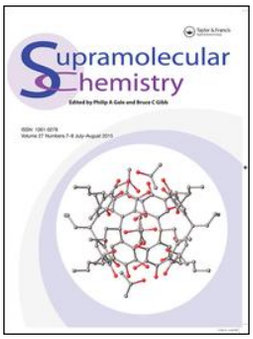


Supramolecular Chemistry

ISSN: 1061-0278 (Print) 1029-0478 (Online) Journal homepage: www.tandfonline.com/journals/gsch20

A novel fluorescent indicator displacement assay for sensing the anticancer drug gefitinib

Yun-Yun Xi, Qing Tang, Ying Huang, Zhu Tao, Sai-Feng Xue, Qingdi Zhou & Gang Wei

To cite this article: Yun-Yun Xi, Qing Tang, Ying Huang, Zhu Tao, Sai-Feng Xue, Qingdi Zhou & Gang Wei (2017) A novel fluorescent indicator displacement assay for sensing the anticancer drug gefitinib, Supramolecular Chemistry, 29:3, 229-235, DOI: 10.1080/10610278.2016.1202413

To link to this article: https://doi.org/10.1080/10610278.2016.1202413

SUPRAMOLECULAR CHEMISTRY, 2017
VOL. 29, NO. 3, 229–235
http://dx.doi.org/10.1080/10610278.2016.1202413

Taylor & Francis
Taylor & Francis Group

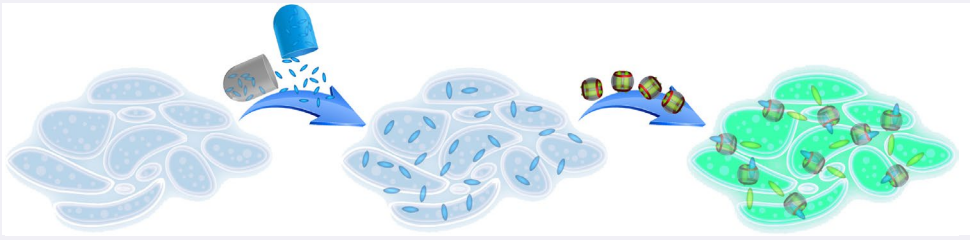
A novel fluorescent indicator displacement assay for sensing the anticancer drug gefitinib

Yun-Yun Xi[a,b], Qing Tang[a], Ying Huang[a,b], Zhu Tao[b], Sai-Feng Xue[b], Qingdi Zhou[c] and Gang Wei[d]

[a]The Engineering and Research Center for Southwest Bio-Pharmaceutical Resources of National Education Ministry of China, Guizhou University, Guiyang, P.R. China; [b]Key Laboratory of Macrocyclic and Supramolecular Chemistry of Guizhou Province, Guizhou University, Guiyang, P.R. China; [c]School of Chemistry, The University of Sydney, Sydney, Australia; [d]Commonwealth Scientific and Industrial Research Organization (CSIRO), Manufacturing, Lindfield, Australia

ABSTRACT

Fluorescent indicator displacement assays have become popular for converting synthetic receptors into optical sensors. We have now shown that a 1:2 host–guest complex between cucurbit[8]uril (Q[8]) and proflavine (PF) can be used as a fluorescent indicator for sensing the anticancer drug gefitinib. The 2PF@Q[8] complex can be used to detect gefitinib with high selectivity using fluorescence spectrometry with a detection limit of 6.63×10^{-8} mol^{-L}. The proposed sensing mechanism was investigated using ^{1}H nuclear magnetic resonance spectroscopy (^{1}H NMR), isothermal titration calorimetry and electrospray ionisation mass spectrometry. The 2PF@Q[8] complex was shown to be suitable for imaging gefitinib in prostate cancer (PC3) cells, which may help to elucidate relevant biological processes at the molecular level. We have developed a novel F-IDA to detect the anticancer drug GEF with high selectivity. The new indicator has excellent selectivity and a low detection limit for GEF. We have also demonstrated that the F-IDA can be used for the practical determination of drugs in living cells.



ARTICLE HISTORY
Received 4 March 2016
Accepted 12 June 2016

KEYWORDS
Cucurbit[8]uril; cell imaging; fluorescent indicator displacement assay; gefitinib

Introduction

In the field of supramolecular chemistry, indicator displacement assays (IDAs) have gained popularity for converting synthetic receptors into optical sensors. IDAs which utilise non-covalent interactions between the indicator or analyte and the host, have been used to sense different analytes, including metal cations and anions, drugs and other biological molecules. In fluorescent IDA assays (F-IDAs), the analyte competes with an indicator for the same binding site on the receptor. When the analyte is added to the indicator–receptor pair, the indicator is released from the receptor, inducing an absorbance and/or fluorescence change (*1*, *2*). Cucurbit[*n*]urils (Q[*n*]s) are synthetic receptors that are composed of glycoluril units linked through methylene bridges. These receptors have highly polar carbonyl portals with hydrophobic cavities and can form remarkably stable complexes with a variety of guest molecules (*3*, *4*). F-IDAs using Q[*n*]s which employ host–guest interactions between the indicator (*5*, *6*) (e.g. an isoquinoline alkaloid or other dye) or analyte and the receptor Q[*n*]s, have been used extensively for sensing or determining non-fluorescent or weakly fluorescent analytes, including ethambutol, sotalol, ranitidine and paraquat (*7*–*13*). This method, which converts Q[*n*]-indicator

CONTACT Ying Huang yhuang7@gzu.edu.cn; Gang Wei Gang.Wei@csiro.au

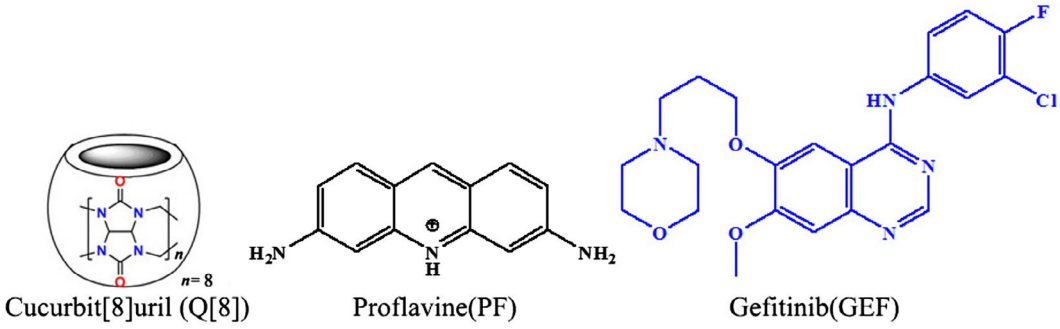


Figure 1. (Colour online) Structures of Q[8], proflavine and gefitinib.

complexes into optical sensors, has recently attracted widespread attention because of its simplicity, rapidity, sensitivity and selectivity.

Gefitinib (Iressa®, GEF) is an anticancer drug that is used in the treatment of locally advanced or metastatic non-small-cell lung cancer in patients who have previously received chemotherapy (*14*). To date, determination of GEF has mainly used high-performance liquid chromatography (HPLC), liquid chromatography–tandem mass spectrometry (HPLC/MS/MS), high-performance liquid chromatography with ultraviolet detection (HPLC-UV) and high-performance liquid chromatography-diode array detector (HPLC-DAD) methods (*15–19*). Because of their high sensitivity, these methods can be adapted to measure drug concentrations in biological media. GEF exhibits no fluorescence in aqueous solution, which makes its determination difficult using direct fluorescence measurement methods. There are numerous reports describing the formation of host–guest complexes between Q[*n*]s and tricyclic basic dyes which are accommodated inside the Q[*n*] forming supramolecular entities (*20, 21*). Proflavine (PF) is a tricyclic basic dye that forms a 1:2 host–guest complex with cucurbit[8]uril (Q[8]) leading to a decrease in fluorescence intensity of PF (*22, 23*). The fluorescence intensity of PF was quenched when Q[8] was added to a solution of PF and recovered when GEF was added to the Q[8]-PF mixture. Our experimental results show that complexation of Q[8] and PF with GEF provides a fluorescent indicator that has potential application in drug determination. We have developed an F-IDA by converting Q[8]-dye complexes into optical sensors that are able to recognise GEF. We then evaluated the ability of our newly developed method to sense GEF in cell imaging experiments (Figure 1).

Experimental

Materials

Q[8] was synthesised according to literature procedure (*24*) and characterised by ^{1}H NMR. PF was obtained from Sigma-Aldrich (Shanghai, China). GEF was obtained from Aladdin (Shanghai, China). All reagents were of analytical reagent grade and were used without further purification. Doubly distilled water was used. PC3 cells were obtained from Key Laboratory of Chemistry for Natural Products of Guizhou Province.

Measurement of absorption and fluorescence spectra

Absorption and fluorescence spectra of the host–guest complexes were recorded at 25 °C using an Agilent-8453 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) and a Varian Cary Eclipse spectrofluorometer (Varian, Inc., Palo Alto, CA, USA), respectively. Stock solutions of Q[8] (1×10^{-4} mol L^{-1}), PF (1×10^{-3} mol L^{-1}) and GEF (1×10^{-3} mol L^{-1}) were prepared in water. Working solutions were prepared by diluting the stock solutions to the required concentrations.

Aqueous solutions of Q[8]-PF complex (2PF@Q[8], 5.00×10^{-6} mol L^{-1}) were prepared for characterisation by absorption and fluorescence spectroscopy. For the fluorescence spectra, increasing concentrations (0–3×10^{-5} mol L^{-1}) of GEF solution were added to the 2PF@Q[8] complex. The photomultiplier gain was set at medium, with 5 nm emission and excitation bandwidths. The maximum excitation and emission wavelengths ($\lambda_{ex}/\lambda_{em}$) were 443 nm/511 nm for the complex of 2PF@Q[8] and GEF. For the absorption spectra, aqueous solutions of GEF (0–3×10^{-5} mol L^{-1}) were added to an aqueous solution of 2PF@Q[8], and the absorbance intensity was monitored at 200–600 nm at room temperature. For each experiment, three replicate measurements were taken.

^{1}H NMR measurements

To analyse the host–guest complexation of 2PF@Q[8] and GEF, 2PF@Q[8] in D_2O (1.0×10^{-4} mol^{-L}, 0.6 mL) was mixed with 1 equivalent of GEF, and the ^{1}H NMR spectrum was recorded at 25 °C using a W NMR-I 500 MHz NMR spectrometer (Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences).

ESI-MS measurements

Stock solutions of Q[8] (1×10^{-4} mol L^{-1}), PF (1×10^{-3} mol L^{-1}) and GEF (1×10^{-3} mol L^{-1}) were prepared in water. Working solutions were prepared by diluting the stock solutions to the required concentrations. The all samples were analysed using the Agilent 1100 LC/MSD Trap VL equipped with an ESI source. (Agilent Technologies Inc.). The ESI-MS spectra were acquired in positive ionisation modes over m/z 200–1800.

ITC measurements

A thermostatically controlled and fully computer-operated ITC (TA Instruments-Waters LLC , America) instrument was used for the microcalorimetric experiments. All microcalorimetric titrations were performed in aqueous solution at atmospheric pressure and 25 °C. Each solution was degassed and thermostatically controlled using a ThermoVac before the titration experiment.

Analytical application

Analytical parameters

To obtain the calibration curves, solutions of GEF (0–300 × 10^{-8} mol^{-L} were added to aliquots of concentrated stock solution in presence of the 2PF@Q[8] complex (5.00×10^{-6} mol L^{-1}) in aqueous solution. In accordance with the recommendations of the International Union of Pure and Applied Chemistry, measurements from a blank solution were recorded ($n \geq 20$) to determine the precision and limit of detection of the method.

Cytotoxicity assays and cell imaging

Cytotoxicity assays of 2PF@Q[8] complex in the PC3 cell line were performed using the CCK-8 method (*25*). PC3 cells were cultured at 37 °C in RPMI-1640 medium supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin. PC3 cells were plated in 96-well plates at a concentration of 8 000 cells per well and pre-incubated for 12 h before cytotoxicity assay. The cells were maintained in growth medium and treated with increasing concentrations (0, 5 and 10 μM) of 2PF@Q[8] complex for 24 h, cytotoxicity assays were performed by adding CCK-8 solution(10 μL) to each well and incubating for 2 h at 37 °C. Finally, the optical density (OD450) was measured using a plate reader (Multiskan Spectrum, Thermo Fisher, USA).

PC3 cells were seeded in a 12-well plate. After 24 h, the cells were incubated with 2PF@Q[8] (5 μM) for 30 min and then washed with RPMI-1640 and visualised using fluorescence imaging. PC3 cells were next incubated with GEF (5 μM) for 30 min, washed with RPMI-1640, and incubated at 37 °C with 2PF@Q[8] complex (5 μM). After incubation for 30 min, the cells were visualised using fluorescence imaging. Cell images were obtained using an IX-71 inverted fluorescence microscope (Olympus, Japan).

Results and discussion

F-IDA for sensing anticancer drug GEF

Aqueous solutions of GEF exhibit no fluorescence. When bound to Q[8] in aqueous solution, GEF formed a host–guest complex. The acridine dye, PF shows strong fluorescence, with a high fluorescence quantum yield (Φ_f = 0.35 ± 0.03), Binding to Q[8] to form a 2PF@Q[8] complex in aqueous solution causes a dramatic fluorescence quenching and reduction in fluorescence quantum yield (Φ_f = 0.18 ± 0.02). The fluorescence spectra of free PF, 2PF@Q[8] complex and 2PF@Q[8]+GEF in aqueous solution are shown in Figure 2(a). In the absence of Q[8], strong fluorescence was observed for free PF (black curve in Figure 2(a)). Upon addition of Q[8], PF was encapsulated in Q[8] to form a 2PF@Q[8] complex and the fluorescence was markedly decreased (red curve in Figure 2(a)). With the addition of GEF, the fluorescence was mostly recovered (blue curve in Figure 2(a)). The inset in Figure 2 shows photographs of free PF, 2PF@Q[8] complex and

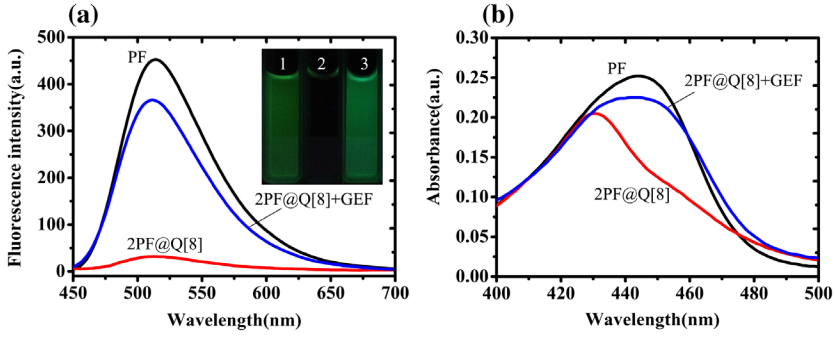


Figure 2. (Colour online) Fluorescence spectra (a) and absorption spectra (b) of free PF (10 μM. black curve), 2PF@Q[8] (5 μM, red curve) and 2PF@Q[8] (5 μM)+GEF (30 μM) (blue curve). Inset: Photographs of 1) PF, 2) 2PF@Q[8] and 3) 2PF@Q[8]+GEF upon excitation at 365 nm using an ultraviolet lamp.

2PF@Q[8] + GEF on excitation at 365 nm using an ultraviolet lamp. A green fluorescence was visible in the presence of GEF whereas no fluorescence was observed when only 2PF@Q[8] was present. The binding behaviour of 2PF@Q[8] towards GEF was further investigated by UV–vis absorption spectroscopy (Figure 2(b)). The absorption spectrum of 2PF@Q[8] showed a decrease in absorption at 444 nm with a violet shift from 444 to 430 nm. Addition of GEF increased the absorption and caused a red shift so that the absorption band resembled that of free PF. These results show that the 2PF@Q[8] complex can be used in an F-IDA to sense the anticancer drug GEF.

Selectivity

To investigate the selectivity of the 2PF@Q[8] complex, examples of ions found in biological systems, drug excipients and drug substances were added to the 2PF@Q[8] solution. Negligible changes in fluorescence were observed following addition of commonly occurring ions (Na^+, Cl^-, K^+, Fe^{3+}, Ca^{2+}, Zn^{2+}), drug excipients (glucose, fructopyranose, lactose, dextrin, starch, magnesium stearate, carboxymethyl cellulose codium, polyvinyl pyrrolidone(PVP) or drugs (sorafenib tosylate, nilotinib, dasatinib, sunitinib) (Figure 3). We also examined whether any of these substances interfere with the determination of GEF (Figure 1S in the Supporting Information). The presence of other analytes, even lapatinib (0.7 μM), did not substantially affect the detection of GEF, indicating that the assay shows excellent selectivity for GEF.

Quantification and sensitivity

Under the optimal experimental conditions described above, a linear correlation ($F = 0.42c + 38.18$, $R = 0.9940$) was observed between the fluorescence intensity and the concentration of GEF over the concentration range 0–300 × 10^{-8} mol^{-L} (Figure 4). The limit of detection of GEF was 6.63 × 10^{-8} mol^{-L}. The newly developed 2PF@Q[8] fluorescent indicator is, therefore, suitable for practical GEF detection.

Mechanism of GEF sensing by probe

^{1}H NMR was used to investigate the mechanism by which the fluorescent indicator 2PF@Q[8] senses GEF. In the ^{1}H NMR spectrum, the H_a, H_b and H_c protons on the acridine ring of PF showed upfield shifts in the Q[8] complex of ~0.4, ~0.6 and ~0.4 ppm, respectively (Figure 5). With the addition of 2.0 equivalent of PF to a Q[8] solution, the H_d proton on the acridine ring showed an upfield shift and merged into the 5–5.5 ppm region, compared with free PF (Figures 5(a) and 5 (b). These data suggest that Q[8] is able

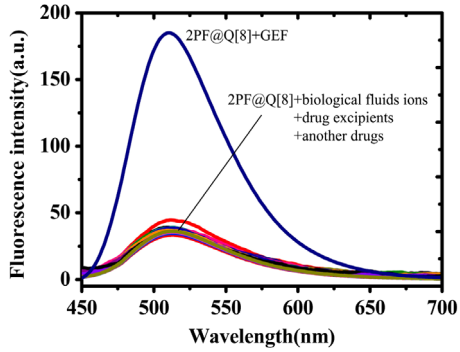


Figure 3. (Colour online) Fluorescence intensity of 2PF@Q[8] (5 μM) + GEF (5 μM) and 2PF@Q[8] (5 μM) with different metal ions (100-fold excess), drug excipients (100-fold excess) and drugs (10 μM).

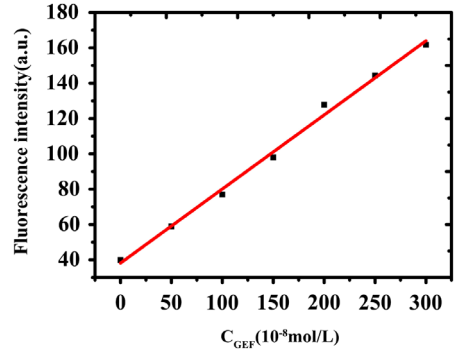


Figure 4. (Colour online) A plot of fluorescence intensity of 2PF@Q[8] (5 μM) with increasing concentrations (0–300 × 10^{-8} mol^{-L}) of GEF in aqueous solution at 511 nm.

to encapsulate two PF molecules to form a 1:2 (2PF@Q[8]) host–guest inclusion complex. When GEF underwent complexation with one equivalent of Q[8], the H_9 and H_{10} protons on the quinazoline ring and the H_{12}, H_{13} and H_{14} protons on the phenyl moiety of GEF showed upfield shifts Figures 5(e) and 5(d), suggesting that the quinazoline ring and phenyl moiety of the guest GEF were also entrapped in the cavity of the host and formed a 1:1 inclusion complex. (*26*) The supramolecular interactions of PF and GEF with Q[8] were further studied by ITC measurements. The results indicated that a 1:2 host–guest complex of PF and Q[8] and 1:1 host–guest complex of GEF and Q[8] were formed. The association constants of the Q[8]-PF and Q[8]-GEF complexes were (2.66 ± 0.62) × 10^6 L mol^{-1} and (3.01 ± 0.42) × 10^6 L mol^{-1} by independent model fitting, respectively (Figure 2S and Table 1S in the Supporting Information).

With the addition of GEF to a 2PF@Q[8] complex, the H_{12}, H_{13} and H_{14} protons on the phenyl moiety of GEF showed upfield shifts but the H_9 and H_{10} protons on the quinazoline moiety of GEF were not noticeably shifted compared with free GEF (Figure 5(c)). The H_a, H_b, H_c and H_d protons on the acridine ring of PF also exhibited upfield

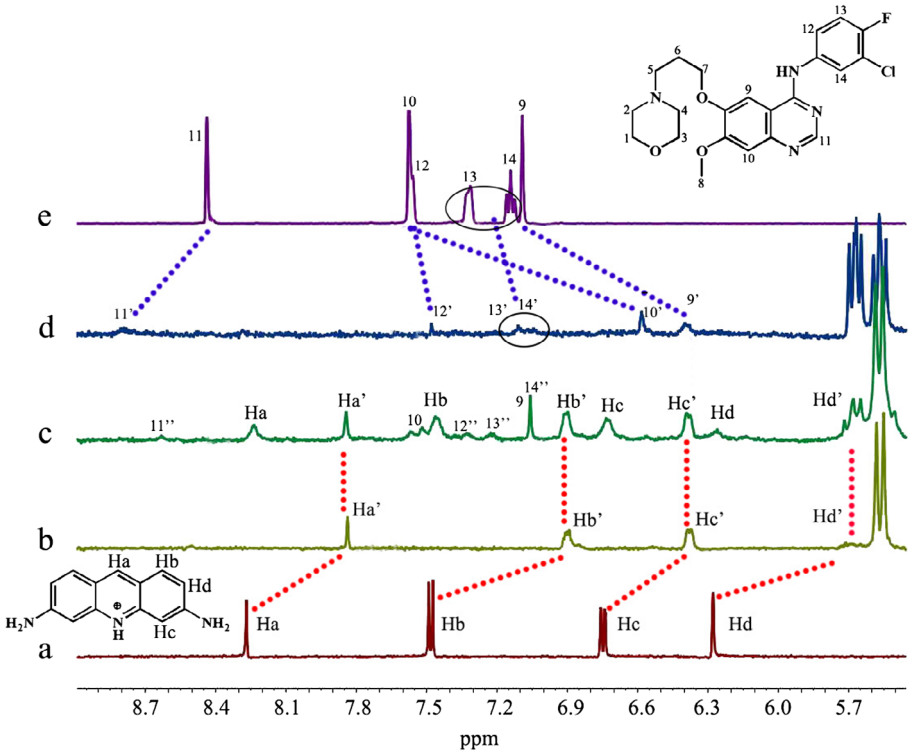
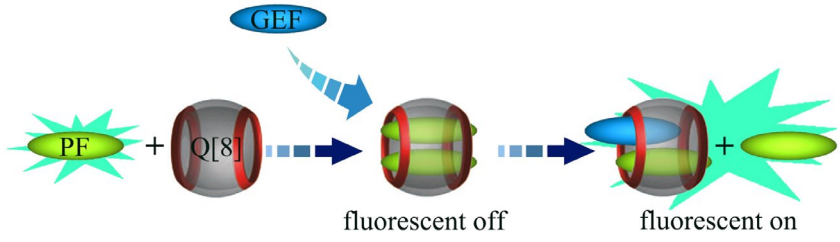


Figure 5. (Colour online) ^{1}H NMR spectra (500 MHz) of (a) PF, (b) Q[8]-PF(1:2), (c) Q[8]-GEF-PF (1:1:2), (d) Q[8]-GEF (1:1) and (e) GEF.



Scheme 1. (Colour online) Possible response mechanism based on interaction of Q[8] with PF and GEF.

shifts and the free H_a–H_d protons reappeared in these ternary complexes (Figure 5(c)). These results demonstrate that the dye molecules were expelled from the Q[8] cavity on addition of GEF and that a 1:1:1 ternary complexes of Q[8], PF and GEF was formed.

The mechanism by which F-IDA senses GEF was further studied by ESI-MS (Figure 3S in the Supporting Information). The ESI-MS spectrum showed doubly-charged species at m/z 888.9 for (Q[8]+GEF + 2H$^+$) of the GEF@Q[8] complex and at m/z 874.5 for (Q[8]+2PF + 2H$^+$) of the 2PF@Q[8] complex. After addition of GEF to the 2PF@Q[8] complex, doubly charged species at m/z 1004.1 for [Q[8]+GEF + PF + Na$^+$+H$^+$], triply charged species at m/z 675.7 for [Q[8]+GEF + PF + K$^+$+2H$^+$] and a peak at m/z 210.1 for [PF + H$^+$] were observed. The ESI-MS data also suggested that the dye molecules were expelled from the Q[8] cavity on introduction of GEF and that a 1:1:1 ternary complex between Q[8] and PF and GEF was formed. A possible mechanism for the interaction of Q[8] and PF with GEF is depicted in Scheme 1.

Application of F-IDA

To demonstrate the applicability of the 2PF@Q[8] complex for fluorescence imaging of GEF in living cells, the cytotoxicity of different concentrations of the 2PF@Q[8] complex in PC3 cells was firstly determined using the CCK-8 assay. Cell viabilities after incubation with 2PF@Q[8] complex (0, 5 and 10 μM) for 24 h are shown in Figure 4S PC3 cell viabilities >90% were observed throughout the test period, indicating low cytotoxicity of the 2PF@Q[8] complex.

We further studied the scope of the 2PF@Q[8] complex for imaging of GEF in living cells. When PC3 cells were incubated with 2PF@Q[8] complex (5 μM) for 30 min and then washed with RPMI-1640, a weak fluorescence was observed in the green channel (Figures 6(a) and 6(b)).

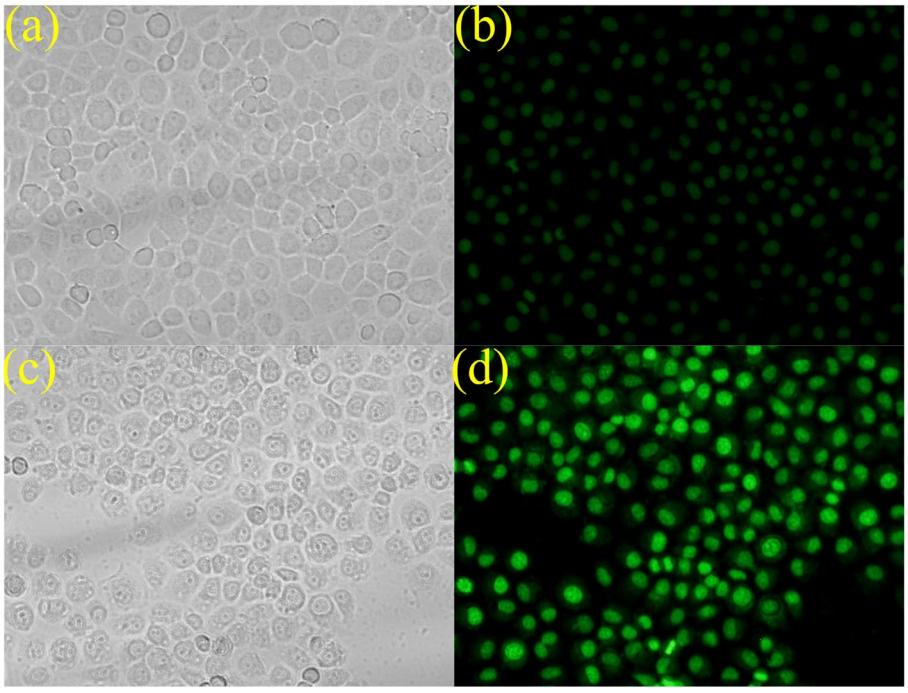


Figure 6. (Colour online) Fluorescence imaging obtained from PC3 cells: (a) brightfield imaging and (b) fluorescence imaging of cells treated with 2PF@Q[8] complex (5 μM); (c) brightfield imaging and (d) fluorescence imaging of 2PF@Q[8] (5 μM) in the presence of GEF (5 μM).

Moreover, when PC3 cells were incubated with GEF (5 μM) for 30 min, washed three times with RPMI-1640 and then incubated with the 2PF@Q[8] (5 μM) probe for 30 min, a strong green fluorescence was observed (Figures 6(c) and 6(d)). The 2PF@Q[8] complex was thus confirmed to be specific for GEF in living cells.

Conclusions

We have developed a novel F-IDA by converting cucurbit[8]uril–PF complexes into optical sensors to detect the anticancer drug GEF with high selectivity. The new indicator has excellent selectivity and a low detection limit for GEF. The proposed sensing mechanism was confirmed using ^{1}H NMR, ITC and ESI-MS. We have also demonstrated that the F-IDA can be used for the practical determination of drugs in living cells using appropriate imaging techniques.

Acknowledgement

The authors thank Dr Liu Yun at Guizhou Provincial College-based Key Lab for Tumor Prevention and Treatment with Distinctive Medicines of Zunyi Medical University for testing cytotoxicity assays.

Funding

This work has been supported by the National Natural Science Foundation of China [grant number 21202026]; the Natural Science Fund of the Science and Technology Department of Guizhou Province [grant number JZ-2014-2005]; the 'Chun-Hui' Fund of Chinese Ministry of Education [grant number Z2015004].

Supplemental material

Supplemental data for this article can be accessed online here: http://dx.doi.org/10.1080/10610278.2016.1202413

References

(1) Wiskur, S.L.; Ait-Haddou, H.; Lavigne, J.J.; Anslyn, E.V. *Acc. Chem. Res.* **2001**, *34*, 963–972.
(2) Nguyen, B.T.; Anslyn, E.V. *Coord. Chem. Rev.* **2006**, *250*, 3118–3127.
(3) Kim, K.; Selvapalam, N.; Ko, Y.H.; Park, K.M.; Kim, D.; Kim, J. *Chem. Soc. Rev.* **2007**, *36*, 267–279.
(4) Day, A.I.; Blanch, R.J.; Arnold, A.P.; Lorenzo, S.; Lewis, G.R.; Dance, I. *Angew. Chem., Int. Ed.* **2002**, *41*, 275–277.
(5) Praetorius, A.; Bailey, D.M.; Schwarzlose, T.; Nau, W.M. *Org. Lett.* **2008**, *10*, 4089–4092.
(6) Nau, W.M.; Ghale, G.; Hennig, A.; Bakirci, H.; Bailey, D.M. *J. Am. Chem. Soc.* **2009**, *131*, 11558–11570.
(7) Wang, G.Q.; Du, L.M.; Guo, Y.H.; Qin, Y.F.; Wang, J.W.; Wu, H. *Anal. Methods* **2013**, *5*, 173–179.
(8) Xing, X.Q.; Zhou, Y.Y.; Sun, J.Y.; Tang, D.B.; Li, T.; Wu, K. *Anal. Lett.* **2013**, *46*, 694–705.
(9) Yao, F.H.; Liu, H.L.; Wang, G.Q.; Du, L.M.; Yin, X.F.; Fu, Y.L. *J. Environ. Sci.* **2013**, *25*, 1245–1251.
(10) Wu, W.Y.; Yang, J.Y.; Du, L.M.; Wu, H.; Li, C.F. *Spectrochim. Acta Part A*. **2011**, *79*, 418–422.

(11) Zhang, H.M.; Yang, J.Y.; Du, L.M.; Li, C.F.; Wu, H. *Anal. Methods* **2011**, *3*, 1156–1162.
(12) Chang, Y.X.; Qiu, Y.Q.; Du, L.M.; Li, C.F.; Guo, M. *Analyst* **2011**, *136*, 4168–4173.
(13) Sun, S.G.; Li, F.S.; Liu, F.Y.; Wang, J.T.; Peng, X.J. *Sci. Rep.* **2014**, *4*, 1–5.
(14) Cohen, M.H.; Williams, G.A.; Sridhara, R.; Chen, G.; McGuinn, W.D.; Morse, D.; Abraham, S.; Rahman, A.; Liang, C.; Lostritto, R.; Baird, A.; Pazdur, R. *Clin. Cancer. Res.* **2004**, *10*, 1212–1218.
(15) Zhao, M.; Hartke, C.; Jimeno, A.; Li, J.; He, P.; Zabelina, Y.; Hidalgo, M.; Baker, S.D. *J. Chromatogr. B.* **2005**, *819*, 73–80.
(16) Faivre, L.; Gomo, C.; Mir, O.; Taieb, F.; Schoemann-Thomas, A.; Ropert, S.; Vidal, M.; Dusser, D.; Dauphin, A.; Goldwasser, F.; Blanchet, B. *J. Chromatogr. B.* **2011**, *879*, 2345–2350.
(17) Wang, L.Z.; Lim, M.Y.X.; Chin, T.M.; Thuya, W.L.; Nye, P.L.; Wong, A.; Chan, S.Y.; Goh, B.C.; Ho, P.C. *J. Chromatogr. B.* **2011**, *879*, 2155–2161.
(18) Zheng, X.; Hu, P.; Liu, J.; Zhao, Q.; Wang, M.Z.; Jiang, J.; Zhong, W. *Chromatographia* **2011**, *74*, 41–49.
(19) Fang, L.; Song, Y.; Weng, X.; Li, F.Z.; Xu, Y.P.; Lin, N.M. *Biomed. Chromatogr.* **2015**, *29*, 1937–1940.
(20) Montes-Navajas, P.; Garcia, H.J. *Photochem. Photobiol. A. Chem.* **2009**, *204*, 97–101.
(21) Baumes, L.A.; Sogo, M.B.; Montes-Navajas, P.; Corma, A.; Garcia, H. *Tetrahedron Lett.* **2009**, *50*, 7001–7004.
(22) Montes-Navajas, P.; Corma, A.; Garcia, H. *Chem. Phys. Chem.* **2008**, *9*, 713–720.
(23) Kemp, S.; Wheate, N.J.; Stootman, F.H.; Aldrich-Wright, J.R. *Supramol Chem.* **2007**, *19*, 475–484.
(24) Kim, J.; Jung, I.S.; Kim, S.Y.; Lee, E.; Kang, J.K.; Sakamoto, S.; Yamaguchi, K.; Kim, K. *J. Am. Chem. Soc.* **2000**, *122*, 540–541.
(25) Liu, Y.; Ding, X.W.; Li, J.H.; Luo, Z.; Hu, Y.; Liu, J.J.; Dai, L.L.; Zhou, J.; Hou, C.J.; Cai, K.Y. *Nanotechnology* **2015**, *26*, 1–14.
(26) Huang, Y.; Hu, Q.H.; Song, G.X.; Tao, Z.; Xue, S.F.; Zhu, Q.J.; Zhou, Q.D.; Wei, G. *RSC Adv.* **2014**, *4*, 3348–3354.