Published in final edited form as:

Chem Commun (Camb). 2018 January 25; 54(9): 1133–1136. doi:10.1039/c7cc08986d.

A Cyanine-based Fluorescent Cassette with Aggregation-induced Emission for Sensitive Detection of pH Changes in Live Cells

Mingxi Fang^a, Shuai Xia^a, Jianheng Bi^a, Travis P. Wigstrom^a, Loredana Valenzano^a, Jianbo Wang^{a,c}, Wafa Mazi^a, Marina Tanasova^a, Fen-Tair Luo^b, and Haiying Liu^a

^aDepartment of Chemistry, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931, USA

^bInstitute of Chemistry, Academia Sinica, Taipei, Taiwan 11529, Republic of China

^cCollege of Biological, Chemical Sciences and Engineering, Jiaxing University, Jiaxing 314001, China

Abstract

An aggregation-induced emission (AIE) cyanine-based fluorescent cassette with a large pseudo-Stokes shift was designed and prepared to sensitively image pH changes in live cells via through-bond energy transfer (TBET) from tetraphenylethene (TPE) donor to cyanine acceptor.

Graphical abstract

A novel fluorescent cassette consisting of TPE donor and cyanine acceptor was developed for sensitive detection of pH changes in live cells.

[†]Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here].
See DOI: 10.1039/x0xx00000x

Conflicts of interest

There are not conflicts to declare.

A variety of fluorescent probes have been developed based on cyanine platforms for detection of metal ions, anions, pH, reactive small molecules, biomacromolecules, and environment-related factors such as temperature, polarity and viscosity because cyanine dyes possess unique near-infrared optical properties, exceptional biocompatibility, and low cytotoxicity to live cells.^{1–7} However, cyanine dyes still suffer from some drawbacks such as low fluorescence quantum yields due to undesired aggregation in aqueous solution, poor photostability and narrow Stokes shifts although some water-soluble cyanine dyes have been prepared to alleviate dye self-aggregation degrees.⁸ In order to overcome these drawbacks, it is very important to develop new near-infrared fluorescent cassettes with excellent photostability, large Stokes shifts, and high turn-on fluorescence brightness. Recently, aggregation-induced emission approach has been employed to effectively overcome fluorescence quenching issues due to dye aggregation in an aqueous solutions, and offered an effective way to make bright fluorescent dyes.^{9–15}

In this communication, we present a simple but very effective way to prepare an AIE cyanine-based fluorescent cassette (**A**) with a large pseudo-Stokes shift, and advantages of visible and near-infrared dual excitations and emissions for sensitive detection of pH in live cells. The cassette **A** was prepared by modifying the central cyclohexenyl chloro group of the cyanine acceptor with a phenol group from TPE donor in basic condition. The fluorescent cassette displays a large pseudo-Stokes of 484 nm via effective TBET from TPE donor to cyanine acceptor. In addition, the cassette possesses significant AIE property in both neutral and acidic conditions, and undergoes significant fluorescence quenching under basic condition because of charge repulsion among negative charges of TPE donor at basic

pH. The fluorescent cassette offers an effective way to visualize pH changes in live cells with dual visible and near-infrared excitation and emission feature.

The cassette **A** was prepared by reacting TPE derivative (**1**) with cyanine dye (IR-780) in dry DMF solution in a basic condition (Scheme 2). The cassette structure was confirmed by NMR analysis and mass spectrometry.

First, we investigated whether the cassettes possess AIE properties by monitoring fluorescence intensity of the cassette TPE donor and cyanine acceptor with increasing amounts of water in an ethanol–water solvent mixture where ethanol and water serves as the solvent and the non-solvent, respectively. Typical AIE behaviour with fluorescence increase is observed during a transition from isolated individual molecules to nano-aggregates at a critical concentration of water. The fluorescent cassette **A** exhibits weak fluorescence of TPE donor and cyanine acceptor in ethanol. However, it shows significant AIE property as the fluorescence intensities of both TPE donor and cyanine acceptor significantly increase when water ratio to ethanol increases from 0% to 70% (Figure 1). The cassette **A** shows a large pseudo-Stokes of 484 nm because of high efficient TBET from TPE donor to cyanine acceptor at excitation of 350 nm (Figure 1, left). Fluorescence intensity of cyanine acceptor at excitation of 720 nm is higher than that at excitation of 350 nm. Lower fluorescence intensity of the cyanine acceptor at excitation of 350 nm may arise from partial loss of energy during TBET process. Fluorescence quantum yield of cassette cyanine acceptor is 6.80% at excitation of 720 nm, and higher than that of cyanine dye IR-780 (5.17%) in the same aqueous solution with 30% ethanol, indicating that the AIE effect enhances cyanine fluorescence intensity.

Next we adjust pH to manipulate aggregation states of the cassette in aqueous solution and investigate pH effect on optical properties of the cassette. At acidic pH 5.0, the cassette shows strong fluorescence. However, increases of pH from 5.0 to 10.7 results in significant fluorescence quenching of both TPE donor and cyanine acceptor, and a slight blue shift in fluorescence peak of the cyanine acceptor. Low fluorescence intensity of the cassette at basic pH arises from the energy loss which was used for rotation the ether bond between the TPE donor and cyanine acceptor, and spin of TPE four phenyl groups because the cassette becomes less aggregated in aqueous solution because of charge repulsion among three negative phenolate ions (Figure 2, right),¹⁶ which was confirmed by dynamic light scattering measurement results (Figures S11-12). In addition, absorbance of cyanine acceptor decreases significantly as pH increases from 5.0 to 10.7 because nanoparticle size of the cassette become smaller with the pH increase (Figures S11-12). Fluorescence intensity of the acceptor at excitation of 720 nm is higher than one at excitation at 350 nm (Figure 3). The cassette **A** shows a quick and reversible responses to pH changes, and can be potentially useful to real-time monitor the consecutive and frequent pH changes.

Density functional theory (DFT –see ESI)¹⁷ was used to further explained the excitation mechanisms observed experimentally. A visual inspection of the structures allows for identification of the following changes, where the oxygen within the ether bond bridging the TPE donor and cyanine acceptor is considered as the reference atom: (i) The C_{cya}-O-C_{TPE} angle between the cyanine and TPE increases by 1° (from 119.2° to 120.2°); (ii) The C_{cya}-

$C_{TPE}-C_{TPE}$ angle referring to a rotation of the TPE with respect to the cyanine is as large as 3° (94.8° vs 97.7°); (iii) The $C_{Cya}-O$ bond length increases from $1.420 \text{ \AA}$ to $1.437 \text{ \AA}$; (iv) A 2° change is also observed in the central sp^2 C-C-C bond in TPE. Overall, these structural modifications confirm the suggested mechanism ruling the decrease in fluorescent activity related to the basic environment. Further confirmation is provided by the changes in electron charge experienced by the aforementioned atoms involved in the $C_{Cya}-O-C_{TPE}$ angle. In fact, a redistribution of charge is experienced in terms of loss of negative charge by the C_{Cya} atom (from $-0.815 |e|$ to $-0.540 |e|$ in acidic and basic conditions, respectively) counterbalanced by a significant gain in negative charge in the C_{TPE} atom (from $-0.066 |e|$ to $-0.176 |e|$ in acidic and basic conditions, respectively) indicating a dramatic change in the conjugation between the TPE donor and the cyanine acceptor. HOMO/LUMO energy gaps were calculated in both acidic and basic conditions. Our computed results (Figure 9S in supporting information) suggest that the HOMO-1/LUMO energy gap best represents the expected electron transition reported experimentally and described as the electronic transition from the TPE donor to the cyanine acceptor. Calculations show that alpha electrons participate into the 350 nm peak while beta electrons contribute to the peak observed at 720 nm. When in acidic conditions, the alpha HOMO-1/LUMO energy gap is calculated to be 326 nm; when in basic conditions, a 388 nm energy gap is observed. Both values are in good agreement with the experimentally reported result of 350 nm. For the beta HOMO-1/LUMO energy gap, values are reported at 853 nm and 719 nm in acidic and basic conditions, respectively. While the latter value is in very good agreement with the position of the main absorption shoulder peak at 720 nm observed experimentally, the former calculated result (853 nm) is overestimated by roughly 10% with respect to main absorption peak at 775 nm of cyanine acceptor (Figure 2). Although this may appear as a dramatic difference, such a disagreement lays within the expected error related to the determination of HOMO/LUMO gaps when the B3LYP functional is adopted. In fact, when converted into eV, the difference between the calculated and the observed energy levels is as small as 0.15 eV. Overall, given the adopted computational model which does not consider either the presence of explicit solvent molecules or counterions, results can be considered to be in reasonably good agreement with experimental evidence. Figure 5 shows the electron transitions witnessed when the HOMO-1/LUMO excitation is considered.

We investigated selectivity of the cassette **A** to pH over different metal ions at pH 10.7 and 5.0. The fluorescence spectra of cassette **A** were conducted in the presence of potential interfering metal ions such as K^+ , Ca^{2+} , Mg^{2+} , Al^{3+} , Zn^{2+} , Fe^{3+} , Mn^{2+} , Hg^{2+} , Co^{2+} and Cu^{2+} ions. The cassette displays highly selective response of pH at 5.0 over those metal ions. However, the presence of Ca^{2+} and Cu^{2+} ions causes slight fluorescence quenching of the cassette at pH 10.7 (Figure 5).

A standard cell viability protocol (MTT assay) was employed to study the cytotoxicity of the cassette on HeLa cells by treating HeLa cells with the cassette for 48 h (Figure 5). The cassette shows low cytotoxicity and good compatibility with greater than 80% viability at concentration of $15 \mu M$.

We investigated whether the cassette can penetrate cellular membrane and stain cells. For this part, the cassette was incubated with HeLa cells, and the confocal fluorescence imaging

was conducted. Fluorescence of cyanine acceptor can be observed at 5 μM concentration of the cassette at excitation of TPE donor or cyanine acceptor. Increase of the cassette concentrations from 5 μM to 20 μM significantly enhances fluorescence of TPE donor and cyanine acceptor at excitation of TPE donor or cyanine acceptor. Cyanine acceptor in cassette **A** shows much stronger fluorescence intensity than TPE donor (Figure 6). In addition, fluorescence intensity of the cyanine acceptor at excitation of the cyanine acceptor is higher than that at the donor excitation, which indicating that TBET from the TPE donor to cyanine acceptor loses partial energy.

In order to investigate whether cassette is capable of determining pH changes in live cells, the fluorescence imaging of live cells with different intracellular pH values was conducted by incubating HeLa cells with 20 μM cassette **A**, and then subsequently with nigericin (5 $\mu\text{g/mL}$) (H^+/K^+ ionophore) in buffers with different pH values ranging from 5.0, 6.0, 7.0, 8.0 to 9.0, respectively where nigericin was used to homogenize the intracellular and extracellular pH.^{18–21} The cassette showed very strong fluorescence intensities of both TPE donor and cyanine acceptor at pH 5.0. However, fluorescence intensities of both TPE donor and cyanine acceptor decreased significantly when intracellular pH increased from 5.0 to 9.0. The TPE donor fluorescence became very weak at pH 7.0 while the cyanine acceptor fluorescence is still strong at excitation of both donor and acceptor. These results indicate that the cassette displays sensitive fluorescence responses to pH changes in live cells in one visible and two near-infrared channels. In addition, the cassette shows ratiometric fluorescence responses to pH changes from 5.0 to 9.0 in live cells as merged images of blue and NIR channels change from purple to weak red at TPE donor excitation (Figure 7).

We also studied the intracellular pH fluctuations caused by oxidative stress. Two redox substances such as hydrogen peroxide and *N*-ethylmaleimide (NEM) were employed to further treat HeLa cells, respectively, and then imaging of the cells was taken after the cells was incubated with the cassette. The hydrogen peroxide treatment results in fluorescence intensity increases in both TPE donor and cyanine acceptor (Figure 8), indicating that hydrogen peroxide causes the intracellular pH to decrease because it is known that hydrogen peroxide treatment induces a significant intracellular acidosis.²² Treatment of HeLa cells with NEM also leads to slight fluorescence enhancement of both PTE donor and cyanine acceptor (Figure 8). It is well-known that GSH decrease can influence the function of Na^+/H^+ antiporter, leading to lower intracellular pH.²³ NEM is commonly employed to reduce GSH levels in live cells.^{24, 25} As a result, NEM treatment of HeLa cells acidify the cells.

Conclusions

We have prepared a TBET-based fluorescent cassette with AIE property consisting of TPE donor and cyanine acceptor through an ether bond connection. The cassette offers sensitive imaging of pH changes in live cells with dual visible and near-infrared excitation and emission capability. It can also detect intracellular pH fluctuations under treatment of HeLa cells with hydrogen peroxide or *N*-ethylmaleimide.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research reported in this publication was partially supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R15GM114751 (to H.Y. Liu) and the National Science Foundation through awards 1048655 (to H.Y. Liu).

Notes and references

1. Sun W, Guo SG, Hu C, Fan JL, Peng XJ. Chem Rev. 2016; 116:7768–7817. [PubMed: 27314280]
2. Manjare ST, Kim Y, Churchill DG. Acc Chem Res. 2014; 47:2985–2998. [PubMed: 25248146]
3. Bhattarai P, Dai ZF. Adv Healthc Mater. 2017; 6
4. Shindy HA. Dyes Pigment. 2017; 145:505–513.
5. Shindy HA. Mini-Reviews in Org Chem. 2012; 9:352–360.
6. Panigrahi M, Dash S, Patel S, Mishra BK. Tetrahedron. 2012; 68:781–805.
7. Shindy HA. Mini-Reviews in Organic Chemistry. 2012; 9:209–222.
8. Wiktorowski S, Rosazza C, Winterhalder MJ, Daltrozzo E, Zumbusch A. Chem Commun. 2014; 50:4755–4758.
9. Wang YF, Zhang TB, Liang XJ. Small. 2016; 12:6451–6477. [PubMed: 27592595]
10. Hong YN. Methods and Applications in Fluorescence. 2016; 4
11. Mei J, Hong YN, Lam JWY, Qin AJ, Tang YH, Tang BZ. Adv Mater. 2014; 26:5429–5479. [PubMed: 24975272]
12. Wang LY, Yang LL, Cao DR. Curr Org Chem. 2014; 18:1028–1049.
13. Kwok RTK, Leung CWT, Lam JWY, Tang BZ. Chem Soc Rev. 2015; 44:4228–4238. [PubMed: 25374162]
14. Ding D, Li K, Liu B, Tang BZ. Acc Chem Res. 2013; 46:2441–2453. [PubMed: 23742638]
15. Mei J, Leung NLC, Kwok RTK, Lam JWY, Tang BZ. Chem Rev. 2015; 115:11718–11940. [PubMed: 26492387]
16. McGrier PL, Solntsev KM, Miao S, Tolbert LM, Miranda OR, Rotello VM, Bunz UHF. Chem Eur J. 2008; 14:4503–4510. [PubMed: 18425736]
17. Frisch, MJ.Trucks, GW.Ortiz, JV.Cioslowski, J., Fox, DJ., editors. Gaussian 09. Gaussian, Inc.; Wallingford CT: 2009. 2009
18. Zhang JT, Yang M, Mazi W, Adhikari K, Fang MX, Xie F, Valenzano L, Tiwari A, Luo FT, Liu HY. ACS Sens. 2016; 1:158–165. [PubMed: 27547822]
19. Zhang JT, Yang M, Li C, Dorh N, Xie F, Luo FT, Tiwari A, Liu HY. J Mater Chem B. 2015; 3:2173–2184.
20. Vegesna GK, Janjanam J, Bi JH, Luo FT, Zhang JT, Olds C, Tiwari A, Liu HY. J Mater Chem B. 2014; 2:4500–4508.
21. Zhang SW, Adhikari R, Fang MX, Dorh N, Li C, Jaishi M, Zhang JT, Tiwari A, Pati R, Luo FT, Liu HY. ACS Sens. 2016; 1:1408–1415. [PubMed: 28845457]
22. Tsai KL, Wang SM, Chen CC, Fong TH, Wu ML. J Physiol (London). 1997; 502:161–174. [PubMed: 9234204]
23. Ciriolo MR, Palamara AT, Incerpi S, Lafavia E, Bue MC, DeVito P, Garaci E, Rotilio G. J Biol Chem. 1997; 272:2700–2708. [PubMed: 9006907]
24. Li YH, Wang YJ, Yang S, Zhao YR, Yuan L, Zheng J, Yang RH. Anal Chem. 2015; 87:2495–2503. [PubMed: 25635470]
25. Giustarini D, Galvagni F, Tesei A, Farolfi A, Zanoni M, Pignatta S, Milzani A, Marone IM, Dalle-Donne I, Nassini R, Rossi R. Free Radic Biol Med. 2015; 89:972–981. [PubMed: 26476010]

Figure 1. Fluorescence spectra of the cassette A at excitation of 350 nm (left) and 720 nm (right) in aqueous solution with different amounts of ethanol.

Figure 2. Absorption (left) and fluorescence (right) spectra of 5 μ M cassette A in buffers with different pH values containing 30% ethanol.

Figure 3. Fluorescence spectra of 5 μ M cassette **A** in buffers with different pH values containing 30% ethanol at excitation of 720 nm (left) and 350 nm (right).

Figure 4.

Acidic conditions: HOMO-1 (c and d), and LUMO (a and b) atomic orbitals; basic conditions: HOMO-1 (f and h), and LUMO (e and g) atomic orbitals. Carbon, hydrogen, oxygen, and nitrogen are indicated as grey, white, red, and blue, respectively.

Figure 5. Fluorescence response of cassette A to pH over metal ions (left) and cytotoxicity of cassette A (right).

Figure 6. Fluorescence images of HeLa cells incubated 5, 10, 15 and 20 μM cassette **A**, respectively. Images were taken by confocal fluorescence microscope at 60x magnification, scale bars = 50 μm .

Figure 7.

Fluorescence images of HeLa cells incubated with 20 μ M cassette **A** in buffers with different pH values of 5.0, 6.0, 7.0, 8.0 or 9.0 containing 5 μ g/mL nigericin. Images were collected by confocal fluorescence microscope at 60x magnification, scale bars = 50 μ m.

Figure 8.

Fluorescence images of HeLa cells incubated 10 μ M cassette **A** before and after further treatment of HeLa cells with 100 μ M hydrogen peroxide and 500 μ M *N*-ethylmaleimide (NEM), respectively. Images were taken by confocal fluorescence microscope at 60x magnification, scale bars = 50 μ m.

Scheme 1.

Fluorescent cassette and its structural response to pH change.

Scheme 2.
Synthetic route to prepare cassette A.