Electronic Supplementary Material (ESI) for Analyst
This journal is © The Royal Society of Chemistry 2012

1 **Electronic Supplementary Information:**
2
3

4 A ligation-triggered highly sensitive fluorescent assay of 5 adenosine-triphosphate based on graphene oxide

6

7 Wenping Zhu, Ziwei Zhao, Zhen Li, Jianhui Jiang*, Guoli Shen, Ruqin Yu*
8
9

10 *State Key Laboratory of Chemo/Bio-Sensing and Chemometrics, College of*
11 *Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, P. R.*
12 *China*

13

14 To whom correspondence should be addressed.

15 Tel: +86 731 88822577.

16 Fax: +86 731 88822577.

17 E-mail address: rqyu@hnu.edu.cn (R.-Q. Yu) jianhuijiang@hnu.edu.cn (J.-H.Jiang)

18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34

This journal is © The Royal Society of Chemistry 2012

35 # Experiment Section

36 ## Chemicals and materials

37 T4 DNA ligase was supplied by Takara Biotechnology Co., Ltd. (Dalian, China).
38 Adenosine triphosphate (ATP), adenosine (A), adenosine diphosphate (ADP),
39 adenosine monophosphate (AMP), uridine triphosphate (UTP), guanosine
40 triphosphate (GTP), and cytidine triphosphate (CTP) were all obtained from Sangon
41 Biotechnology Co. Ltd (Shanghai, China). DNA oligonucleotides used in this work
42 were carefully designed by our team and synthesized from Sangon Biotechnology Co.
43 Ltd (Shanghai, China). Thermodynamic parameters and secondary structures of all
44 oligonucleotides were calculated using bioinformatics software
45 (http://www.bioin-fo.rpi.edu/applications/). The sequences of oligonucleotides are
46 given in Table S1. The buffer containing 66 mM Tris-HCl (pH = 7.6), 6.6 mM $MgCl_2$,
47 and 10 mM dithiothreitol was used for ligation reaction and the detection buffer was
48 10 mM Tris-HCl, 100 mM NaCl, and 5 mM $MgCl_2$ (pH 8.0). GO was synthesized by
49 a modified Hummers' method. [1] All other reagents were of analytical grade and used
50 freshly without further purification. Deionized and sterilized water (resistance > 18.2
51 MΩ.cm) was used throughout.

52 **Table S1** Synthesized oligonucleotides (5'-3') used in the experiments
53

T1	5'-FAM-TGACTGTGAGCTTGACGTGAATG-3'
P1	5'-PO_3^{2-}-GCTCACAGTCA-3'
P2	5'-CATTCACGTCAA-3'
P3	5'-CATTCACGTCAAGCTCACAGTCA-3'

54 **Instrumentation**

55 Fluorescence detection was carried out on a Hitachi F-7000 fluorescence
56 spectrometer (Hitachi Ltd., Japan). The optical path length of a quartz fluorescence
57 cell was 1.0 cm. The instrument settings were chosen as follows: λ_{ex} = 494 nm
58 (bandpass 5 nm), λ_{em} = 520 nm (bandpass 5 nm), PMT detector voltage = 950 V. All
59 fluorescence measurements were performed under room temperature unless otherwise
60 indicated.

61 **Assay procedures**

62 The two half DNA probes (P1 and P2) and dye-labeled template (T1) were firstly
63 diluted in reaction buffer from stock solution. In a typical assay, a reaction mixture
64 (22 μL) containing 0.12 μM P1, 0.12 μM P2 and 0.12 μM T1 was heated to 90°C for
65 5 min then cooled to room temperature. Next, varying concentrations of ATP (2 μL)
66 and T4 DNA ligase (1 μL) at a final concentration of 0.4 U/μL were added to induce
67 the ligation reaction. The reaction was carried out at room temperature for 30 min.
68 Subsequently, the reaction mixture was kept at 45°C for 15 min followed by the
69 addition of 8 μL GO (100 μg/mL) and 67 μL detection buffer and kept at 45°C for
70 another 10 min. Then, the fluorescence was measured and recorded on a Hitachi
71 F-7000 fluorescence spectrometer. During the experimental process, the temperature
72 was carefully controlled in order to obtain the accurate data.

73 ## References

74 1 Y. G. Li and Y. Y. Wu, *J. Am. Chem. Soc.*, 2009, **131**, 5851-5857.