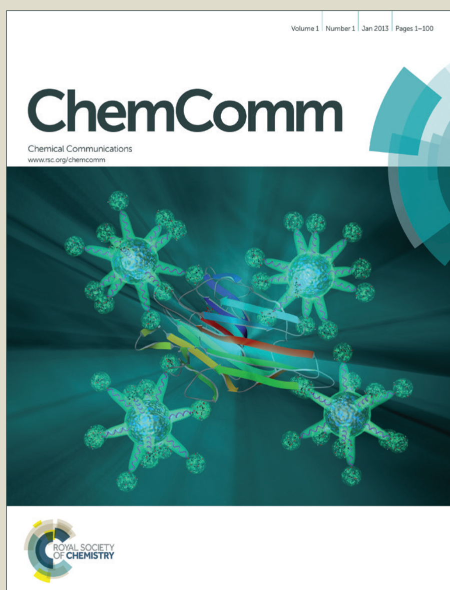
ChemComm

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

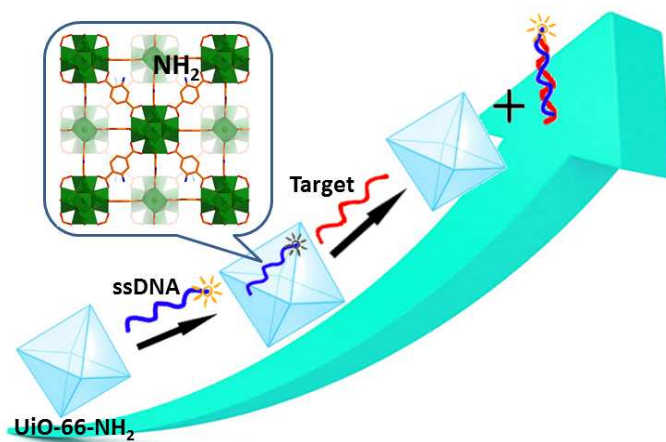
Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Graphical Abstract

Amine-functionalized metal-organic framework (MOF) as a DNA sensing platform, with possible hydrogen bond interaction between DNA and MOF, has been developed.



ChemComm Accepted Manuscript

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Amine-functionalized metal-organic framework as a sensing platform for DNA detection

Hao-Tian Zhang,[a] Jian-Wei Zhang,[ab] Gang Huang,[a] Zi-Yi Du[b] and Hai-Long Jiang*[a]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

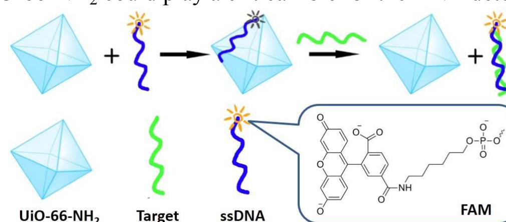
DOI: 10.1039/b000000x

An amine-functionalized metal-organic framework (MOF) has been employed as an effective fluorescent sensing platform for DNA detection and is capable of distinguishing complementary and mismatched target sequences with high sensitivity and selectivity.

The detection of DNA sequences is of particular interest and importance in genetics, pathology, criminology, pharmacogenetics, food safety, and so on.[1] The polymerase chain reaction (PCR) method suffers from high cost, risk of contamination, and false-negative results, although it is well-known for DNA amplification and sequencing and has extensive application in modern biological and medical sciences.[2] Another mature technique of gene chip is widely employed for high-throughput DNA detection, while it requires high-cost instrumentation for fluorescent signal readout and sophisticated numerical algorithms for data explanation.[3] Therefore, it is necessary to develop simple, rapid, sensitive and cost-effective approaches for this purpose. In recent years, lots of endeavors have been devoted to developing homogeneous fluorescence assays, most of which are based on fluorescence resonance energy transfer (FRET) via fluorophore-quencher pairs for the detection of DNA sequences, including carbon nanostructures with different forms, Au nanoparticles (NPs) and other nanomaterials.[4] Although they have been proven to be effective fluorescent platforms, the respective drawbacks limit their practical use; the preparation of the detection agent in many systems is time-consuming, tedious or labor-intensive, and some of them cannot be prepared on a large scale or suffer from stability issues.[4a-c,5]

On the other hand, metal-organic frameworks (MOFs), constructed by metal ions/clusters and organic linkers, are a class of crystalline porous materials.[6] In recent two decades, MOFs have captured widespread research interest due to their intriguing structural topologies and potential applications as functional materials in wide fields.[7-10] Particularly, MOF have been demonstrated to be fluorescent sensors for the detection and recognition of various cations, anions, vapors and small molecules based on their fluorescence response.[10] However, to the best of our knowledge, very rare MOFs have been studied for the detection of DNA or biomolecules.[11]

The organic linkers involved in MOFs usually have conjugated π-electron system and offer source for possible hydrogen bonds that allow suitable interaction between MOF and single-stranded DNA (ssDNA). Therefore, MOFs could be reasonably able to recognize DNA molecules via fluorescence change, similar to previously reported detection agents. In this work, we have developed an amine-functionalized MOF, UiO-66-NH_2 (UiO = University of Oslo), as an efficient biosensor for the detection of DNA with high selectivity. The principle for this assay is proposed in Scheme 1. The free ssDNA with a fluorophore (FAM) at its 5' end has strong fluorescence emission at 518 nm (λ_{ex} = 480 nm). The electrostatic attraction such as π…π stacking or hydrogen bond interactions between aromatic nucleotide bases in the ssDNA and UiO-66-NH_2 allows them to attach or close proximity together, which results in the substantial fluorescence quenching of FAM (off-state), possibly due to the photoinduced electron transfer.[12] The absorption spectrum of MOF dispersed in Tris-HCl buffer (pH: 7.42) exhibits two absorption peaks at 217 nm and 328 nm (Fig. S3), suggesting that there is no spectra overlap and thus no FRET occurs between MOF and ssDNA. Upon the introduction of target ssDNA (tDNA) into the system, double-stranded DNA (dsDNA) detaches from UiO-66-NH_2. As a result, the energy transfer process is inhibited and the fluorescence of ssDNA is recovered (turn-on state). It is proposed that hydrogen bond interaction between ssDNA and amino group in UiO-66-NH_2 could play a critical role for the DNA detection.



Scheme 1 Proposed principle for the fluorophore-labeled DNA detection by a MOF, UiO-66-NH_2, as a sensing platform.

The UiO-66-NH_2 is constructed by Zr^{IV} and 2-amino-1,4-benzenedicarboxylic acid (NH_2-BDC) with great chemical and thermal stability. The framework built up from $Zr_6O_4(OH)_4$ oxoclusters linked together by 12 NH_2-BDC ligands, formulated $Zr_6O_4(OH)_4(BDC\text{-}NH_2)_6$, features a 3D network involving tetrahedral and octahedral cages of 6 and 11 Å, respectively, accessible through microporous windows (4−6 Å) (Fig. 1A-C). Powder X-ray diffraction (XRD) profiles demonstrate its phase purity and great water stability. The scanning electron microscopy (SEM) image indicates that the sizes of UiO-66-NH_2 particles are in 50-100 nm (Fig. 1D), suitable for dispersion in the

ChemComm Accepted Manuscript

aqueous solution. N_2 sorption isotherms confirm their permanent porosity and the BET surface area is 615 m^2/g (Fig. S2).

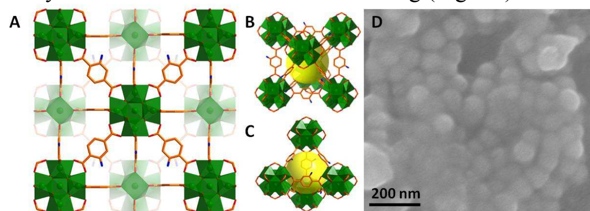


Fig. 1 A) The 3D structure of UiO-66-NH_2 and it contains B) large octahedral and C) small tetrahedral cages. The Zr, C, O and N atoms are represented by olive, orange, red and blue, respectively, and $Zr_6O_4(OH)_4$ clusters are shaded in olive polyhedra. D) A SEM image for UiO-66-NH_2.

To demonstrate the feasibility of employing UiO-66-NH_2 as a fluorescent sensing platform for DNA detection, an oligonucleotide sequence associated with human immunodeficiency virus (HIV) was employed as a model system. As displayed in Fig. 2, the FAM-ssDNA probe (P_{HIV}) exhibit fairly strong fluorescence emission due to the presence of fluorescein-based dye, FAM. The introduction of UiO-66-NH_2 leads to around 56% drop of the fluorescence intensity, suggesting that MOF has interaction with ssDNA and quenches the fluorescence effectively. Significantly, the MOF-P_{HIV} composite presents remarkable fluorescent enhancement with recovery up to 70% upon its incubation with complementary target T_1 (tDNA), revealing the release of ssDNA due to its binding to tDNA. Actually, the fluorescence of P_{HIV} is nearly not affected by the tDNA without MOF and the MOF itself has no fluorescence contribution in the investigated wavelength range.

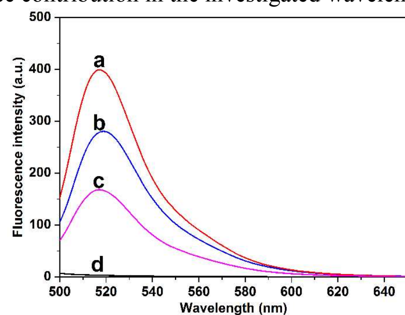


Fig. 2 Fluorescence spectra of P_{HIV} (50 nM) in different systems (λ_{ex} = 480 nm): (a) P_{HIV}; (b) P_{HIV}+UiO-66-NH_2+T_1 (150 nM); (c) P_{HIV}+UiO-66-NH_2; (d) UiO-66-NH_2 in the absence of P_{HIV}.

The influence of amount of MOF introduced in the system shows that the fluorescence quenching efficiency increases while the recovery efficiency decreases along with more MOF used ranging from 0-50 µL. More MOFs nearly do not affect the both efficiencies (Fig. 3A). It is understandable because more MOFs would result in more efficient adsorption of ssDNA and thus maximize the fluorescence quenching to some extent. In this case, some tDNA may also be adsorbed on MOFs and the binding between ssDNA and tDNA is suppressed, thus to reduce the release of ssDNA and the fluorescence recovery. Therefore, 20 µL MOF as an optimized volume was used in this study otherwise specified. In addition, the amount of tDNA has positive influence on the fluorescence recovery of MOF-ssDNA. The fluorescence recovery efficiency increases when more tDNA, ranging from 10-150 nM, are introduced into the system, while more tDNA would not cause the change any more (Fig. 3B).

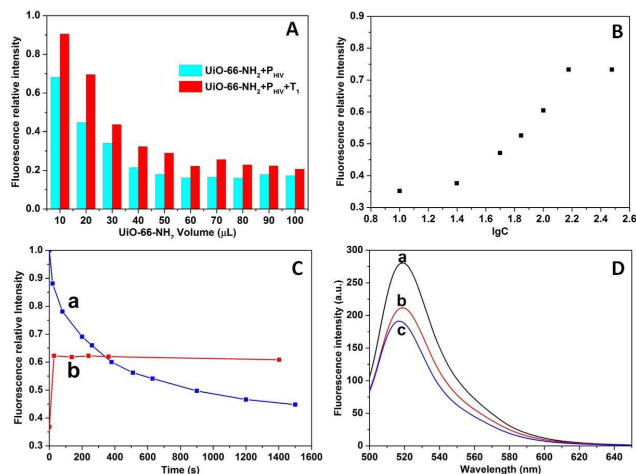


Fig. 3 Influence of the amount of A) UiO-66-NH_2 or B) T_1 on the fluorescence quenching efficiency (P_{HIV}: 50 nM). C) Kinetic behavior study: (a) fluorescence quenching of P_{HIV} (50 nM) by UiO-66-NH_2 and (b) fluorescence recovery of P_{HIV}-UiO-66-NH_2 by T_1 (150 nM) as a function of incubation time. D) Fluorescence intensity of P_{HIV}+UiO-66-NH_2 under λ_{ex} = 480 nm upon introducing different targets: (a) T_1; (b) single-base mismatched target T_2 to ssDNA (c) mismatched target T_3 to ssDNA. All measurements were done in Tris-HCl buffer with 5 mM Mg^{2+} (pH: 7.42).

To further understand the kinetics of the fluorescence quenching and recovery, the time-dependent fluorescence intensity of P_{HIV} by UiO-66-NH_2 and MOF-P_{HIV} composite with tDNA as a function of incubation time has been examined (Fig. 3C). In the absence of tDNA, the curve has a rapid decrease in the first 5 min and reaches equilibrium at around 20 min, suggesting that UiO-66-NH_2 can absorb ssDNA effectively and quickly. Moreover, it seems the trend that more MOFs make the equilibrium more quickly reached (Fig. S4). The introduction of tDNA allows the fluorescence recovery in a faster kinetics. Upon the addition of tDNA, the fluorescence intensity increases rapidly and reaches equilibrium in around 3 min, suggesting that T_1 can detach ssDNA from UiO-66-NH_2 effectively in very short time. Rapid adsorption and detachment of ssDNA make UiO-66-NH_2 promising for fluorescent sensing of DNA molecules.

In addition to the complementary target T_1, mismatched sequences (single-base mismatched T_2 and mismatched T_3 to ssDNA) have also been introduced into the system to investigate the discrimination ability of the sensing platform (Fig. 3D). It is observed that the fluorescence intensity of the system in the presence of T_1, T_2 and T_3, respectively exhibits 67%, 26%, and 14% enhancement compared to that in the absence of target. The fluorescence intensity is clearly associated with matching level of base pairs between ssDNA and target. These results indicate that the sensing platform is able to distinguish the complementary and unmatched target sequences, no matter the only single-base or total mismatching.

To understand the role of the amino group in UiO-66-NH_2 for the sensing property, another MOF bearing the same structure but without amino group, UiO-66, has been examined. Unexpectedly, although the introduction of UiO-66 leads to around 42% drop of the fluorescence intensity, a bit less than that with UiO-66-NH_2 (56%), the fluorescence intensity of the system in the presence of T_1 and T_2, respectively exhibits 58% and 56% enhancement compared to that in the absence of target, indicating that UiO-66 cannot distinguish the complementary and the single-base

ChemComm Accepted Manuscript

This journal is © The Royal Society of Chemistry [year]

mismatching targets (Fig. S5). Given the negatively charged backbone of ssDNA,[13] the zeta potentials of UiO-66 and UiO-66-NH_2 of +1.28 and -5.54 mV, respectively, indicates that UiO-66 has weak electrostatic interaction with ssDNA while there could exist a bit electrostatic repulsion between UiO-66-NH_2 and ssDNA. Meanwhile, UiO-66 has similar structure and conjugated π-electron system to UiO-66-NH_2. In this context, what interaction is able to offset the electrostatic repulsion and enables the successful adsorption of ssDNA on the surface of UiO-66-NH_2? It is well known that DNA double helix formed depending on the hydrogen bonds between amino groups of bases on two single-stranded DNAs. Accordingly, we may infer that there are hydrogen bonds between the amino groups in UiO-66-NH_2 and bases in ssDNA, resulting in the adsorption of ssDNA on UiO-66-NH_2. In contrast, UiO-66 without amino group does not form hydrogen bonds with ssDNA, which could be responsible for its indistinguishableness for different target DNAs. The Infrared (IR) absorption spectrum of MOF-P_{HIV} composite shows the N-H stretching vibration at 3460 and 3376 cm^{-1} (Fig. S6), which present blue shift compared to the characteristic peaks of the unbounded NH_2 group at 3500 and 3386 cm^{-1} in pristine UiO-66-NH_2.[14] The peak shift further supports the existence of hydrogen bond interaction between the amino group in UiO-66-NH_2 and ssDNA bases.[15]

In conclusion, our results indicate that an amine-functionalized MOF, UiO-66-NH_2, can afford an effective fluorescence sensing platform for DNA detection. The sensing system can distinguish complementary and mismatched DNA sequences down to single-base mismatch with high selectivity and good reproducibility. In contrast, the UiO-66 with similar structure in the absence of amino group cannot realize such function. For the first time, the hydrogen bond interaction between MOF and ssDNA has been proposed to account for DNA detection. Given the high stability, the facile and scalable synthesis with cheap reactants, the UiO-66-NH_2-based assay holds great promise for practical application in clinical sample analysis.

This work is supported by the NSFC (Grants 21371162 and 51301159), the 973 Program (Grant 2014CB931803), Research Fund for the Doctoral Program of Higher Education of China (20133402120020), the Recruitment Program of Global Experts and the Fundamental Research Funds for the Central Universities (WK2060190026).

Notes and references

[a]*Hefei National Laboratory for Physical Sciences at the Microscale, Department of Chemistry, University of Science and Technology of China, Hefei, Anhui 230026, P.R. China Fax: +86-551-63607861; Tel: +86-551-63607861; E-mail: jianglab@ustc.edu.cn*

[b]*College of Chemistry and Chemical Engineering, Gannan Normal University, Ganzhou 341000, P.R. China*

† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

1 D. Gresham, D. M. Ruderfer, S. C. Pratt, J. Schacherer, M. J. Dunham, D. Botstein and L. Kruglyak, *Science*, 2006, **311**, 1932.
2 (a) K. B. Mullis, F. A. Faloona, *Methods Enzymol*, 1987, **155**, 335; (b) A. Gopi, S. M. Madhavan, S. K. Sharma and S. A. Sahn, *Chest*, 2007, **131**, 880.
3 (a) M. Ramsey, *Nature Biotechnol.*, 1998, **16**, 40; (b) R. Moeller and W. Fritzsche, *IEE Proc. Nanobiotechnol*, 2005, **152**, 47.
4 (a) B. Dubertret, M. Calame and A. J. Libchaber, *Nat. Biotechnol.*, 2001, **19**, 365; (b) D. J. Maxwell, J. R. Taylor and S. Nie, *J. Am. Chem. Soc.*, 2002, **124**, 9606; (c) R. H. Yang, J. Y. Jin, Y. Chen, N. Shao, H. Z. Kang, Z. Xiao, Z. W. Tang, Y. R. Wu, Z. Zhu and W. H. Tan, *J. Am. Chem. Soc.*, 2008, **130**, 8351; (d) L. B. Zhang, T. Li, B. L. Li, J. Li and E. K. Wang, *Chem. Commun.*, 2010, **46**, 1476; (e) C. H. Lu, H. H. Yang, C. L. Zhu, X. Chen and G. N. Chen, *Angew. Chem., Int. Ed.*, 2009, **48**, 4785; (f) S. J. He, B. Song, D. Li, C. F. Zhu, W. P. Qi, Y. Q. wen, L. H. Wang, S. P. Song, H. P. Fang and C. H. Fan, *Adv. Funct. Mater.*, 2010, **20**, 453; (g) H. L. Li, Y. W. Zhang, L. Wang, J. Q. Tian and X. P. Sun, *Chem. Commun.*, 2011, **47**, 961; (h) H. L. Li, Y. W. Zhang, Y. L. Luo and X. P. Sun, *Small*, 2011, **7**, 1562.
5 (a) H. L. Li and X. P. Sun, *Chem. Commun.*, 2011, **47**, 2625; (b) L. Wang, Y. W. Zhang, J. Q. Tian, H. L. Li and X. P. Sun, *Nucleic Acids Res.*, 2011, **39**, 37.
6 (a) G. Férey, C. Mellot-Draznieks, C. Serre and F. Millange, *Acc. Chem. Res.*, 2005, **38**, 217; (b) S. Horike, S. Shimomura and S. Kitagawa, *Nat. Chem.*, 2009, **1**, 695; (c) J. R. Long and O. M. Yaghi, *Chem. Soc. Rev.*, 2009, **38**, 1213; (d) H.-C. Zhou, J. R. Long and O. M. Yaghi, *Chem. Rev.*, 2012, **112**, 673.
7 (a) H. Wu, W. Zhou and T. Yildirim, *J. Am. Chem. Soc.*, 2009, **131**, 4995; (b) K. Sumida, D. L. Rogow, J. A. Mason, T. M. McDonald, E. D. Bloch, Z. R. Herm, T.-H. Bae and J. R. Long, *Chem. Rev.*, 2012, **112**, 724; (c) M. P. Suh, H. J. Park, T. K. Prasad and D.-W. Lim, *Chem. Rev.*, 2012, **112**, 782; (d) J.-R. Li, J. Sculley and H.-C. Zhou, *Chem. Rev.*, 2012, **112**, 869; (e) Y.-X. Tan, Y.-P. He and J. Zhang, *ChemSusChem.*, 2012, **5**, 1597; (f) Y. Hu, W. M. Verdegaal, S. H. Yu and H. L. Jiang, *ChemSusChem.*, 2014, **7**, 734.
8 (a) J. S. Seo, D. Whang, H. Lee, S. I. Jun, J. Oh, Y. J. Jeon and K. Kim, *Nature*, 2000, **404**, 982; (b) L. Q. Ma, C. Abney and W. B. Lin, *Chem. Soc. Rev.*, 2009, **38**, 1248; (c) D. Farrusseng, S. Aguado and C. Pinel, *Angew. Chem., Int. Ed.*, 2009, **48**, 7502; (d) H.-L. Jiang and Q. Xu, *Chem. Commun.*, 2011, **47**, 3351; (e) Y. Fu, D. Sun, Y. Chen, R. Huang, Z. Ding, X. Fu and Z. Li, *Angew. Chem. Int. Ed.*, 2012, **51**, 3364; (f) J. Gascon, A. Corma, F. Kapteijn, F. X. Llabrés and I. Xamena, *ACS Catal.*, 2014, **4**, 361.
9 (a) Z. Q. Wang and S. M. Cohen, *Chem. Soc. Rev.*, 2009, **38**, 1315; (b) S. C. Sahoo, T. Kundu and R. Banerjee, *J. Am. Chem. Soc.*, 2011, **133**, 17950; (c) J. D. Rocca, D. M. Liu and W. B. Lin, *Acc. Chem. Res.*, 2011, **44**, 957; (d) P. Horcajada, R. Gref, T. Baati, P. K. Allan, G. Maurin, P. Couvreur, G. Férey, R. E. Morris and C. Serre, *Chem. Rev.*, 2012, **112**, 1232; (e) X. Zhao, F. Liu, L. Zhang, D. Sun, R. Wang, Z. Ju, D. Yuan and D. Sun, *Chem. Eur. J.*, 2014, **20**, 649.
10 (a) B. L. Chen, S. C. Xiang and G. D. Qian, *Acc. Chem. Res.*, 2010, **43**, 1115; (b) H.-L. Jiang, Y. Tatsu, Z.-H. Lu and Q. Xu, *J. Am. Chem. Soc.*, 2010, **132**, 5586; (c) Y. Takashima, V. M. Martinez, S. Furukawa, M. Kondo, S. Shimomura, H. Uehara, M. Nakahama, K. Sugimoto and S. Kitagawa, *Nat. Commun.*, 2011, **2**, 168; (d) L. E. Kreno, K. Leong, O. K. Farha, M. Allendorf, R. P. Van Duyne and J. T. Hupp, *Chem. Rev.*, 2012, **112**, 1105; (e) S.-R. Zhang, D.-Y. Du, J.-S. Qin, S.-J. Bao, S.-L. Li, W.-W. He, Y.-Q. Lan, P. Shen and Z.-M. Su, *Chem. Eur. J.* 2014, **20**, 3589; (f) Z. C. Hu, B. J. Deibert and J. Li, *Chem. Soc. Rev.*, 2014, DOI: 10.1039/C4CS00010B.
11 (a) X. Zhu, H. Y. Zheng, X. F. Wei, Z. Y. Lin, L. H. Guo, B. Qiu and G. N. Chen, *Chem. Commun.*, 2013, **49**, 1276; (b) S. Liu, L. Wang, J. Q. Tian, Y. L. Luo, G. H. Chang, A. M. Abdullah, A. O. Al-Youbi and X. P. Sun, *ChemPlusChem.*, 2012, **77**, 23.
12 (a) Y. J. Cui, Y. F. Yue, G. D. Qian and B. L. Chen, *Chem. Rev.*, 2012, **112**, 1126; (b) M. A. Fox and M. Chanon, *Photoinduced electron transfer, ed.*, Elsevier, Amsterdam, 1988; (c) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515; (d) B. Valeur, *Molecular Fluorescence: Principles and Applications*, Wiley-VCH, Weinheim, 2001.
13 J. F. Zhai, H. L. Li and X. P. Sun, *RSC Adv.*, 2011, **1**, 36.
14 M. Kandiah, M. H. Nilsen, S. Usseglio, S. Jakobsen, U. Olsbye, M. Tilset, C. Larabi, E. A. Quadrelli, F. Bonino and K. P. Lillerud, *Chem. Mater.*, 2010, **22**, 6632.
15 (a) L.-S. Teo, C.-Y. Chen and J.-F. Kuo, *Macromolecules*, 1997, **30**, 1793; (b) T. Kondo and C. Sawatari, *Polymer*, 1996, **37**, 393.

ChemComm Accepted Manuscript