Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

A Graphene Oxide Enhanced Fluorescence Anisotropy Strategy for DNAzyme-Based Assay of Metal Ions

Yan Yu,[a] Yue Liu,[b] Shu Jun Zhen*[b] and Cheng Zhi Huang*[a, b]

[a] *College of Pharmaceutical Science, Southwest University,* [b] *Education Ministry Key Laboratory on Luminescence and Real-Time Analysis, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China.*

*Corresponding author: *zsj@swu.edu.cn*; *chengzhi@swu.edu.cn*.

Principle of GO amplifying FA Strategy.

It is known that the anisotropy value, *r*, is sensitive to the rotational motion changes of the fluorescent molecule-linked object. It can be described by the Perrin equation:[1]

$$r = \frac{r_0}{1 + (\tau/\theta)} \qquad (1)$$

$$\theta = \eta V / RT \qquad (2)$$

so

$$\frac{1}{r} = \frac{1}{r_0} + \frac{\tau RT}{r_0 \eta V} \qquad (3)$$

where r is the observed anisotropy, r_0 is the fundamental anisotropy in the absence of rotational diffusion, θ is the rotational correlation time for the diffusion process, τ is excited state lifetime, R is the gas constant, T is the temperature in Kelvin, η is the viscosity of the solution and V is the effective volume of the rotating unit. The anisotropy of a fluorophore is proportional to its rotational relaxation time, which in turn depends on its molecular volume (molecular mass). Therefore, a small molecule in solution rotates fast and has small FA while larger molecules will have larger anisotropy due to their hindered motion.

EXPERIMENTAL SECTION

1. Apparatus.

Fluorescence anisotropy was measured with an F-2500 fluorescence spectrophotometer equipped with a polarization filter (Hitachi, Tokyo, Japan). A vortex mixer QL-901 (Haimen, China) was employed to blend the solution and a constant-temperature water-base boiler (Jiangsu, China) was employed to control the temperature.

2. Materials.

The sequences of DNAzyme (Cu-Enz) and its substrate strand (Cu-Sub) were 5'-GGT AAG CCT GGG CCT CTT TCT TTT TAA GAA AGA AC-3' and 5'- AGC TTC

TTT CTA ATA CGG CTT ACC - TAMRA - 3', respectively. Three control sequences were: Ctrl1, 5'-TAMRA- AAA AAA AAA AAA AAA A - 3'; Ctrl2, 5'-TAMRA-CCG TGG GGC AAT TTC TCG GG-3'; Ctrl3, 5'-TAMRA-ACC TGG GGG AGT ATA TA-3'. All of the above sequences were synthesized using a standard procedure, and purified by HPLC from Songon Inc (Shanghai, China). Graphene oxide was purchased from Nanjing XFNANO Materials Tech Inc (Nanjing, China) and suspended in water via sonication. Ascorbic acid was obtained from Chuandong Chemical Industrial Inc (Chongqing, China). NaCl, $BaCl_2$, $CaCl_2$, $CdCl_2$, $CoCl_2$, $CuCl_2$, $FeCl_2$, $HgCl_2$, KCl, $MgCl_2$, $MnCl_2$, $Pb(NO_3)_2$, and $ZnCl_2$ were of analytical reagent grade. Ultrapure water was obtained from a Millipore water (18.0MΩ cm^{-1}) purification system.

3. Preparation of the hybrid of Cu-Enz and Cu-Sub.

To form the hybrid consisting of Cu-Enz and Cu-Sub, a final Cu-Sub concentration of 3.33 μM and a Cu-Enz concentration of 6.67 μM were annealed in 1.5 M NaCl and 50 mM HEPES, pH 7.0. The mixture was heated to 80 ^oC for 2 min and allowed to cool naturally to room temperature for 60 min. The resultant DNA hybrid was stored at 4 ^oC in dark.

4. Fluorescence anisotropy measurements.

Briefly, 20 μL of buffer (1.5 M NaCl, 50 mM HEPES, pH 7.4), 10 μL of DNA hybrid, 10 μL of ascorbic acid (2mM), 10 μL of Cu^{2+} solution with a certain concentration were sequentially added into an individual tube, and keep the temperature at 37 ^oC. 15 min later, 40 μL of GO solution (1 mg/mL) was added to the mixture and followed by adding certain ultrapure water to make a final volume of 500 μL for another 10 min incubation. Fluorescence anisotropy measurements were carried out on the F-2500 fluorescence spectrophotometer with an excitation wavelength of 543 nm at room temperature and emission was detected at 580 nm.

The anisotropy, *r*, of the test solution was calculated by

$$r = \frac{I_{VV} - G \times I_{VH}}{I_{vv} + 2G \times I_{VH}} \quad (4)$$

and

$$G = \frac{I_{HV}}{I_{HH}} \quad (5)$$

where *I* represents the intensity of the fluorescence signal and the subscripts define the orientation H for horizontal and V for vertical of the excitation and emission polarizers, respectively. *G* is the grating factor of the fluorescence spectrophotometer, which is used to correct for the wavelength response to polarization of the emission optics and detectors.[2]

5. Detection of Cu^{2+}

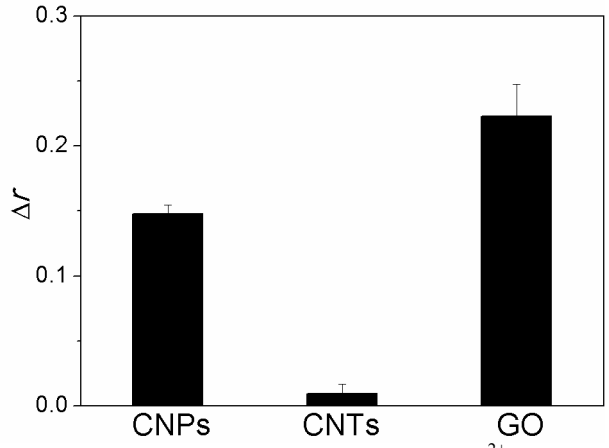

Fig. S1 Fluorescence anisotropy changes (Δ*r*) induced by 20 nM Cu^{2+} with addition of different carbon materials.

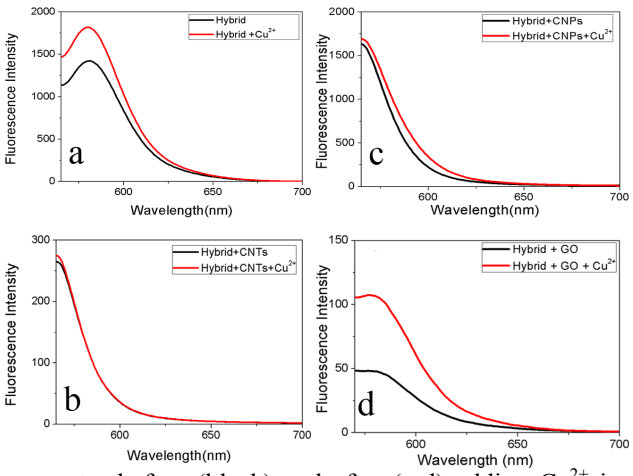

Fig. S2 The fluorescence spectra before (black) and after (red) adding Cu^{2+} in the reaction solution of Cu-Enz/Cu-Sub with subsequently incubation with None (a), CNTs (b), CNPs (c), GO (d).

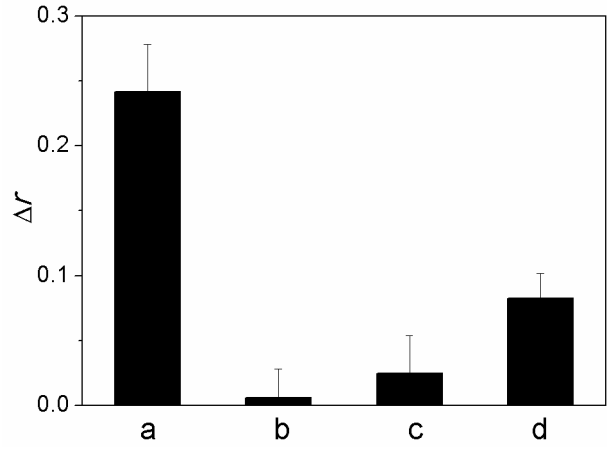

Fig. S3 Fluorescence anisotropy changes (Δ*r*) of different substrates catalyzed by Cu-Enz: Cu-Sub +GO +20 nM Cu^{2+}(a); Ctrl1 +GO +20 nM Cu^{2+}(b); Ctrl2 +GO +20 nM Cu^{2+}(c); Ctrl3 +GO +20 nM Cu^{2+}(d).

References

1 D. M. Jameson and J. A. Ross, Chem. Rev. , 2010, 110, 2685.
2 D. Zhang, H. Shen, G. Li, B. Zhao, A. Yu, Q. Zhao and H. Wang, Anal. Chem., 2012, 84, 8088.