Journal Name

Dynamic Article Links ►

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Polysaccharide-Porphyrin-Fullerene Supramolecular Conjugates as a Photo-driven DNA Cleavage Reagent

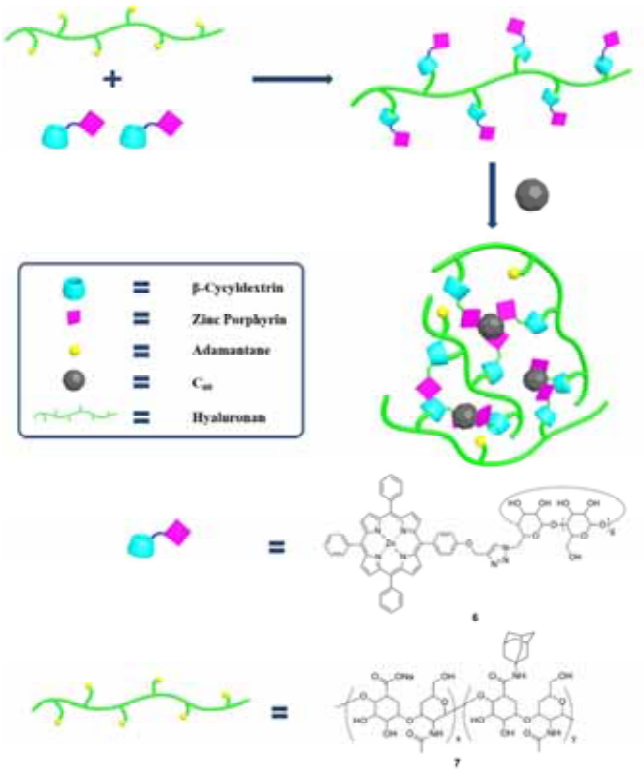
Yong Chen,[1,2‡] Di Zhao[1,‡] and Yu Liu[1,2]*

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

DOI: 10.1039/b000000x

Two water-soluble polysaccharide-porphyrin-fullerene supramolecular conjugates were constructed from the non-covalent incorporation of triphenyl Zn-porphyrin-modified β-cyclodxetrin, adamantyl-modified hyaluronate and C_{60}. Significantly, these supramolecular conjugates, which existed as cross-linked or discrete nanoparticles with a diameter of 50-200 nm, can completely cleave the closed supercoiled DNA to the nicked DNA under the light irradiation.

The construction of nanometer-scaled functional materials based on porphyrins and their derivatives are being intensely investigated due to their attractive electronic, photochemical, and photophysical properties.[1-3] In the past two decade, porphyrin-based nano-materials with various topology, such as spheres,[4] wires,[1,5,6] rings,[7] have been widely reported. Among them, porphyrin-fullerene conjugate has attracted more and more attentions owing to its good properties in photo-induced electron transfer and photodynamic therapy.[8–13] On the other hand, cyclodextrin (CD) and hyaluronic acid (HA) are widely regarded as two important polysaccharide building blocks in the construction of bioactive supramolecular assemblies. Cyclodextrins, a class of cyclic oligosaccharides with six to eight D-glucose units linked by 1,4-glucose bonds, are water-soluble, non-toxic, commercial available with low price, and their hydrophobic cavities can bind various inorganic/organic/biological molecules and ions in both aqueous solution and the solid state to form functional supramolecular systems.[14] In addition, hyaluronic acid, an anionic glycosaminoglycan, has been widely used for various medical applications such as drug delivery,[15] tissue engineering,[16] and bioimaging diagnosis[17] due to its biocompatibility, biodegradability, non-toxicity, non-immunogenicity and non-inflammatory. Interestingly, HA can be also used as a targeting agent for cancer therapy because various tumor cells are known to over-express HA receptors such as cluster determinant 44 (CD44)[18] and receptor for hyaluronate-mediated motility (RHAMM).[19] Therefore, one can hypothesize that the combination of polysaccharide with porphyrin-fullerene conjugate may bring a breakthrough in many fields of chemistry and biomaterial science. Herein, we wish to report a facile method to construct water-soluble nanometer-scaled supramolecular conjugate, composed of polysaccharide (β-CD and adamantyl-modified HA), triphenyl Zn-porphyrin and C_{60} (Scheme 1). Compared with the reported porphyrin-fullerene conjugates, the inherent advantages of this supramolecular conjugate are (1) the presence of numerous CD units can greatly improve the water solubility and the biocompatibility of porphyrin-fullerene conjugates. (2) the introduction of hyaluronate will enable the targeting property of porphyrin-fullerene conjugate (3) the supramolecular non-covalent conjugation among polysaccharide, porphyrin and fullerene can provide a convenient and versatile approach to overcome the disadvantage of the direct covalent conjugation that usually requires a large amount of organic solvents, a long time course, many times of separation and purification, and does harm to environment. Interestingly, these polysaccharide-porphyrin-fullerene supramolecular conjugates showed the good DNA cleavage abilities under the light irradiation.



Scheme 1. Construction of polysaccharide-porphyrin-fullerene supramolecular conjugate.

The grafting of adamantyl groups on the backbone of HA was confirmed by FT-IR spectra (Figure S1), where the stretching

vibration band of carbonyl group (amido bond) at 1557 cm^{-1} in **7** was stronger than that in the unmodified HA, attributing to the partial amidation of carboxylic acid groups in HA. The degree of substitution (D_S) of adamantyl groups in **7**, defined as the ratio of adamantyl groups per 100 carboxyl units of HA, was quantitatively calculated as 25.0% from the integration ratio of characteristic peaks of adamantane at 1.4~1.8 ppm and HA at 1.9 ppm (-CH_3) in ^{1}H NMR spectra (Figure S2). In addition, the UV-Vis spectra of supramolecular conjugates **6**/C_{60} and **6**/**7**/C_{60} exhibited the broad bands at 265 and 339 nm ascribed to C_{60} (Figure 1a).[20] In aqueous solution, the UV-Vis spectrum of **6** showed a maximum at 430 nm assigned to the Soret band of porphyrin unit, and this maximum shifted to a longer wavelength (439 nm for **6**/C_{60} and 441 nm for **6**/**7**/C_{60}) in the UV-Vis spectrum of **6**/C_{60} or **6**/**7**/C_{60}, accompanied by a slight bathochromic shift of Q-band, indicating the electronic interaction between **6** and C_{60}.[21] Moreover, the broad absorption at 650-800 nm in the UV-Vis spectrum of **6**/C_{60} or **6**/**7**/C_{60} also indicated the charge transfer (CT) interactions between fullerene and porphyrin.[22] In addition, the fluorescence emission of **6**/C_{60} and **6**/**7**/C_{60} also revealed the significant quenching (Figure 1b) as compared with that of free **6**, indicating an efficient electron transfer between porphyrin and C_{60}.[6] These phenomena jointly demonstrated the fullerene/porphyrin interactions.

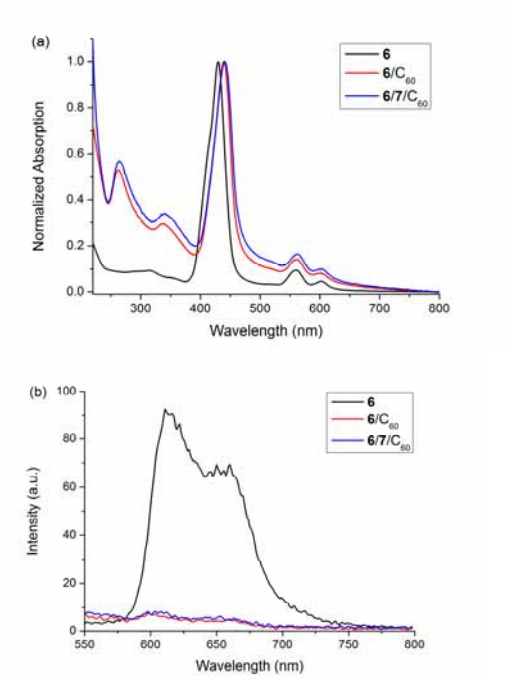


Figure 1. (a) Normalized UV-Vis spectra of **6**, **6**/C_{60} and **6**/**7**/C_{60} in water at 25 °C; (b) Fluorescence spectra of **6**, **6**/C_{60} and **6**/**7**/C_{60} in water at 25 °C.

Considering the solubility difference of C_{60} in DMSO and toluene, the content of C_{60} in supramolecular conjugate was determined by UV-Vis spectroscopy. A certain amount of supramolecular conjugate was dissolved in DMSO, the insoluble substance was separated by centrifugation and dissolved in toluene. The UV-Vis spectra of DMSO and toluene solution were recorded (Figure S3), and the content of C_{60} in **6**/**7**/C_{60} was calculated as 5.98 × 10^{-5} mol·g^{-1}. In addition, the molar ratio between **6** and **7** in **6**/**7**/C_{60} was determined by ^{1}H NMR spectra (Figure S4). Since the ^{1}H NMR signals in range of 3.0 to 6.0 ppm, which were assigned to the glucose groups in both **6** and **7**, were complex and overlapped with each other, we only analyzed the signals in low field and high field, which were assigned to the porphyrin group in **6** and the adamantyl group in **7**, respectively, to calculate the molar ratio between **6** and the each adamantyl unit of **7** in **6**/**7**/C_{60} as 1:1.

The morphological information of supramolecular conjugates came from the electronic microscopy measurements. Figure 2 showed the TEM images of **6**/C_{60} and **6**/**7**/C_{60}. Therein, **6**/C_{60} existed as the irregular nanoparticles with a diameter of 50 to 130 nm, and these nanoparticles tended to crosslink with the neighboring ones (Figure 2a). In contrast, **6**/**7**/C_{60} existed as the spherical nanoparticles with a diameter of 50 to 200 nm and did not show the obvious crosslinking and transmogrification (Figure 2b). A similar phenomenon also was observed in the AFM images. As shown in Figure 3a, **6**/C_{60} mainly existed as the cross-linked aggregates, while **6**/**7**/C_{60} mainly existed as the discrete nanoparticles. These phenomena demonstrated that the introduction of HA prevented the crosslinking of neighboring **6**/C_{60} aggregates to some extent.

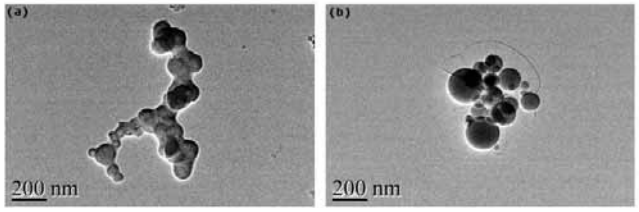


Figure 2. TEM images of (a) **6**/C_{60} and (b) **6**/**7**/C_{60}.

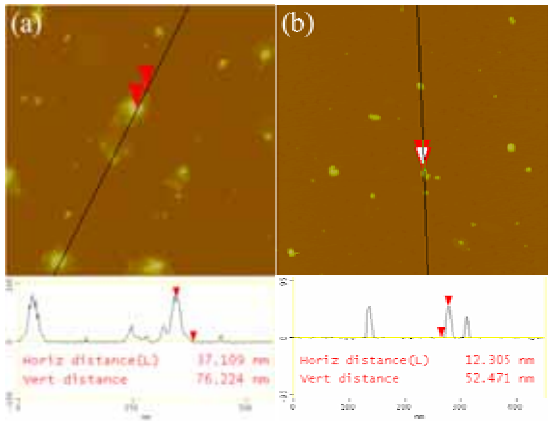


Figure 3. AFM images of (a) **6**/C_{60} and (b) **6**/**7**/C_{60}.

The photo-induced DNA cleavage ability of supramolecular conjugates was detected by the agarose gel electrophoresis assay. As shown in Figure 4, both **6**/C_{60} and **6**/**7**/C_{60} showed the significant DNA cleavage abilities, and **6**/**7**/C_{60} gave the higher DNA cleavage ability than **6**/C_{60}. For example, the closed supercoiled DNA (form I) was completely cleaved to the nicked DNA (form II) by **6**/**7**/C_{60} at a mass concentration of 300 ng·μL^{-1} (Figure 4, lane 3), while the mass concentration of **6**/C_{60} should be 700 ng·μL^{-1} to achieve the same consequence (Figure 4, lane 10). The control experiments showed that the content of C_{60} in **6**/**7**/C_{60} was 5.6 times lower than the corresponding value in **6**/C_{60},

we deduced that the DNA cleavage ability of **6**/**7**/C_{60} should be fairly higher than that of **6**/C_{60}.

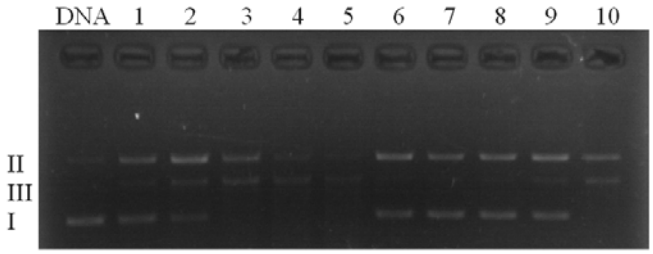


Figure 4. Agarose gel electrophoresis assay of pBR 322 DNA (5 ng·μL^{-1}). [**6**/**7**/C_{60}] = 50, 100, 300, 500, 700 ng·μL^{-1} from lanes 1 to 6, respectively; [**6**/C_{60}] = 50, 100, 300, 500, 700 ng·μL^{-1} from lanes 6 to 10, respectively.

Conclusions

In summary, several water-soluble polysaccharide-porphyrin-fullerene supramolecular conjugates, which have the application potential as DNA reagents, have been constructed from β-CD, triphenylporphyrin and adamantyl-modified hyaluronate. As an example of the possible applications, these supramolecular conjugates displayed a good ability of cleaving DNA under the visible light irradiation. Considering the good photophysical and photochemical properties of porphyrin and fullerene as well as the targeting capability of hyaluronate, this achievement would not only provide a new access of modifying polysaccharide with functional groups but also extend the possible applications of polysaccharide-based supramolecular species in many fields of pharmaceutical chemistry and biological technology.

Acknowledgement

We thank the 973 Program (2011CB932502) and the NNSFC (91227107, 21432004 and 21272125) for financial support.

Notes and references

[1]*Department of Chemistry, State Key Laboratory of Elemento-Organic Chemistry,* [2]*Collaborative Innovation Center of Chemical Science and Engineering, Nankai University, Tianjin 300071, P. R. China.*
Email: yuliu@nankai.edu.cn

† Electronic Supplementary Information (ESI) available: synthesis, characterization and the experiment details. See DOI: 10.1039/b000000x/

‡ These two authors contributed equally to this work.

1 (a) Fathalla, M.; Neuberger, A.; Li, S.-C.; Schmehl, R.; Diebold, U.; Jayawickramarajah, J. *J. Am. Chem. Soc.* **2010**, *132*, 9966–9967. (b) Fathalla, M.; Li, S.-C.; Diebold, U.; Alb, A.; Jayawickramarajah, J. *Chem. Commun.* **2009**, 4209–4211. (c) Zhang, N.; Chu, X.; Fathalla, M.; Jayawickramarajah, J. *Langmuir* **2013**, *29*, 10796–10806.

2 Lifschitz, A. M.; Young, R. M.; Mendez-Arroyo, J.; Stern, C. L.; McGuirk, C. M.; Wasielewski, M. R.; Mirkin, C. A. *Nature Commun.* **2015**, *6*, 6541.

3 (a) Sun, M.; Zhang, H.-Y.; Liu, B.-W.; Liu, Y. *Macromolecules* **2013**, *46*, 4268–4275. (b) Li, Z.-Q.; Zhang, Y.-M.; Chen, Y.; Liu, Y. *Chem. Eur. J.* **2014**, *20*, 8566–8570. (c) Liu, Y.; Yang, Z.-X.; Chen, Y.; Song, Y.; Shao, N. *ACS Nano* **2008**, *2*, 554–560.

4 (a) Zhang, H.; Zhang, B.; Zhu, M.; Grayson, S. M.; Schmehl, R.; Jayawickramarajah, J. *Chem. Commun.* **2014**, *50*, 4853–4855. (b) Charalambidis, G.; Kasotakis, E.; Lazarides, T.; Mitraki, A.; Coutsolelos, A. G. *Chem. Eur. J.* **2011**, *17*, 7213–7219.

5 Li, Z.-Q.; Zhang, Y.-M.; Guo, D.-S.; Chen, H.-Z.; Liu, Y. *Chem. Eur. J.* **2013**, *19*, 96–100.

6 Xu, H.; Zheng, J. *Macromol. Chem. Phys.* **2010**, *211*, 2125–2131.

7 (a) Liu, P.; Neuhaus, P.; Kondratuk, D. V; Balaban, T. S.; Anderson, H. L. *Angew. Chem. Int. Ed.* **2014**, *53*, 7770–7773. (b) Sprafke, J. K.; Kondratuk, D. V; Wykes, M.; Thompson, A. L.; Hoffmann, M.; Drevinskas, R.; Chen, W.-H.; Yong, C. K.; Kärnbratt, J.; Bullock, J. E.; Malfois, M.; Wasielewski, M. R.; Albinsson, B.; Herz, L. M.; Zigmantas, D.; Beljonne, D.; Anderson, H. L. *J. Am. Chem. Soc.* **2011**, *133*, 17262–17273. (c) Hoffmann, M.; Wilson, C. J.; Odell, B.; Anderson, H. L. *Angew. Chem. Int. Ed.* **2007**, *46*, 3122–3125.

8 (a) Yan, Q.-F.; Luo, Z.-Y.; Cai, K.; Ma, Y.-G.; Zhao, D.-H. *Chem. Soc. Rev.* **2014**, *43*, 4199-4221. (b) Zhu, Y.-Y.; Wang, G.-T.; Li, Z.-T. *Curr. Org. Chem.* **2011**, *15*, 1266-1292. (c) Wróbel, D.; Graja, A. *Coord. Chem. Rev.* **2011**, *255*, 2555– 2577. (d) Bottari, G.; de la Torre, G.; Torres, T. *Acc. Chem. Res.* **2015**, *48*, 900-910. (e) D'Souza, F.; Ito, O. *Chem. Commun.* **2009**, 4913-4928.

9 (a) Hou, J.-L.; Yi, H.; Shao, X.; Li, C.; Wu, Z.; Jiang, X.; Wu, L.-Z.; Tung, C.-H.; Li, Z. *Angew. Chem. Int. Ed.* **2006**, *45*, 796-800. (b) Feng, K.; Yu, M.-L.; Wang, S.-M.; Wang, G.-X.; Tung, C.-H.; Wu, L.-Z . *Chemphyschem* **2013**, *14*, 198-203. (c) Zhou, C.-S.; Liu, Q.-L.; Xu, W.; Wang, C.-R.; Fang, X.-H. *Chem. Commun.* **2011**, *47*, 2982-2984.

10 (a) Wang, S.-S.; Qu, Y.-P.; Li, S.-J.; Ye, F.; Chen, Z.-B.; Yang, X.-N. *Adv. Funct. Mater.* **2015**, *25*, 748-757. (b) Liang, Z.; Dzienis, K. L.; Xu, J.; Wang, Q. *Adv. Funct. Mater.* **2006**, *16*, 542-548.

11 (a) Zheng, J.-Y.; Tashiro, K.; Hirabayashi, Y.; Kinbara, K.; Saigo, K.; Aida, T.; Sakamoto, S.; Yamaguchi, K. *Angew. Chem. Int. Ed.* **2001**, *40*, 1858-1861. (b) Charvet, R.; Yamamoto, Y.; Sasaki, T.; Kim, J.; Kato, K.; Takata, M.; Saeki, A.; Seki, S.; Aida T. *J. Am. Chem.Soc.* **2012**, *134*, 2524-2527. (c) Zhao, H.-Y.; Zhu, Y.-Z.; Chen, C.; Zheng, J.-Y. *Polymer* 2014, 55, 1913-1916.

12 (a) D'Souza, F.; Amin, A. N.; El-Khouly, M. E.; Subbaiyan, N. K.; Zandler, M. E.; Fukuzumi, S. *J. Am. Chem. Soc.* **2012**, *134*, 654-664. (b) Calderon, R. M. K.; Valero, J.; Grimm, B.; de Mendoza, J.; Guldi D. M. *J. Am. Chem. Soc.* **2014,** *136,* 11436-11443. (c) Shirakawa, M.; Fujita, N.; Shinkai, S. *J. Am. Chem. Soc.* **2004**, *126*, 9902-9903. (d) Zhang, C.; Wang, Q.; Long, H.; Zhang, W. *J. Am. Chem. Soc*. **2011**, *133*, 20995-21001. (e) Zhang, J.; Tan, J.; Ma, Z.; Xu, W.; Zhao, G.; Geng, H.; Di, C.; Hu, W.-P.; Shuai, Z.-G.; Singh, K.; Zhu D.-B. *J. Am. Chem. Soc.* **2013**, *135*, 558−561.

13 (a) Moreira, L.; Calbo, J.; Illescas, B. M.; Aragó, J.; Nierengarten, I.; Delavaux-Nicot, B.; Ortí, E.; Martín, N.; Nierengarten, J.-F. *Angew. Chem. Int. Ed.* **2015**, *54*, 1255-1260. (b) Umeyama, T.; Tezuka, N.; Kawashima, F.; Seki, S.; Matano, Y.; Nakao, Y.; Shishido, T.; Nishi, M.; Hirao, K.; Lehtivuori, H.; Tkachenko, N. V.; Lemmetyinen, H.; Imahori, H. *Angew. Chem. Int. Ed.* **2011**, *50*, 4615-4619. (c) Lim, G. N.; Maligaspe, E.; Zandler, M. E.; D'Souza, F. *Chem. Eur. J.* **2014**, *20*, 17089-17099.

14 Chen, Y.; Liu, Y. *Chem. Soc. Rev.* **2010**, *39*, 495-505.

15 (a) Kakehi, K.; Kinoshita, M.; Yasueda, S. *J. Chromatogr. B* **2003**, *797*, 347–355. (b) Oh, E. J.; Park, K.; Kim, K. S.; Kim, J.; Yang, J.-A.; Kong, J.-H.; Lee, M. Y.; Hoffman, A. S.; Hahn, S. K. *J. Control. Release* **2010**, *141*, 2–12. (c) Oh, E. J.; Kim, J.-W.; Kong, J.-H.; Ryu, S. H.; Hahn, S. K. *Bioconjug. Chem.* **2008**, *19*, 2401–2408. (d) Han, S.-Y.; Han, H. S.; Lee, S. C.; Kang, Y. M.; Kim, I.-S.; Park, J. H. *J. Mater. Chem.* **2011**, *21*, 7996–8001.

16 Toole, B. P. *Nat Rev Cancer* **2004**, *4*, 528–539.

17 (a) Lee, H.; Lee, K.; Kim, I. K.; Park, T. G. *Biomaterials* **2008**, *29*, 4709–4718. (b) Kim, J.; Kim, K. S.; Jiang, G.; Kang, H.; Kim, S.; Kim, B.-S.; Park, M. H.; Hahn, S. K. *Biopolymers* **2008**, *89*, 1144–1153.

18 (a) Annabi, B.; Thibeault, S.; Moumdjian, R.; Béliveau, R. *J. Biol. Chem.* **2004**, *279*, 21888–21896. (b) Choi, K. Y.; Min, K. H.; Na, J. H.; Choi, K.; Kim, K.; Park, J. H.; Kwon, I. C.; Jeong, S. Y. *J. Mater. Chem.* **2009**, *19*, 4102–4107. (c) Choi, K. Y.; Chung, H.; Min, K. H.; Yoon, H. Y.; Kim, K.; Park, J. H.; Kwon, I. C.; Jeong, S. Y. *Biomaterials* **2010**, *31*, 106–114.

19 (a) Platt, V. M.; Szoka, F. C. *Mol. Pharm.* **2008**, *5*, 474–486. (b) Li, N.; Chen, Y.; Zhang, Y.-M.; Yang, Y.; Su, Y.; Chen, J.-T.; Liu, Y.

Sci. Rep. **2014**, *4*, 4164. (c) Yang, Y.; Zhang, Y.-M.; Chen, Y.; Chen, J.-T.; Liu, Y. *J. Med. Chem.* **2013**, *56*, 9725–9736.
20 Liu, Y.; Liang, P.; Chen, Y.; Zhang, Y.-M.; Zheng, J.-Y.; Yue, H. *Macromolecules* **2005**, *38*, 9095–9099.
21 (a) Kuciauskas, D.; Lin, S.; Seely, G. R.; Moore, A. L.; Moore, T. A.; Drovetskaya, T.; Reed, C. A.; Boyd, P. D. W.; Gust, D. *J. Phys. Chem.* **1996**, *100*, 15926–15932. (b) Baran, P. S.; Khan, A. U.; Schuster, D. I.; Wilson, S. R.; York, N.; Monaco, R. R. *J. Am. Chem. Soc.* **1997**, *119*, 8363–8364. (c) Dietel, E.; Hirsch, A.; Eichhorn, E.; Rieker, A.; Hackbarth, S.; Roder, B.; Röder, B. *Chem. Commun.* **1998**, 1981–1982. (d) Tashiro, K.; Aida, T.; Zheng, J.-Y.; Kinbara, K.; Saigo, K.; Sakamoto, S.; Yamaguchi, K. *J. Am. Chem. Soc.* **1999**, *121*, 9477–9478.
22 (a) Imahori, H.; Tkachenko, N. V; Vehmanen, V.; Tamaki, K.; Lemmetyinen, H.; Sakata, Y.; Fukuzumi, S. *J. Phys. Chem. A* **2001**, *105*, 1750–1756. (b) Bell, T. D. M.; Ghiggino, K. P.; Jolliffe, K. A.; Ranasinghe, M. G.; Langford, S. J.; Shephard, M. J.; Paddon-Row, M. N. *J. Phys. Chem. A* **2002**, *106*, 10079–10088. (c) Tkachenko, N. V; Lemmetyinen, H.; Sonoda, J.; Ohkubo, K.; Sato, T.; Imahori, H.; Fukuzumi, S. *J. Phys. Chem. A* **2003**, *107*, 8834–8844. (d) Wang, Y.; Xu, H.; Zhang, X. *Adv. Mater.* **2009**, *21*, 2849–2864.