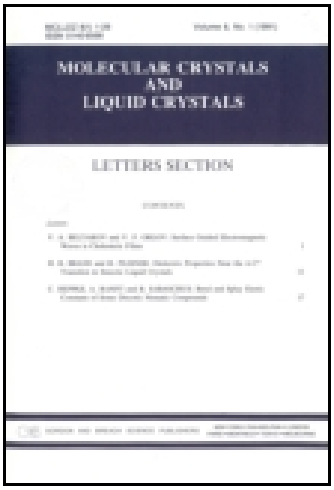

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Preparation of Conjugated Polymer Dots as a Fluorescence Turn-On Assay for Bovine Serum Albumin by Interaction with Graphene Oxide

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Preparation of Conjugated Polymer Dots as a Fluorescence Turn-On Assay for Bovine Serum Albumin by Interaction with Graphene Oxide

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Fluorescent conjugated polymers with various monomer compositions were synthesized via Suzuki coupling polymerization and spherically-shaped conjugated polymer dots (CPdot) with uniform size were prepared via conventional reprecipitation technique. Chemically modified graphene oxide (GO) was prepared to use as a component of sensor platform for protein detection. GO showed an excellent interaction with CPdot on its surface via hydrophobic interaction, which, in turn, induced the quenching of the fluorescence of CPdot. Upon exposure to bovine serum albumin (BSA), the quenched fluorescence was recovered, resulting from the release of CPdot from the complex with GO, as BSA adsorbed preferentially on the GO surface.

Keywords Sensors; fluorescence; graphene oxide; bovine serum albumin; conjugated polymer dot

Introduction

Fluorescent conjugated polymers with interesting optical properties have attracted great attention in the applications of chemo- and biosensors [1]. The electronic structure of conjugated polymers enables the easy transport of excitation energy along the rigid backbone to energy receptors, producing amplification of fluorescence signals [2].

Considerable effort in the manipulation of nanostructured materials with integrated functions has been made for a variety of applications, such as imaging, optoelectronics,

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and nanomedicine [3]. One of the results, conjugated polymer nanospheres are fluorescent probes that demonstrate extraordinary fluorescence brightness, excellent photostability, and high emission rate. It has been reported that polymer nanospheres show excellent optical performance, good biocompatibility through surface functionalization, and successful bioconjugation [4].

GO is known to be a two-dimensional nanomaterial that can be easily obtained by exfoliation into monolayer sheets. GO has oxygen-containing functional groups such as epoxides, alcohols, and carboxylic acids, and thus, can be uniformly dispersible in water with many applications [5]. Because GO sheets in aqueous dispersion show negative charges with a hydrophobic backbone, positively charged molecules can be adsorbed on the GO surface through electrostatic and $\pi-\pi$ interactions.

The ability of GO for *inter*molecular $\pi-\pi$ interaction, besides the electrostatic interaction, provides a platform to induce aggregation with CPdots. Herein, we demonstrate the intermolecular interaction-induced aggregation of fluorescent CPdots on a GO surface, resulting in fluorescence quenching. The aggregated, quenched CPdots can be easily released from the GO surface because of the more favorable interaction between GO and BSA, which, in turn, triggers the recovery of fluorescence of the CPdots. To our knowledge, this is the first protocol that describes an interaction between conjugated polymer nanospheres and GO to use as a sensor platform for BSA.

Experimental Details

Materials

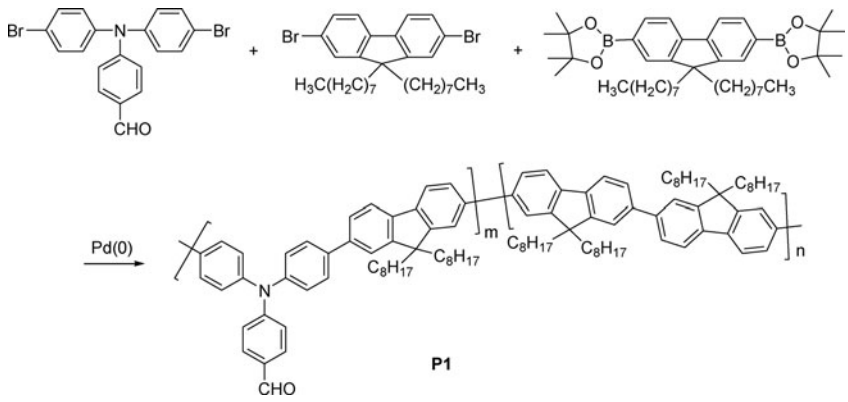
9,9-Dioctyl-2,7-dibromofluorene, 9,9-dioctylfluorene-2,7-diboronic acid bis(1,3-propanediol)ester, and tetrakis(triphenylphosphine)-palladium(0) were purchased from Sigma-Aldrich and used further purification. BSA was purchased from Sigma-Aldrich. 4-(Bis(4-bromophenyl)amino)benzaldehyde was synthesized using previously published methods [6]. GO was synthesized from graphite powder based on Hummer's method [7].

Characterization

^{1}H NMR was obtained on a Bruker DRX-300 spectrometer (Korea Basic Science Institute). The elemental analysis was determined with a CE Instruments EA-1110 elemental analyzer. FT-IR analysis was measured from Bruker Temsor 27 spectrometer. The particle image and size were observed by FE-SEM (Hitachi S-4800). The image of atomic force microscope (AFM) was recorded on VEECO Nanoman. UV-vis absorption spectra were recorded on a PerkinElmer Lambda 35 spectrometer. Photoluminescence spectra were taken from a Varian Cary Eclipse equipped with a xenon lamp excitation source.

Synthesis of P1

4-(Bis(4-bromophenyl)amino)benzaldehyde (0.215 g, 0.50 mmol), 9,9-dioctyl-2,7-dibromofluorene (0.274 g, 0.50 mmol), and 9,9-dioctylfluorene-2,7-diboronic acid bis(1,3-propanediol)ester (0.670 g, 1.2 mmol) were dissolved in a mixture of THF (10 mL) containing aqueous 2 M potassium carbonate solution (4 mL). After addition of tetrakis(triphenylphosphine) palladium(0) (3.5 mg, 0.003 mmol), the reaction mixture was stirred under argon at 100°C for 48 h. After reaction, the reaction mixture was cooled and slowly added to methanol (500 mL), and the precipitate was isolated by filtration



Scheme 1. Synthetic route and chemical structure of **P1**.

and washed with methanol. The product was extracted with acetone for 48 h in a Soxhlet apparatus to remove oligomers and catalyst residues., yielding whitish yellow powders (yield 0.45 g, 41%). Other polymers were synthesized according to the same procedure and only difference lied in the monomer feed ratios. ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 9.8$ (s), 7.7−6.8 (m), 4.2−3.7 (m), 3.7−3.2 (m), 3.0−2.6 (m), 2.5−1.8 (m), 1.6−1.0 (m) ppm. FT-IR (KBr pellet): 3028 (C−H), 1698 (C=O), 1592 (C=C), 1461 (C=C), 1275 cm^{-1} (C−N). Anal. Calcd. For $C_{50.4}H_{69.0}N_{0.4}O_{0.4}$: C, 88.2%; H, 10.1%; N, 0.80%. Anal. Found. For C, 87.5%; H, 9.7%; N, 0.78%.

Preparation of CPdot [8]

The green-emitting conjugated polymer nanoparticles in aqueous solution were prepared by the reprecipitation method. In a typical preparation, **P1** was first dissolved in THF to make a 1 mg/mL stock solution. 1 mL of the solution was quickly added to 12 mL of Milli-Q water in a bath sonicator. THF was removed by nitrogen stripping. The solution

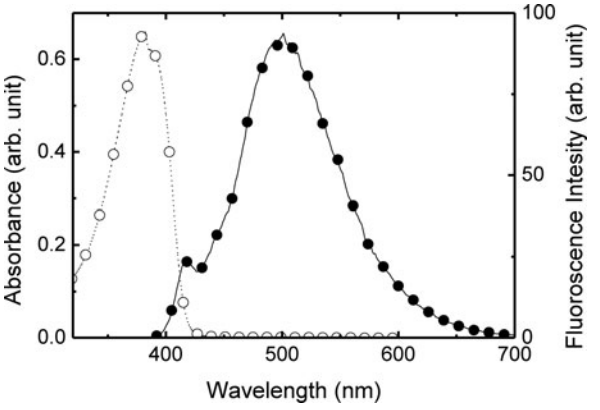
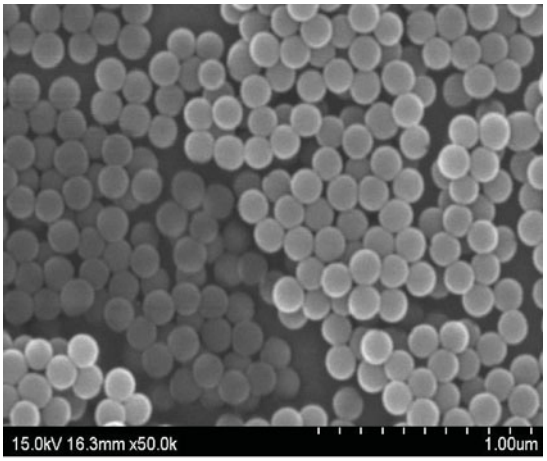
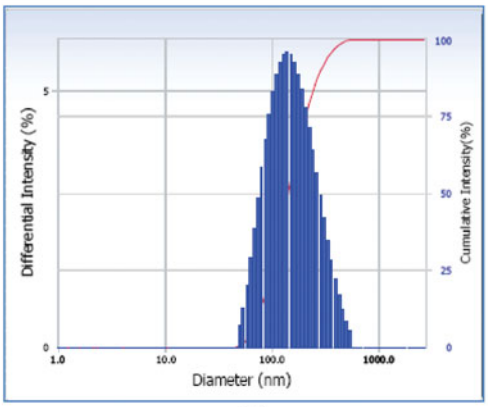


Figure 1. Absorption (○) and fluorescence spectra (●) of **P1** in THF solution. Excitation wavelength $\lambda_{ex} = 380$ nm.



(a)



(b)

Figure 2. (a) FE-SEM image and (b) dynamic light scattering (DLS) data of CPdot-**P1** in aqueous solution.

was filtered using a 0.45 μm filter. The conjugated polymer nanoparticle dispersions were clear and stable for two weeks without any aggregation.

Results and Discussion

The synthetic route and chemical structure of polymer are illustrated in Scheme 1. The **P1** was synthesized via a reaction with 4-(bis(4-bromophenyl)amino)benzaldehyde, 9,9-ditoctylfluorene-2,7-diboronic acid bis(1,3-propanediol)ester, and 9,9-dioctyl-2,7-dibromofluorene with various monomer ratio using Suzuki coupling polymerization. **P1** was soluble in common organic solvents, such as THF, chloroform, and DMF. The molar composition (m : n) of **P1** was determined by elemental analysis and found to be 0.40 : 0.60. GPC measurement showed that the number-average molecular weight of **P1** was found to be 10570 and weight-average molecular weight to be 49470 using THF as an eluent.

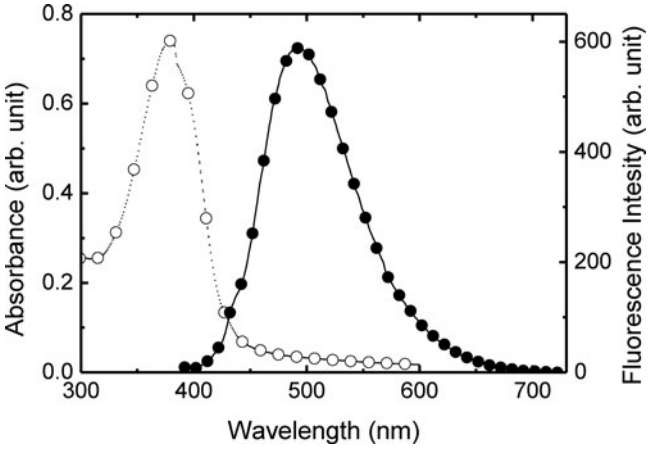
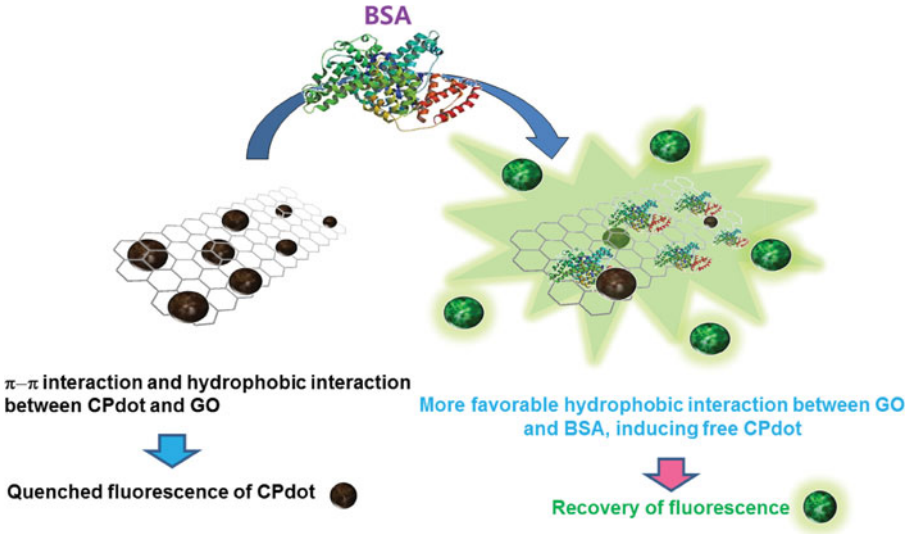


Figure 3. Absorption (○) and emission (●) spectra of CPdot-**P1** in aqueous solution. Excitation wavelength corresponds to the absorption maximum.

The optical absorption and emission spectra of **P1** in THF solutions are shown in Figure 1. **P1** in THF solution shows absorption at 380 nm and broad emission centered around 500 nm.

Green emitting CPdot of **P1** (CPdot-**P1**) can be obtained via a reprecipitation method by adding its THF solution into water under sonication, which enables the formation of nanospheres with a mean diameter of 120 nm measured by SEM and DLS (Figure 2). The optical properties of **P1** in THF solution and CPdot in water are illustrated in Figure 3. The absorption and emission maximum of **P1** was observed at 380 nm and 500 nm, respectively, with strong bluish-green emission in THF solution (Figure 1). Similar absorption and emission were found in the CPdot-**P1** in water, which exhibited an absorption (380 nm) and an emission (496 nm).



Scheme 2. Schematic illustration for detection of BSA using interaction between CPdot and GO.

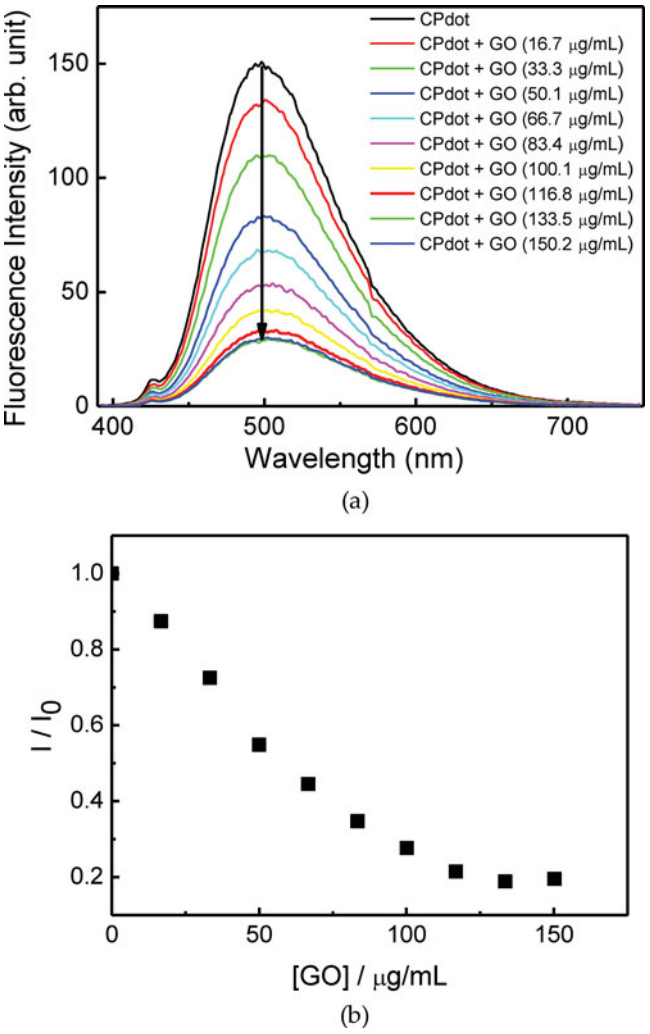


Figure 4. Changes in emission intensity of CPdot-**P1** in the presence of various concentration of GO. $\lambda_{ex} = 380$ nm. I_0 and I correspond to the emission intensities at 492 nm before and after addition of GO, respectively.

GO was successfully prepared from graphite powder based on Hummer's method. It showed a nearly single-layer with a topographic height of <2 nm. The chemically synthesized GO was readily water-dispersible, mainly because of the presence of hydrophilic groups such as epoxy, hydroxyl 3420 cm^{-1}), and carboxylic groups (1740 and 1250 cm^{-1}) at the surface, which was confirmed by FT-IR.

The working principle of our strategy is schematically represented in Scheme 2. The detection assay is composed of GO and fluorescent CPdot-**P1**. When the fluorescent CPdot solution is exposed to GO nanosheets, the fluorescence of the CPdot is completely quenched via non-covalent hydrophobic and $\pi-\pi$ interactions between CPdot and GO. In the presence of BSA, GO exhibits intriguing interaction with BSA, which is much stronger than the interaction between CPdot and GO. As a result, the green fluorescence of CPdot-**P1** is

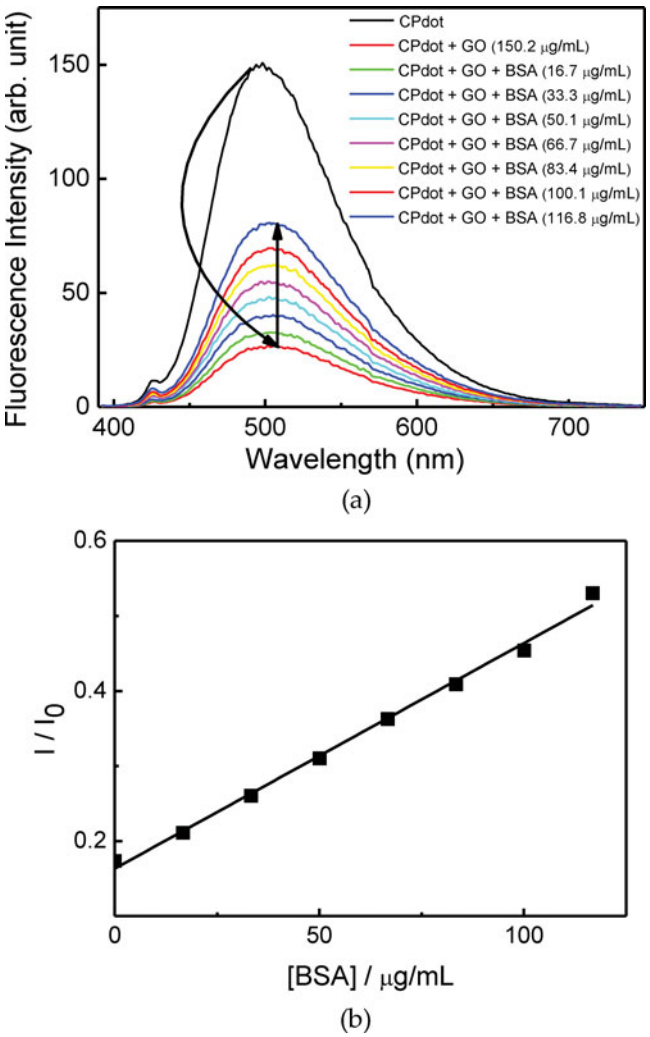


Figure 5. Changes in emission intensity of CPdot-**P1** and GO mixture in the presence of various concentration of BSA. [GO] = 150.2 μg/mL, λ_{ex} = 380 nm. I_0 and I correspond to the emission intensities at 492 nm before and after addition of BSA, respectively.

recovered gradually to an extent that depends on the concentration of BSA added. Thus the observed emission enhancement enables us to obtain fluorescence turn-on assay for BSA.

Upon addition of GO, the fluorescence of CPdot-**P1** was gradually quenched, because of the strong photoinduced electron or energy transfer between CPdot and GO as shown in Figure 4 [9]. The spectral response clearly indicates strong electrostatic and hydrophobic $\pi-\pi$ interactions between CPdots and GO sheets.

Addition of BSA into CPdot-GO solution revealed a gradual recovery about 53% in the emission intensity of the solution with negligible emission shift (Figure 5). Complete, 100% recovery of initial intensity of CPdot-**P1** was not attained, presumably due to the presence of remaining GO. It is known that the BSA has a more favorable interaction with GO via both hydrophobic and electrostatic interactions [10]. The enhancement of

emission was considerably dependent on the ratio of CPdot to GO. GO of 150.2 μg/mL was found to be optimal concentration for the subsequent enhancement of emission. The increase in the fluorescence shows a linear relationship with the concentration of BSA as shown in Figure 5b. A control experiment showed that CPdot-**P1** was barely responsive to BSA in the absence of GO, and thus, exhibited negligible emission enhancement without GO, indicating the importance of GO to attain the noticeable emission response.

Thus this sensing platform which involves CPdots and GO can be applied to other biologically-relevant species using control of interaction.

Conclusion

Conjugated polymer with triphenylamine units was synthesized to emit greenish blue emission and was used to prepare polymer nanodots. It was found that BSA could modulate the hydrophobic and $\pi-\pi$ interaction between CPdot and GO. The addition of BSA protein to CPdot-GO resulted in fluorescence turn-on mode, because of the more favorable and stronger interaction of GO and BSA protein, demonstrating that the CPdot-GO interaction could be an efficient tool in the development of a fluorescence turn-on sensor for protein detection.

Funding

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References

[1] (a) Skotheim, T. A., Elsenbaumer, R. L., Reynolds, J. R., In *Handbook of Conducting Polymers, 2nd ed.*, Eds, Marcel Dekker, New York, 1997. (b) Hadziioannou, G., van Hutten, P. F., In *Semiconducting Polymers*, Eds, Wiley-VCH: Weinheim, Germany, 2000.

[2] (a) Thomas, S. W., Joly, G. D., & Swager, T. M. (2007). *Chem Rev.*, *107*, 1339. (b) Rose, A., Zhu, Z., Madigan, C. F., Swager, T. M., & Bulovic V. (2005). *Nature*, *434*, 876. (c) Ho, H.A., Najari, A., & Leclerc, M. (2008). *Acc Chem Res*, *41*, 168. (d) McQuade, D. T., Pullen, A. E., & Swager, T. M. (2000). *Chem Rev.*, *100*, 2537. (e) Kwak, C. K., Kim, D. G., Kim, T. H., Lee, C.-S., Lee, M., & Lee, T. S. (2010). *Adv. Funct. Mater.*, *20*, 3847. (f) Kwon, N. Y., Kim, D., Jang, G., Lee, J. H., So, J.-H., Kim, C.-H., Kim, T. H., & Lee, T. S. (2012). *ACS Appl. Mater. Interfaces*, *4*, 1429. (g) Kim, D., Jang, G., Kim, J., Seo, S., Park, W. H., & Lee, T. S. (2012). *Macromol. Rapid Commun. 33*, 1510.

[3] (a) Jin, Y., & Gao, X. (2009). *Nat. Nanotechnol.*, *4*, 571. (b) Hu, S.-H., & Gao, X. (2010). *J. Am. Chem. Soc.*, *132*, 7234. (c) Zhang, J., Tang, Y., Lee, K., & Ouyang, M. (2010). *Science*, *327*, 1634. (d) Jones, M. R., Osberg, K. D., Macfarlane, R. J., Langille, M. R., & Mirkin, C. A. (2011). *Chem. Rev.*, *111*, 3736.

[4] (a) Wu, C., Bull, B., Szymanski, C., Christensen, K., & McNeill, J. (2008). *ACS Nano*, *2*, 2415. (b) Howes, P., Green, M., Levitt, J., Suhling, K., & Hughes, M. (2010). *J. Am. Chem. Soc.*, *132*, 3989. (c) Kaeser, A., & Schenning, A. P. H. J. (2010). *Adv. Mater.*, *22*, 2985. (d) Pecher, J., & Mecking, S. (2010). *Chem. Rev.*, *110*, 6260. (e) Wu, C., Schneider, T., Zeigler, M., Yu, J., Schiro, P. G., Burnham, D. R., McNeill, J. D., & Chiu, D. T. (2010). *J. Am. Chem. Soc.*, *132*, 15410. (f) Chan, Y.-H., Jin, Y., Wu, C., & Chiu, D. T. (2011). *Chem. Commun.*, *47*, 2820. (g) Chan, Y.-H., Wu, C., Ye, F., Jin, Y., Smith, P. B., & Chiu, D. T. (2011). *Anal. Chem.*, *83*, 1448. (h) Jin, Y., Ye, F., Zeigler, M., Wu, C., & Chiu, D. T. (2011). *ACS Nano*, *5*, 1468.

[5] (a) Dikin, D. A., Stankovich, S., Zimney, E. J., Piner, R. D., Dommett, G. H. B., Evmenenko, G., Nguyen, S. T., & Ruoff, R. S. (2007). *Nature*, *448*, 457. (b) Allen, M. J., Tung, V. C., & Kaner, R. B. (2010). *Chem. Rev.*, *110*, 132. (c) Novoselov, K. S., Geim, A. K., Morozov, S. V., Jiang, D., Zhang, Y., Dubonos, S. V., Grigorieva, I. V., & Firsov, A. A.(2004). *Science*, *306*, 666. (d) Li, D., & Kaner, R. B. (2008). *Science*, *320*, 1170.
[6] Zhang, Z.-G., Zhang, K.-L., Liu, G., Zhu, C.-X., Neoh, K.-G., & Kang, E.-T. (2009) *Macromolecules*, *42*, 3104.
[7] Hummers, W. S., & Offeman, R. E. (1958). *J. Am. Chem. Soc.*, *80*, 1339.
[8] Wu, C., Hansen, S. J., Hou, Q., Yu, J., Zeigler, M., Jin, Y., Burnham, D. R., McNeill, J. D., Olson, J. M., & Chiu, D. T. (2011). *Angew. Chem. Int. Ed.*, *50*, 3430.
[9] (a) Balapanuru, J., Yang, J. X., Xiao, S., Bao, Q., Jahan, M., Polavarapu, L., Wei, J., Xu, Q. H., & Loh, K. P. (2010). *Angew. Chem., Int. Ed.*, *49*, 6549. (b) Xu, Y., Malkovskiy, A., & Pang, Y. (2011). *Chem. Commun.*, *47*, 6662.
[10] (a) Jisha, V. S., Arun, K. T., Hariharan, M., & Ramaiah, D. (2010). *J. Phys. Chem. B*, *114*, 5912. (b) He, X. M., & Carter, D. C. (1992). *Nature*, *358*, 209.