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COMMUNICATION

Luminescent Carbon Nanoparticles as a Donor for the FRET-based Detection of Oligonucleotide Hybridization

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Accepted 00th January 2012Ying Li,^a Chunhua Luo,^{*b} Chunli Jiang,^a Rong Huang,^a Yitang Wang,^a Hui Peng^{*a,c} and Jadranka Travas-Sejdic^c

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Luminescent Carbon Nanoparticles were prepared by electrochemical oxidation of graphite rods and used as a donor for the detection of ODN hybridization based on the FRET. The hybridization event was recognized by the different FRET efficiency due to different interaction abilities of single-stranded and double-stranded ODN to the polymer.

Quantum dots (QDs) based on semiconductors have been widely investigated, which gives rise to very promising applications in biosensing and bioimaging^{1, 2}. However, strict synthesis conditions, high prices and known toxicity greatly limits their practical applications¹. Thus, significant efforts have been paid to synthesis of QDs with appropriate optical properties but based on alternative materials with lower toxicity, for example silicon nanoparticles.³⁻⁵

Luminescent carbon nanoparticles (CNs) are unconventional type of QDs, that have drawn considerable attention in recent years due to easy preparation⁶⁻⁸ and comparable photoluminescence properties with conventional semiconductor QDs^{9, 10}. Up to now, various methods have been developed for the preparation of luminescent CNs. Examples include CNs derived from candle soot and carbohydrates^{11, 12}, hydrothermally synthesised from organic precursors^{7, 10, 13, 14}, CNs obtained by ultrasonic treatment of active carbon⁸, synthesized from organogel precursors under UV irradiation at room temperature¹⁵ and electrochemical oxidation of carbon fiber¹⁶ and graphite.¹⁷

The biosensors based on Förster resonance energy transfer (FRET) have been widely developed, including using semiconductor QDs as donors.^{18, 19} Although luminescent CNs have been successfully used in bioimaging^{20, 21} and detection of heavy metal ions^{10, 22}, there is few report related to luminescent CNs as a donor for FRET based oligonucleotide (ODN) sensor. A reason is that carbon nanomaterials normally show quenching effect of a dye chromophore on a dye-labelled single-stranded oligonucleotide (ssODN) due to the π - π stacking interaction²³. However, Mondal et al. recently characterized the surfactant bilayer protected core-shell carbon nanoparticles based on the strategy of FRET,²⁴ showing the great potential of CNs as a donor.

Here we successfully demonstrate a concept that luminescent CNs can be used as donors for the homogeneous detection of ODN hybridization based on FRET, as shown in Scheme 1. In order to achieve efficient FRET, a cationic polymer, poly(diallyldimethylammonium chloride) (PDADMAC), was used to modulate the distance between negatively charged luminescent CNs and dye-labelled single-stranded ODN (ssODN) probes through the electrostatic interaction. Unlike the traditional FRET based ODN sensors which generally need labeling of two nucleic acids or dual modification of the same strand, in this design covalent immobilization of the probe molecules is not required and ODN sequence specificity was achieved with minimal probe modification.



Scheme 1. Principle of ODN hybridization detection system based on the luminescent CNs/Cy3-labelled ODN FRET. Negatively charged CNs and Cy3-ODN form a sandwich structure through the electrostatic interaction in the presence of the cationic polymer, PDADMAC.

Luminescent CNs were first prepared by electrochemical oxidation of graphite rod in a 30 mL of PBS buffer solution containing 1.0 g of KNO_3 at room temperature. The product was purified by centrifugation and dialysis (see Support Information for experimental details). The prepared CNs shows an absorption band around 350 nm and an emission dependent on the excitation energy: as the excitation wavelength is increased, the emission peak position shifts to longer wavelengths and the intensity decreases (see Fig. S3 in Support Information). Fig. 1A and 1B gives the TEM image of as prepared CNs. Fig. 1A shows the large scale view of the CNs. The high resolution TEM image (Fig. 1B) illustrates that the size of small CNs is about 5 nm. FT-IR was used to characterize the prepared CNs. The FT-IR spectra clearly identified the carboxyl groups, both through the very broad 3300 cm^{-1} O-H stretching absorption

and the 1642 cm^{-1} C=O stretching vibration (see Fig. S2 in Support Information). The zeta potential of CNs was measured to be -8.7 mV , indicating the surface of prepared CNs is negatively charged due to the carboxyl groups.

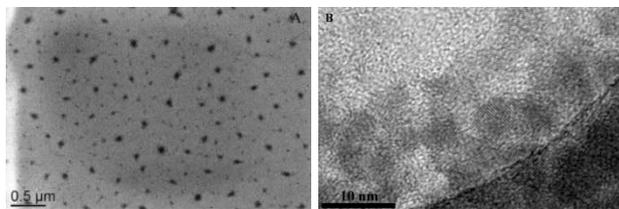


Fig. 1 (A) TEM and (B) high resolution TEM images of prepared CNs;

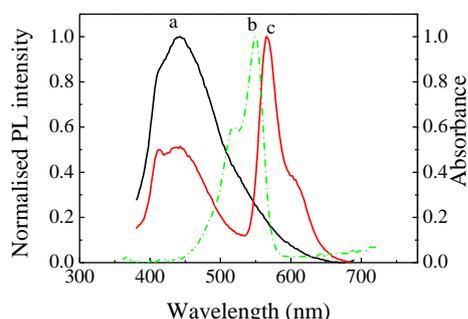


Fig. 2 Normalized spectra of (a) emission of C-dots excited at 360 nm, (b) absorption of Cy3-ODN, (c) emission of CNs /PDADMAC /Cy3-ODN excited at 360 nm. All spectra were recorded in diluted PBS buffer. The concentration of CNs, PDADMAC and Cy3-ODN are 0.003 OD (optical density at 360 nm), 0.17% (w/w) and 163.8 nM, respectively.

Due to the negatively charged surface of CNs, the addition of cationic polymer, PDADMAC, could result in the formation of the CNs/PDADMAC (CN^+) complex. The zeta potential of CNs/PDADMAC complex was measured to be 40.2 mV , showing that the surface CNs is covered by PDADMAC through the electrostatic interaction and led to the surface charge changed from negative to positive. Although the addition of PDADMAC changes the surface charge of CNs, it has little effect on the emission properties of CNs (see Fig. S4 in Support Information). The CN^+ shows an emission peak at 445 nm in diluted PBS buffer solution upon excitation at 360 nm (Fig. 2, curve a). Cy3-labelled ssODN (Cy3-ODN) was chosen as the acceptor because Cy3 is widely used in ODN labelling. However it is rarely used in FRET-based assays as its emission peak overlaps with most of the commercially available QDs. The absorption spectrum of Cy3-ODN is also shown in Fig. 2, curve b. The absorption spectrum of Cy3-ODN partly overlaps with the emission spectra of CN^+ , which is essential for efficient FRET taking place between CN^+ and Cy3 molecules. In fact, in a solution of CN^+ and Cy3-labelled ssODN, excitation of CN^+ at 360 nm results in efficient FRET to Cy3, as shown in Fig. 2, curve c (note: there is negligible direct emission from Cy3 upon excitation at 360 nm). Furthermore, a good separation between the emission peaks was achieved. The calculated FRET efficiency is 82% according to $E = 1 - F_{\text{DA}}/F_{\text{D}}$, where F_{DA} and F_{D} are integrated fluorescence intensities of CN^+ in the presence or absence of the acceptor Cy3, respectively.²⁵

As mentioned before, carbon based nanomaterials are normally used as quenchers for the biosensing. So the

quenching effect of prepared CNs was also investigated, as shown in Fig. S5. When excited upon 460 nm , the fluorescence intensity of Cy3 obviously decreased with the increase of amount of CNs. While in the presence of PDADMAC, the fluorescence intensity of Cy3 first increased after the addition of CNs, and then slowly decreased with the further increase in the amount of CNs (Fig. S6). Interpretation of this is that the increased fluorescence intensity of Cy3 after the initial addition of CNs is due to the formation of a sandwich structure of the CNs/PDADMAC/Cy3-ODN which greatly weakens the quenching effect of CNs, as the CNs and Cy3-ODN are separated by PDADMAC. Furthermore, CNs are luminescent when the excitation wavelength of 460 nm is employed, as shown in Fig. S2, which then results in the FRET between CNs and Cy3. The increase in the fluorescence intensity indicates that the distance between the CNs and Cy3-ODN in such 'sandwich' complex is appropriate for FRET to occur. The further increase in the amount of CNs causes the excess of CNs to directly interact with Cy3-ODN, leading to the decrease in fluorescence intensity. These results illustrate that PDADMAC plays an important role in the occurrence of FRET between CNs and Cy3-ODN.

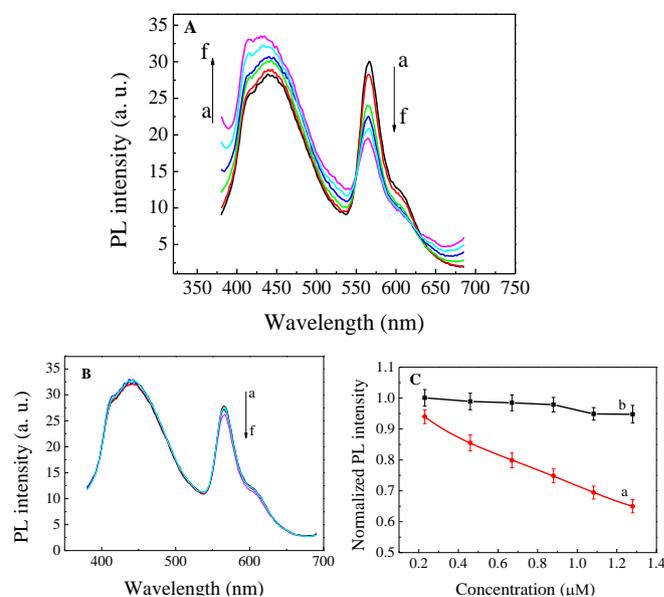


Fig. 3 Emission spectra of CNs /PDADMAC /Cy3-ODN after incubation with different concentration of (A) complementary ODN target and (B) noncomplementary ODN. a: $0\text{ }\mu\text{M}$; b: $0.23\text{ }\mu\text{M}$; c: $0.67\text{ }\mu\text{M}$; d: $0.88\text{ }\mu\text{M}$; e: $1.08\text{ }\mu\text{M}$; f: $1.28\text{ }\mu\text{M}$. (C) The dependence of normalized fluorescence intensity changes of Cy3-ODN probe on the concentrations of the complementary (curve a) or non-complementary ODN (curve b) samples. The concentration of CNs, PDADMAC and Cy3-ODN are 0.003 OD (optical density at 360 nm), 0.17% (w/w) and 163.8 nM, respectively.

In order to investigate how the presence of target ODN affected the FRET efficiency between CN^+ and Cy3-ODN probe, the complex of CN^+ and Cy3-ODN probe was incubated with different concentrations of complementary ODNs at room temperature, and the spectra are represented in Fig. 3A. With an increase in the complementary ODN concentration, the fluorescence intensity of the CN^+ at 445 nm increased while the fluorescence intensity of the Cy3 molecules decreased. These results clearly illustrate that the event of hybridization has an effect on the FRET efficiency. One likely reason is that relatively flexible single strand ODN strongly interact with the

cationic polymer chain, which is in conformation of a random coil, through the electrostatic and hydrophobic interactions that bring them in intimate contact. On the formation of stiffer ODN duplex structure the distance between the CN⁺ and dye increases resulting in decrease of FRET efficiency. Additionally, Liu et al. illustrated that hydrophobic interactions also play an important role for the efficient FRET besides electrostatic interaction.²⁶ After hybridization, the hydrophobic bases in the double-stranded ODN are packed inside the helix, which minimizes external hydrophobic interactions and thus contribute to a decrease of the FRET efficiency.²⁶

To further illustrate the capability of suggested sensing platform for the hybridization detection, the hybrid was also incubated with different concentrations of noncomplementary ODNs. As seen in Fig. 3B, the incubation with noncomplementary ODN causes very small decrease in the fluorescence intensity. For comparison, Fig. 3C presents the dependence of the normalized FRET intensity changes ODN on the concentrations of complementary (curve a) and non-complementary ODNs (curve b). It is clear that complementary ODN leads to a much larger change in the fluorescence intensity of Cy3 molecules than corresponding concentrations of non-complementary ODN. This result clearly demonstrates that the developed sensing platform can recognize ODN hybridization efficiently. Additionally, the FRET efficiency in Fig. 3B is lower than Fig. 3A due to the different patches of CNs samples used. This indicates the experimental conditions need to be further optimized including the synthesis of CNs and accurate determination of the CNs concentration.

In summary, luminescent CNs were prepared by electrochemical oxidation of graphite rods. A sensing platform for ODN hybridization based on FRET has been designed and demonstrated by using the CNs as a donor. A cationic polymer, PDADMAC, was used to modulate the distance between negatively charged CNs and dye-labelled single-stranded ODN probes through the electrostatic interaction. The cationic polymer not only contributes to the efficient energy transfer occurring, but also weakens the quenching effect of CNs on the dye. The differential interaction of single-stranded and double-stranded ODN with CN⁺ results in differential changes of FRET efficiency, which is used here to recognize the hybridization event. This platform illustrates that luminescent CNs can be used as a donor for homogeneous ODN assays that has all the advantages of a solution based fluorescence detection method, but requires only minimal ODN modification. For fully understanding the selectivity of suggested sensing platform, the work about the detection of one- and two –mismatched non-complementary ODN is undergoing.

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Notes and references

^a Key Laboratory of Polar Materials and Devices, Ministry of Education, East China Normal University, Shanghai, P. R. China

^b National Laboratory for Infrared Physics, Shanghai Institute of Technical Physics of the Chinese Academy of Sciences, Shanghai, China. Email: chunhua_luo@hotmail.com

^c Polymer Electronic Research Centre, The University of Auckland, Private Bag 92019, Auckland, New Zealand. Email: h.peng@auckland.ac.nz

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