Supporting Information

Biostable L-DNAzyme for Sensing of Metal Ions in Biological Systems

Liang Cui[1], *Ruizi Peng*[1], *Ting Fu*[1], *Xiaobing Zhang*[1]*, *Cuichen Wu*[1,3], *Huapei Chen*[1],

Hao Liang[1], *Chaoyong James Yang*[2] *and Weihong Tan*[1,3]*

[1]Molecular Science and Biomedicine Laboratory, State Key Laboratory of Chemo/Bio-Sensing and Chemometrics, College of Chemistry and Chemical Engineering, College of Biology, Collaborative Innovation Center for Chemistry and Molecular Medicine, Hunan University, Changsha 410082, China.

[2]State Key Laboratory of Physical Chemistry of Solid Surfaces, Key Laboratory for Chemical Biology of Fujian Province, The MOE Key Laboratory of Spectrochemical Analysis & Instrumentation, Department of Chemical Biology, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, P. R. China

[3]Department of Chemistry and Physiology and Functional Genomics, Center for Research at the Bio/Nano Interface, Shands Cancer Center, University of Florida, Gainesville, FL 32611-7200, USA

To whom correspondence should be addressed. E-mail: tan@chem.ufl.edu, xbzhang@hnu.edu.cn

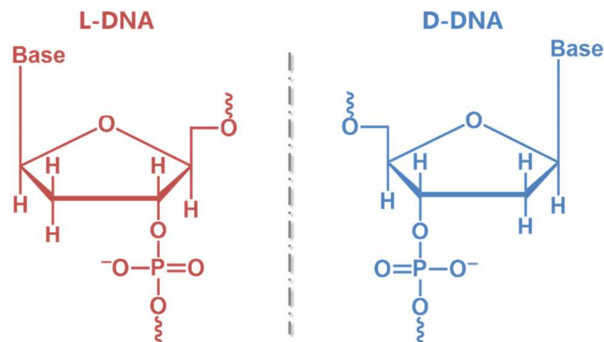

Figure S1. Mirror structure of L-DNA (left) and D-DNA (right).

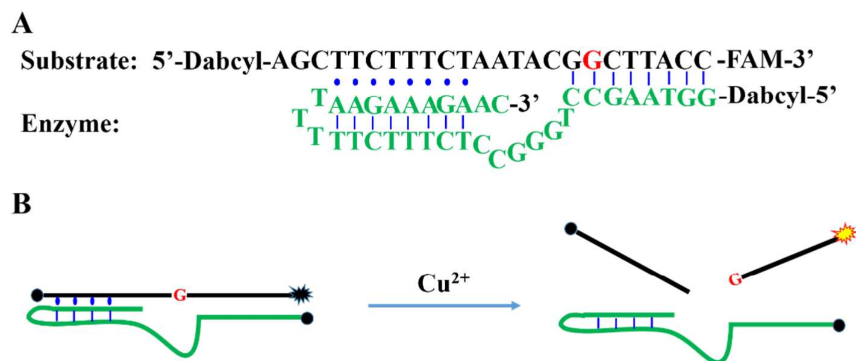

Figure S2. (A) Sequence of Cu^{2+}-dependent DNAzyme. (B) The substrate was labeled with a FAM fluorophore at the 3'-end and a quencher at the 5'-end, while the enzyme contained a 5'-quencher. This dual-quencher approach was employed to efficiently suppress background signals. Initially, the FAM emission was quenched by the nearby quenchers. In the presence of Cu^{2+}, the substrate was irreversibly cleaved at the cleavage site, leading to an increased fluorescence signal.

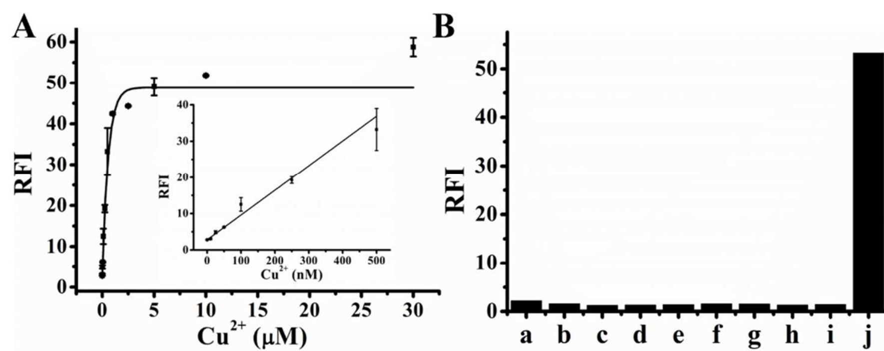

Figure S3. Response of Cu^{2+}-dependent L-DNAzyme to different concentrations (0, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10 and 30 μM) of Cu^{2+} (A) and the control metal ions (B) a-j: blank, Cd^{2+}, Co^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+} and Cu^{2+}. RFI = relative fluorescence intensity. While the $[Cu^{2+}]$ = 30 μM, the other metal ions $[M^{n+}]$ = 300 μM.

Cu^{2+}-dependent DNAzyme

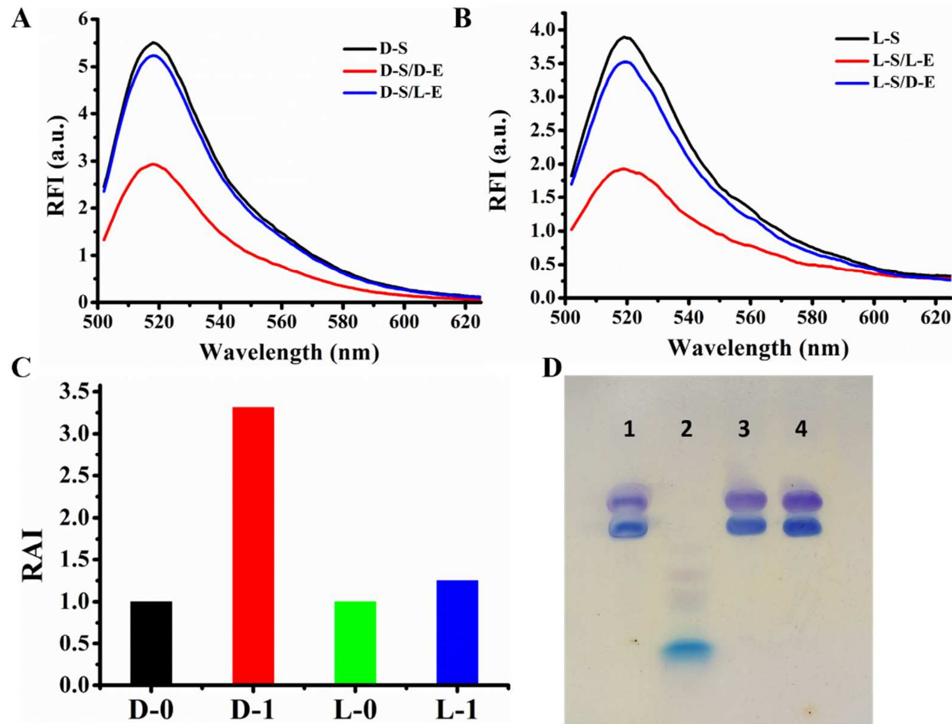

Figure S4. (A) Fluorescence spectra of Cu^{2+}-dependent D-S, D-S/D-E, and D-S/L-E. (B) Fluorescence spectra of Cu^{2+}-dependent L-S, L-S/L-E, and L-S/D-E. (C) Response of Cu^{2+}-dependent D-S and L-S to SSB using fluorescence anisotropy. While D-S exhibited a 3.5-fold enhancement in anisotropy signal in the presence of SSB, L-S did not induce any change. RAI = relative anisotropy intensity; D0/D1, D-substrate in the absence (0) or presence (1) of SSB; L0/L1, L-substrate in the absence (0) or presence (1) of SSB. (D) Denaturing PAGE of the Cu^{2+}-dependent L-/D-DNAzyme in the absence or presence of DNase I. D-DNAzyme without (Lane 1) or with (Lane 2) DNase I; L-DNAzyme without (Lane 3) or with (Lane 4) DNase I.

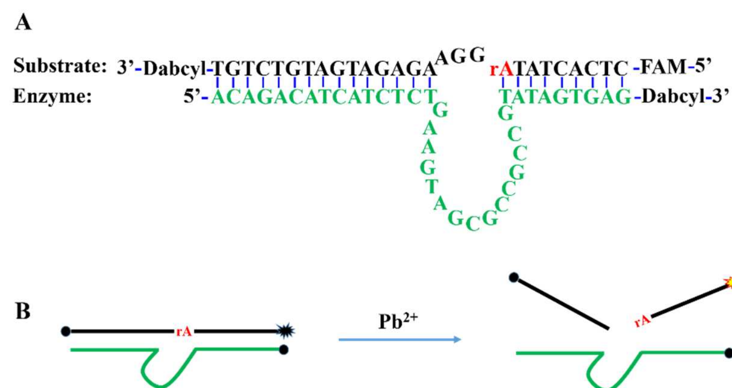

Figure S5. (A) Sequence of Pb^{2+}-dependent DNAzyme. (B) The working principle agrees with that of Cu^{2+}-dependent DNAzyme, as described in Figure S2B. In the presence of Pb^{2+}, the substrate was irreversibly cleaved at the cleavage site, leading to an increasing fluorescence signal.

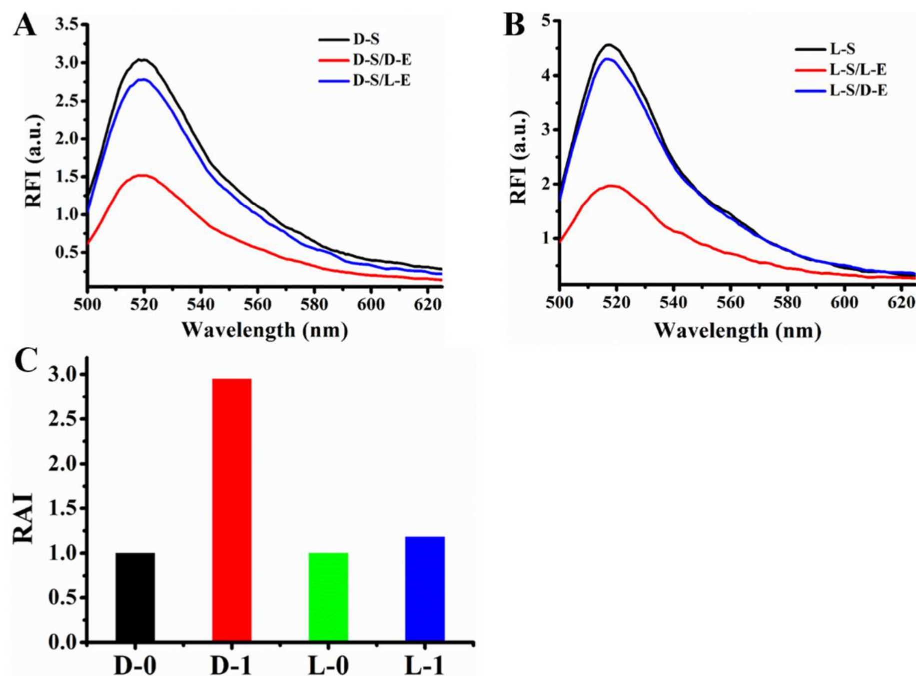

Figure S6. (A) Fluorescence spectra of Pb^{2+}-dependent D-S, D-S/D-E, and D-S/L-E. (B) Fluorescence spectra of Pb^{2+}-dependent L-S, L-S/L-E, and L-S/D-E. (C) Response of Pb^{2+}-dependent D-S and L-S to SSB using fluorescence anisotropy. While D-S exhibited a 3-fold enhancement in anisotropy signal in the presence of SSB, L-S did not induce any change. D0/D1, D-substrate in the absence (0) or presence (1) of SSB; L0/L1, L-substrate in the absence (0) or presence (1) of SSB.