# Supplemental Information

## Fluorescence Assay Based on Thioflavin T-induced Conformation Switch of G-quadruplex for TET1 Detection

Xue Chen,[a, b, 1] Ying Cheng,[a, 1] Yafen Wang,[c] Jing Tang,[a] Fang Wang,[*a,b] Zilin Chen[* a, b]

[a] Key Laboratory of Combinatorial Biosynthesis and Drug Discovery, Ministry of Education, Hubei Province Engineering and Technology Research Center for Fluorinated Pharmaceuticals, and Wuhan University School of Pharmaceutical Sciences, Wuhan, 430071, China

[b] State Key Laboratory of Transducer Technology, Chinese Academy of Sciences, Beijing, 100080, China

[c] College of Chemistry and Molecular Sciences, Key Laboratory of Biochemical Polymers of Ministry of Education. The Institute for Advanced Studies, Hubei Province Key Laboratory of Allergy and Immunology, Wuhan University, Wuhan, Hubei, 430072, P.R. China.

[1] These authors have contributed equally to this work.

* Corresponding author.

School of Pharmaceutical Sciences, Wuhan University, Wuhan, 430071, China.

E-mail address: fwang@whu.edu.cn, chenzl@whu.edu.cn

## Experimental procedures

**Apparatus and reagents.** HPLC-purified 5hmC-DNA and G-quadruplex sequence were obtained from Takara Biotechnology Co., Ltd. (Dalian, China). The base sequences are as follows: 5hmC-DNA: 5'-GGG TAG GGC GGG TTG GGC$^{hm}$ CGG AGA ATG TTT TCA TTC TCC$^{hm}$ GGC CCA AC-3'; G-quadruplex: 5'-GGG TAG GGC GGG TTG GGC-3'. The synthesized oligonucleotides were dissolved in deionized water and stored at -20°C. To ensure the formation of hairpin structure, each oligonucleotide was heated to 95°C for 5 min and slowly cooled down to room temperature before use. ThT (3,6-dimethyl-2-(4-dimethylaminopenyl) benzo-thiazoliumcation) was purchased from Sigma–Aldrich (MO, USA). Recombinant TET1 protein was purchased from Epigentek (Cat.No.E12002, USA) and stored at -80°C. Restriction endonuclease MspI (Cat.No.R0106L) and 10 × Cutsmart buffer (Cat.No.B7204S), rSAP (Cat.No.M0371L) and 10 × Cutsmart buffer were supplied by New England Biolabs (Ipswich, MA). DNA Clean & Concentrator™-5 Kit was obtained from ZYMO RESEARCH (Shanghai, China). DL-dithiothreitol (DTT), α-ketoglutaric acid (α-KG) and ferrous alum were purchased from Aladdin (Shanghai, China). ATP was sourced from Biosharp (Guangzhou, China). S1 Nuclease (Cat.No.EN0321) was purchased from Thermo Scientific. AidQuick PAGE Extraction Kit used for extraction DNA from the band was purchased from Aialab (Beijing, China). All other chemicals were purchased from Sinopharm Group (Shanghai, China). All solutions were prepared with Milli-Q water (18.2 MΩ·cm).

All fluorescence emission spectra were measured on LS-55 (PerkinElmer, USA) with excitation at 450 nm and emission at 450-600 nm. Excitation slits was set to 5nm and emission slits was set to 15nm. Polyacrylamide gel electrophoresis (PAGE) was imaged on Gel Documentation system (Huifuxingye, Beijing, China). LC-MS data were collected with the Agilent 1220 Infinity LC combined with the 6120 Single Quadrupole mass spectrometer (Agilent Technologies).

**TET1 enzymatic reaction.** 1 μM 5hmC-DNA was incubated with a certain concentration of TET1 in reaction buffer (50 mM HEPES, 100 mM NaCl, 75 μM

$Fe(NH_4)_2(SO_4)_2$, 2 mM ascorbate, 1 mM DTT, 1 mM ATP and 1 mM α-KG, pH 8.0) at 37 °C for 6 h. The reaction matrix was then purified by using DNA Clean & Concentrator™-5 Kit with an instruction manual. The purified DNA product was followed by incubating with 5 U MspI in 1 × Cutsmart buffer (50 mM KAc, 20 mM Tris-HAc, 10 mM $Mg(Ac)_2$ and 100 μg/mL BSA, pH 7.9) at 37 °C for overnight.

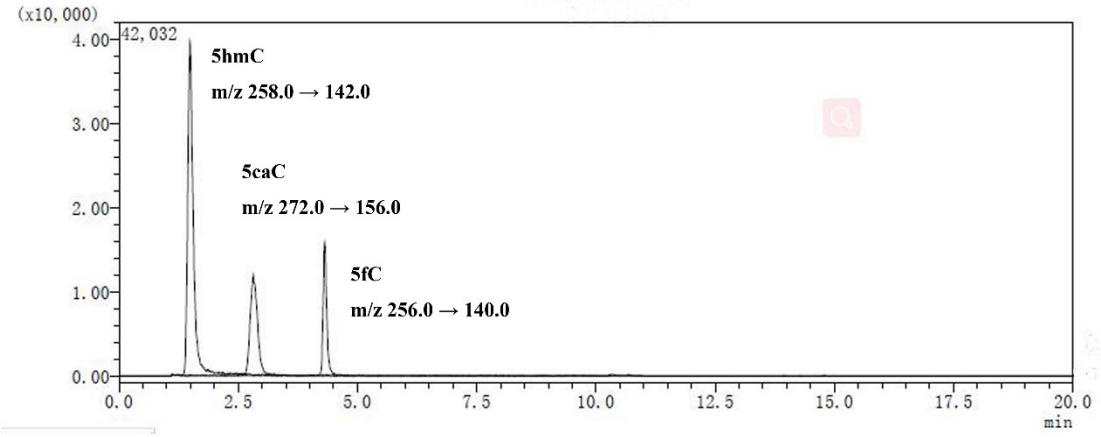
**Fluorescence Detection.** The above reaction mixture was prepared in reaction buffer (50 mM Tris-HCl, 50 mM KCl, PH 7.2), and 25 μM ThT was added for inducing the formation of special G-quadruplex structure for 10 min. Then the fluorescence measurement was taken and the fluorescence emission spectra was recorded in the range from 450 nm to 600 nm.

**Gel electrophoresis.** Different samples were analyzed by polyacrylamide gel electrophoresis (PAGE) in TBE buffer (89 mM Tris, 89 mM boricacid, 2.0 mM EDTA, pH 8.0) at 350 V constant voltage for 90 min at room temperature. Then, the gel was stained with GelRed nucleic acid gel for 10 min. At last, gels were imaged.

**Samples preparation for LC-MS.** 23 μL samples (containing 600ng DNA) were denatured at 95 °C for 10 min and immediately ice for 2 min. Then, 1 μL S1 Nuclease (10 U/μL) and 6 μL 5 × S1 Buffer were added at 37 °C for overnight. 3.5 μL 10 × CIAP buffer, 1 μL CIAP (2 U/μL) and 1 μL VPDE (0.001 U) were added into the above reaction mixture at 37 °C for 4 h. At last, centrifuge at 12,000 rpm for 5 min and take the supernatant for LC-MS.

**Figure S1 Separation and quantification of 5hmC, 5fC and 5caC by LC-MS/MS.**

**(A)** Detection of 5hmC, 5fC and 5caC in multiple reaction monitoring (MRM) mode.




**(B)** Standard curves for 5hmC, 5fC and 5caC generated for quantification.

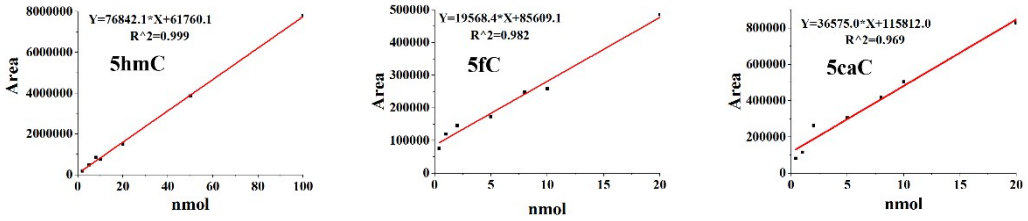
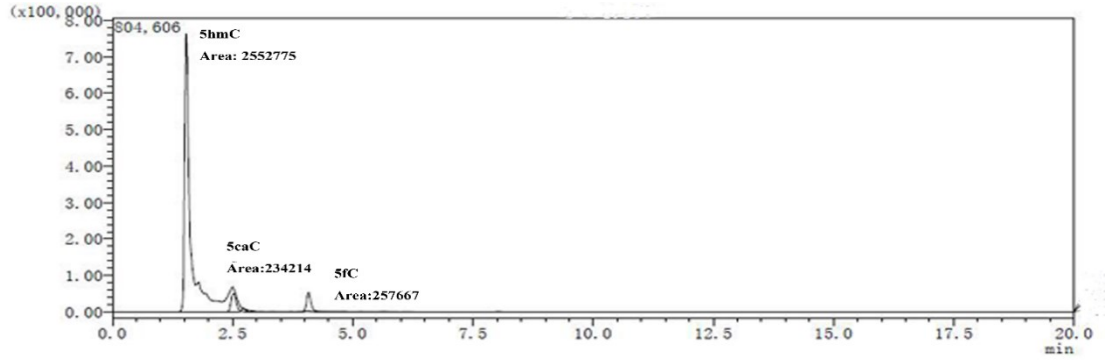


**Figure S2 Determination of 5hmC, 5fC and 5caC-containing substrates after enzymatic digestion.**

Optimal conditions: 4.5μM oligo DNA, 0.0105 μg/μL TET1, 50 mM HEPES, 100 mM NaCl, 75 μM $Fe(NH_4)_2(SO_4)_2$, 2 mM ascorbate, 1 mM DTT, 1 mM ATP and 1 mM α-KG, pH 8.0, 37 °C, 6h.



**Tabe S1** LC-MS data for quantification of 5hmC, 5fC and 5caC and determination of the oxidation efficiency for TET1.

| Samples | 5hmC | 5fC | 5caC | Total amount (nmol) | TET1 oxidation capacity | Average |
|---|---|---|---|---|---|---|
| 1 | 28.15 | 8.8 | 3.83 | 40.78 | 30.97% | |
| 2 | 28.1 | 9.31 | 2.71 | 40.12 | 29.96% | 31.52% |
| 3 | 22.07 | 4.28 | 6.91 | 33.26 | 33.63% | |

**Table S2** After PAGE and AidQuick PAGE Extraction Kit for extraction DNA, LC-MS data for quantification of 5hmC, 5fC and 5caC and determination of the oxidation efficiency for TET1.

| Samples | 5hmC | 5fC | 5caC | Total amount (nmol) | TET1 oxidation capacity | Average |
|---|---|---|---|---|---|---|
| 1 | 32.42 | 8.77 | 3.29 | 44.48 | 27.11% | |
| 2 | 57.80 | 13.23 | 7.35 | 78.38 | 26.27% | 27.13% |
| 3 | 59.56 | 12.34 | 10.82 | 82.72 | 28.00% | |

**Table S3** Comparison of different methods for TET1 detection.

| Method | Linear range | Detection limit | Reference |
|---|---|---|---|
| LC-MS | 10-30 μg/mL | not mentioned | [14-15] |
| TET activity assay kit (fluorometric) | 1-10 ng/μL | 1 ng/μL | Epigentek Group Inc. |
| Electrochemiluminescence biosensor | 1-10 μg/mL | 0.37 μg/mL | [16] |
| Fluorescence Assay | 0.0105-0.0525 μg/μL | 0.0054 μg/μL | this work |