

A Graphene Oxide Nanoprobe for Sensitive Fluorescent Telomerase Activity Analysis

Author details:

Bin Zhu, Ruiguo Cao, Yuanyuan Jiang, Qi Li, Qiang Zhang, Gu Yuan, Jingjian Li*, Dongsheng Xu*

Beijing National Laboratory for Molecular Sciences, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, P. R. China

**** Corresponding author. Tel.: +86 10 62758115; fax: +86 10 62760360***

E-mail address: lijj@pku.edu.cn, dsxu@pku.edu.cn

lijj@pku.edu.cn , lijj@pku.edu.cn

ABSTRACT

A novel fluorescent biosensor with ultra-sensitivity and selectivity for telomerase detection has been designed based on oligonucleotide-adsorption to graphene oxide (GO). This PCR-free method employs fluorescent ssDNA as a probe, which exhibits minimal background fluorescence in the present of GO. In the test procedure, telomeric primer DNA was incubated with cancer cell extract and dNTP molecules for 18 hours and then added to the fluorescent probe solution. If telomerase is present and active, this telomeric primer will be elongated and hybridised with the fluorescent DNA probe, causing strong fluorescence emission due to the formation of double helix DNA and its detachment from the surface of GO. If telomerase is absent or inactive (e.g. due to heating in the incubation solution), the telomeric primer will not be elongated, preventing hybridisation with the fluorescent DNA probe. Therefore, in the absence of telomerase, fluorescence is quenched by GO, enabling detection of telomerase activity with a high signal-to-

background ratio. Using this GO-based fluorescence method, the telomerase activity detection limits were 5 cells and 50 cells under $0.2 \text{ mg}\cdot\text{mL}^{-1}$ and $0.5 \text{ mg}\cdot\text{mL}^{-1}$ GO concentration, respectively.

Keywords

graphene oxide; telomerase activity; fluorescent probe; ultra-sensitivity

1. Introduction

Telomerase is an essential cellular ribonucleoprotein (RNP) that stabilises telomere length by adding TTAGGG repeats onto the telomeric ends of the chromosomes using reverse transcription and intrinsic RNA as a template [1-4]. Over 85% of all known human tumours exhibit high telomerase activity compared to neighbouring normal cells. Therefore, it is regarded as a biomarker for cancer diagnosis as well as a therapeutic target [5-7]. To date, many telomerase analysis protocols have been developed, including the telomeric repeat extension protocol [8], telomeric repeat amplification protocol (TRAP) [9], homogeneous chemiluminescent assay (HPA) [10], RT-PCR analysis of human telomerase reverse transcriptase (hTERT) gene expression [11] and rolling-circle mechanism telomeric arrays (RCA) [12]. Among these methods, polymerase chain reaction (PCR)-based TRAP is the most sensitive and widely-used telomerase activity detection assay. Although powerful, TRAP is limited by its requirement for expensive equipment and reagents, such as PCR instrumentation and Taq polymerases. In addition, it is susceptible to PCR-derived artefacts, especially when screening compounds for telomerase inhibition. Furthermore, the exponential amplification of products increases the risk of false positives and complicates the accurate quantification of telomerase activity [13]. In the last decade, the advancement of nanotechnologies has facilitated the development of many other methods for detecting telomerase activity [14, 15]. These methods include colorimetric detection by DNAzyme [16, 17], quartz crystal microbalance methods [18], electrical and surface plasmon resonance methods [19, 20]

51 chemiluminescence [21] and electrochemiluminescence methods [22] based on nanoprobe,
52 electrochemical impedance spectroscopy (EIS) [23], electrochemical methods by using a ligands bound
53 to tetraplex DNA [24] as well as using Au nanoparticles amplification means [25]. These new methods
54 are PCR-free and most of them incorporate nanoscale elements. Moreover, in addition to consider the
55 DNA extension in TRAP, these new methods still maintained the unique composition and structure of
56 telomeric repeat sequences. However, the sensitivity of these methods is usually insufficient without
57 amplification by PCR process. (See supporting information S1.)

58 Herein, we report a novel method to detect telomerase activity using a sensitive fluorescent probe
59 based on DNA-adsorption to graphene oxide. Graphene consists of a single layer of carbon atoms in a
60 closely-packed, honeycomb two-dimensional lattice and possesses exceptional electronic properties and
61 enormous potential for applications. It has been actively studied as a chemical sensor material since
62 2004, when it was first isolated [26-31]. In recent years, it has shown particular promise as a component
63 in biotechnology applications such as DNA sequencing [32]. Yang et al. [33] and Fan et al. [34]
64 independently proposed that water-soluble graphene oxide (GO) could selectively adsorb single stranded
65 DNA (ssDNA) probes. This strong adsorption of ssDNA was attributed to the π - π stacking interaction
66 between the ring structures in nucleobases and the hexagonal cells of graphene. In their work, a
67 graphene oxide (GO)-based multicolour fluorescent DNA nanoprobe was reported. Due to the high
68 signal-to-background ratio between ssDNA and dsDNA with GO, this nanoprobe allowed rapid,
69 sensitive, and selective detection of DNA targets in homogeneous solution. Utilising this low cost
70 unique multifunctional material, they detected various target molecules, such as target DNA, Hg^{2+} , Ag^+ ,
71 and ATP [35]. Willner et al. also developed multiplexed aptasensors and amplified DNA detection
72 based on the functional GO by coupling exonuclease III, Exo III, to system, and applied it into logic gate
73 operation [36]. Using the extraordinarily high quenching efficiency of GO, we developed a PCR-free
74 assay for telomerase activity detection based on the conformational change of a DNA probe from single
75 strand to double helix after elongation by telomerase. This new assay achieves higher sensitivity than the
76 TRAP method.

77 2. Experiment section

78 2.1. Chemicals and Materials

79 Water-soluble graphene oxide (GO) was synthesised using the chemical Hummers method [37-39].
80 The GO product was dialysed in distilled water to completely remove metal ions and acids. After
81 centrifugation for 10 min at 8000 rpm, the supernatants containing GO were collected and brought to a
82 concentration of 1 mg/mL.

83 Two kinds of polyacrylamide gel electrophoresis (PAGE) -purified DNA fragments were used for
84 telomerase activity detection, both of which were synthesised and provided by Sangon. Co. Ltd.
85 (Shanghai, China). The sequences were as follows: P1: 5'-CCCTAACCCTAACCCTAACCCTAACTC-
86 FAM-3' (27 bp); P2: 5'-AATCCGTCGAGCAGAGTT-3' (18 bp). The P1 sequence was used as the
87 fluorescent probe with a complementary sequence to the telomeric repeat sequence, while P2 was the
88 telomerase primer. All these probes and primers were prepared as a 1 μ M concentration in 20 mM Tris-
89 HCl buffer (100 mM NaCl, 5 mM KCl and 5 mM MgCl₂, pH 7.4).

90 All other chemical agents were purchased from Sigma-Aldrich (USA) and Sinopharm Chemical
91 Reagent Beijing Co., Ltd. Ultrapure water with electric resistance >18 M Ω was supplied from a
92 Millipore Milli-Q water purification system (Billerica, MA, USA).

93 2.2. Telomerase Extracts

94 The commercial TRAPeze telomerase detection kit S7700 was purchased from Millipore and was
95 used to provide telomerase-positive cells and a TRAP control group result. The telomerase was
96 extracted from positive control cells (10⁶ cells) from the S7700 kit. The cells were broken down by 200
97 μ L of 1 $\times$ CHAPS Lysis Buffer, whose sediment was removed by centrifugation (12,000 rpm, 20 min);
98 next, 160 μ L of liquid supernatant was repacked as 10 μ L of mother liquor and stored at -86 $^{\circ}$ C. Before
99 being used, the mother liquor was 20 $\times$ diluted by 1 $\times$ CHAPS Lysis Buffer, generating 250 cells $\cdot\mu$ L⁻¹ of
100 telomerase extracts.

101 2.3. Preparation of oligonucleotide-adsorbing GO probes

102 Telomeric primer P2 was first incubated in a mixed solution for 18 hrs. The mixed solution contain
103 1 μ L of dNTP, 1 μ L 50 μ mol $\cdot$ L⁻¹ of P2 DNA, 5 μ L of 10 $\times$ TRAP Reaction Buffer, 2 μ L of different
104 quantities of telomerase cell extracts (1, 5, 10, 50, 100, 500 cells and 500 cells heat-treated at 95 $^{\circ}$ C for
105 10 min), and a number of PCR-Grade Water to bring the total volume to 50 μ L. The incubated products
106 were then mixed with fluorescent probe P1 and GO at various concentrations (1mg $\cdot$ mL⁻¹, 0.5 mg $\cdot$ mL⁻¹,
107 0.2 mg $\cdot$ mL⁻¹, 0.1 mg $\cdot$ mL⁻¹) to achieve a final concentration of 50 nM DNA in each solution for 30 min
108 at room temperature.

109 2.4. Apparatus

110 Atomic force microscopy (AFM) was conducted using an SPA400 equipped with an SPI3800N
111 controller microscope instrument (Seiko Instruments Inc., Japan) in tapping mode to simultaneously
112 collect the height and phase data from GO. The XPS spectrum of GO was measured using an Axis Ultra
113 (Kratos Analytical Ltd., Japan), and the Raman spectrum was collected on a Renishaw inVia system
114 (Renishaw, UK).

115 The fluorescence profiles of the DNA probe and primer mixtures were obtained using a Hitachi F-
116 4500 fluorometer (Hitachi Co. Ltd., Japan) with an excitation wavelength of 480 nm.

117 PCR was carried out in a Mastercycler (Eppendorf Co. Ltd., Germany). Electrophoresis was
118 performed in a Hoefer Minive (Pharmacia Biotech, Japan) and was used for the detection of PCR
119 products in order to compare our method with TRAP. SYBR Green I used in electrophoresis was
120 purchased from Invitrogen Ltd, while Taq DNA Polymerase ET101 (5 unit/ μ L) and DNA Ladder
121 Marker MD100 were purchased from Tiangen Biotech (Beijing) Co., Ltd.

122 2.5. Characterisation of GO

123 An AFM image and XPS spectra of the synthesised GO are shown in Fig. 1. It can be seen that the
124 GO sheet size was less than 1 μ m, with a height of 1.19 ± 0.11 nm, demonstrating that the GO is a
125 single-layer carbon material. The GO was also characterised by Raman. (See Figure S-1 of supporting
126 information for more details)

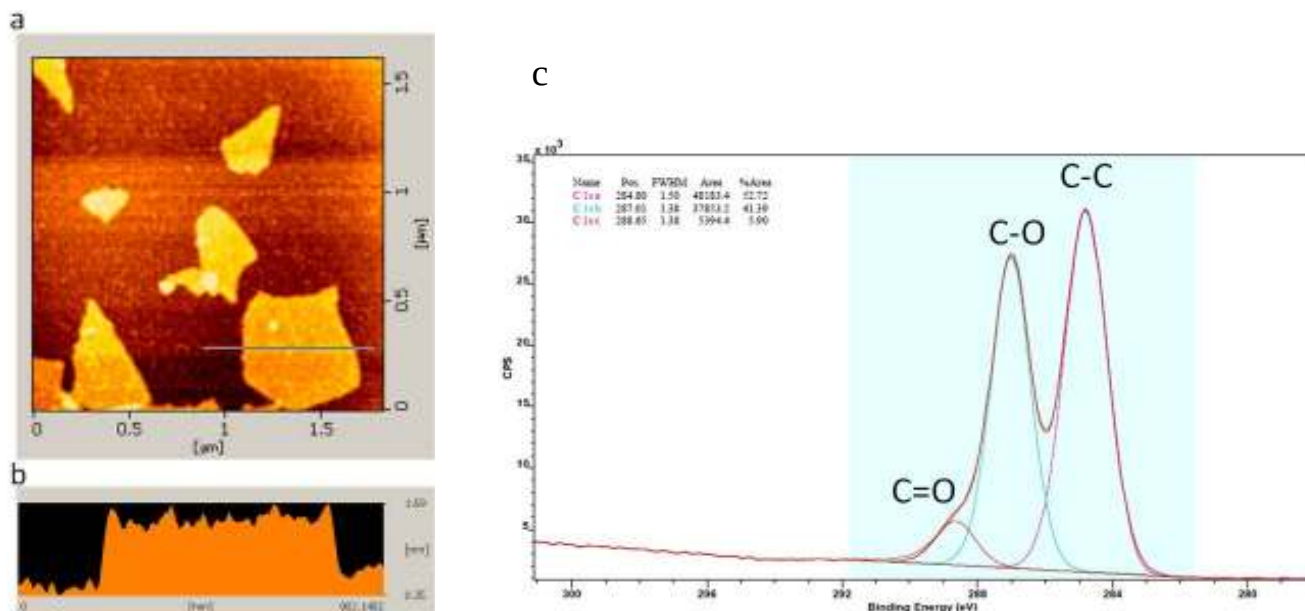


Fig. 1. (a) AFM image of GO on mica; (b) the cross-sectional profile of the AFM image along the black line; (c) XPS spectra of GO, shows the C-C bond, C-O bond and C=O bond.

XPS is a powerful surface technique that can provide information about the state and environment of atoms in a sample. The C1s core level of XPS has been used to distinguish sp^2 and sp^3 hybridisation in carbon nanomaterials [40]. The C1s XPS spectrum of GO clearly indicates a considerable degree of oxidation with three components that correspond to carbon atoms in different functional groups [41]: the non-oxygenated ring C (284.80 eV, sp^2 hybridised), the C in C–O bonds (287.01 eV), and the carbonyl C (288.62 eV). D and G bands of GO were found in the Raman spectrum (See Figure S-1 of supporting information). The D band at 1320 cm^{-1} arises from sp^3 -hybridised carbon, and the peak at 1602 cm^{-1} represents the E_{2g} zone centre mode of the crystalline graphite [42]. There was also a 2D band at 2600 cm^{-1} . The relative intensity ratio of the D and G lines (I_D/I_G ratio) was 1.005, which was proportional to the number of defect sites in graphite carbon. All the information above indicated that these graphite materials were GO sheets with significant edge-plane-like defective sites on the surface [43–47].

3. Results and discussion

3.1. Fluorescence emission spectra of different mixed systems

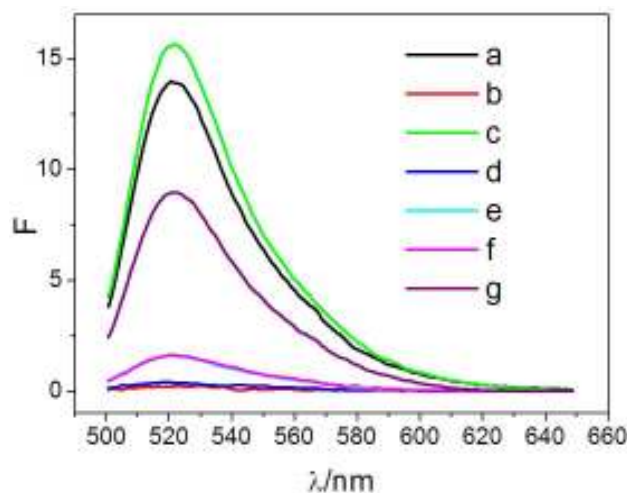
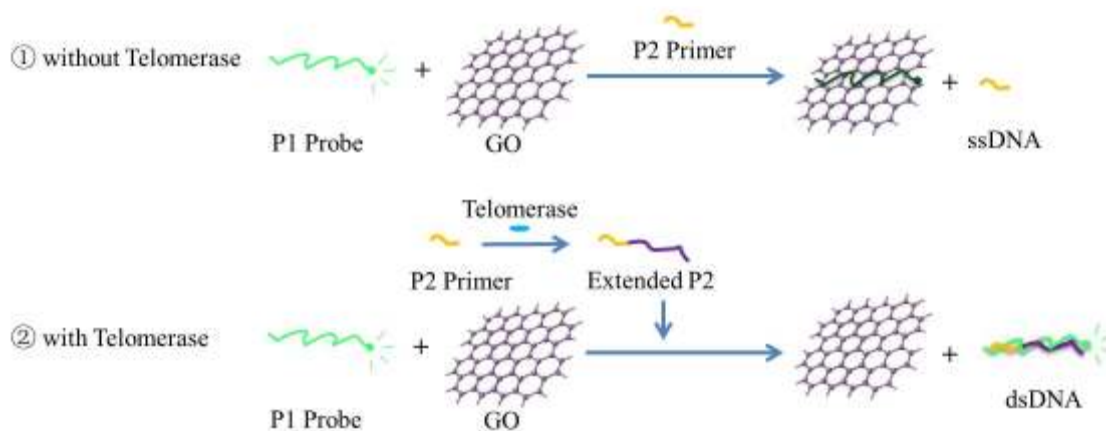


Fig.2. Fluorescence emissions of FAM of 50 nM P1 DNA in different systems: line **a**: only P1 DNA; line **b**: P1 and GO; line **c**: P1 and P2 DNA; line **d**: P1 with 500 cell extracts and GO; line **e**: P1, P2 DNA and GO; line **f**: P1 and P2 incubated with inactivated 500 cell extracts for 18 hrs and GO; line **g**: P1 and P2 incubated with 500 cell extracts for 18 hrs and GO. The excitation wavelength was 480 nm.

Figure 2 displays the fluorescence emission spectra of different mixed systems for $0.5 \text{ mg} \cdot \text{mL}^{-1}$ GO. Spectrum **a** is the fluorescence emission of the P1 probe itself, while spectrum **b** shows that GO can totally quench the fluorescence of P1. Spectrum **c** indicates that the P2 primer can enhance the fluorescence of the P1 probe by diluting the concentration of P1 and reducing the probe's self-quenching effect. Spectra **d** and **e** show that GO can also quench the ssDNA probe's fluorescence in the presence of P2 primer or cell extracts (telomerase) separately. These results suggest that these two elements were both necessary for fluorescence recovery in the mixture solution. Even in the presence of P2 primer after incubation with inactivated 500-cell extracts, as spectrum **f** shows, the system's fluorescence was still quite weak. Only when P2 primers were incubated with 500-cell extracts, the P1 probe could be hybridised with the extended primer forming a dsDNA and released from the GO surface, resulting in a high fluorescence signal (Fig. 2. line **g**). The experiment described above can clearly distinguish differences in telomerase activity based on fluorescence recovery levels.

162 This PCR-free method harnesses the special oligonucleotide-adsorption properties of GO by
 163 employing fluorescent ssDNA P1 as an analysis probe. This probe was adsorbed in the presence of GO
 164 because of the π - π stacking interaction between the ring structures in nucleobases and the hexagonal
 165 cells of grapheme, which lead to the fluorescence quenching of ssDNA P1 and exhibited minimal
 166 background fluorescence. In contrast, when the primer DNA P2 was extended by telomerase in cell
 167 extracts after incubation and added to the probe solution, strong emission was observed attributed to the
 168 hybridization of extended primer DNA P2 and P1 probe which was release from the GO (see Scheme. 1,
 169 ②). In other experimental conditions, for example, without incubation of cell extracts or incubation with
 170 inactivated telomerase at 95°C, fluorescence emission was weak (see Scheme. 1, ①). Therefore, only
 171 when P2 primer was extend by active telomerase and hybridize with P1 probe to a double-helix DNA
 172 and that broke away from the surface of GO, which gives rise to the strong fluorescence. The quenching
 173 of fluorescence from the P1 probe by GO in the absence of telomerase provides a high signal-to-
 174 background ratio for telomerase activity detection.



175
 176 **Scheme 1.** Illustration of the GO-based fluorescence method for telomerase activity detection.

177 ① DNA P1 probe fluorescence emission was weak in the situation primer P2 without
 178 incubation of cell extracts or incubation with inactivated telomerase at 95°C; ②: When
 179 primer P2 was extended by telomerase in cell extracts after incubation and added to the P1
 180 probe solution, strong emission was observed.

3.2. Telomerase-activity assay under different GO concentrations

In order to find suitable experimental conditions for telomerase activity detection, we tested various GO concentrations, including $1 \text{ mg} \cdot \text{mL}^{-1}$, $0.5 \text{ mg} \cdot \text{mL}^{-1}$, $0.2 \text{ mg} \cdot \text{mL}^{-1}$ and $0.1 \text{ mg} \cdot \text{mL}^{-1}$. Adsorption and quenching efficiency varied under these different concentrations, as exhibited in the fluorescence spectra from the P1+P2+GO mixtures. The normalised fluorescence factor f is introduced to support the discussion of telomerase-activity detectability under different GO concentrations.

$$f = (F_x - F_{\text{P1+P2+GO}}) / (F_{\text{P1+P2}} - F_{\text{P1+P2+GO}}) \quad (1)$$

This factor is defined by equation 1, in which F_x is the fluorescence emission of P1 DNA and P2 primers incubated with specific concentrations of cell extracts at specific GO concentrations; $F_{\text{P1+P2+GO}}$ is the fluorescence background of P1, P2 DNA at specific concentrations of GO and $F_{\text{P1+P2}}$ is the fluorescence emission of P1 and P2 DNA. After such normalisation, the detection of telomerase activity using this GO-based fluorescence method can be compared at different GO concentrations.

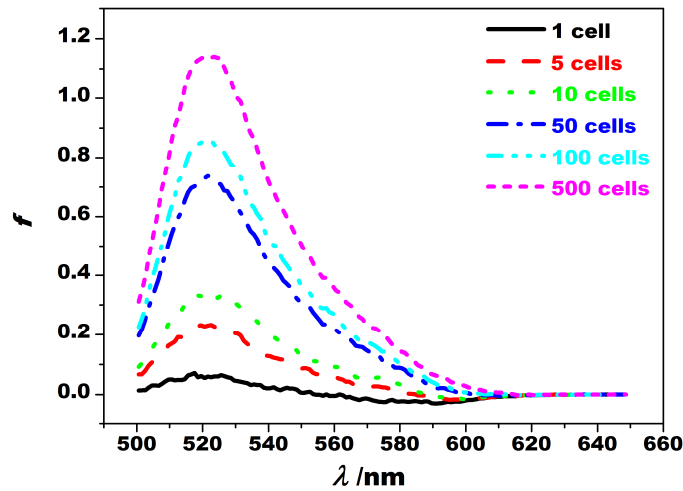


Fig.3. Fluorescence factor f of P1 DNA with $0.5 \text{ mg} \cdot \text{mL}^{-1}$ GO and P2 primer incubated with various concentrations of telomerase (1, 5, 10, 50, 100, and 500-cell extracts). The excitation wavelength was 480 nm.

Fluorescence signals were collected while systematically varying GO and telomerase concentrations (See Figure S-2 of supporting information for more details). For example, at a 0.2

199 $\text{mg} \cdot \text{mL}^{-1}$ GO concentration, fluorescence signals were collected after incubating P2 primer with
200 telomerase extracted from between 1 and 500 cells (Figure 3). Figure 4 (a) shows the work curves of
201 different GO concentrations in the $f - \text{Log}(\text{cell number})$ diagram, demonstrating that $0.5 \text{ mg} \cdot \text{mL}^{-1}$ and
202 $0.2 \text{ mg} \cdot \text{mL}^{-1}$ were appropriate GO concentrations. Generally, fluorescence signals were low when the
203 GO concentration was too high (such as $1 \text{ mg} \cdot \text{mL}^{-1}$) because of the strong quenching effect. Conversely,
204 the fluorescence background was too high when the GO concentration was low (such as $0.1 \text{ mg} \cdot \text{mL}^{-1}$),
205 generating measuring errors for telomerase activity detection. When the GO concentration was in
206 between these two extremes (such as $0.5 \text{ mg} \cdot \text{mL}^{-1}$ and $0.2 \text{ mg} \cdot \text{mL}^{-1}$), better and linear working curves
207 were obtained. Table 1 displays the slopes and R^2 of the work curves at different GO concentrations.
208 Moreover, inactivated telomerase comparisons were carried out at each GO concentration, as seen in
209 Figure 4 (b). After 10 min of 95°C heat inactivation, telomerase is unable to extend telomeric primer P2
210 and the weak fluorescence signals of P1 can be easily distinguished from activated telomerase.

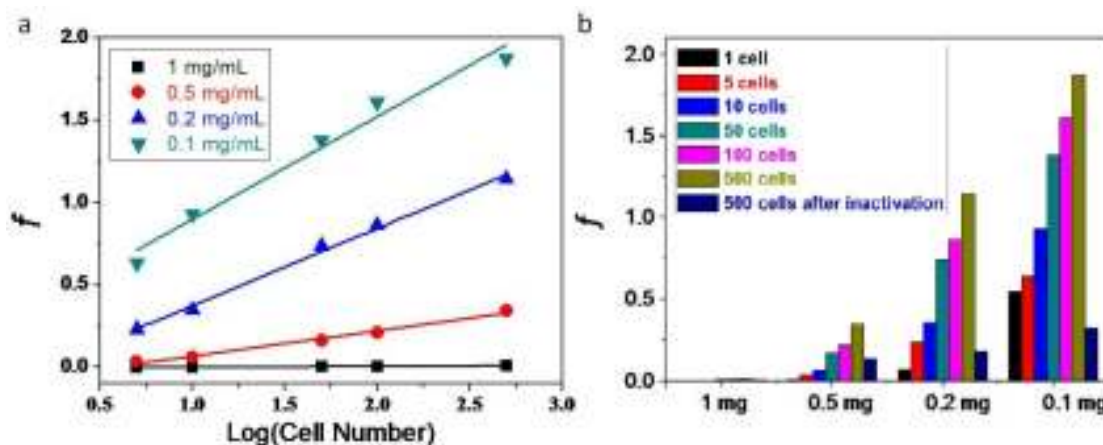


Fig.4. Calibration curve for telomerase activity detection under different GO concentrations (a) and inactivated telomerase control (b). Square: $1 \text{ mg} \cdot \text{mL}^{-1}$, circle: $0.5 \text{ mg} \cdot \text{mL}^{-1}$, triangle: $0.2 \text{ mg} \cdot \text{mL}^{-1}$, inverse triangle: $0.1 \text{ mg} \cdot \text{mL}^{-1}$.

Table 1. Slopes and R^2 of the calibration curves for telomerase activity detection at various GO concentrations

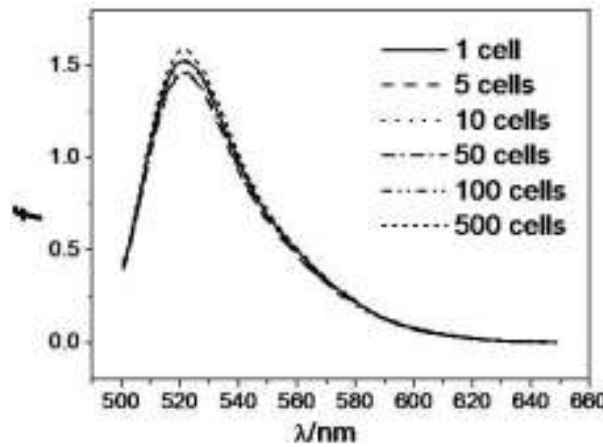
GO concentration/ $\text{mg} \cdot \text{mL}^{-1}$	Slope	R^2
1	0.0052	0.981

0.5	0.154	0.985
0.2	0.468	0.993
0.1	0.623	0.968

217

218 3.3. Control experiment

219 To understand the role of GO in this telomerase activity detection method, control studies were
 220 carried out in the absence of GO. As seen in Figure 5 in the supporting information, normalised
 221 fluorescence curves at different telomerase concentrations were nearly the same, demonstrating that GO
 222 was the critical element in our fluorescence method. This effect is attributed to distinction in adsorption
 223 between ssDNA and dsDNA, which amplify differences in the fluorescence signal before and after
 224 telomeric extension and result in a high signal/background ratio. We conclude that this GO-based
 225 fluorescence method for telomerase activity detection provides the best results at $0.5 \text{ mg} \cdot \text{mL}^{-1}$ or 0.2
 226 $\text{mg} \cdot \text{mL}^{-1}$ GO concentrations. The detection limits of this assay were 50 cells and 5 cells for $0.5 \text{ mg} \cdot \text{mL}^{-1}$
 227 and $0.2 \text{ mg} \cdot \text{mL}^{-1}$, respectively, which is lower than that of the fluorescent QD method [20]. Though 1
 228 cell could be detected at $0.1 \text{ mg} \cdot \text{mL}^{-1}$ GO concentration, further testing is needed due to the deviation
 229 from linearity.



230

231 **Fig. 5.** Fluorescence factor f of control groups in the absence of GO.

232 Finally, we compared our assay to the TRAP method following the specifications of the S7700 kit.
 233 After PCR and electrophoresis, TRAP product bands were observed in the 500 cell extract lane and

quantitation controls, while no products were observed in the inactivated control and CHAPS control (for details, see Figure S-3 in supporting information). The TRAP method requires 5 to 6 hours and has a detection limit of several hundred cells. Therefore, the need for more sensitive and convenient methods is still an important concern in the field of telomerase activity detection.

4. Conclusion

In summary, we have utilised the high fluorescence quenching ability of GO toward ssDNA to design a novel fluorescent sensor for multiplexed, ultra-sensitive and specific telomerase activity detection. This GO-based telomerase sensor has several advantages compared to the TRAP method. Principally, GO can be synthesised in large quantities and the DNA probe is only labeled with a single dye, reducing the cost for telomerase activity detection. Secondly, this method is PCR-free and easy to execute, which can avoid false positive results. Thirdly, GO-based detection improves sensitivity by quenching the fluorescence of the probe DNA, minimising the background fluorescence and resulting in an optimised detection limit of several-cell telomerase extracts. Although the telomeric primer extension in this method was time consuming, it is still highly promising due to its considerable sensitivity. Therefore, this GO-based method may provide opportunities to develop low cost and ultra-sensitive telomerase detection protocols using the conformational changes of DNA probes and the multi-functional nanomaterial GO. We expect that this will guide new research and enable important and widespread applications in many other areas of biological analysis.

Appendix A. Supplementary data. Supplementary data associated with this article can be found in the online version at doi:

References

1. C. W. Greider, E. H. Blackburn, *Cell* 43 (1985) 405-413.
2. V. A. Zakian, *Annu. Rev. Genet.* 23 (1989) 579-604.
3. C.W. Greider, *BioEssays* 12 (1990) 363-369.
4. C.W. Greider, *Curr. Opin. Cell Biol.* 3 (1991) 444-451.

- 259 5. P.J. Perry, J.R. Arnold, T.C. Jenkins, *Expert. Opin. Investig. Drugs* 10 (2001) 2141-2156.
- 260 6. S. Kim, P. Kaminker, J. Campisi, *Oncogene* 21 (2002) 503-511.
- 261 7. J. W. Shay, W.E. Wright, *Nat. Rev. Drug Discov.* 5 (2006) 577-584.
- 262 8. G. B. Morin, *Cell* 59 (1989) 521-529.
- 263 9. N. W. Kim, M. A. Platyszek, K. R. Prowse, C. B. Harley, M. D. West, P. L. C. Ho, G. M.
- 264 Coviello, W. E. Wright, S. L. Weinrich, J.W. Shay, *Science* 266 (1994) 2011-2015.
- 265 10. D. B. Lachey, *Anal. Biochem.* 263 (1998) 57-61.
- 266 11. A. Tchirhov, C. Rolhion, J. L. Kemeny, B. Irthum, S. Puget, T. Khalil, O. Chinot, F. Kwiatkowski,
- 267 B. Pe´risse, P. Vago, P. Verrelle, *Br. J. Cancer* 88 (2003) 516-520.
- 268 12. J. Nosek, A. Rycovska, A. M. Makhov, J. D. Griffith, L. Tomaska, *J. Bio. Chem.*, 280 (2005)
- 269 10840- 10845.
- 270 13. J. Fajkus, *Clinica Chimica Acta* 371 (2006) 25-31.
- 271 14. Y. Weizmann, F. Patolsky, E. Katz, I. Willner, *ChemBioChem.* 5 (2004) 943-948.
- 272 15. U. Eskiocak, D. Ozkan-Ariksoysal, M. Ozsoz, H. A. Öktem, *Anal. Chem.* 79 (2007) 8807-8811.
- 273 16. R. Freeman, E. Sharon, C. Teller, A. Henning, Y. Tzfati, I. Willner, *ChemBioChem.* 11 (2010)
- 274 2362-2367.
- 275 17. Y. Li, X. Li, X.T. Ji, X.M. Li, *Biosens. Bioelectron.* 26 (2011) 4095-4098.
- 276 18. V. Pavlov, I. Willner, A. Dishon, M. Kotler, *Biosens. Bioelectron.* 20 (2004) 1011-1021.
- 277 19. C. Maesawa, T. Inaba, H. Sato, S. Iijima, K. Ishida, M. Terashima, R. Sato, M. Suzuki, A. Yashima,
- 278 S. Ogasawara, H. Oikawa, N. Sato¹, K. Saito, T. Masuda, *Nucleic Acids Res.* 31 (2003) e4.
- 279 20. E. Sharon, R. Freeman, M. Riskin, N. Gil, Y. Tzfati, I. Willner, *Anal. Chem.* 82 (2010) 8390-8397.
- 280 21. V. Pavlov, Y. Xiao, R. Gill, A. Dishon, M. Kotler, I. Willner, *Anal. Chem.* 76 (2004) 2152-2156.
- 281 22. X. Zhou, D. Xing, D. Zhu, L. Jia, *Anal. Chem.* 81 (2009) 255-261.
- 282 23. W. Yang, X. Zhu, Q. Liu, Z. Lin, B. Qiu, G. Chen, *Chem. Commun.* 47 (2011) 3129-3131.
- 283 24. S. Sato, H. Kondo, T. Nojima, S. Takenaka, *Anal. Chem.* 77 (2005) 7304-7309.
- 284 25. G. Zheng, W. L. Daniel, C. A. Mirkin, *J. Am. Chem. Soc.* 130 (2008) 9644-9645.

- 285 26. K. S. Novoselov, A. K. Geim, S. V. Morozov, D. Jiang, Y. Zhang, S. V. Dubonos, I. V. Grigorieva,
286 A. A. Firsov, *Science* 306 (2004) 666-669.
- 287 27. F. Schedin, A.K. Geim, S.V. Morozov, E. W. Hill, P. Blake, M. I. Katsnelson, K. S. Novoselov,
288 *Nature Mater.* 6 (2007) 652-655.
- 289 28. N. Mohanty, V. Berry, *Nano Lett.* 8 (2008) 4469-4476.
- 290 29. Z. Wang, X. Zhou, J. Zhang, F. Boey, H. Zhang, *J. Phys. Chem. C* 113 (2009) 14071-14075.
- 291 30. Y. Zhou, Q. Bao, L. A. L. Tang, Y. Zhong, K. P. Loh, *Chem. Mater.* 21 (2009) 2950-2956.
- 292 31. Z. Tang, H. Wu, J. R. Cort, G. W. Buchko, Y. Zhang, Y. Shao, I. A. Aksay, J. Liu, Y. Lin, *Small* 6
293 (2010) 1205-1209.
- 294 32. S. Garaj, W. Hubbard, A. Reina, J. Kong, D. Branton, J. A. Golovchenko, *Nature* 467 (2010) 190-
295 194.
- 296 33. C.H. Lu, H.H. Yang, C.L. Zhu, X. Chen, G.N. Chen, *Angew. Chem.* 121 (2009) 4879-4881.
- 297 34. Y. Wen, F. Xing, S. He, S. Song, L. Wang, Y. Long, D. Li, C. Fan, *Chem. Commun.* 46 (2010)
298 2596-2598.
- 299 35. S.J. He, B. Song, D. Li, C. Zhu, W. Qi, Y. Wen, L. Wang, S. Song, H. Fang, C. Fan, *Adv. Funct.*
300 *Mater.* 20 (2010) 453-459.
- 301 36. X. Liu, R. Aizen, R. Freeman, O. Yehezkeli, I. Willner, *ACS Nano*, 6(2012), 3553-3563.
- 302 37. W. Hummers, J. Offeman, *J. Am. Chem. Soc.* 80 (1958) 1339-1339.
- 303 38. N.I. Kovtyukhova, *Chem. Mater.* 11 (1999) 771-778.
- 304 39. M. Zhou, Y. Zhai, S. Dong, *Anal. Chem.* 81 (2009) 5603-5613.
- 305 40. J. Díaz, G. Paolicelli, S. Ferrer, F. Comin, *Phys. Rev. B* 54 (1996) 8064-8069.
- 306 41. S. Stankovich, D.A. Dikin, R.D. Piner, K.A. Kohlhaas, A. Kleinhammes, Y. Jia, Y.Wu, S.T.
307 Nguyen, R.S. Ruoff, *Carbon* 45 (2007) 1558-1565.
- 308 42. F. Tuinstra, J. L. Koenig, *J. Chem. Phys.* 53 (1970) 1126-1130.
- 309 43. M. Zhou, Y. Wang, Y. Zhai, J. Zhai, W. Ren, F. Wang, S. Dong, *Chem. Eur. J.* 15 (2009) 6116-
310 6120.

- 311 44. H.L. Guo, X.F. Wang, Q.Y. Qian, F.B. Wang, X.H. Xia, ACS Nano. 3 (2009) 2653-2659.
- 312 45. S. Wang, P. K. Ang, Z. Wang, A.L.L. Tang, J.T.L. Thong, K.P. Loh, Nano Lett. 10 (2010) 92-98.
- 313 46. D.R. Dreyer, S. Park, C.W. Bielawski, R S. Ruoff, Chem. Soc. Rev. 39 (2010) 228-240.
- 314 47. Choudhary et al., J Biosens Bioelectron 2013, 4:4

Figure captions

Fig. 1. (a) AFM image of GO on mica; (b) the cross-sectional profile of the AFM image along the black line; (c) XPS spectra of GO, shows the C-C bond, C-O bond and C=O bond.

Fig. 2. Fluorescence emissions of FAM of 50 nM P1 DNA in different systems, line **a**: only P1 DNA; line **b**: P1 and GO; line **c**: P1 and P2 DNA; line **d**: P1 with 500 cell extracts and GO; line **e**: P1, P2 DNA and GO; line **f**: P1 and P2 incubated with inactivated 500 cell extracts for 18 hrs and GO; line **g**: P1 and P2 incubated with 500 cell extracts for 18 hrs and GO. The excitation wavelength was 480 nm.

Fig. 3. Fluorescence factor f of P1 DNA with $0.5 \text{ mg} \cdot \text{mL}^{-1}$ GO and P2 primer incubated with various concentrations of telomerase (1, 5, 10, 50, 100, and 500 cell extracts). The excitation wavelength was 480 nm.

Fig. 4. Calibration curve for telomerase activity detection under different GO concentrations (a) and inactivated telomerase control (b). Square: $1 \text{ mg} \cdot \text{mL}^{-1}$, circle: $0.5 \text{ mg} \cdot \text{mL}^{-1}$, triangle: $0.2 \text{ mg} \cdot \text{mL}^{-1}$, inverse triangle: $0.1 \text{ mg} \cdot \text{mL}^{-1}$.

Fig. 5. Fluorescence factor f of control groups in the absence of GO.

Scheme 1. Illustration of the GO-based fluorescence method for telomerase activity detection. ① DNA P1 probe fluorescence emission was weak in the situation without incubation of cell extracts or incubation with inactivated telomerase at 95°C ; ②: When primer P2 was extended by telomerase in cell extracts after incubation and added to the probe solution, strong emission was observed.