Aptamer-Decorated Self-Assembled Aggregation-Induced Emission Organic Dots for Cancer Cell Targeting and Imaging

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Supporting Information

ABSTRACT: A facile and simple one-step method was developed to fabricate aptamer-decorated self-assembled organic dots with aggregation-induced emission (AIE) characteristics. With integration of the advantages of AIE aggregates with strong emission and the cell-targeting capability of aptamers, the as-prepared Apt-AIE organic nanodots can specifically target to cancer cells with good biocompatibility, high image contrast, and photostability. On the basis of this universal method, a variety of versatile organic fluorescent nanoprobes with high brightness, specific recognition, and clinical-transitional potential could be facilely constructed for biological sensing and imaging applications.

Fluorescence imaging is a highly sensitive and low-cost approach for early cancer detection, tumor margin visualization and therapeutic effect evaluation.1−3 Following the rapid development of nanotechnology in the past few decades, various nanomaterials with excellent optical properties have been developed as fluorescence nanoprobes for cancer diagnosis.4−6 In comparison with traditional organic molecules, nanoparticles generally show higher brightness, better photostability, and larger absorption coefficients, which make them capable of enhancing the sensitivity and versatility of fluorescence-based detection and imaging. Unfortunately, most fluorescent nanoparticles, such as semiconductor quantum dots, are composed of highly toxic heavy metal cations, which raises the concerns of long-term toxicity and limits the further clinical transition.7 In terms of biocompatibility, organic fluorescent nanoparticles consisting of organic molecules or semiconducting polymers encapsulated inside or decorated on the matrix of biocompatible polymers are generally considered more favorable than the inorganic nanoparticles.8,9

Great efforts have been made to develop organic dye-doped nanoparticles with dramatically enhanced photostability and biocompatibility.10 The brightness of as-prepared organic nanoparticles, however, tends to decrease along with the increase of dye doping concentration. This fluorescence decrease has been known as aggregation-caused quenching (ACQ), which presents a dilemma for fabricating organic nanoparticles.11 A breakthrough in luminescent materials has been made in 2001. Tang et al. reported a kind of propeller-shaped molecules like hexaphenylsilole and tetraphenylethylene with aggregation-induced emission (AIE) characteristics, in which the AIE luminogens (AIEgens) generally exhibit no or weak emission in solution but enhanced emission in aggregated or solid states.12 Since then, a variety of AIEgens have been developed.13−17 More importantly, the strong solid-state emission of AIEgens provides great opportunity for fabricating highly bright organic nanoparticles (denoted as AIE dots) without destructing their emission.18−21

The use of recognition motifs, including folic acid, peptides, and antibodies, for specific tumor accumulation and active binding is considered as one of the most promising approaches for targeting cancers.22 Aptamers are single-stranded DNA or RNA oligonucleotides that can bind to their targets, such as small molecules, proteins, cells, and drugs, with high affinity.

Cite This: Anal. Chem. 2018, 90, 1063−1067
DOI: 10.1021/acs.analchem.7b03933
Received: September 26, 2017
Accepted: December 25, 2017
Published: December 25, 2017
and selectivity through folding into distinct secondary and tertiary structures. Benefiting from their oligonucleotide properties, aptamers possess several advantages over antibodies, such as their relatively small size, lack of immunogenicity, ease of synthesis and various modifications. All these merits make aptamers as promising recognition motifs to replace antibodies for cancer imaging and therapy. Considering the high photostability of AIE dots and the specific targeting capabilities of aptamers, it would be highly desirable to develop aptamer-decorated AIE nanoprobes and apply them in biological sensing and biomedical imaging.

In this work, we attempt to develop a facile and simple method to fabricate aptamer-anchored AIE-dots (also termed as Apt-AIE dots) based on self-assembly approach. As demonstrated in Scheme 1, the Apt-AIE dots were prepared by a one-pot and one-step self-assembly method by mixing of the AIEgens, PEG-lipids, lipids, and aptamer-cholesterol under sonication. The hydrophobic lipid segments tend to embed themselves in the aggregates of hydrophobic AIEgens while the hydrophilic PEG chains extend themselves into the aqueous phase to render the AIE dots with good water dispersibility and enhanced colloidal stability. The aptamer was covalently linked with cholesterol, which served as an anchor to be firmly inserted into the lipid layer via hydrophobic interactions.

2TPE-4E, a red emissive AIEgens, was synthesized to fabricate the Apt-AIE dots. The synthetic route for 2TPE-4E was depicted in Scheme S1. The optical properties of 2TPE-4E were exhibited in Figure 1. Unlike conventional luminogens with large \( \pi \)-conjugation, 2TPE-4E displayed typical AIE feature with very weak emission in good solvents but strong red fluorescence in the aggregated states (Figure 1A). The photoluminescent (PL) intensity remained at a low level when the water fraction \( (f_w) \) of the water/THF mixture was below 50 vol \%. However, a significant PL enhancement was observed when more water was added. 2TPE-4E reached its maximal emission intensity at \( f_w \) of 95 vol \%, which was \( \sim 20 \)-fold higher than that in the pure THF (Figure 1B).

The Apt-AIE dots were then prepared by simply mixing 2TPE-4E with the cholesterol-tagged aptamer during the hydration of the lipid-nanoparticles formulation. The absorption and the emission spectra of the 2TPE-4E Apt-AIE dots with and without aptamer-anchoring were recorded in HEPES buffer, respectively, and compared with the 2TPE-4E molecules measured in chloroform solution (Figure 2A,B).

The chloroform solution of 2TPE-4E exhibited an absorption peak at 480 nm. The absorption maximum of the 2TPE-4E Apt-AIE dots was 520 nm. This 40 nm red-shift in absorption was possibly due to the 2TPE-4E received stronger intermolecular \( \pi-\pi \) interactions inside the nanoparticles (Figure 2A). The emission maximum of 2TPE-4E Apt-AIE dots appeared at 630 nm, which holds particular advantages of low autofluorescence from the background in biological media. On the other hand, the 2TPE-4E molecules in chloroform were almost non-fluorescent as the active intramolecular rotation of TPE. Noteworthy, the absorbance and emission profiles of as-prepared Apt-AIE dots was only slightly changed after the aptamers were decorated on the surface of 2TPE-4E Apt-AIE dots. Also, the Apt-AIE dots showed a large Stokes shift of 110 nm (abs = 520 nm; em = 630 nm), which greatly minimized the self-absorption that are typically observed in conventional organic dyes. The amount of 2TPE-4E loaded in the Apt-AIE dots has also been optimized. As indicated in Figure S5, the PL intensity of the Apt-AIE dots increased along with the mass of the

Figure 1. (A) Photoluminescence (PL) spectra of 2TPE-4E in the mixtures with different \( f_w \). The insert is the molecular structure of 2TPE-4E. (B) The plot of relative PL intensities \( (I/I_0) \) versus \( f_w \) where \( I_0 \) is the PL intensity of 2TPE-4E at 630 nm. Concentration: 10 \( \mu \)M; ex = 480 nm. The insert is the photographs of 2TPE-4E in the THF/water mixture with different water fractions \( (f_w) \) taken under 365 nm UV light.

Figure 2. Characterizations of Apt-AIE dots. (A) Normalized absorption and (B) normalized fluorescence spectra of 2TPE-4E molecules in chloroform and Apt-AIE dots with or without aptamer in HEPES buffer. (C) Dynamic light scattering (DLS) and (D) zeta potential measurements of the Apt-AIE dots with or without aptamer.
2TPE-4E loaded. The AIE dots reached its maximum emission intensity when the 2TPE-4E mass was 62.5 μg. As a result, we chose 62.5 μg as the optimal amount for the following AIE dots preparation. The average amount of 2TPE-4E encapsulated in each AIE dot was calculated to be ~1000 according to Prof. Liu’s method, and the average number of aptamer on each nanoparticles was calculated to be ~100.

The morphology of Apt-AIE dots was further investigated using transmission electron microscopy (TEM). The TEM images indicated that the Apt-AIE dots were in spherical shape with an average size of ~50 nm (Figure S6). The aptamer-anchored AIE-dots were further characterized by dynamic light scattering (DLS) and zeta potential measurements (Figure 2C,D). The hydrodynamic diameter of 2TPE-4E loaded AIE dots with and without aptamer modification revealed average hydrodynamic diameters of ~68 nm and ~58 nm, respectively. The diameter of Apt-AIE dots from DLS analysis increased by about 10 nm compared with that of 2TPE-4E AIE dots, suggesting that the attachment of aptamers increased the hydration diameter of the nanoparticles. According to the previous report, the length of 20-mer DNA was about 5.6 nm. The length of the 38-mer AS1411 aptamer should be about 10 nm, which was consistent with experimental results. The average particle size estimated by DLS was also larger than that measured by TEM because the nanoparticles had a certain extent of swelling and hydration in water, thus the DLS result showed the hydration diameters of the Apt-AIE dots. As depicted in Figure 2D, the surface of 2TPE-4E AIE dots were negatively charged (~23 mV), which was ascribed to the phosphate group on the hydrophilic head with negative charge. After modification with aptamers, the zeta potential of the Apt-AIE dots further decreased to approximately ~28 mV, evidently because of the attachment of negatively charged DNA strands on the surface of 2TPE-4E AIE dots.

The stability of the Apt-AIE dots was evaluated under various conditions. The fluorescence and colloid stability of as-prepared Apt-AIE dots was evaluated under several biologically relevant conditions, including change of pH from 3 to 10, change of ionic strength of buffer (0–2 M NaCl) (Figure S7), and in the presence of oxidizing/reductive species as well as in the presence of various ions under the conditions of room temperature with light exposure (Figure S8). All results revealed that the Apt-AIE dots stayed stable with no sign of aggregation or loss of fluorescence, supporting the high stability of the Apt-AIE dots.

AS1411, an aptamer with 26-mer single-strand DNA which can selectively bind to nucleolin, a nucleolar phosphoprotein overexpressed on the surface of several cancer cell lines (such as breast cancer cells, lung cancer cells, etc.), was selected as a recognition motif anchored on the Apt-AIE dots. To demonstrate the targeting capability of Apt-AIE dots, two cancer cell lines, MCF-7 (human breast cancer cell line) and A549 cells (human lung cancer cell line), and one normal healthy cell line, 293T cells (human embryonic kidney cell line), were selected and incubated with Apt-AIE dots and 2TPE-4E AIE dots, separately. The fluorescence of the treated cells was recorded by using confocal laser scanning microscopy (CLSM) and flow cytometry. As the CLSM images illustrated in Figure 3A, Figure S9, and Figure S10, the cancer cells (MCF-7 cells and A549 cells) treated with Apt-AIE dots were highly fluorescent when compared to those cells treated with the 2TPE-4E AIE dots. On the other hand, no fluorescent intensity was detected in the normal 293T cells treated with 2TPE-4E AIE dots and even for the Apt-AIE dots (Figure S11). These results demonstrated the Apt-AIE dots possessed stronger binding affinity to the cancer cells with overexpression of nucleolin (MCF-7 cells and A549 cells) than the normal cells (293T). In addition, the fluorescence intensity of the treated cells was further analyzed by flow cytometry (Figure 3B and Figure S12). The amplified fluorescence signal intensities of MCF-7 and A549 cells bound by Apt-AIE dots suggested that the aptamer AIE dots were only specifically internalized by MCF-7 cells and A549 cells but not by 293T cells. The flow cytometric analysis results were consistent with the CLSM data, further proving that the Apt-AIE dots could target the nucleolin-overexpressed cancer cells with high selectivity and efficiency. The subcellular distribution and location of the Apt-AIE dots in MCF-7 cells was further investigated by colocalization study. The MCF-7 cells were co-stained with Apt-AIE dots (in red) and LysoTracker Green (in green), a commercial lysosome-specific dye (Figure S13). The red fluorescence completely overlapped with the green fluorescence from the lysosome, clearly indicating that most Apt-AIE dots were accumulated in the lysosomes. Therefore, Apt-AIE dots are very promising for cancer-cell-specific imaging and delivery. The receptor blocking experiment on MCF-7 also confirm the specific targeting ability of as-prepared Apt-AIE dots (Figure S14).
Moreover, biocompatibility is the main concern for the imaging agent during its use in biomedical applications. The cytotoxicity of the Apt-AIE dots after 24 or 72 h of incubation with different cell lines, including MCF-7 cells, A549 cells, and 293T cells, was evaluated using the CCK-8 assay (Figure 3C, Figures S15–S19). The results demonstrated that the Apt-AIE dots possessed low cytotoxicity toward the three kinds of cell lines at different concentrations, suggesting that as-prepared Apt-AIE dots were biocompatible and suitable for the uses in bioimaging. To further confirm the potential of the AIE dots to serve as an efficient fluorescent imaging probe, we evaluated its photostability. As shown in Figure S20, the photostability of the AIE dots was much higher than the traditional ACQ rubrene dye. The fluorescent signals of AIE dots were still detectable even after 30 min irradiation continuously. Also, the hybridization between Apt-AIE dots and their complementary DNA (cDNA) was studied (Figure S21), which further demonstrated the potential nucleic hybridization application of as-prepared Apt-AIE dots.

In summary, we developed a simple one-pot self-assembly method to fabricate aptamer-decorated AIE dots for cancer cell targeting and imaging. As-prepared aptamer-decorated AIE-dots showed specific targeting ability, high stability, and good biocompatibility. More promisingly, various cholesterol-modified aptamers could be anchored onto the surface of AIE-dots through this general approach. By combining the diversity of aptamers with the rich category of AIEgens, a variety of biocompatible organic fluorescent nanoprobes with specific recognition, high sensitivity, and tracking capability can be facilely constructed, paving the way for long-term, real-time and dynamic sensing, tracking and imaging applications.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.7b03933.

SYNTHESIS AND CHARACTERIZATION OF 2TPE-4E, TEM IMAGE, STABILITY TEST, CONFOCAL IMAGES, AND FLOW CYTOMETRIC RESULTS OF A549 CELLS AND 293T CELLS, COLocalIZATION EXPERIMENTS, CELL VIABILITY, AND PHOTOSTABILITY COMPARISON (PDF)

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Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was partially supported by the National Basic Program of China (973 Program; Grants 2013CB834701 and 2013CB834702), the University Grants Committee of Hong Kong (Grant AoE/P-03/08), the Research Grants Council of Hong Kong (Grants 16301614, 16305015, 16308016, N_HKUST604/14, and A-HKUST605/16), and the Innovation and Technology Commission (Grants ITC-CNERC14SC01 and RE:ITCPD/17-9). B.Z.T. is grateful for the support from the Guangdong Innovative Research Team Program of China (Grant 201101C0105067115) and the Science and Technology Plan of Shenzhen (Grant JCYJ20160229205601482). P.Z. is grateful for the support from the National Natural Science Foundation of China (Grant 81501591).

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