Supporting Information

Multiple Roles of Cu(II) in Catalyzing Hydrolysis and Oxidation of β-Lactam Antibiotics

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Text S1. Chemicals.

AMX, CFX hydrate, CFD and CFP were obtained from Sigma-Aldrich at >90% purity. AMP sodium salt, PG sodium salt, and CFR were obtained from Fisher Scientific at the highest purity. All chemicals were used without further purification. 2-(*N*-Morpholino)ethanesulfonic acid (MES), 4-morpholinepropanesulfonic acid (MOPS), 2-(cyclohexylamino)ethanesulfonic acid (CHES), cupric sulfate ($CuSO_4 \cdot 5H_2O$), hydrochloric acid (HCl), sodium hydroxide (NaOH), humic acid (HA), ethylenediamine tetraacetic acid (EDTA), *tert*-butyl alcohol (TBA) and bathocuproine disulfonic acid disodium salt hydrate were obtained from Fisher Scientific or Acros Organics at analytical grade. Deionized (DI) reagent water (resistivity >18 ΩM) was produced from a Millipore Milli-Q Ultrapure Gradient A10 purification system. All stock solutions of β-lactam antibiotics were prepared in DI water at 1.0 g/L, stored at 5 ^{o}C before use, and renewed weekly. The stock solution of Cu^{II} was prepared by dissolving $CuSO_4 \cdot 5H_2O$ in acidic DI water (pH 3.0, adjusted by HCl).

Text S2. LC/MS Method Conditions.

The mobile phase gradient elution was with 0.1% formic acid in water (A) and pure methanol (B) at a flow rate of 0.2 mL/min: 5% B was kept for 2 min first, then ramped to 18% B over 10 min, kept for 5 min, ramped to 30% B over 9 min and kept for 8 min, and finally ramped back to the initial mobile phase composition. Electrospray positive ionization at fragmentor voltage of 70 eV with mass scan range of *m/z* 50-1000 was employed. The drying gas was set at 6 L/min at 350 ^{o}C, capillary voltage 4000 V, and nebulizer pressure 25 psig.

Table S1. Characteristics of the water samples

Sample	pH	UV254	TOC (mg/L)	Cu (μM)
SW1	7.31	0.043	6.4	0.32
SW2	7.26	0.021	4.4	0.11

Note: SW1: river water; SW2: lake water.

Table S2. HPLC method parameters for detection of β-lactam antibiotics

Chemical	Mobile Phase	Wavelength (nm)	Flow Rate (mL/min)
CFX	water containing 0.75% acetic acid/ methanol (75:25 v/v)	262	1
CFR	water containing 0.75% acetic acid/ methanol (85:15 v/v)	262	1
CFD	water containing 0.75% acetic acid/ methanol (70:30 v/v)	262	1
CFP	water containing 0.75% acetic acid/ methanol (85:15 v/v)	262	1
CFT	water containing 0.75% acetic acid/ methanol (70:30 v/v)	262	1
AMP	water containing 0.75% acetic acid/ methanol (70:30 v/v)	230	1
AMX	water containing 0.75% acetic acid/ methanol (85:15 v/v)	230	1
PG	water with 10 mM H_3PO_4 /acetonitrile (35:65 v/v)	220	1

Table S3. First-order hydrolysis rate constants of CFX and PG

Reaction Conditions	k (s^{-1})	
	CFX	PG*
pH 11.0	7.89×10^{-6}	7.82×10^{-5}
Zn^{II}	$8.33\times10^{-6,a}$	$2.89\times10^{-4,b}$

* Data from Ref (1).

[a] Hydrolysis of CFX catalyzed by 5 mM Zn^{II}

[b] Hydrolysis of PG catalyzed by 1 mM Zn^{II}

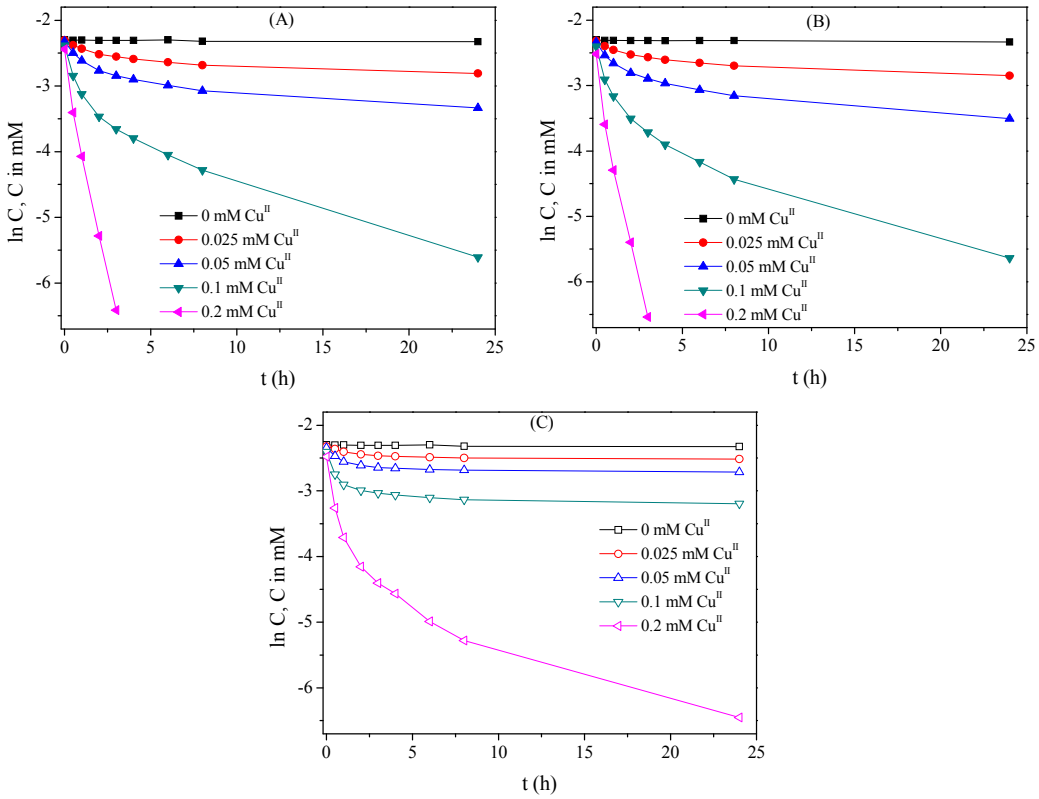


Figure S1. Reaction of CFX with different amounts of Cu^{II} under different oxygenation conditions: (A) oxygen-rich (open to ambient air during the reaction); (B) oxygen gas purging during the reaction; (C) oxygen-limited (nitrogen gas purging before the reaction and closed to ambient air during the reaction). Reaction conditions: $[CFX]_0 = 0.1$ mM, pH 7.0 and 22 °C.

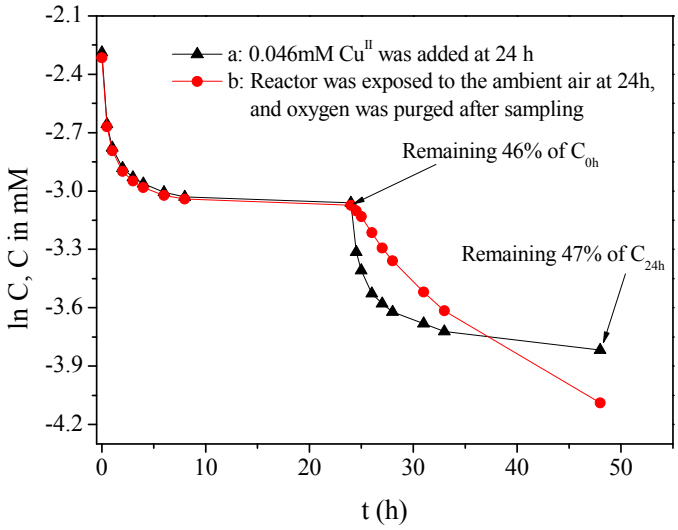


Figure S2. Reaction of CFX and Cu^{II} under oxygen-limited conditions - Effects of (a) the addition of Cu^{II}; and (b) exposure to oxygen after 24 h of reaction. $[CFX]_0 = 0.1$ mM, $[Cu^{II}]_0 = 0.1$ mM, pH 7.0 and 22 °C.

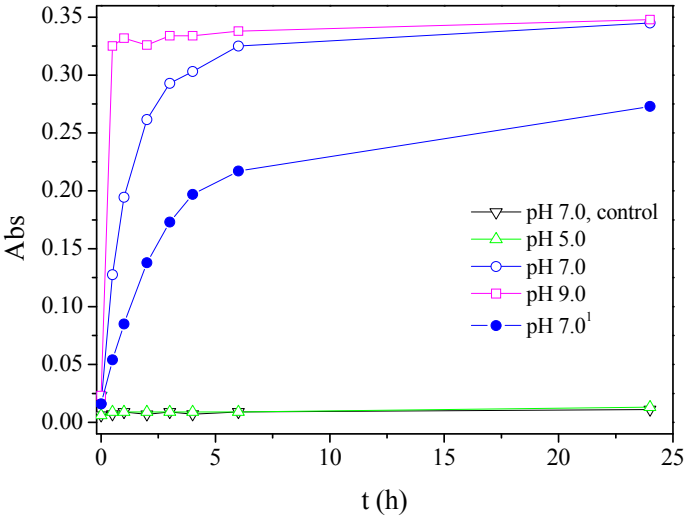


Figure S3. Accumulation of Cu^{I} during the reaction of Cu^{II} with CFX at different pHs under the oxygen-limited condition. $[CFX]_0 = 0.1$ mM, $[Cu^{II}]_0 = 0.05$ mM. Control experiment: CFX only without the addition of Cu^{II}. Note [1]: Reaction of Cu^{II} with CFX under the oxygen-rich condition.

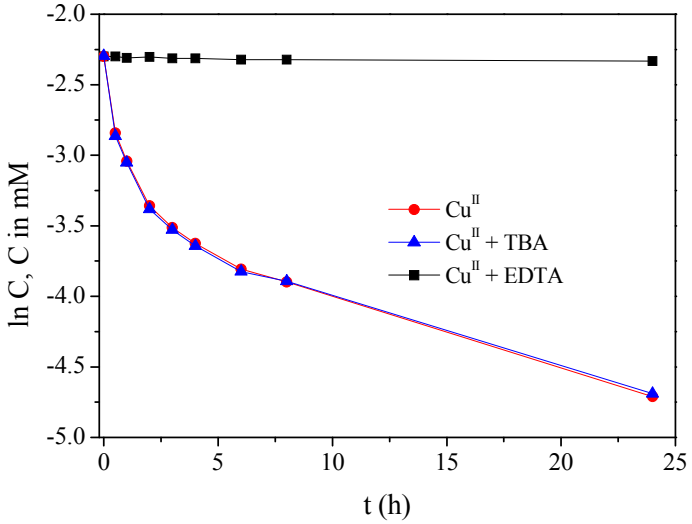


Figure S4. Effect of TBA and EDTA on the Cu^{II}-catalyzed degradation of CFX in the oxygen-rich condition. $[CFX]_0 = [Cu^{II}]_0 = 0.1$ mM, [TBA] = 50 mM, [EDTA] = 5 mM, pH 7.0 and 22 °C.

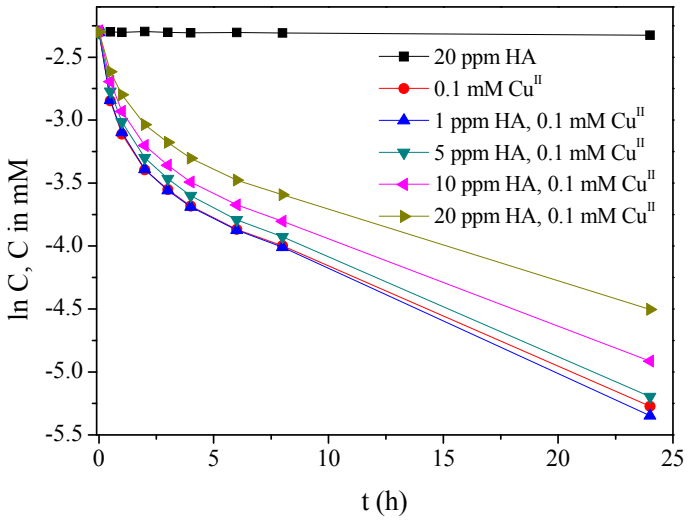


Figure S5. Effect of humic acid (HA) on the Cu^{II}-catalyzed degradation of CFX under the oxygen-rich condition. $[CFX]_0 = [Cu^{II}]_0 = 0.1$ mM, pH 7.0 and 22 °C.

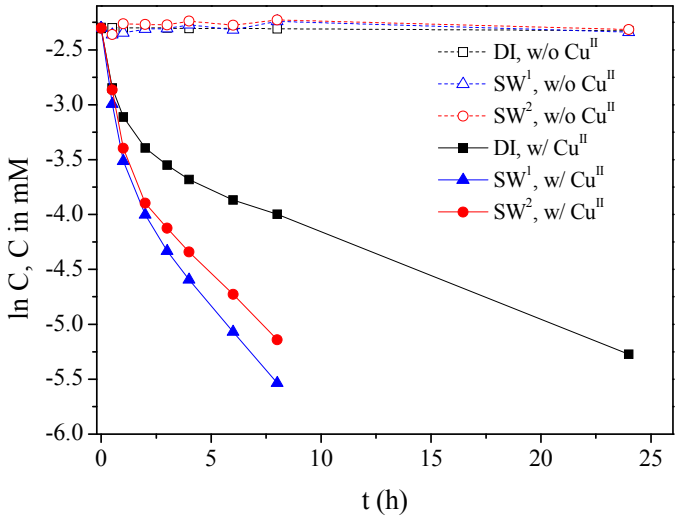
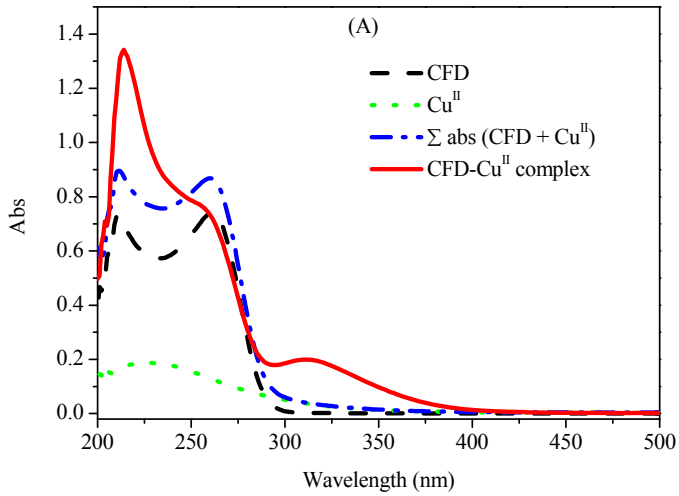


Figure S6. Cu^{II}-catalyzed degradation of CFX in DI water and surface water (SW) samples. $[CFX]_0 = [Cu^{II}]_0 = 0.1$ mM, 22 °C. DI: pH = 7.01; SW1: river water, pH = 7.31; SW2: lake water, pH = 7.26.



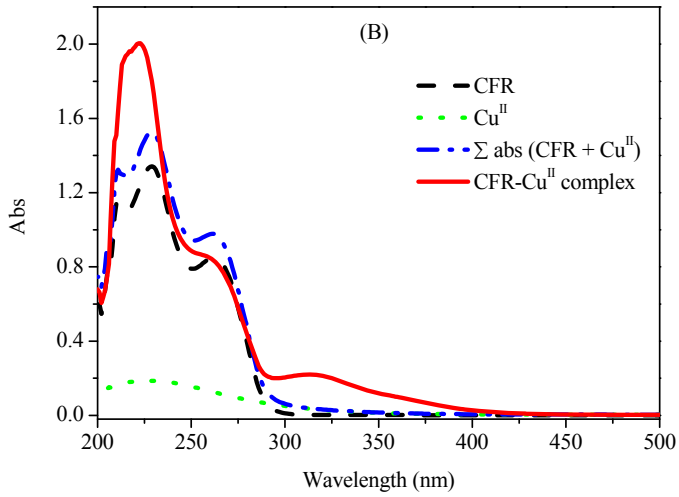


Figure S7. (continued next page) -

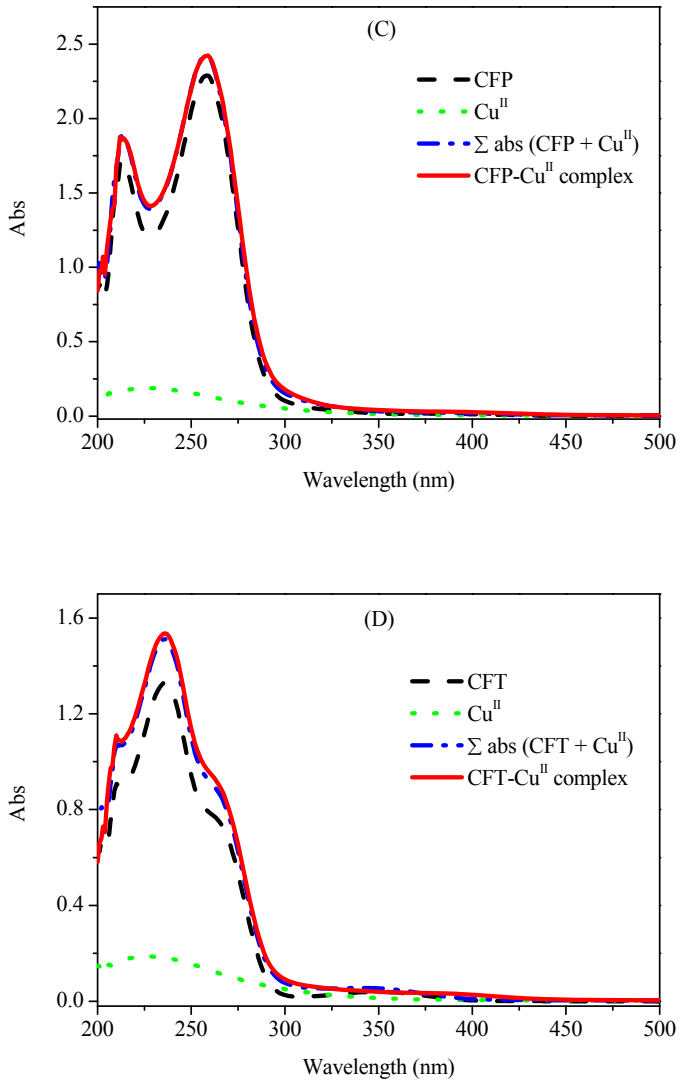


Figure S7. Ultraviolet absorption spectra of free cephalosporins, free Cu^{II}, and Cu^{II}-cephalosporin complex at pH 7. (A) CFD; (B) CFR; (C) CFP; and (D) CFT. $[cephalosporin]_0 = [Cu^{II}]_0 = 0.1$ mM, pH 7.0 and 22 °C.

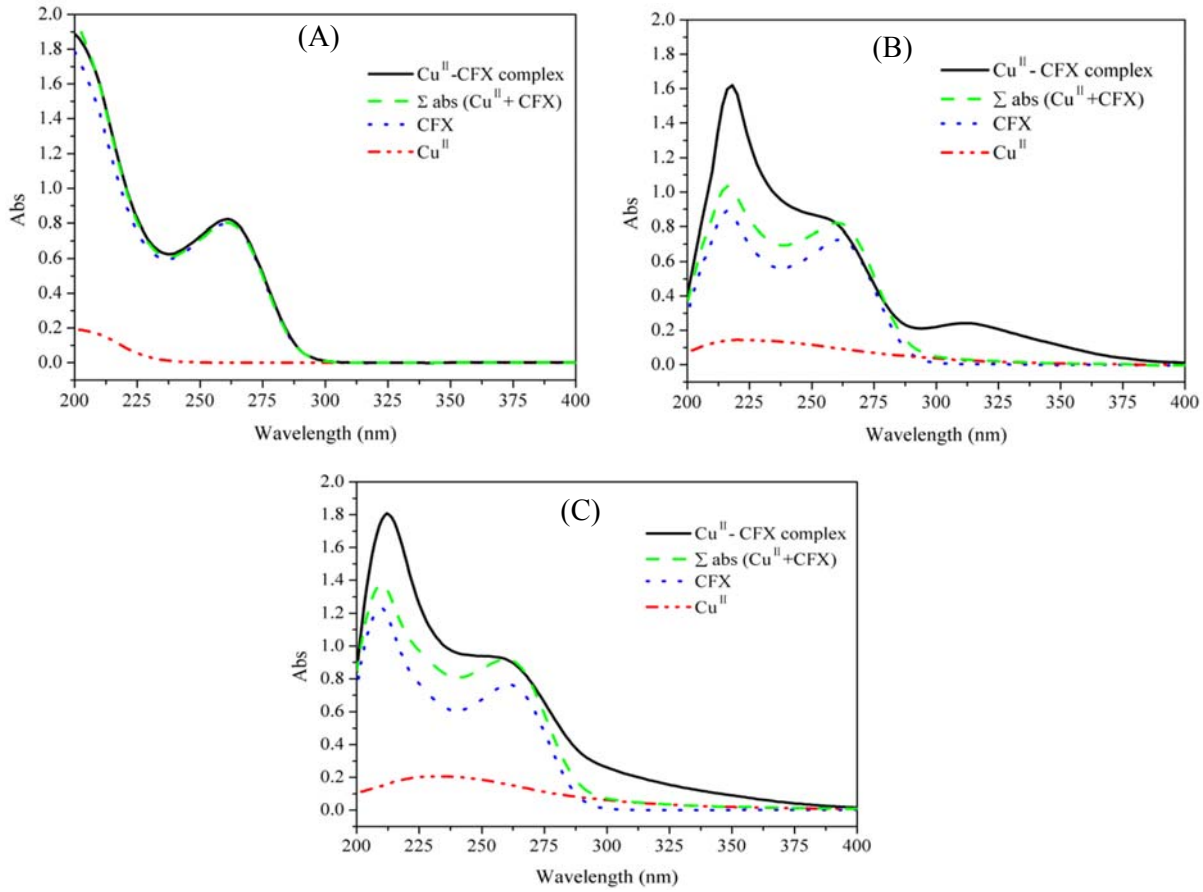


Figure S8. Effect of pH on the complex of CFX and Cu^{II}. (A) pH 5.0; (B) pH 7.0; and (C) pH 9.0. $[CFX]_0 = [Cu^{II}]_0 = 0.1$ mM.

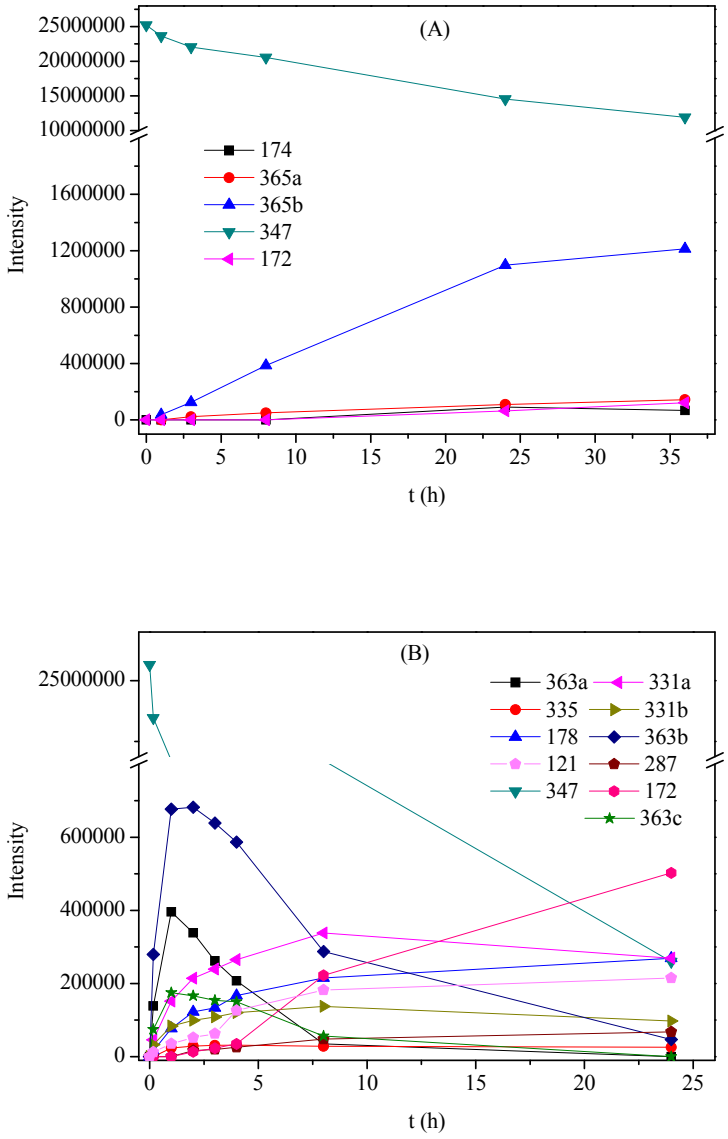


Figure S9. Degradation products of CFX in the presence of Zn^{II} (A) and Cu^{II} (B). $[Cu^{II}]_0$ = 0.1 mM, $[Zn^{II}]_0$ = 5 mM, pH 7.0 and 22 °C.

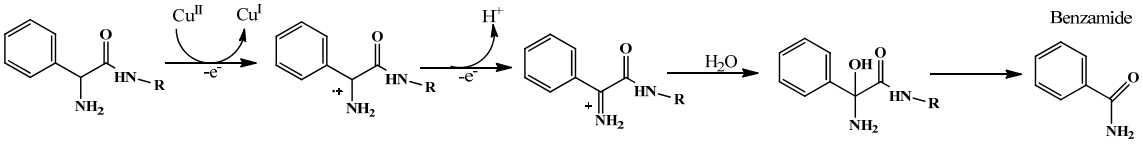


Figure S10. Proposed reaction scheme for oxidation of phenylglycine side chain by Cu^{II}.

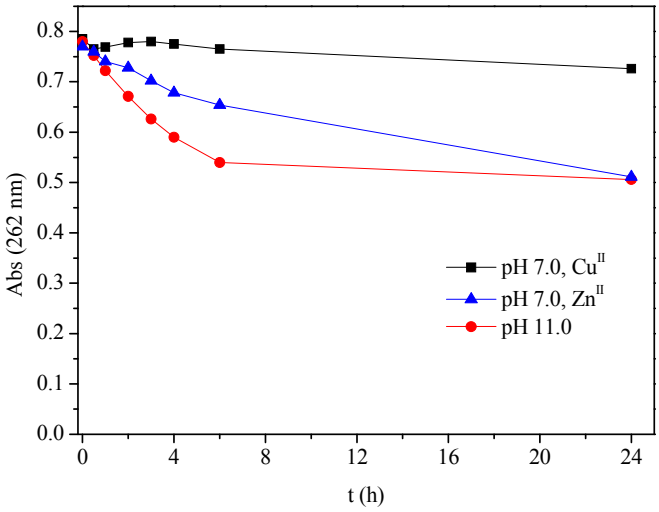
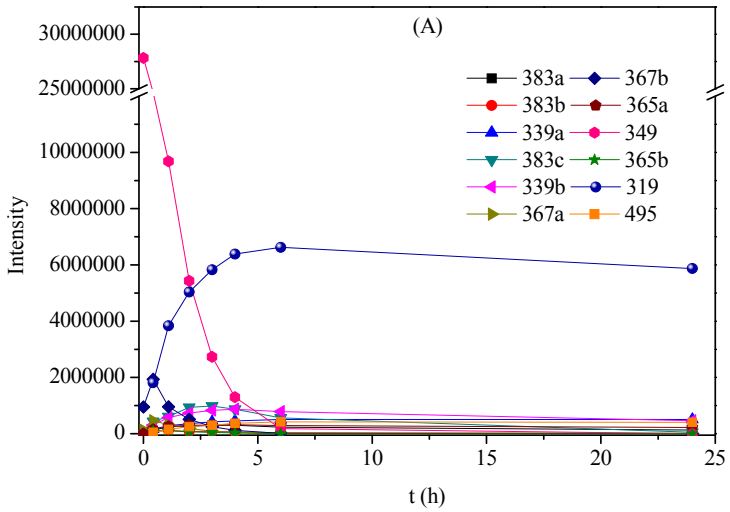


Figure S11. Absorbance change at 262 nm during the degradation of CFX in the presence of Zn^{II} or Cu^{II}, or at pH 11.0. $[CFX]_0 = 0.1$ mM, $[Cu^{II}]_0 = 0.1$ mM, $[Zn^{II}]_0 = 5$ mM. The reaction with Cu^{II} was conducted under the oxygen-rich condition.



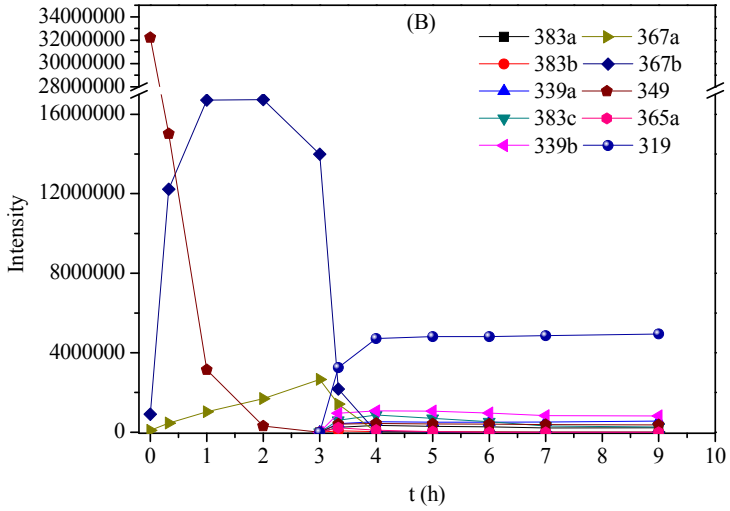


Figure S12. (continued next page) -

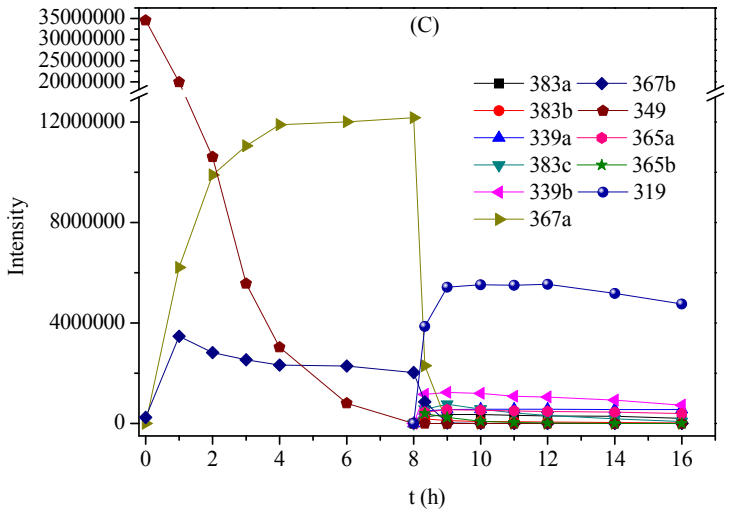


Figure S12. Degradation products of AMP (A) and ampicilloic acid (B, and C) catalyzed by Cu^{II} in the presence of oxygen. (B) Degradation of AMP at pH 11.5 for 3 h, followed by the adjustment of pH to 7.0, and the addition of MOPS and Cu^{II}; (C) Hydrolysis of AMP catalyzed by 1 mM Zn^{II} for 8 h, followed by the addition of Cu^{II}. $[AMP]_0$ = 0.1 mM, $[Cu^{II}]_0$ = 0.1 mM, pH 7.0 and 22 °C.

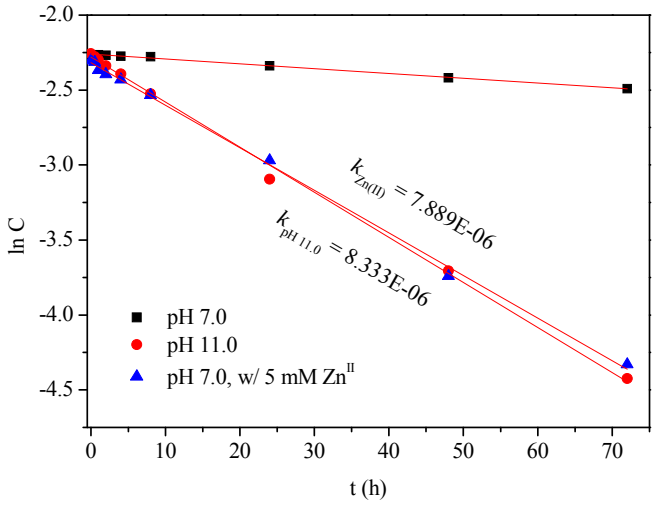


Figure S13. Hydrolysis of CFX at alkaline pH or in the presence of Zn^{II}. The rate constant *k* is in unit of s^{-1}.

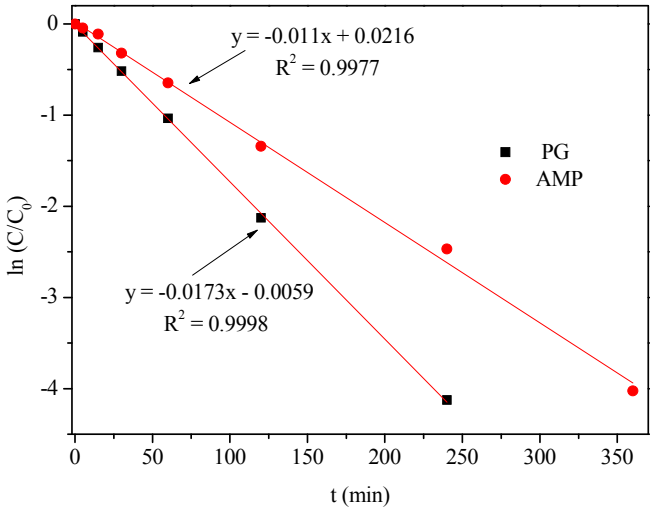


Figure S14. Hydrolysis of PG and AMP in the presence of 1.0 mM Zn^{II}. $[antibiotics]_0$ = 0.1 mM, pH 7.0.

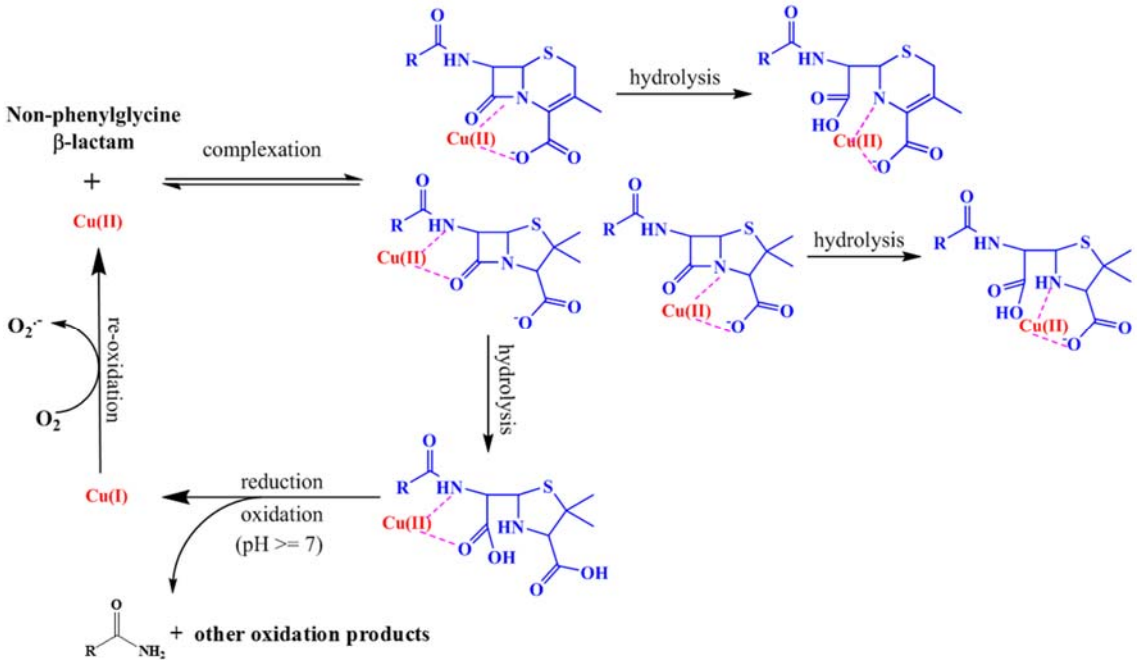


Figure S15. Proposed mechanisms for the Cu^{II}-catalyzed degradation of non-phenylglycine-type β-lactam antibiotics.

References

(1) Chen, J.; Sun, P.; Zhou, X.; Zhang, Y.; Huang, C.-H. Cu(II)–Catalyzed Transformation of Benzylpenicillin Revisited: The Overlooked Oxidation. *Environ. Sci. Technol.* **2015,** *49*, (7), 4218-4225.