

A robust fluorescence probe for detection of telomerase activity in-vitro and imaging in living cells via telomerase-triggering primer extension to desorb DNA from graphene oxide

Fenglei Gao^{a,1}, Yao Yao^{a,1}, Jing Wu^{a,1}, Lin Cui^b, Yu Zhang^a, Deqin Geng^{a,*}, Daoquan Tang^{a,*} and Yanyan Yu^{a,*}

Jiangsu Key Laboratory of New Drug Research and Clinical Pharmacy, School of Pharmacy, Xuzhou Medical University, 221004, Xuzhou, China.

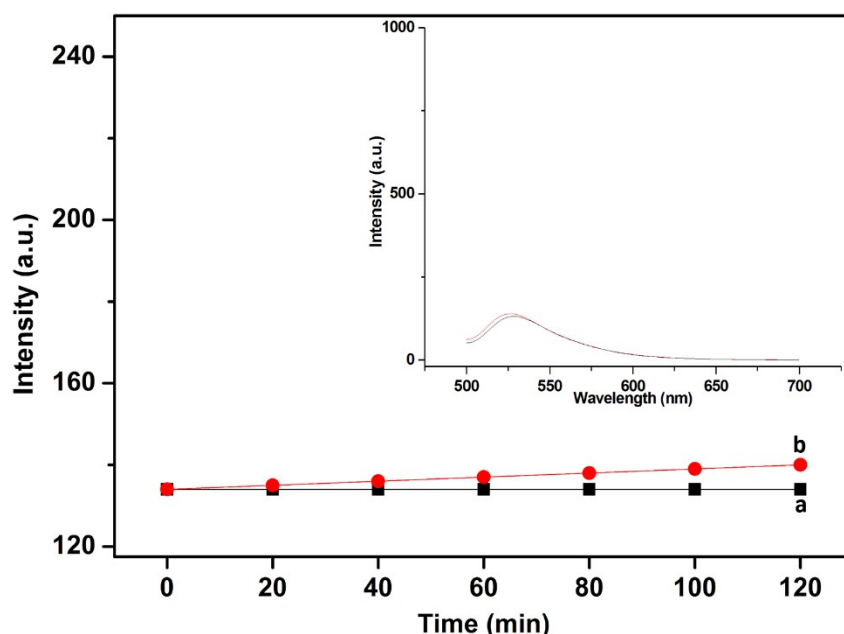


Fig. S1 Plots of fluorescence intensity of the GO nanoprobe in PBS before (a) and after (b) addition of the Hela cell extract pretreated with 2 µg RNase A vs. incubation time.

Inset: fluorescence spectra corresponding to a and b at 120 min.

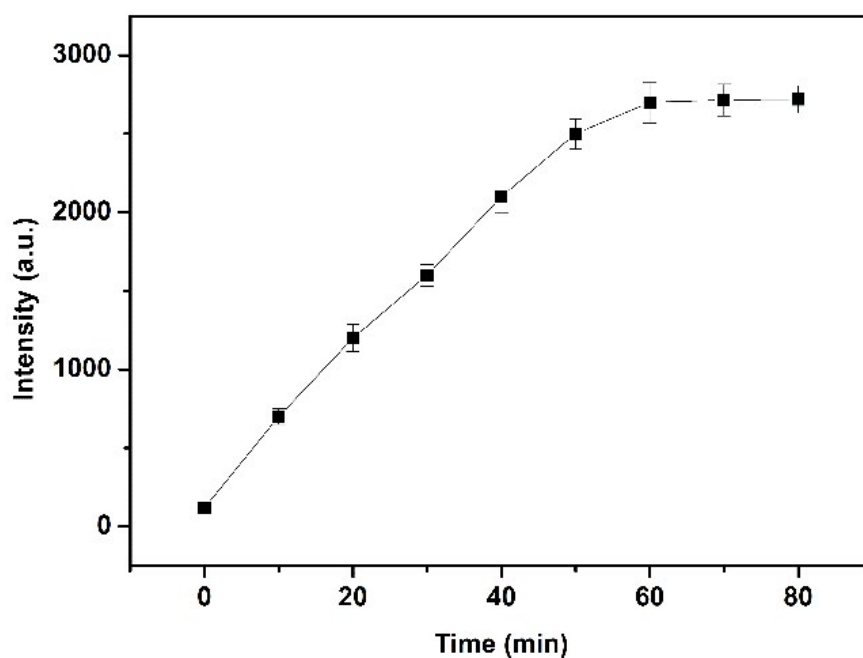


Fig.S2 The fluorescence intensity vs. incubation time of the GO nanoprobe with the cell lysates and dNTPs.

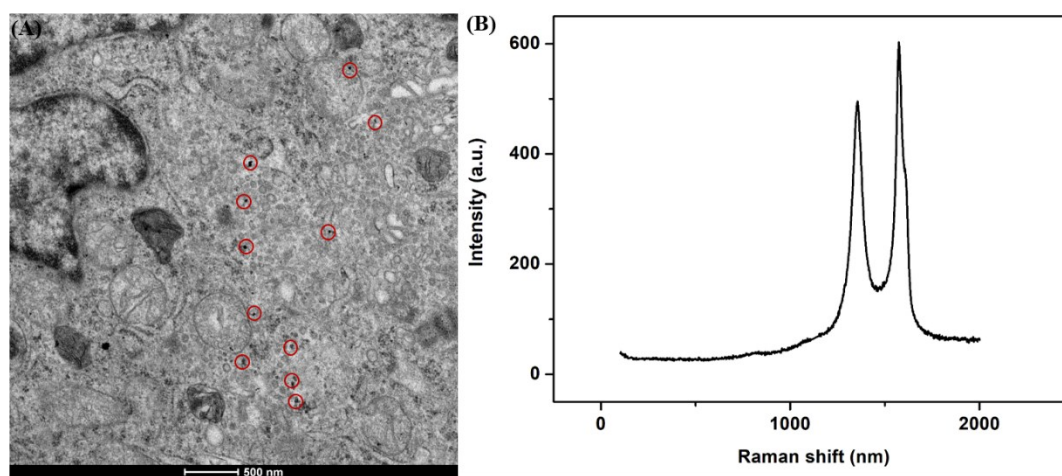


Fig.S3 (A) TEM images of a fixed HeLa cell, treated with GO probes; (B) Raman spectra of the black particle in A.

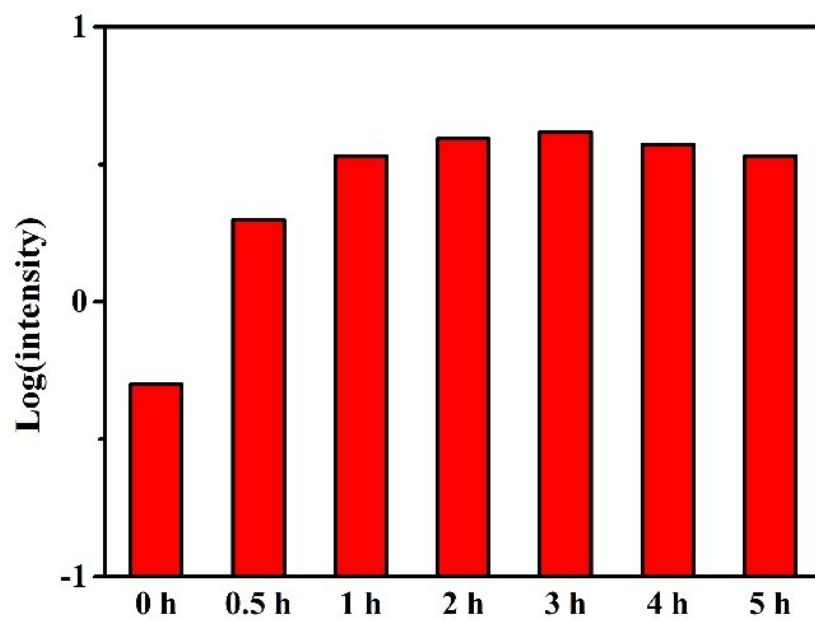


Fig. S4 Flow cytometric detection of HeLa cells (0.5 mL, 1.0×10^5 /mL) after incubation with 100 μ L GO nanoprobe (0.1 mg/mL) at 37 °C for different time.

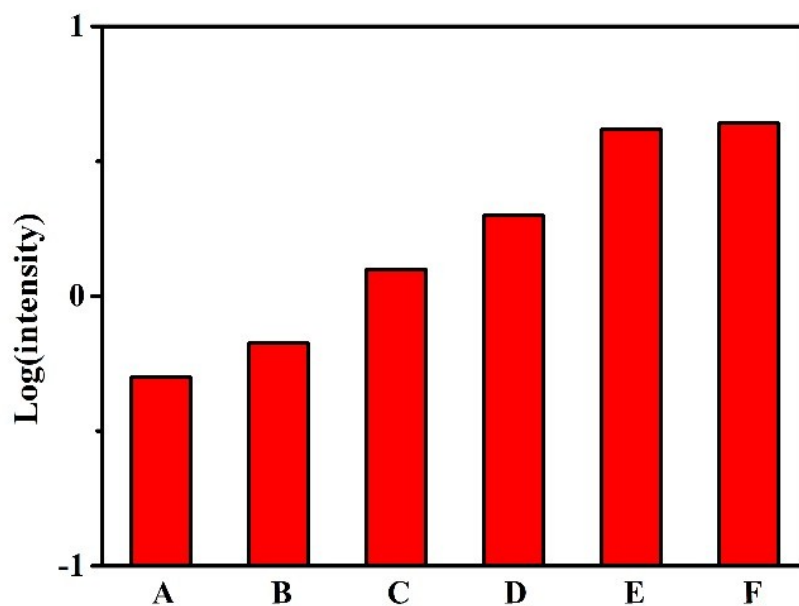


Fig. S5 Flow cytometric detection of HeLa cells (0.5 mL, 1.0×10^6 /mL) after incubation with 0, 20, 50, 80, 100 and 200 μ L GO nanoprobe (0.1 mg/mL) (from A to F) at 37 °C for 3 h.

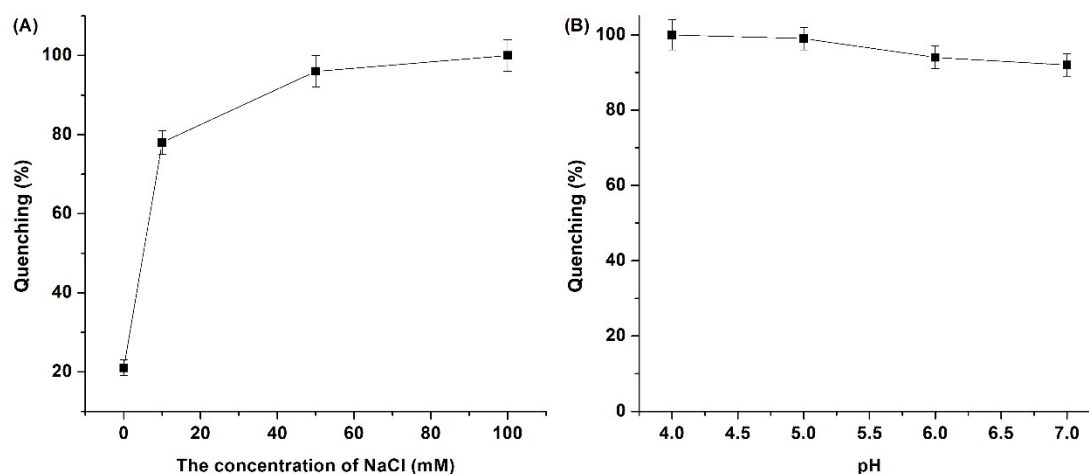


Fig. S6 Quenching efficiency as a function of varying concentrations of NaCl (A) and pH (B) with the FAM-DNA/TS primer/GO probe.

As shown in Fig. S6A, in absence of salt, the quenching efficiency was less than 30% for FAM-DNA/TS primer/GO probe, confirming that the binding was quite weak. Significant quenching was observed with even 10 mM NaCl. At higher NaCl concentrations, the quenching efficiencies were progressively better. For example, with 100 mM NaCl, the quenching was close to 100% for the FAM-DNA/TS primer.

The significantly increased acidity in subcellular compartments of cancer cells such as lysosomes (pH 4.5–5.0). However, the pH of cytoplasm in cancer cells and normal cells was 7.2. As previous works reported, nanoparticles would enter cell via cytoplasm transport, finally locate in cytoplasm[1]. In addition, since FAM is a pH-sensitive fluorophore and its quantum yield is close to zero at pH's lower than 4, a direct comparison of the quenching efficiency is difficult at low pH[2]. Therefore, the pH effect was studied by an indirect method. Four buffers were prepared ranging from pH 4 to 7, and the FAM-DNA/TS primer was mixed with GO in the buffers containing also 50 mM NaCl. After incubation at room temperature for 1 h, the samples were centrifuged at 15 000 rpm for 20 min, and all the GO was precipitated. The supernatant solution containing only the free FAM-DNA/TS primer was collected and diluted 10 times in a pH 7.2 buffer for fluorescence measurement. As can be observed in Fig. S6B,

the binding was more effective at lower pH. So the lower pH did not affect the binding of FAM-DNA/TS primer with GO.

References:

- [1]J. Tian, L. Ding, H.-J. Xu, Z. Shen, H. Ju, L. Jia, L. Bao, J.-S. Yu, *J. Am. Chem. Soc.*, 2013, **135**, 18850-18858.
- [2]M. Wu, R. Kempaiah, P. J. Jimmy Huang, V. Maheshwari, J. W. Liu, *Langmuir*, 2011, **27**, 2731–2738.