Specific detection of cancer cells through aggregation-induced emission of a light-up bioprobe†

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A cancer cell specific aptamer was labeled with an aggregation-induced emission (AIE) probe for the first time. Using it as a light-up bioprobe, a specific cancer cell detection method is developed.

Cancer is a group of diseases involving abnormal cell growth. Cancer cells grow and divide at a very rapid and unregulated pace, and could invade or spread to other parts of the body.1

Highly sensitive and selective detection of cancer cells is of great importance for both early diagnosis of cancer and investigation of cancer metastasis.2 In recent years, a number of cancer cell detection methods have been developed, which include the fluorescence,3 chemiluminescence,4 electrochemical,5 surface-enhanced Raman scattering,6 surface plasmon resonance,7 colorimetric,8 and electrochemiluminescence9 methods. Compared with other methods, the fluorescence-based method has drawn growing attention due to its high sensitivity, rapid response, and simple manipulation.

Aggregation-induced emission (AIE) is a novel photophysical phenomenon which has attracted considerable interest in recent years.10 The molecules with AIE characteristics are non-emissive in the monomeric form but become highly emissive in the aggregated form as a result of the restriction of intramolecular rotations, which could significantly reduce the energy dissipation through nonradiative means.11 Based on this observation, a number of AIE probes have been developed for applications in bioanalysis and biosensing.12 The AIE probes are usually propeller-shape molecules. Tetrakis(4-phenylphenoxy)ethylene (TPE) is one of the most important AIE probes, which has been widely used for the construction of novel biosensing techniques.13

Aptamers are single-stranded DNA or RNA oligonucleotides which can specifically and tightly bind to a variety of targets, such as metal ions, small molecules, proteins, and even entire viruses and cells.14 Compared with other recognition molecules such as antibodies, aptamers are more stable, inexpensive and can be synthesized and labeled chemically with ease.15 As a result, a variety of aptamer-based biosensing methods have been developed.14,16 In recent years, specific aptamers against entire cancer cells have been reported.17 Using those aptamers as novel and powerful affinity recognition ligands, several aptamer-based cancer cell detection methods have been developed.3–9

In this work, a cancer cell specific aptamer was labeled with an AIE probe for the first time. Using it as a light-up bioprobe, a specific cancer cell detection method is developed. Ramos cells (human Burkitt’s lymphoma cells) were selected as the model cancer cells. The overall sensing strategy is schematically illustrated in Scheme 1. A Ramos cell specific aptamer with both ends labeled with TPE probes (TPE-aptamer) was designed and successfully synthesized. The TPE-aptamer showed only very weak emission in an aqueous buffer solution. Upon the addition of Ramos cells, specific binding between the aptamer and the Ramos cells resulted in restrictions of the intramolecular rotations of the

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TPE molecules. Turn-on emission of the TPE-aptamer was observed, and a specific fluorescence cancer cell detection method is therefore established.

The synthetic route for the TPE-labeled Ramos cell specific aptamer (TPE-aptamer) is shown in Scheme 2. A succinimidyl ester-modified TPE derivative (compound 1) was prepared (Fig. S1–S6, ESI†). The aptamer with amino functional groups labeled on both ends was coupled directly with two molecules of compound 1. The resulting crude product was purified by reverse-phase HPLC. Fig. S7 (ESI†) shows the HPLC chromatograms of the crude product. The peaks 1–4 are the unreacted aptamer (with absorption at 260 nm only), single-TPE-labeled aptamer (with absorption at both 260 and 350 nm), dual-TPE-labeled aptamer (with absorption at both 260 and 350 nm), and unreacted TPE (with absorption at 350 nm mainly), respectively. Peak 3 was collected, and pure TPE-aptamer was obtained.

Fig. S8 (ESI†) shows the UV-vis absorption spectra of the aptamer, compound 1 and the TPE-aptamer, respectively. It was observed that the TPE-aptamer contained the characteristic absorption of both the aptamer (maximum absorption at 260 nm) and compound 1 (maximum absorption at 350 nm). The results clearly indicate that the aptamer has been successfully labeled with the TPE probe.

Fig. S9 (ESI†) shows the emission spectra of compound 1 and the TPE-aptamer in aqueous and organic solvents. In DMSO, compound 1 (a hydrophobic molecule) mainly existed in the monomeric form and showed no AIE emission. While in a DMSO-water solvent mixture (v/v = 1/99), compound 1 had a strong tendency to self-aggregate due to the strong hydrophobic interactions among the probe molecules, thus strong compound 1 AIE emission was observed. In sharp contrast, the TPE-aptamer showed only very weak emission in water but strong emission in the DMSO-water solvent mixture (v/v = 99/1). These results clearly suggest that the TPE-aptamer had better solubility in an aqueous solution. Since the TPE molecules were covalently labeled to the aptamer (an anionic polymer), the negatively charged oligonucleotide phosphate backbone diminished the tendency of TPE self-aggregation.

To investigate further the properties of the TPE-aptamer, control experiments were conducted. The TPE-aptamer was digested into small fragments by nuclease S1. Fig. S10 (ESI†) shows that, in an aqueous solution, after the digestion of the TPE-aptamer with nuclease S1, a significant increase of TPE AIE emission was observed. The results confirm that the aptamer conjugated TPE molecules mainly existed in the monomeric form, and the negatively charged oligonucleotide effectively diminished the tendency of the TPE probe self-aggregation. After the digestion with nuclease S1, the TPE probe was released, and strong hydrophobic interactions among the probe molecules caused strong AIE emission.

The Ramos cell specific aptamer can specifically recognize the immunoglobulin heavy mu chain on the surface of the Ramos cells. Upon the addition of Ramos cells to the solution of TPE-aptamer, specific binding between the aptamer and the Ramos cells resulted in restrictions of the intramolecular rotations of the TPE molecules. And strong AIE emission of the TPE-aptamer was thus observed. Fig. 1 and Fig. S11 (ESI†) show that, in the presence of 2.5 μM of TPE-aptamer, the emission intensity of the aptamer increased gradually with the addition of increasing amounts of the Ramos cells (0–20 000). A linear relationship was obtained at a Ramos cell range of 0–5000. The linear regression equation is $E = 0.067A + 84.55$ (correlation coefficient $R^2 = 0.999$), where $E$ is the emission intensity of the TPE-aptamer at 470 nm and $A$ is the amount of Ramos cells. Our assay is quite sensitive compared with the previously reported fluorometric methods. Without the use of any signal amplification, 100 Ramos cells could be easily detected.

![Scheme 2: Synthetic route for the TPE-aptamer.](image_url)

**Fig. 1** (a) Changes in emission spectrum of the TPE-aptamer with the amount of Ramos cells added (0, 100, 200, 500, 1000, 2000, 3000, 4000, 5000, 10 000, 15 000 and 20 000, respectively). (b) Plot of the changes in emission intensity of the TPE-aptamer at 470 nm against the amount of Ramos cells added. Inset: Expanded linear region of the curve.
The selectivity of the assay was studied. Five cancer cell lines (HeLa, CCRF-CEM, MCF-7, A549 and HepG2) and one normal cell line (L929) were selected. Fig. 2 shows that, under the same experimental conditions, 20,000 Ramos cells could induce obvious AIE emission increase of the TPE-aptamer. In contrast, the other cells of the same amount could not induce noticeable emission increase. The results clearly indicate that the assay is highly selective for Ramos cells.

The assay was also tested in complex sample mixtures. TPE-aptamer (2.5 μM) and Ramos cells of different amounts were added to human serum (80%), and the sample mixtures were tested. Fig. S12 (ESI†) shows that the more Ramos cells were spiked, the larger emission intensity increase of the TPE-aptamer was obtained. The results clearly indicate that the assay could also be used in complex sample mixtures.

The assay could also be used for the specific fluorescence imaging of cancer cells. Ramos, CCRF-CEM and HeLa cells were tested. Fig. 3 shows that, in the presence of 2.5 μM TPE-aptamer and 10,000 of Ramos cells, the AIE emission of the TPE-aptamer could be easily observed. Under the same experimental conditions, CCRF-CEM and HeLa cells could not give noticeable AIE fluorescence. The results clearly suggest that the assay could also be used for the specific fluorescence imaging of cancer cells. In addition, a cytotoxicity study of the TPE-aptamer was conducted (Fig. S13, ESI†). The results clearly indicate that our probe is nontoxic to the cells.

In summary, a cancer cell specific aptamer is labeled with an AIE probe for the first time, and a selective detection method for cancer cells based on aggregation-induced emission is developed. A TPE labeled Ramos cell specific aptamer (TPE-aptamer) was synthesized. The TPE-aptamer shows very weak emission in an aqueous buffer solution. Upon the addition of Ramos cells, specific binding between the aptamer and Ramos cells resulted in restrictions of intramolecular rotations of the TPE probe molecules. Strong AIE emission of the TPE-aptamer was observed, and a selective Ramos cell detection method is established.

Our assay has several important features. First, it offers a convenient “mix-and-detect” approach, which is simple and fast. Second, it is based on the fluorescence “turn-on” mode, which could considerably reduce the likelihood of false-positive signals associated with the “turn-off” assay. Third, without the use of any signal amplification, highly sensitive cancer cell detection could be easily accomplished. Fourth, our assay could be used in complex sample mixtures, and also be used for the specific fluorescence imaging of cancer cells. Fifth, the AIE probe has certain unique characteristics, the fluorescence turn on does not rely on energy transfer or nanomaterials. We envision that the principle of our assay could be employed for the development of novel AIE and nucleic acid aptamer based sensing techniques for the detection of various targets.

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Notes and references


