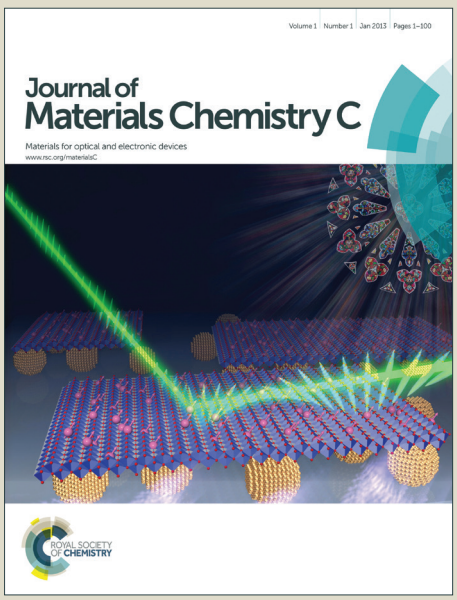
Journal of Materials Chemistry C

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/materialsC

Journal Name

RSCPublishing

COMMUNICATION

Development of a conjugated polymer-based fluorescent probe for selective detection of HOCl

Cite this: DOI: 10.1039/x0xx00000x

Han Wang,[abc] Ying Li,[b] Ying Chen,[bc] Lianshan Li,[*b] Tao Fang,[*a] and Zhiyong Tang[*b]

Received 00th January 2012,
Accepted 00th January 2012

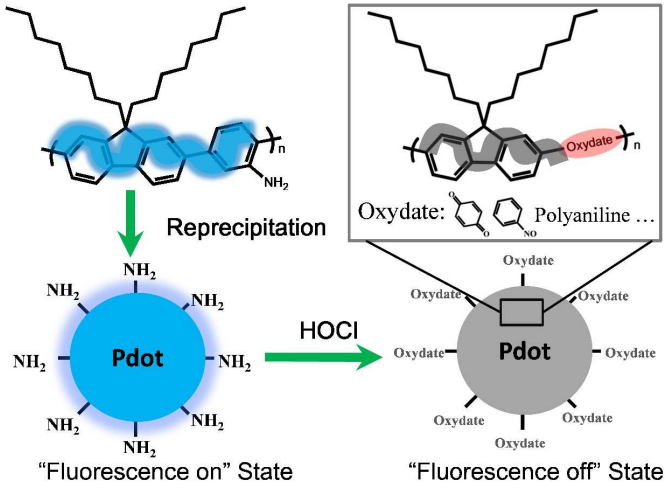
DOI: 10.1039/x0xx00000x

www.rsc.org/

A novel fluorescent probe is fabricated by integrating an aniline unit into the semiconducting conjugated polymer (SCP) nanoparticles. As-prepared probe shows a remarkable fluorescence "turn-off" response to hypochlorous acid (HOCl) due to the conjugation perturbation caused by selective oxidation of aniline group by HOCl. This work provides a new method for highly selective detection of HOCl by using conjugated polymer nanoparticles.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) play an essential role in a wide variety of biological events.[1] Among the various types of ROS, hypochlorous acid (HOCl) is one the most important ROS, which is centrally linked to innate host defence and plays a vital role in killing a wide range of pathogens.[2] On the other hand, oxidative stress due to excessive generation of ROS is implicated in many human diseases,[3] and it is well known that neutrophil-derived HOCl contributes to inflammation-associated tissue injury, such as hepatic ischemia reperfusion injury, atherosclerosis, lung injury, and rheumatoid arthritis.[4] Because of the pathophysiological importance of HOCl, a number of methods for detection of HOCl have been developed.[2b, 5] Synthetic dye-based small molecule fluorescent probes including fluorescein, rhodamine and cyanine are among the most versatile fluorophores currently used for detection of HOCl.[2b, 5c, 5e, 6] It should be pointed out that although several dye-base small molecule probes for HOCl detection have been attempted, only a few meet the requirement of (1) high brightness and good photo-stability, (2) remarkable sensitivity and selectivity for HOCl, and (3) suitability for biological application *in vitro* and *in vivo*.

Semiconducting π-conjugated polymers (SCPs) are optically and electrically active materials with many applications, ranging from electronic devices and sensors to tissue engineering.[7] Recently, SCPs have been further constructed into nanoparticles as a new class of photo stable fluorescent nanomaterials.[8] Recent research has demonstrated that SCPs nanoparticles exhibit excellent characteristics as fluorescent probes, including their extraordinary fluorescence brightness, fast emission rate, excellent photostability, and nonblinking and nontoxic features.[8a] Compared with conventional organic dyes and inorganic semiconducting quantum dots, these materials are bright, completely organic and have no heavy metal ion-induced toxicity to living organism.[9] More intriguingly, as for SCP nano-probes, one single interaction can quench a large number of fluorophores, and the complete fluorescence quenching would be observed upon interaction with even a single analyte molecule.[10] As a result of all these favourable properties, development of fluorescent sensing materials based on SCPs is capturing special attention of researchers. For instance, Chiu *et al.* demonstrated successful formation of SCP nanoparticle based bioconjugates and obtained their extraordinary brightness compared to commercially available dye-tagged antibodies.[11] McNeill's group first demonstrated the formation of hydrophobic SCP nanoparticles that exhibit salient features as promising imaging probes.[12] It is worth mentioning that although there has been steady progress in creating fluorescent SCP nanoparticle as highly sensitive chemical/biological sensors, there are no reports until now to design and synthesize SCP nanoparticle for selective detection of ROS/RNS.



Scheme 1 Chemical structure and HOCl-induced oxidation of SCP fluorescence probes for HOCl detection

Journal of Materials Chemistry C Accepted Manuscript

In this communication, we report the synthesis and characterization of photostable and bright SCP nanoparticles consisting of amine functionalized polyfluorene derivative (poly [2, 7-(9, 9'-dioctylfluorene)-co-alt-2, 5-phenylamine]), and demonstrate that the aniline units can be selectively oxide by HOCl (Scheme 1). The oxidation of aniline units disturbed the conjugation in SCP backbone, leading to remarkable fluorescence quenching of the conjugated polymer. To our best knowledge, this is the first example of using SCP nanoparticles as fluorescence probe for highly selective detection of HOCl.

The π-conjugated polymer, poly [2, 7-(9, 9'-dioctylfluorene)-co-alt-2, 5-phenylamine], were synthesized by Suzuki cross-coupling polymerization of 9, 9'-dioctylfluorene-2, 7-bis(trimethyleneboronate) with 2, 5-dibromo-phenylamine in yield of 64%. The chemical structure and molecular weight were confirmed by ^{1}HNMR (Supporting Information Fig. S1) and GPC (Gel Permeation Chromatography) with molecular weight (M_n) of 11.0 kg mol^{-1} (Fig. S2). The optical properties of the polymer solution in THF were then investigated. The UV-Vis spectrum shows an absorption peak at 363 nm, while the photoluminescence (PL) spectrum exhibits strong blue emission at 419 nm (Fig. S3), which are similar with those of fluorene containing SCPs.[13]

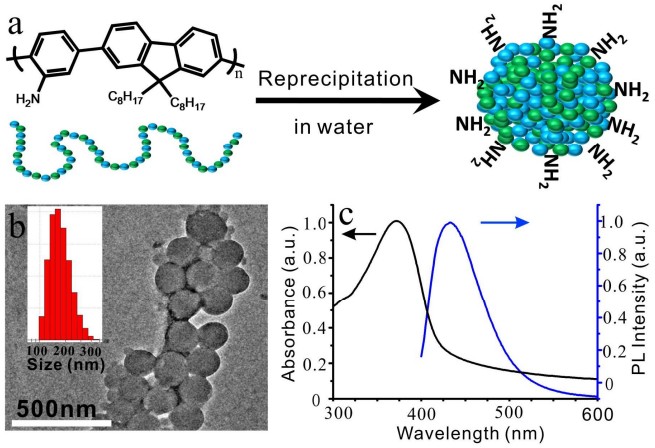


Fig. 1 (a) A sketch of formation of water-dispersed SCP nanoparticles, (b) TEM image and hydrodynamic size distribution of SCP nanoparticles determined by DLS (insert), (c) UV-Vis and PL spectra of SCP nanoparticles

Notably, as synthesized polymer could be simply precipitated in water to afford water-dispersible nanoparticles. (Fig. 1a) Interestingly, the uniform nanoparticles are obtained without the aid of any surfactants or coating regents, which is ascribed to the hydrophilic amine groups of polymer. The size and morphology of the SCP nanoparticles are first characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM). TEM image reveals the spherical morphology of the SCP nanoparticles with uniform diameters of around 200 nm (Fig. 1b), and the narrow size distribution of SCP nanoparticles is also confirmed by DLS measurement (insert in Fig. 1b). The SCP nanoparticles have absorption with maxima at 373 nm and are strongly fluorescent upon excitation at 373 nm, with emission maxima at 430 nm (Fig. 1c). Compared with free polymer in THF solvent, both absorption and emission characteristics shift to longer wavelength, indicating that close association among conjugate backbones in the SCP nanoparticles gives rise to an increase in π-π interaction. Fluorescence decay kinetic (Figure S4) was tested and excited-state lifetime was extracted from the kinetics traces using custom software. The lifetime of SCP particle was determined to be 2 ns.

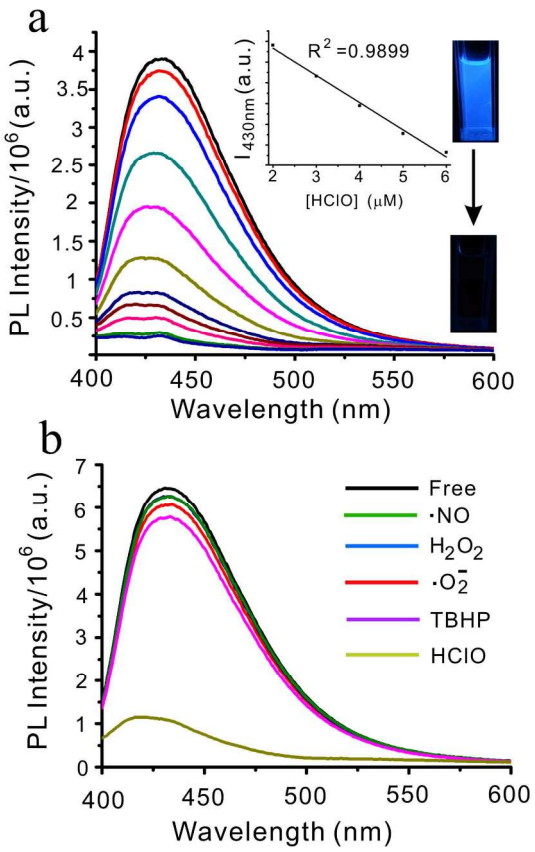


Fig. 2 (a) PL spectra changes of SCP nanoparticles (1 μM) as titration of HOCl (1, 2, 3, 4, 5, 6, 7, 8 ,9, 10, 12, 15 μM) and PL intensity at 430 nm as a function of added OCl$^-$ (insert) (b) PL spectra of SCP nanoparticles before and after addition of various ROS (•NO: 15 μM; H_2O_2: 200 μM; $\cdot O_2^-$: 200 μM; TBHP: 200 μM; HOCl: 15 μM).

With the water dispersible SCP nanoparticles in hands, we next investigated the sensitivity and selectivity of these nanoparticles for HOCl detection. The absorption and PL spectra of SCP nanoparticles with HOCl were first monitored. The absorption peak of the nanoparticles in water at 373 nm decrease upon addition of HOCl, indicating the efficient oxidation of SCP nanoparticles in water. (Fig S5a) However, the absorption decrease of SCP in THF after addition of HOCl is not so remarkable probably due to the poor solubility of water in THF. (Fig S5b) Since oxidation of SCPs can disturb the conjugation and further affect the emission properties, we next test the PL spectra of SCP nanoparticles after addition of different amount of HOCl. SCP nanoparticles are strongly blue fluorescent. (Fig. 2a) When HOCl is titrated from 0 to 15 μM, the PL emission decreases remarkably and quickly. This can be ascribed to conjugation breakage caused by the oxidation of aniline units on the backbone of SCPs (Scheme 1). DLS measurement after oxidation shows that the diameter of SCP nanoparticles remain 150-200 nm, which means that the PL quenching is not because of the aggregation. (Fig. S6) Meanwhile, upon addition of HOCl to the solution of SCP nanoparticles, the PL emission shift to a shorter wavelength (blue shift), (Fig. 2) which also confirm the reduced conjugation in the polymer chains (Scheme 1). The PL intensity at 430 nm as a function of HOCl concentration is recorded, and

Journal of Materials Chemistry C Accepted Manuscript

This journal is © The Royal Society of Chemistry 2012

a nearly linear relationship in the range of 2-6 μM is achieved (insert in Fig. 2a). The minimum amount of HOCl that can be detected is evaluated to be 0.5 μM (limit of detection, LOD), which is comparable to that of the previously reported rhodamine-based dye probe.[6d] All spectroscopic changes are discerned immediately upon the addition of HOCl (less than 5 min), indicating that the oxidation reaction occurs rapidly at room temperature. The addition of HOCl to the probe solution results in an instantaneous PL quenching that is easily detectable by the naked eye under UV irradiation (insert in Fig. 2a). In contrast, other typical ROS including H_2O_2, •NO, $•O^{2-}$ and tert-butyl hydroperoxide (TBHP) produce almost no PL quenching (Fig. 2b). Thus, these amine functionalized SCP nanoparticles appear to be highly sensitive and selective for detection of HOCl. In addition, we examined the pH-dependence of SCP nanoparticles in the detection of HOCl. Results show that the SCP nanoparticles display an efficient fluorescence response to HOCl in the pH range of 6.0–8.0 (Fig. S7), which suggests that SCP nanoparticles are very suitable for biological applications.

Moreover, the photo-stability of the SCP nanoparticles was investigated. The PL intensity of the SCP nanoparticles decreases only 5% in 2.5 hours and becomes stable afterwards, (Fig. S8) indicating that the SCP nanoparticles are chemically- and photo-stable. Such improvement in photo and chemical stability are a notable feature of the present sensing system toward HOCl compared with dye-based small molecule system [6e, 6f].

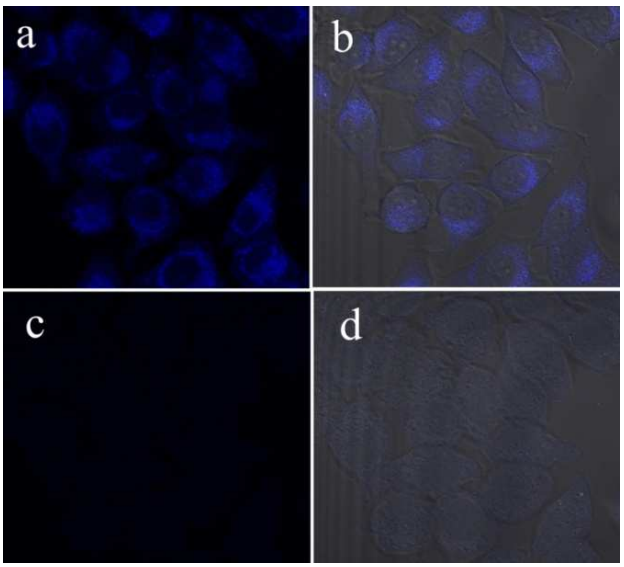


Fig. 3 (a, b) Fluorescence and merged images of HeLa cells treated with only probes (1 μM) for 2h. (c, d) Fluorescence and merged images of HeLa cells treated with probe for 2 h and then incubated with HOCl (15 μM) for another 30 min.

Encouraged by its high sensitivity and selectivity, we applied SCP nanoparticle probes to track intracellular HOCl levels *via* fluorescence microscopy. HeLa cells were first treated with SCP nanoparticles (1μM, 2 h), followed by washed three times with phosphate-buffered saline (PBS,10 mM, pH 7.12). The HeLa cells treated with SCP nanoparticles exhibit a very strong intracellular fluorescence (Fig 3a and 3b), indicating that SCP nanoparticle probes can penetrate the cell membrane and be used for *in vivo* imaging of HOCl in living cells potentially. When HeLa cells pre-treated with SCP nanoparticles in a buffer medium are incubated with HOCl (15 μM, 0.5 h), a significant fluorescence quenching could be discerned (Fig. 3c and 3d). These are consistent with the observations in titration experiments, indicating that the SCP nanoparticles could readily detect the level of HOCl in living cells with the switching-off fluorescent method.

Cell viability assay(Fig. S9) indicated that the cells growth on treated with SCP nanoparticles medium had high cell viability (>95%). The Calcein AM/PI staining assay demonstrated there were almost no red (dead) cells after incubating with the probe (Fig. 4), indicating that the probe had no toxicity to cells. It needs to point out that the HeLa cell remains their morphology after treatment with the SCP nanoparticles. The SCP nanoparticles not affect the cell growth and proliferation. The results showed that SCP nanoparticles are suitable for the detection. This result is consistent with previous report that SCP nanoparticles have very low cytotoxicity.[9]

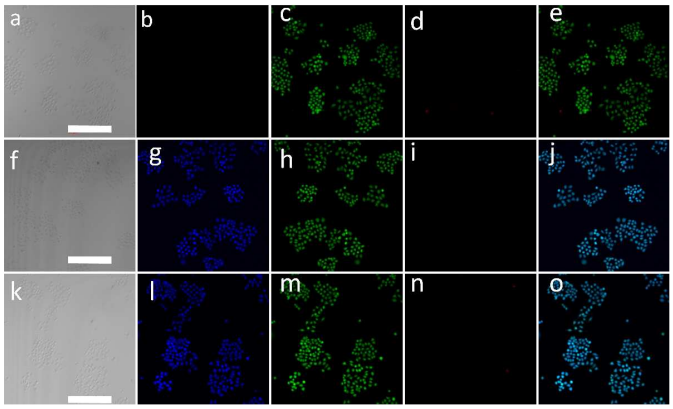


Fig. 4 Calcein Acetoxymethyl Ester (Calcein AM)/ Propidium Iodide (PI) Assay. (a) normal Hela cells, (g) cells treated with 1 μM probes, (l) cells treated with 2 μM probes. (a, f, k) Optical micrographs, (b, g, i) cells treated with probes appeared blue fluorescence, (c, h, m) live cells appeared green fluorescence, (d, i, n) dead cells appeared red fluorescence, (e, j, o) merged images. The scale bar equal to 100 μm.

To explore the sensing mechanism of SCP nanoparticles to HOCl, FT-IR spectroscopy is further used to characterize the oxidation products. The spectrum obtained for pristine polymer clearly shows the characteristic N-H stretching band of the amine group (3250-3500 cm^{-1}) and C-H stretching of octyl group (2800-3000 cm^{-1}), (Fig. S10c) indicating successful polymerization of PF and PPA monomers. After 1 equivalent of HOCl is added, the double peak of N-H stretching dramatically decreases, indicating the oxidation of amine group. (Fig. S10d) Further increase of the HOCl to 5 equivalents, the double peak of N-H stretching is replaced by a broad peak centred at 3400 cm^{-1}, which might be ascribed to the protonation of imine group. Simultaneously, the phenyl peak (at 1601 cm^{-1}) broadens, shifts, and merges with the peak for the newly formed C=N bond (1645 cm^{-1}). (Fig. S10e) To further investigate the oxidation mechanism, HPLC/MS was used to analyze the oxidation products of aniline. (Fig. S11) The HPLC spectrum shows more than ten peaks, which means the products are complicated. MS analysis shows that numerous products with different molecular weight are obtained. Although most of the peaks are from undefined products, a

Journal of Materials Chemistry C Accepted Manuscript

few of them can be ascribed to certain products. For example, the peak with molecular weight of 108 might from *p*-benzoquinone and peaks with molecular weight more than 300 might be ascribed to supramolecular structures. It is widely reported that polyaniline (PANI) are formed by oxidation of aniline monomers in aqueous medium. The reaction itself seems to be simple, yet it represents interplay of complex multilevel processes. Various supramolecular structures of the final product are obtained, depending on the conditions of the reaction, but the mechanism of their formation has not been fully elucidated. [14] Based on the above characterization and discussion, we hypothesize that the conjugation of the SCP was disturbed during the oxidation process thus shortening the conjugation in SCP chains, leading to the fluorescence quenching. (Scheme 1)

In conclusion, we have developed a novel semiconducting conjugated polymer-based luminescent nanoparticle probe for detection of HOCl with high selectivity and sensitivity over other typical ROS. Different from dye-based small molecule fluorescent probes, this novel type of probes operate through PL quenching caused by oxidation induced conjugation breakage in the conjugate polymers. The probes show a fast response time and operates efficiently under physiological conditions, which is of crucial importance for biological imaging studies. Besides the rapid and specific response to HOCl in solution, application of the sensing materials for detection of HOCl in living cells is also successfully demonstrated. This research represents the first example of the potential, but still unexplored, applicability of bio-compatible SCP nanoparticle probes to highly sensitive detection of ROS.

This work was supported by the National Research Fund for Fundamental Key Project (2014CB931801, Z. Tang), the National Natural Science Foundation for Distinguished Youth Scholars of China (21025310, Z. Tang) and the National Natural Science Foundation of China (51472054, L. S. Li)

Notes and references

[a] Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, P. R. China. E-mail: fangt@ihb.ac.cn
[b] CAS Key Laboratory of Nanosystem and Hierarchy Fabrication, National Center for Nanoscience and Technology, Beijing 100190, P. R. China. E-mail: zytang@nanoctr.cn, lils@nanoctr.cn
[c] Graduate University of Chinese Academy of Sciences, Beijing 100049, P.R. China

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c000000x/

1 (a) S. G. Rhee, *Science*, 2006, **312**, 1882. (b) J. M. McCord, *Science*, 1974, **185**, 529. (c) Y. Cui, Z. Lu, L. Bai, Z. Shi, W. Zhao and B. L. Zhao, *Eur. J. Cancer*, 2007, **43**, 2590. (d) K. J. Barnham, C. L. Masters and A. I. Bush, *Nat. Rev. Drug Discovery*, 2004, **3**, 205.

2 (a) M. Emrullahoğlu, M. Üçüncü and E. Karakuş, *Chem. Commun*, 2013, **49**, 7836. (b) Y. Koide, Y. Urano, K. Hanaoka, T. Terai and T. Nagano, *J. Am. Chem. Soc.*, 2011, **133**, 5680. (c) S.-R. Liu and S.-P. Wu, *Org. Lett.*, 2013, **15**, 878.

3 (a) Q. A. Best, N. Sattenapally, D. J. Dyer, C. N. Scott and M. E. McCarroll, *J. Am. Chem. Soc.*, 2013, **135**, 13365. (b) N. Guengoer, A. M. Knaapen, A. Munnia, M. Peluso, G. R. Haenen, R. K. Chiu, R. W. L. Godschalk and F. J. van Schooten, *Mutagenesis* 2010 **25**, 149. (c) Q. Xu, K.-A. Lee, S. Lee, K. M. Lee, W.-J. Lee and J. Yoon, *J. Am. Chem. Soc.*, 2013, **135**, 9944.

4. (a) T. Hasegawa, E. Malle, A. Farhood and H. Jaeschke, *Am. J. Physiol. Gastrointest. Liver Physiol.*, 2005, **289**, 760. (b) A. Daugherty, J. L. Dunn, D. L. Rateri and J. W. Heinecke, *J. Clin. Invest.*, 1994, **94**, 437. (c) S. Hammerschmidt, N. B€uchler and H. Wahn, *Chest*, 2002, **121**, 573. (d) S. M. Wu and S. V. Pizzo, *Arch. Biochem. Biophys.*, 2001, **391**, 119.

5 (a) X. Chen, X. Tian, I. Shin and J. Yoon, *Chem. Soc. Rev.*, 2011, **40**, 4783. (b) X. Chen, K.-A. Lee, E.-M. Ha, K. M. Lee, Y. Y. Seo, H. K. Choi, H. N. Kim, M. J. Kim, C.-S. Cho, S. Y. Lee, W.-J. Lee and J. Yoon, *Chem. Commun.*, 2011, **47**, 4373. (c) X. Cheng, H. Jia, T. Long, J. Feng, J. Qin and Z. Li, *Chem. Commun.*, 2011, **47**, 11978. (d) T. Guo, L. Cui, J. Shen, R. Wang, W. Zhu, Y. Xu and X. Qian, *Chem. Commun.*, 2013, **49**, 1862. P. Panizzi, M. Nahrendorf, M. Wildgruber, P. Waterman, J. Figueiredo, E. Aikawa, J. McCarthy, R. Weissleder and S. A. Hilderbrand, *J. Am. Chem. Soc.*, 2009, **131**, 15739.

6 (a) L. Yuan, W. Lin, J. Song and Y. Yang, *Chem. Commun.*, 2011, **47**, 12691. (b) S. Kenmoku, Y. Urano, H. Kojima and T. Nagano, *J. Am. Chem. Soc.*, 2007, **129**, 7313. (c) W. Lin, L. Long, B. Chen and W. Tan, *Chem.Eur. J.*, 2009, **15**, 2305. (d) X. Lou, Y. Zhang, Q. Li, J. Qin and Z. Li, *Chem. Commun*., 2011, **47**, 3189. (e) A. Toutchkine, D. V. Nguyen and K. M. Hahn, Org. Lett., 2007, 9, 2775. (f) G. Chen, F. Song, J. Wang, Z. Yang, S. Sun, J. Fan, X. Qiang, X. Wang, B. Doub and X. Peng, *Chem. Commun*., 2012, 48, 2949

7 (a) C. Zhu, L. Liu, Q. Yang, F. Lv and S. Wang, *Chem. Rev.*, 2012, **112**, 4687. (b) J. Pecher and S. Mecking, *Chem. Rev.*, 2010, **110**, 6260.

8. (a) C. Wu and D. T. Chiu, *Angew. Chem. Int. Ed.*, 2013, **52**, 3086. (b) L. H. Feng, C. L. Zhu, H. X. Yuan, L. B. Liu, F. T. Lv and S. Wang, *Chem. Soc. Rev.*, 2013, **42**, 6620. (c) J. Yoon, J. Kwag, T. J. Shin, J. Park, Y. M. Lee, Y. Lee, J. Park, J. Heo, C. Joo, T. J. Park, P. J. Yoo, S. Kim and a. J. Park, *Adv. Mater.*, 2014, **26**, 4559.

9 (a) L. Feng, L. Liu, F. Lv, G. C. Bazan and S. Wang, *Adv. Mater.*, 2014, **26**, 3926. (b) X. L. Feng, G. M. Yang, L. B. Liu, F. T. Lv, Q. Yang, S. Wang and D. B. Zhu, *Adv. Mater.*, 2012, **24**, 637.

10 (a) S. W. Thomas, G. D. Joly and T. M. Swager, *Chem. Rev.*, 2007, **107**, 1339. (b) A. Álvarez-Diaz, A. Salinas-Castillo, M. Camprubí-Robles, J. M. Costa-Fernández, R. Pereiro, R. Mallavia and A. Sanz-Mede, *Anal. Chem.*, 2011, **83**, 2712.

11 (a) F. Ye, C. Wu, Y. Jin, M. Wang, Y. Chan, J. Yu, W. Sun, S. Hayden and D. T. Chiu, *Chem. Commun.*, 2012, **48**, 1778. (b) Y. Jin, F. Ye, C.Wu, Y. Chan and D. T. Chiu, *Chem. Commun.*, 2012, **48**, 3161. (c) X. Zhang, Y. Jin, F. Ye, C.Wu, Y. Chan and D. T. Chiu, *ACS Nano*, 2012, **6**, 5429.

12. (a) C. Wu, B. Bull, C. Szymanski, K. Christensen and J. McNeill, *Angew. Chem. Int. Ed.*, 2009, **48**, 2741. (b) J. Yu, C. Wu, Z. Tian and J. McNeill, *Nano Lett.*, 2012, **12**, 1300.

13. (a) P. Zalar, Z. B. Henson, G. C. Welch, G. C. Bazan and T.-Q. Nguyen, *Angew. Chem. Int. Ed.*, 2012, **124**, 7613. (b) B. Liu, W.-L. Yu, Y.-H. Lai and W. Huang, *Chem. Mater.*, 2001, **13**, 1984.

14. (a) M. Trchová, I. Šeděnková, E. N. Konyushenko, J. Stejskal, P. Holler, and G. Ćirić-Marjanović，*J. Phys. Chem. B* 2006, **110**, 9461 (b) Z. D. Zujovic, L. Zhang, G. A. Bowmaker, P.A. Kilmartin, and J. Travas-Sejdic, *Macromolecules* 2008, **41**, 3125 (c) J. Stejskal, I.

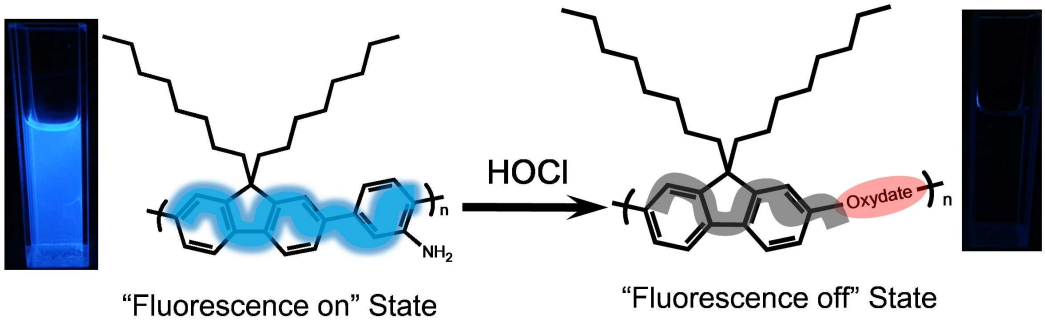
Journal of Materials Chemistry C Accepted Manuscript

 This journal is © The Royal Society of Chemistry 2012

Sapurina, M. Trchová, and E. N. Konyushenko, *Macromolecules* 2008, **41**, 3530

Journal of Materials Chemistry C Accepted Manuscript

A novel fluorescent probe based on semiconducting conjugate polymer (SCP) nanoparticles is fabricated, which demonstrates remarkable fluorescence "turn-off" response to hypochlorous acid (HOCl) due to the conjugation perturbation caused by selective oxidation of aniline groups by HOCl.



Journal of Materials Chemistry C Accepted Manuscript