications for the combination advantages of AIE dyes and dextran.

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References

- [1] M. Bruchez, M. Moronne, P. Gin, S. Weiss, A.P. Alivisatos, Semiconductor Nanocrystals as Fluorescent Biological Labels, *Science* 281 (1998) 2013-2016.
- [2] W.C. Chan, S. Nie, Quantum Dot Bioconjugates for Ultrasensitive Nonisotopic Detection, *Science* 281 (1998) 2016-2018.
- [3] X. Zhang, S. Wang, M. Liu, B. Yang, L. Feng, Y. Ji, L. Tao, Y. Wei, Size Tunable Fluorescent Nano-graphite Oxides: Preparation and Cell Imaging Applications, *Phys. Chem. Chem. Phys.* 15 (2013) 19013-19018.
- [4] X. Zheng, M. Liu, J. Hui, D. Fan, H. Ma, X. Zhang, Y. Wang, Y. Wei, Ln 3+-doped hydroxyapatite nanocrystals: controllable synthesis and cell imaging, *Phys. Chem. Chem. Phys.* 17 (2015) 20301-20307.
- [5] X. Zhang, S. Wang, C. Zhu, M. Liu, Y. Ji, L. Feng, L. Tao, Y. Wei, Carbon-dots Derived from Nanodiamond: Photoluminescence Tunable Nanoparticles for Cell Imaging, *J. Colloid Interf. Sci.* 397 (2013) 39-44.
- [6] X. Zhang, X. Zhang, S. Wang, M. Liu, Y. Zhang, L. Tao, Y. Wei, Facile Incorporation of Aggregation-induced Emission Materials into Mesoporous Silica Nanoparticles for Intracellular Imaging and Cancer Therapy, *ACS Appl. Mater. Interf.* 5 (2013) 1943-1947.
- [7] Q. Wan, K. Wang, H. Du, H. Huang, M. Liu, F. Deng, Y. Dai, X. Zhang, Y. Wei, A rather facile strategy for the fabrication of PEGylated AIE nanoprobes, *Polym. Chem.* 6 (2015) 5288-5294.
- [8] Q. Wan, K. Wang, C. He, M. Liu, G. Zeng, H. Huang, F. Deng, X. Zhang, Y. Wei, Stimulus Responsive Cross-linked AIE-active polymeric Nanoprobes: Fabrication and Biological Imaging Application, *Polym. Chem.* 6 (2015) 8214-8221.
- [9] X. Zhang, S. Wang, L. Xu, Y. Ji, L. Feng, L. Tao, S. Li, Y. Wei, Biocompatible Polydopamine Fluorescent Organic Nanoparticles: Facile Preparation and Cell Imaging, *Nanoscale* 4 (2012) 5581-5584.
- [10] M. Liu, J. Ji, X. Zhang, X. Zhang, B. Yang, F. Deng, Z. Li, K. Wang, Y. Yang, Y. Wei, Self-polymerization of dopamine and polyethyleneimine: novel fluorescent organic nanoprobes for biological imaging applications, *J. Mater. Chem. B* 3 (2015) 3476-3482.
- [11] A.M. Smith, H. Duan, A.M. Mohs, S. Nie, Bioconjugated quantum dots for in vivo molecular and cellular imaging, *Adv. Drug Delivery Rev.* 60 (2008) 1226-1240.
- [12] A.M. Breul, M.D. Hager, U.S. Schubert, Fluorescent monomers as building blocks for dye labeled polymers: synthesis and application in energy conversion, biolabeling and sensors,

Chem. Soc. Rev. 42 (2013) 5366-5407.

[13] R. Hu, N.L. Leung, B.Z. Tang, AIE macromolecules: syntheses, structures and functionalities, Chem. Soc. Rev. 43 (2014) 494-4562.

[14] Z. Wang, S. Chen, J.W. Lam, W. Qin, R.T. Kwok, N. Xie, Q. Hu, B.Z. Tang, Long-Term Fluorescent Cellular Tracing by the Aggregates of AIE Bioconjugates, J. Am. Chem. Soc. 135 (2013) 8238-8245.

[15] A. Shao, Y. Xie, S. Zhu, Z. Guo, S. Zhu, J. Guo, P. Shi, T.D. James, H. Tian, W.-H. Zhu, Far-Red and Near-IR AIE-Active Fluorescent Organic Nanoprobes with Enhanced Tumor-Targeting Efficacy: Shape Specific Effects, Angew. Chem. Int. Ed 127 (2015) 7383-7388.

[16] K. Li, Z. Zhu, P. Cai, R. Liu, N. Tomczak, D. Ding, J. Liu, W. Qin, Z. Zhao, Y. Hu, Organic Dots with Aggregation-Induced Emission (AIE Dots) Characteristics for Dual-Color Cell Tracing, Chem. Mater. 25 (2013) 4181-4187.

[17] H. Shi, R.T. Kwok, J. Liu, B. Xing, B.Z. Tang, B. Liu, Real-Time Monitoring of Cell Apoptosis and Drug Screening Using Fluorescent Light-Up Probe with Aggregation-Induced Emission Characteristics, J. Am. Chem. Soc. 134 (2012) 17972-17981.

[18] H. Shi, J. Liu, J. Geng, B.Z. Tang, B. Liu, Specific Detection of Integrin $\alpha\beta 3$ by Light-Up Bioprobe with Aggregation-Induced Emission Characteristics, J. Am. Chem. Soc. 134 (2012) 9569-9572.

[19] X. Zhang, X. Zhang, L. tao, Z. Chi, J. Xu, Y. Wei, Aggregation induced emission-based fluorescent nanoparticles: Fabrication methodology and biomedical application, J. Mater. Chem. B 2 (2014) 4398-4414.

[20] X. Zhang, K. Wang, M. Liu, X. Zhang, L. Tao, Y. Chen, Y. Wei, Polymeric AIE-based nanoprobes for biomedical applications: recent advances and perspectives, Nanoscale 7 (2015) 11486-11508.

[21] X. Zhang, X. Zhang, B. Yang, L. Liu, J. Hui, M. Liu, Y. Chen, Y. Wei, Aggregation-induced Emission Dyes Based Luminescent Silica Nanoparticles: Facile Preparation, Biocompatibility Evaluation and Cell Imaging Applications, RSC Adv. 4 (2014) 10060-10066.

[22] J. Luo, Z. Xie, J.W.Y. Lam, L. Cheng, H. Chen, C. Qiu, H.S. Kwok, X. Zhan, Y. Liu, D. Zhu, B.Z. Tang, Aggregation-induced emission of 1-methyl-1, 2, 3, 4, 5-pentaphenylsilole, Chem. Commun. 37 (2001) 1740-1741.

[23] Q. Li, Z. Li, AIE probes towards biomolecules: the improved selectivity with the aid of graphene oxide, Sci. China Chem. (2015) DOI: 10.1007/s11426-11015-15517-11424

[24] J. Liang, G. Feng, R.T.K. Kwok, D. Ding, B. Tang, B. Liu, AIEgen based light-up probes for live cell imaging, Sci. China Chem. (2015) doi: 10.1007/s11426-11015-15470-11422.

[25] A. Qin, Y. Zhang, N. Han, J. Mei, J. Sun, W. Fan, B.Z. Tang, Preparation and self-assembly of amphiphilic polymer with aggregation-induced emission characteristics, Sci. China Chem. 55 (2012) 772-778.

[26] J. Yang, J. Huang, Q. Li, Z. Li, Blue AIEgens: approaches to control the intramolecular conjugation and the optimized performance of OLED devices, J. Mater. Chem. C (2016) DOI: 10.1039/C1035TC03262H

[27] W. Guan, J. Lu, W. Zhou, C. Lu, Aggregation-induced emission molecules in layered matrices for two-color luminescence films, Chem. Comm. 50 (2014) 11895-11898.

[28] W. Guan, W. Zhou, C. Lu, B.Z. Tang, Synthesis and Design of Aggregation-Induced

Emission Surfactants: Direct Observation of Micelle Transitions and Microemulsion Droplets, *Angew. Chem. Int. Ed.* 54 (2015) 15160-15164.

[29] Z. Li, W. Guan, C. Lu, X.-R. Zhou, S.-Z. Luo, Y. You, J. Ouyang, Hydrophobicity-induced prestaining for protein detection in polyacrylamide gel electrophoresis, *Chem. Comm.* 52 (2016) 2807-2810.

[30] X. Zhang, X. Zhang, B. Yang, S. Wang, M. Liu, Y. Zhang, L. Tao, Aggregation-Induced Emission Materials Based Fluorescent Organic Nanoparticles: Facile PEGylation and Cell Imaging Applications, *RSC Adv.* 3 (2013) 9633-9636.

[31] Z. Chi, X. Zhang, B. Xu, X. Zhou, C. Ma, Y. Zhang, S. Liu, J. Xu, Recent advances in organic mechanofluorochromic materials, *Chem. Soc. Rev.* 48 (2012) 3878-3896.

[32] M. Liu, X. Zhang, B. Yang, F. Deng, Z. Huang, Y. Yang, Z. Li, X. Zhang, Y. Wei, Ultrabright and biocompatible AIE dye based zwitterionic polymeric nanoparticles for biological imaging, *RSC Adv.* 4 (2014) 35137-35143.

[33] X. Zhang, X. Zhang, B. Yang, J. Hui, M. Liu, Z. Chi, S. Liu, J. Xu, Y. Wei, Facile Preparation and Cell Imaging Applications of Fluorescent Organic Nanoparticles that Combine AIE Dye and Ring-opening Polymerization, *Polym. Chem.* 5 (2014) 318-322.

[34] X. Zhang, X. Zhang, B. Yang, J. Hui, M. Liu, Z. Chi, S. Liu, J. Xu, Y. Wei, A Novel Method for Preparing AIE Dye based Cross-linked Fluorescent Polymeric Nanoparticles for Cell Imaging, *Polym. Chem.* 5 (2014) 683-688.

[35] X. Zhang, X. Zhang, B. Yang, J. Hui, M. Liu, W. Liu, Y. Chen, Y. Wei, PEGylation and Cell Imaging Applications of AIE Based Fluorescent Organic Nanoparticles via Ring-opening Reaction, *Polym. Chem.* 5 (2014) 689-693.

[36] X. Zhang, X. Zhang, B. Yang, M. Liu, W. Liu, Y. Chen, Y. Wei, Polymerizable Aggregation Induced Emission Dye Based fluorescent Nanoparticles For Cell Imaging Applications *Polym. Chem.* 5 (2014) 356-360.

[37] X. Zhang, X. Zhang, B. Yang, M. Liu, W. Liu, Y. Chen, Y. Wei, Fabrication of Aggregation Induced Emission Dye-based Fluorescent Organic Nanoparticles via Emulsion Polymerization and Their Cell Imaging Applications, *Polym. Chem.* 5 (2014) 399-404.

[38] M. Liu, X. Zhang, B. Yang, F. Deng, Y. Yang, Z. Li, X. Zhang, Y. Wei, Preparation and Bioimaging Applications of AIE Dye Cross-linked Luminescent Polymeric Nanoparticles, *Macromol. Biosci.* 14 (2014) 1712-1718.

[39] M. Liu, X. Zhang, B. Yang, L. Liu, F. Deng, X. Zhang, Y. Wei, Polylysine Crosslinked AIE Dye Based Fluorescent Organic Nanoparticles for Biological Imaging Applications, *Macromol. Biosci.* 14 (2014) 1260-1267.

[40] M. Li, Y. Hong, Z. Wang, S. Chen, M. Gao, R.T. Kwok, W. Qin, J.W. Lam, Q. Zheng, B.Z. Tang, Fabrication of Chitosan Nanoparticles with Aggregation-Induced Emission Characteristics and Their Applications in Long-term Live Cell Imaging, *Macromol. Rapid Commun.* 34 (2013) 767-771.

[41] X. Zhang, X. Zhang, B. Yang, M. Liu, W. Liu, Y. Chen, Y. Wei, Facile Fabrication and Cell Imaging Applications of Aggregation induced Emission Dye Based Fluorescent Organic Nanoparticles, *Polym. Chem.* 4 (2013) 4317-4321.

[42] G. Israeli-Lev, Y.D. Livney, Self-assembly of hydrophobin and its co-assembly with hydrophobic nutraceuticals in aqueous solutions: Towards application as delivery systems, *Food Hydrocolloid* 35 (2014) 28-35.

- [43] A.Z.M. Badruddoza, Z.B.Z. Shawon, W.J.D. Tay, K. Hidajat, M.S. Uddin, Fe³⁺/cyclodextrin polymer nanocomposites for selective heavy metals removal from industrial wastewater, *Carbohydr. Polym.* 91 (2013) 322-332.
- [44] D. Liu, Z. Li, Y. Zhu, Z. Li, R. Kumar, Recycled chitosan nanofibril as an effective Cu (II), Pb (II) and Cd (II) ionic chelating agent: adsorption and desorption performance, *Carbohydr. Polym.* 111 (2014) 469-476.
- [45] M. Liu, X. Zhang, B. Yang, Z. Li, F. Deng, Y. Yang, X. Zhang, Y. Wei, Fluorescent nanoparticles from starch: Facile preparation, tunable luminescence and bioimaging, *Carbohydr. Polym.* 121 (2015) 49-55.
- [46] W.W. Ngah, L. Teong, M. Hanafiah, Adsorption of dyes and heavy metal ions by chitosan composites: A review, *Carbohydr. Polym.* 83 (2011) 1446-1456.
- [47] P.J. Polowsky, S. Janaswamy, Hydrocolloid-based nutraceutical delivery systems: Effect of counter-ions on the encapsulation and release, *Food Hydrocolloid* 43 (2015) 658-663.
- [48] A. Shpigelman, Y. Shoham, G. Israeli-Lev, Y.D. Livney, β -Lactoglobulin-naringenin complexes: Nano-vehicles for the delivery of a hydrophobic nutraceutical, *Food Hydrocolloid* 40 (2014) 214-224.
- [49] R. Penalva, I. Esparza, M. Agüeros, C.J. Gonzalez-Navarro, C. Gonzalez-Ferrero, J.M. Irache, Casein nanoparticles as carriers for the oral delivery of folic acid, *Food Hydrocolloid* 44 (2014) 399-406.
- [50] T.A. Scott Jr, E.H. Melvin, Determination of dextran with anthrone, *Analytical Chemistry* 25 (1953) 1656-1661.
- [51] R. Gibbons, M. Nygaard, Synthesis of insoluble dextran and its significance in the formation of gelatinous deposits by plaque-forming streptococci, *Archives of oral biology* 13 (1968) 1249-1251.
- [52] A. Matsumoto, S. Ikeda, A. Harada, K. Kataoka, Glucose-responsive polymer bearing a novel phenylborate derivative as a glucose-sensing moiety operating at physiological pH conditions, *Biomacromolecules* 4 (2003) 1410-1416.
- [53] H. Qi, M. Liu, L. Xu, L. Feng, I. Tao, Y. Ji, X. Zhang, Y. Wei, Biocompatibility Evaluation of Aniline Oligomers with Different End-functional Groups, *Toxicol. Res.* 2 (2013) 427-433.
- [54] X. Zhang, W. Hu, J. Li, L. Tao, Y. Wei, A Comparative Study of Cellular Uptake and Cytotoxicity of Multi-walled Carbon Nanotube, Graphene Oxide, and Nanodiamond, *Toxicol. Res.* 1 (2012) 62-68.
- [55] X. Zhang, M. Liu, X. Zhang, F. Deng, C. Zhou, J. Hui, W. Liu, Y. Wei, Interaction of Tannic Acid with Carbon Nanotubes: Enhancement of Dispersibility and Biocompatibility, *Toxicol. Res.* 4 (2015) 160-168.
- [56] X. Zhang, H. Qi, S. Wang, L. Feng, Y. Ji, L. Tao, S. Li, Y. Wei, Cellular responses of aniline oligomers: a preliminary study, *Toxicol. Res.* 1 (2012) 201-205.
- [57] X. Zhang, S. Wang, M. Liu, J. Hui, B. Yang, L. Tao, Y. Wei, Surfactant-dispersed Nanodiamond: Biocompatibility Evaluation and Drug Delivery Applications, *Toxicol. Res.* 2 (2013) 335-346.