Electronic Supplementary Information (ESI) for Chemical Communications

Ultrasensitive fluorescence detection of bleomycin via exonuclease III-aided DNA recycling amplification

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Experimental

Materials and reagents: Bleomycin (BLM) sulfate was purchased from Melone Pharmaceutical Co., Ltd. (Dalian, China). Dactinomycin, mitomycin, daunorubicin and $FeCl_2$ were purchased from Sigma–Aldrich. Exonuclease III (Exo III) and NEB buffer 1 were purchased from New England Biolabs (Ipswich, MA, USA). Phosphate buffer saline (PBS) was prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4. DNA hybridization buffer contained 10 mM PBS (pH 7.4), 137 mM NaCl, and 2.5 mM Mg^{2+}. BLM detection buffer was the mixture of 10 mM PBS, 0.5 M NaCl, and 0.1 M $NaClO_4$ (pH 8.0). Human serum samples were kindly provided by the Jiangsu Cancer Hospital (Nanjing, China). A mixture containing equal volumes of human serum sample and BLM detection buffer was used for recovery testing. DNA oligonucleotides were synthesized and purified by Takara Biotechnology Co., LTD. (Dalian, China), and stored in DNA hybridization buffer. The

sequences of these oligonucleotides are expressed as follows:

Hairpin DNA: 5'-GAGGGATAGAATATAA**GC***AAAAAAAAAA***GC**TTATATTCT

ATCCCTCAG-3'

Probe 1 (P_1): 5'-FAM-TTTTTTTTG-3'
– BHQ

Probe 2 (P_2): 5'-FAM-TT**GC**TT-BHQ-3'

Here FAM and BHQ are fluorescent dye (carboxyfluorescein) and its quencher, respectively.

Fluorescent assay: The BLM-Fe(II) solution was prepared by mixing BLM sulfate with $FeCl_2$ in 1:1 molar ratio. After a series of 20 μL BLM-Fe(II) solution with various concentrations were added into the mixture of 20 μL of hairpin DNA (1 μM) and 20 μL P_1 (1 μM), 100 μL NEB buffer 1 and 5 U Exo III, the digestion and Exo III-assisted DNA recycling reactions were performed in 37 ^{o}C for 30 min. The reactions were then terminated through heating at 75 ^{o}C for 5 min. Fluorescence measurements were conducted by scanning from 500 to 620 nm with a step of 1 nm at an excited wavelength of 496 nm on a F900 fluorescence spectrometer (Edinburgh Instruments Ltd., UK). The whole assay needs about 40 min.

Gel electrophoresis: A 20 % polyacrylamide gel electrophoresis analysis was carried out in 1×Tris-Borate-EDTA (pH 8.3) at a constant voltage of 110 V for about 2 h. After ethidium bromide staining, gels were scanned using a Molecular Imager Gel Doc XR (BIO-RAD, USA)

Structure of bleomycin

BLM contains four functional domains (Fig. S1): The metal binding domain for recognizing DNA and activating oxygen, the bithiazole and C-terminal substituent for DNA binding, the linker region for efficient DNA cleavage, and the carbohydrate moiety for metal binding and DNA efficient cleavage. Based on this unique properties, BLM-Fe has been reported to selectively respond to the characteristic 5'-GC-3' and 5'-GT-3' sequence in the minor groove of DNA. BLM-mediated DNA degradation can be initiated by C4'-H atom abstraction from deoxyribose, which leads to efficient cleave of the hairpin DNA.[S1,S2]

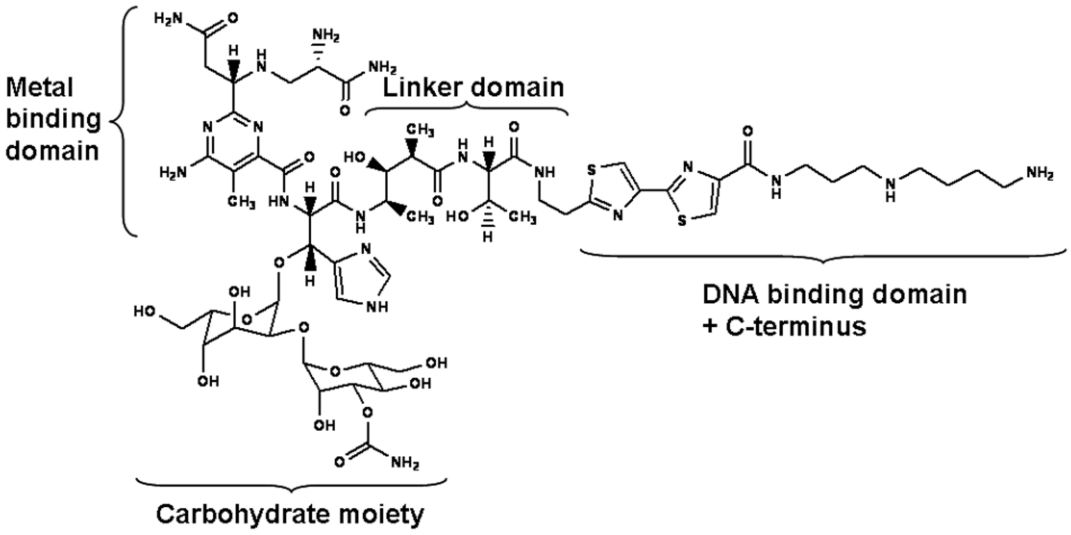

Fig. S1 Structure of bleomycin with four major domains.

BLM detection without Exo III amplification

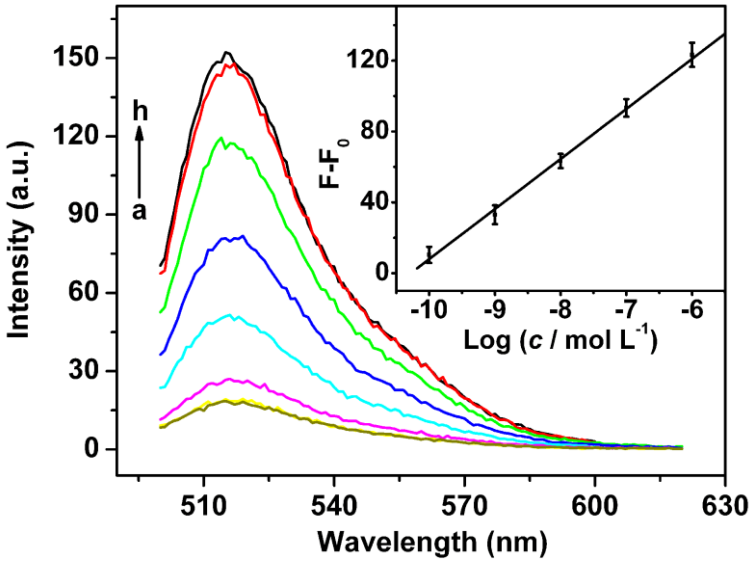

Fig. S2 Fluorescence spectra for 0, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} mol L^{-1} BLM-Fe(II) (from a to h) in the presence of P_2. Inset: linear calibration of $F\text{-}F_0$ *vs* logarithm value of BLM-Fe(II) concentration.

Supplementary References

S1 Y. Akiyama, Q. Ma, E. Edgar, A. Laikhter and S. M. Hecht, *J. Am. Chem. Soc.*, 2008, **130**, 9650–9651

S2 R. A. Giroux and S. M. Hecht, *J. Am. Chem. Soc.*, 2010, **132**, 16987–16996.