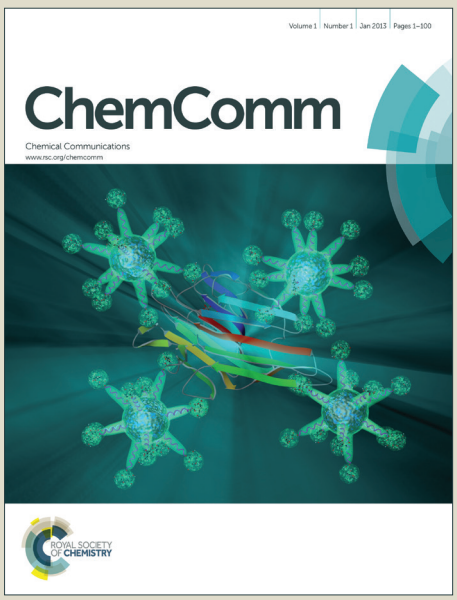
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Journal Name

RSCPublishing

COMMUNICATION

Fluorescent Probe for Specific Detection of Cysteine in Lipid Dense Region of Cells

Cite this: DOI: 10.1039/x0xx00000x

Received xxxxxxxxxx
Accepted xxxxxxxxxx

DOI: 10.1039/x0xx00000x

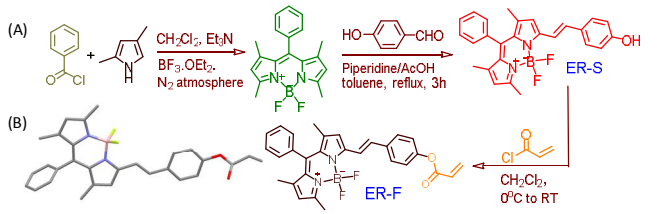
www.rsc.org/

Firoj Ali,[a] Anila H. A.,[a] Nandaraj Taye,[b] Rajesh G. Gonnade,[c] Samit Chattopadhyay,*[b] Amitava Das*[a]

A new Cysteine (Cys) specific chemodosimetric reagent (ER-F) is used in imaging of endogenous Cys localized in the lipid dense region of the live Hct116 cells and the release of Cys within HepG2 cells from a drug following a biochemical transformation. Silica surface, modified with ER-F, could be used for quantitative estimation of Cys present in aqueous solution (pH 7.2) and in human blood plasma (HBP).

For eukaryotic cells, Endoplasmic Reticulum (ER)ð a lipid dense region, plays a central role in biosynthesis of lipids and proteins.[1] Oxidized glutathione (GSH) and Cys are believed to participate in disulfide interchange reactions for ER-resident as well as newly made proteins.[2] It has been argued that the homeostasis of the redox state in the ER depends on the flux of small disulfides, secreted together with their reduced counterparts, primarily GSH and Cys, which are being released during the process of protein disulfide bond formation.[2] Cys is the important precursor for synthesis of GSH, which plays crucial roles in maintaining cellular antioxidant immune system.[3] Apart from these, Cys is also involved in various biological activities like cellular detoxification and metabolism.[4,5] A nuance in Cys concentration in cells or in HBP affect crucial biological processes. For example diseases like haematopoiesis, leucocyte loss, hair depigmentation caused by decreased Cys level,[5,6] while its elevated level is responsible for neurotoxicity, cardiovascular and Alzheimerôs diseases.[7] Thus, any reagent that allows specific detection and quantification of Cys in biological fluids as well as that allows imaging of endogenous Cys in live cells is of immense importance, as this has a direct relevance for diagnostic application. Such a reagent is even more significant if it is capable of detecting subtle changes in Cys distribution in ER, as this would help in probing protein modification in the ER through a conversion of Cys to formylglycine.[8] Among various analytical techniques, high-performance liquid chromatography (HPLC) with post column derivatisation and a spectrophotometric assay using 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB; Ellman's Reagent) are most common for estimation of Cys in bio-fluids or in protein residues.[9] Use of HPLC technique involves skilled manpower, expensive instrumentation and time consuming analysis process, while Ellmanôs reagent is sensitive to O_2/OH^- and produces strongly absorbing 4-nitrothiophenolate on reaction with various amino acids (AAs) and protein residues having sulfhydryl group. Thus, this reagent fails to delineate between Cys from Hcy/GSH or Cys/Hcy/GSH residue with free ï SH functionality present in a protein. Further, none of these two procedures is appropriate for imaging application. Some recent reports on chemodosimetric reagents with α,β-unsaturation reveal that such receptors fail to distinguish between Cys and the Cys residue in protein molecules having free sulfhydryl (-SH) group.[10] Receptors that work on the cleavage of -Sï S- or ï alkoxy bond cleavage, induced by ï SH group, fail to distinguish between Cys and Hcy/GSH.[11] Considering these limitations, a receptor that is specific for Cys and capable of showing instant fluorescence *ON* response is highly desirable. However such example remained elusive until recently, when Peng ***et al.*** reported a fluorescent probe for specific detection of Cys.[12] Also Strongin ***et al.*** and Yoon ***et al.*** developed an acrylate based reagent for Cys detection.[13]

Herein, we have described a new molecular probe **ER-F** that is specific towards Cys and capable of detection Cys localized in lipid dense region in live Hct116 cells as well as in bio-fluids like HBP samples. Moreover, this reagent could be used for imaging the release of Cys during metabolism of the drug N-acetyl cysteine in live HepG2 cells and for developing a test strip based technique for quantitative estimation of Cys in HBP.



Scheme 1: (A) Methodologies adopted for the synthesis of **ER-F**; (B) Single-crystal X-ray structure of probe **ER-F** [Hydrogen atoms are omitted for clarity].†

Methodology for synthesis of this new chemodosimetric reagent **ER-F** is shown in Scheme 1. Analytical as well as spectroscopic data confirmed the proposed molecular structure and desired purity of **ER-F**.[Å] Single crystal X-ray structure for **ER-F** also confirmed its proposed molecular structure. All studies were performed in aqueous HEPES-CH_3CN (9:1, v/v) medium at physiological pH 7.2, unless mentioned otherwise.

Electronic spectrum recorded for **ER-F** showed three distinct band maxima at ~335, ~535 and ~575 nm in aq. buffer medium.[Å] Band at ~575 nm and 535 nm were attributed to the 0-0 and 0-1 vibrational band respectively, of a strong S_0-S_1 transition.[14] Relatively weak and shorter wavelength bands (< 400 nm) were assigned for the S_0-S_2 and S_0-S_3 transitions. Modest detectable shifts to longer wavelength were observed for bands at ~535 and ~575 nm on increase in solvent polarity. This confirmed some ICT nature of these transitions with alkoxy/phenoxy moiety for **ER-F**/**ER-S** as donor and BODIPY core as acceptor.[Å] Two weak emission bands at 565 and 610 nm were observed ($\Phi_{\lambda Ext}^{530nm}$ = 0.004,[Å] Φ: integrated quantum yield) on excitation at 530 nm. While, a weak emission band was observed at 610 nm ($\Phi_{\lambda Ext}^{575nm}$ = 0.001) on excitation at 575 nm. Shifts of these emission bands to longer wavelength were relatively more prominent than that was observed for electronic spectra, which suggested that ICT states became more prominent in their excited state.[15]

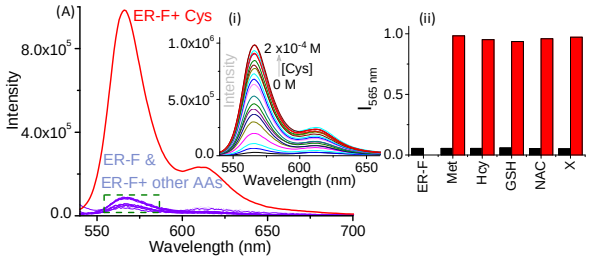
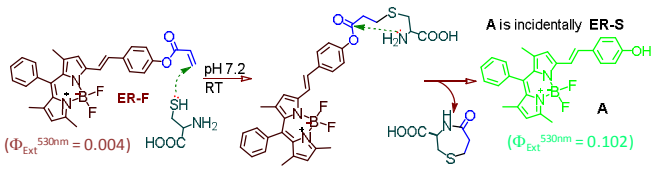


Fig 1: (A) Change in emission spectrum of **ER-F** (10 μM) in absence and presence of different AAs (0.2 mM; AA: Cys, Met, Hcy, GSH, N-acetyl cysteine (NAC) and X (X = His, Leu, Phe, Try, Tyr, Val , Ala, Arg, Gly, Glu, Pro, Ser, Asp, Glu, Thr, Iso & Lys)); Insets: (i) emission titration profile for **ER-F** (10 μM) with varying [Cys] (0-200 μM) and (ii) changes in emission intensity of **ER-F** (10 μM) induced by Cys (50 μM) in presence of large excess (0.2 mM) of other AAs like Met, Hcy, GSH, NAC and X. Red bar and black bar represent emission response in presence and absence of Cys, respectively. Studies were performed in aq. HEPES buffer-CH_3CN (9:1, v/v; pH 7.2) medium; $\lambda_{Ext}/\lambda_{Em}$: 530/565 nm.

In order to explore the potential bio analytical application of the probe **ER-F**, we checked emission responses of **ER-F** (10 μM) in presence of 20 mole equivalence of various AAs and certain anionic analytes (e.g. CN^-, SCN^- etc.) in an essentially aq. buffer medium (Fig. 1).[Å] No detectable change in emission spectrum for **ER-F** was observed for all these analytes that we had used for this study, except Cys. An apparent switch *ON* emission response was observed for Cys with maxima at 565 ($\Phi_{\lambda Ext}^{530nm}$ = 0.102) nm. Literature reports reveal that for unsubstituted BODIPY,[16] band around 610 nm is absent and this further confirms the ICT nature of this transition. Interference studies were performed by recording emission spectra of **ER-F** (10 μM) with Cys (50 μM) in presence of large excess (200 μM) of other AAs and anions under identical condition (Fig. 1). Results of such studies confirmed insignificant interference from these analytes mentioned. A close look at the ESI-Ms data tends to suggest that reagent **ER-F** reacts with Cys to produce **ER-S**. To confirm this, we further isolated and purified the product of the reaction between **ER-F** and Cys. ^{1}H NMR and ESI-Ms spectral data of the isolated product clearly confirmed the formation of **ER-S**.[Å] Importantly, electronic and emission spectra for this pure isolated product was found to be identical with **ER-S** in aq. buffer medium and this further corroborated our proposition. Nucleophilic conjugate addition reaction involving free sulfhydryl group of Cys moiety was anticipated with α,β-unsaturated ester functionality of **ER-F** to yield an intermediate, which underwent an intramolecular cyclization reaction to eliminate (R)-5-oxo-1,4-thiazepane-3-carboxylic acid and regenerate **ER-S** and accounted for the luminescence *ON* response (Scheme 2).



Scheme 2. Proposed mechanistic pathway for the reaction between the reagent **ER-F** and Cys (Φ values are reported at 565 nm).

A linear dependency of the pseudo first order rate (k_{obs}) constants (k_{obs} = k_c[Cys] + c, where k_c: rate constant for the overall reaction) and c is an intercept; Fig. 2A) on [Cys] was observed by monitoring luminescence changes at 565 nm (λ_{Ext}: 530 nm) (Fig. 2A).[Å] This clearly suggested that the rate determining step for this reaction involved Cys. Negligible intercept also suggested absence of any detectable side reaction that could contribute to this observed luminescence changes.

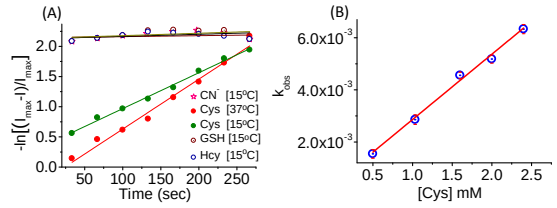


Fig. 2: (A) Representative (-ln(I_{Max}-I) / I_{Max}] vs. time) plots for evaluation of k_{obs} for **ER-F** (10 μM) & Cys/Hcy/GSH/CN^- (2 mM) at specified temperature and these plots clearly revealed no change in solution luminescence for Hcy/GSH/CN^- was observed; (B) plot of k_{obs} vs. [Cys] for evaluating the overall rate constant for the reaction between **ER-F** and Cys. All kinetic studies were performed in aq HEPES-CH_3CN (9:1, v/v; pH 7.2) medium & $\lambda_{Ext}/\lambda_{Em}$: 530/565 nm.

This helped us in evaluating k_c as 2.51x10^{-3} s^{-1} at 15°C. These results confirmed that the chemical transformation associated with the luminescence enhancement at 565 nm, involved Cys in the slow step of the reaction and led to the generation of **ER-S** with much improved Φ value.

Hcy, GSH and NAC are most common analytes which interfere with Cys recognition that involve free -SH functionality. For the present study, Hcy, GSH and N-acetyl cysteine (NAC) failed to participate in such reaction presumably due to their unfavourable pK_a values.[Å10g,17] Interestingly, no change in fluorescence for **ER-F** was observed on addition of Bovine serum albumin (BSA), a protein molecule that has free sulfhydryl$_{Cys}$ group. Cys residue in BSA lacks the free ï NH_2 functionality and thus falils to participate

in the intramolecular cyclization reaction for regeneration of **ER-S**. Thus, above discussions confirmed that the fluorescence *ON* response at ~ 565 and ~ 610 nms could only be achieved for reaction between **ER-F** and Cys havingï SH and ï NH_2 moieties (Scheme 2). Feasibility of such a mechanistic pathway is also discussed in earlier reports.[13,18] Apart from unfavourable pK_a, this could have also contributed to the inactivity of NAC towards the reagent **ER-F**.

Good linear fit of the B-H plot, obtained from the data available from the emission titration profile, confirmed a 1:1 reaction stoichiometry for the reaction between **ER-F** and Cys.[Å] Further, pH studies confirmed that the probe **ER-F** was stable for the pH range 4.5 ï 9.0 and this supported the basis for performing all our studies in physiologically relevant pH (7.2).[Å] Lowest detection limit was evaluated as 15 nM.[Å] The concentration level of Cys in HBP sample of a healthy person is typically in the range of 240ï 360 μM.[7b,19] Considering this, reagent **ER-F** is sensitive enough for analysis of Cys in real HBP sample and this led us to explore such a possibility. Diluted HBP (200 μl) was added to the reagent **ER-F** (10 μM) solution (aq. HEPES-CH_3CN medium (9:1, v/v; pH 7.2) at RT and emission measurements were performed after the solution mixture was allowed to equilibrate for 2 min.

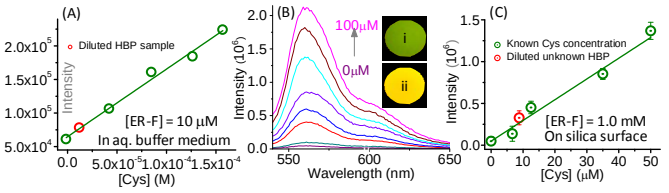


Fig. 3: (A) and (C) Calibration plots for emission intensity vs. [Cys] for **ER-F** (λ_{Mon} = 565 nm & λ_{Ext} = 530 nm) in presence of known (**O**) [Cys] and (**O**) unknown [Cys] in HBP sample; (B) solid state emission spectra for silica surface modified with **ER-F** (0.1 mM) and exposed to varying [Cys], Insets: snap shot of visually detectable change in luminescence of the silica surface modified with **ER-F** on irradiation with 365 nm light in (i) absence and presence all other AAs (except Cys) & NAC and in (ii) presence of on Cys; aq. HEPES buffer-CH_3CN (9:1, v/v; pH 7.2) solution was used for studies in (A) and Cys solution in pure aq. HEPES buffer medium was used for studies described in (B) and (C).

Emission intensity of such solution was measured and $[Cys]_{Free}^{HBP[dilute]}$ was evaluated by comparing observed intensity with the standard calibration curve (Fig. 3A & 3C). This on multiplication with the appropriate dilution factor, revealed the exact $[Cys]_{Free}^{HBP}$ ((310 ± 4) μM) in HBP sample[Å] and this value was within the allowed limit for a healthy human being.[7b]

Possibility of developing test strip for detection of Cys was also explored. Acetonitrile solution of **ER-F** was drop casted on silica TLC strips and was dried at 35°C.[Å] These plates were exposed to different AAs (100 μM) solution (aq. HEPES buffer, pH 7.2). Results clearly revealed (Fig. 3B: inset) that the plate exposed to Cys showed visually detectable yellow luminescence, while no such change was observed for all other AAs and NAC. Interestingly, luminescence spectra recorded for such strips dipped in pure aq. buffer solutions (pH 7.2) of varying [Cys] helped us in developing a calibration plot (Fig. 3B & 3C), which could be utilized for quantitative estimation of Cys in an HBP sample.[Å] Thus, this result confirmed that this reagent could be utilized for developing test strip for qualitative and quantitative estimation of Cys in HBP samples.

MTT assay studies confirmed insignificant toxicity of the reagent **ER-F** towards Hct116 cells.[Å] This encouraged us to explore the option of using this reagent for mapping endogenous Cys in Hct116 cells. Detailed procedure for MTT assay and imaging studies using CLSM are discussed in supporting information section. It is evident from Fig. 4(IB) & 4(IG) that the bright-red fluorescence images were observed for Hct116 cells that were treated only with **ER-F** (1 μM), which confirmed that the intracellular Cys reacted with probe **ER-F** and accounted for the formation of **ER-S**. To confirm this, further experiments were performed. Hct116 cells were washed thoroughly after pre-treated with excess NEM (1 mM), an effective thiol blocker, and then these cells were exposed to the reagent **ER-F**. Quenched intracellular red fluorescence (Fig. 4(IE) & 4(IH)) clearly established that this was arose solely due to the chemodosimetric reaction between endogenous Cys and **ER-F**.

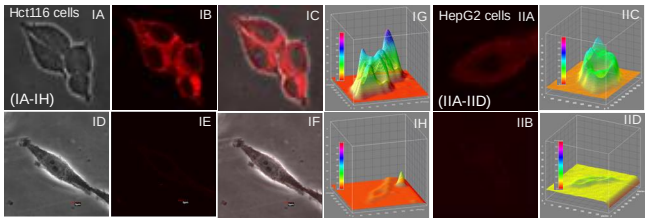


Fig. 4: (I) CLSM images of Hct116 cells (IA-IH), cells incubated with 1 μM of **ER-F**, (IA): bright field images, (IB): dark field image and (IC): overlay images of (IA) & (IB); Cells pre-treated with 1 mM of NEM then incubated with 1 μM of **ER-F**, (ID): bright field image, (IE): dark field image, (IF): overlay of (ID) & (IE). (IG) and (IH) were 3D intensity plot of (IB) & (IE) respectively; (II) CLSM images of live HepG2 cells: (IIA) & (IIB) dark field images of cells treated with 1 mM NEM, washed and then treated with (IIA) or without (IIB) 25 μM NAC for 1h, followed by incubation of 1 μM of **ER-F** for 20 min and (IIC) & (IID) are respective 3D intensity profile plot of images (II)A & (II)B. (II)A & (II)C revealed the generation of Cys from NAC by aminoacylase-I present in HepG2 cells, $\lambda_{Ext}/\lambda_{Em}$: 530/573 nm.

To explore the suitability of the reagent **ER-F** for probing any intracellular enzymatic transformations, HepG2 cells with higher abundance of aminoacylase-I were exposed to NAC, a well known drug used as Cys supplement. HepG2 cells were first pre-treated with NEM and after thorough washing; cells were further exposed to NAC. Red fluorescence (Fig. 4(IIA & IIC)) of these HepG2 cells exposed to NAC and the complete absence of any intracellular red fluorescence in control experiment (Fig. 4(IIB & IID)) clearly confirmed the generation of Cys from NAC by enzymatic action of aminoacylase-I.

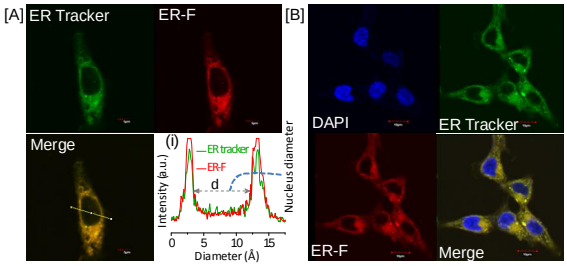


Fig. 5: (A) Hct116 cells stained with 1 μM of **ER-F** in presence of ER tracker green; (i) Intensity profile of ROIs across cells: red line represent intensity of **ER-F** and green line indicate intensity for ER Tracker green; (B) co-localization experiment:

Cells were co-stained with **ER-F**, ER tracker green and DAPI; $\lambda_{Ext}/\lambda_{Em}$: 530/573 nm.

CLSM images shown in Fig. 5 reveal that this reagent **ER-F** as well as the product (**ER-S**) is preferentially localized in the lipid dense region, Endoplasmic Reticulum (ER) of Hct116 cells. Results of the co-localization studies with ER-tracker green (ER-specific reagent) and DAPI (nuclei specific reagent, with little or no cytoplasmic labelling) as well as the high Pearsonôs co-localization coefficient of 0.9682 for this co-localization study confirmed this (Fig. 5). Thus, this reagent offered us an option of mapping endogenous Cys in the ER region of Hct116 cells. To the best of our knowledge such reports are scarce.

In summary, we have developed a new chemodosimetric imaging probe **ER-F** for detection of spatial and temporal distribution of Cys as well as its *in-situ* generation during the enzymatic action of aminoacylase-I in NAC in ER region of live HepG2 cells. Interference studies confirmed specificity of this reagent towards Cys among all other AAs, including Hcy, GSH and NAC. Notably this reagent could even be used for developing a modified silica strip for qualitative and quantitative estimation of Cys present in human bio-fluid like HBP without any interference from other derivatives of cysteine (like NAC, BSA). Example of such a versatile Cys-specific regent is rare in contemporary literature.

A.D. acknowledges SERB (India) Grant (SB/S1/IC-23/2013) and CSIR-NCL (MLP 028226) for funding. F.A, N.T & AHA acknowledge CSIR & UGC for their research fellowships.

Notes and References

[a]Organic Chemistry Division, CSIR-National Chemical Laboratory, Pune-411008, India; E-mail: a.das@ncl.res.in; Tel: +91 2025902385.

[b]Chromatin and Disease Biology Lab; National Centre for Cell Science; Pune 411007, India, Email: samit@nccs.res.in.

[c]Center for Materials Characterization, CSIR-National Chemical Laboratory, Pune-411008, India.

Electronic Supplementary Information (ESI) available: Invention disclosure no.: 2015-INV-0021. Details of synthesis, X-ray crystal data for **ER-F**, CCDC: 1420800, photophysical studies, imaging studies. See DOI: 10.1039/c000000x/

1. (a) J. C. Bardwell, K. McGovem and J. Beckwith, *Cell*, 1991, **67**, 581-589; (b) M.-J. Gething and J. Sambrook, *Nature*, 1992, **355**, 33-35; (c) M. LaMantia and W. Lennarz, *Cell*, 1993, **74**, 899-908.
2. (a) C. Hwang, A. J. Sinskey and H. F. Lodish, *Science*, 1992, **257**, 1496-1502; (b) S. Carelli, A. Ceriotti, A. Cabibbo, G. Fassina, M. Ruvo and R. Sitia, *Science*, 1997, **277**, 1681-1684.
3. (a) R. O. Ball, G. Courtney-Martin and P. B. Pencharz, *J. Nutr*., 2006, **136**, 1682S-1693S; (b) V. I. Lushchak, *J. Amino Acids*, 2012, doi:10.1155/2012/736837.
4. C. E. Paulsen and K. S. Carroll, *Chem. Rev*., 2013, **113**, 4633ï 4679.
5. K. G. Reddie and K. S. Carroll, *Curr. Opin. Chem. Biol*., 2008, **12**, 746ï 754.
6. (a) E. Weerapana, C. Wang, G. M. Simon, F. Richter, S. Khare, D. M. B. D. Dillon, D. A. Bachovchin, K. Mowen, D. Baker and B. F. Cravatt, *Nature*, 2010, **468**, 790ï 795; (b) D. M. Townsend, K. D. Tew and H. Tapiero, *Biomed. Pharmacother*., 2003, **57**, 145-155; (c) S. Shahrokhian, *Anal. Chem*., 2001, **73**, 5972 - 5978.
7. (a) X. F. Wang and M. S. Cynader, *J. Neurosci*., 2001, **21**, 3322-3331; (b) U. G. Reddy, H. Agarwalla, N. Taye, S. Ghorai, S. Chattopadhyay and A. Das, *Chem. Commun.,* 2014, **50**, 9899-9902.
8. T. Dierks, B. Schmidt and K. V. Figura, *Proc. Natl. Acad. Sci*., 1997, **94**, 11963ï 11968.
9. (a) P. W. Riddles, R. L. Blakeley and B. Zerner, *Methods Enzymol.*, 1983, **91**, 49-60; (b) N. Ercal, P. Yang and N. Aykin, *J. Chromatogr. Biomed. Appl.,* 2001, **753**, 287-292; (c) S. D. Fei, J. H. Chen, S. Z. Yao, G. H. Deng, D. L. He and Y. F. Kuang, *Anal. Biochem*., 2005, **339**, 29-35; (d) T. Inoue and J. R. Kirchhoff, *Anal. Chem*., 2002, **74**, 1349-1354; (e) Y. Sato, T. Iwata, S. Tokutomi and H. Kandori, *J. Am. Chem. Soc*., 2005, **127**, 1088-1089; (f) X. Guan, B. Hoffman, C. Dwivedi and D. P. Matthees, *J. Pharm. Biomed. Anal*., 2003, **31**, 251-261.
10. (a) L. Yi, H. Li, L. Sun, L. Liu, C. Zhang and Z. Xi, *Angew. Chem. Int. Ed*., 2009, **48**, 4034-4037; (b) X. Chen, S.-K. Ko, M. J. Kim, I. Shin and J. Yoon, *Chem. Commun*., 2010, **46**, 2751-2753; (c) X. Zhou, X. Jin, G. Sun and X. Wu, *Chem. Eur. J*., 2013, **19**, 7817-7824; (d) H. S. Jung, K. C. Ko, G.-H. Kim, A.-R. Lee, Y.-C. Na, C. Kang, J. Y. Lee and J. S. Kim, *Org. Lett*., 2011, **13**, 1498-1501; (e) V. Hong, A. A. Kislukhin and M. G. Finn, *J. Am. Chem. Soc*., 2009, **131**, 9986-9994; (f) L. E. Santos-Figueroa, M. E. Moragues, E. Climent, A. Agostini, R. Martĕnez-Ma´nẽez and F. Sanceno´n, *Chem. Soc. Rev*., 2013, **42**, 3489-3613; (g) H. A. Anila, U. G. Reddy, F. Ali, N. Taye, S. Chattopadhyay and A. Das, *Chem. Commun*., 2015, DOI: 10.1039/C5CC04876A.
11. (a) S. R. Malwal, A. Labade, A. S. Andhalkar, K. Sengupta and H. Chakrapani, *Chem. Commun*., 2014, **50**, 11533-11535; (b) B. Zhu, X. Zhang, Y. Li, P. Wang, H. Zhangb and X. Zhuang, *Chem. Commun*., 2010, **46**, 5710-5712; (c) R. R. Nawimanage, B. Prasai, S. U. Hettiarachchi and R. L. McCarley, *Anal. Chem*., 2014, **86**, 12266ī 12271.
12. H. Li, J. Fan, J. Wang, M. Tian, J. Du, S. Sun, P. Sun and X. Peng, *Chem. Commun*., 2009, 5904-5906.
13. (a) X. Yang, Y. Guo and R. M. Strongin, *Angew. Chem. Int. Ed*., 2011, **50**, 10690-10693; (b) Z. Guo, S. W. Nam, S. Park and J. Yoon, *Chem. Sci*., 2012, **3**, 2760ï 2765.
14. R. Hu, E. Lager, A. Aguilar-Aguilar, J. Liu, J. W. Y. Lam, H. H. Y. Sung, I. D. Williams, Y. Zhong, K. S. Wong, E. Penĕa-Cabrera and B. Z. Tang, *J. Phys. Chem. C*, 2009, **113**, 15845ï 15853.
15. M. Baruah, W. Qin, C. Flors, J. Hofkens, R. A. L. Valle´e, D. Beljonne, M. V. d. Auweraer, W. M. De Borggraeve and N. Boens, *J. Phys. Chem. A,* 2006, **110**, 5998-6009.
16. (a) M. Emrullahoĵ lu, M. Üçüncü and E. Karak, *Chem. Commun.,* 2013, **49**, 7836-7838; (b) S. Saha, H. Agarwalla, H. Gupta, M. Baidya, E. Suresh, S. K. Ghosh and A. Das, *Dalton Trans*., 2013, **42**, 15097ï 15105.
17. C. A. S. Regino and D. E. Richardson, *Inorg. Chim. Acta*, 2007, **360**, 3971ï 3977.
18. (a) Q. Zhang, D. Yu, S. Ding and G. Feng, *Chem. Commun*., 2014, **50**, 14002-14005; (b) B. Liu, J. Wang, G. Zhang, R. Bai and Y. Pang, *ACS Appl. Mater. Interfaces*, 2014, **6**, 4402ī 4407.
19. (a) P. Das, A. K. Mondal, N. B. Chandar, M. Baidya, H. B. Bhatt, B. Ganguly, S. K. Ghosh and A. Das, *Chem. Eur. J*., 2012, **18**, 15382-15393; (b) S. Sreejith, K. P. Divya and A. Ajayaghosh, *Angew. Chem., Int. Ed*., 2008, **47**, 7883-7887.

