

Supporting Information

Real-time activity assays of β -lactamases in living bacterial cells: application to the inhibition of antibiotic-resistant *E. coli* strains

Ying Ge, Ya-Jun Zhou, Ke-Wu Yang,* Yi-Lin Zhang, Yang Xiang, and Yue-Juan Zhang

General methods	2
1-1. L1 enzyme expression and purification	2
1-2. <i>E. coli</i> cells preparations for <i>in vivo</i> UV-Vis studies	2
1-3. Plating colony tests for L1 <i>E. coli</i> cells	2
1-4. Measurements and analyses of IC ₅₀	3
Supporting figures	4
References	7

General methods

1-1. L1 enzyme expression and purification

L1 enzyme was overexpressed and purified based on the previously described^[2]. L1 plasmids were transformed into *E. coli* BL21 (DE3) cells, and the transformation mixtures were spread into lysogeny broth (LB) plates containing 25 µg/mL kanamycin and chloromycetin. A single colony was transferred into 50 mL of LB containing 25 µg/mL kanamycin and chloromycetin, and the culture was allowed to shake overnight at 37 °C. The overnight culture (10 mL) was transferred into 4 × 1 L of LB containing 25 µg/mL kanamycin and chloromycetin. The resulting culture was grown at 37 °C with shaking at 150 rpm until an OD₆₀₀ of 0.6–0.8 was reached. Protein production was induced by making the cultures 1 mM in IPTG. The cells were allowed to shake for 3 h at 37 °C, and then the cells were harvested by centrifugation for 40 min at 6000 rpm and 4 °C. The pellets were resuspended in 25 mL of 50 mM Tris (pH 8.5) containing 500 mM NaCl. The cells were lysed by Ultrasonic. The insoluble components were removed by centrifugation for 30 min at 18000 rpm. The supernatant was dialyzed versus 50 mM Tris (pH 8.5) for 24 h. After centrifugation for an additional 30 min at 18000 rpm to remove the insoluble components, the cell lysate was loaded onto a Q-sepharose column that had been equilibrated with 50 mM Tris (pH 8.5). Proteins were eluted using a linear gradient from 50 mM Tris (pH 8.5) to 50 mM Tris (pH 8.5) containing 500 mM NaCl. The purified L1 protein was identified by SDS–PAGE from column fractions.

1-2. *E. coli* cells preparations for *in vivo* UV-Vis studies

The plasmids *pET26b* (+) transformed into *Escherichia coli* cells. The kanamycin and chloromycetin resistance genes was contained in the plasmids. *E. coli* cells were inoculated into 5 mL lysogeny broth, LB, media in the presence of 25 µg/mL kanamycin were grown with shaking (150 rpm) at 37 °C until cells reached OD₆₀₀ = 0.5-0.6. At that time, 100 µM IPTG was added for metallo-beta-lactamase induction and cells were grown for 2 hours at 37 °C with shaking (150 rpm). Cell cultures were centrifuged at 4000 rpm for 10 min at 4 °C, the supernatant was discarded and the cell pellets were washed thoroughly by re-suspending them in 1 mL buffer (50 mM sodium phosphate, pH 7.0), and were then pelleted again by centrifugation (4000 rpm, for 10 min at 4 °C). This process was repeated 3 times and finally cells were re-suspended in buffer to OD₆₀₀ = 0.15 for UV-Vis studies. Different concentrations of each inhibitor and 80 µM of cefazolin were added for estimation of the IC₅₀ values. Supernatants from the cell suspensions were collected by centrifugation (10000 rpm for 10 min at 4 °C) and then filtered through 0.22 µm filters.

1-3. Plating colony tests for L1 *E. coli* cells

We obtained 10⁵ fold dilutions from stock suspensions of L1 *E. coli* and *E. coli* cells (OD₆₀₀ =

0.15). Then, we inoculated an LB-agar (with and without kanamycin) plates with 10 μ L drops of each dilution for the average number of colonies in triplicate. The drops were allowed to dry on the plate and the plate was incubated at 37 °C overnight.

1-4. Measurements and analyses of IC₅₀

We calculated the percentage of the inhibition seen 110-330 minutes, when the substrate in the absence of inhibitor was almost completely hydrolyzed, after the initiation of the reaction using the equation below which was according to the method introduced by Dalvit et al^[3]. (Equation 1):

$$\% \text{ inhibition} = 100 \times [1 - ([S_T] - [S_I]) / ([S_T] - [S_O])] \text{ [Equation 1]}$$

Where $[S_T]$ is the initial concentration of the substrate and $[S_O]/[S_I]$ is the real-time concentration of the substrate in the absence or presence of inhibitor, respectively.

IC₅₀ can be obtained by fitting the data of % inhibition vs. inhibitor concentration to Equation 2:

$$\% \text{ inhibition} = 100 \times [1 - 1 / (1 + ([I]/IC_{50})^n)] \text{ [Equation 2]}$$

where $[I]$ is the concentration of the inhibitor and n is the cooperativity factor.

Supporting figures

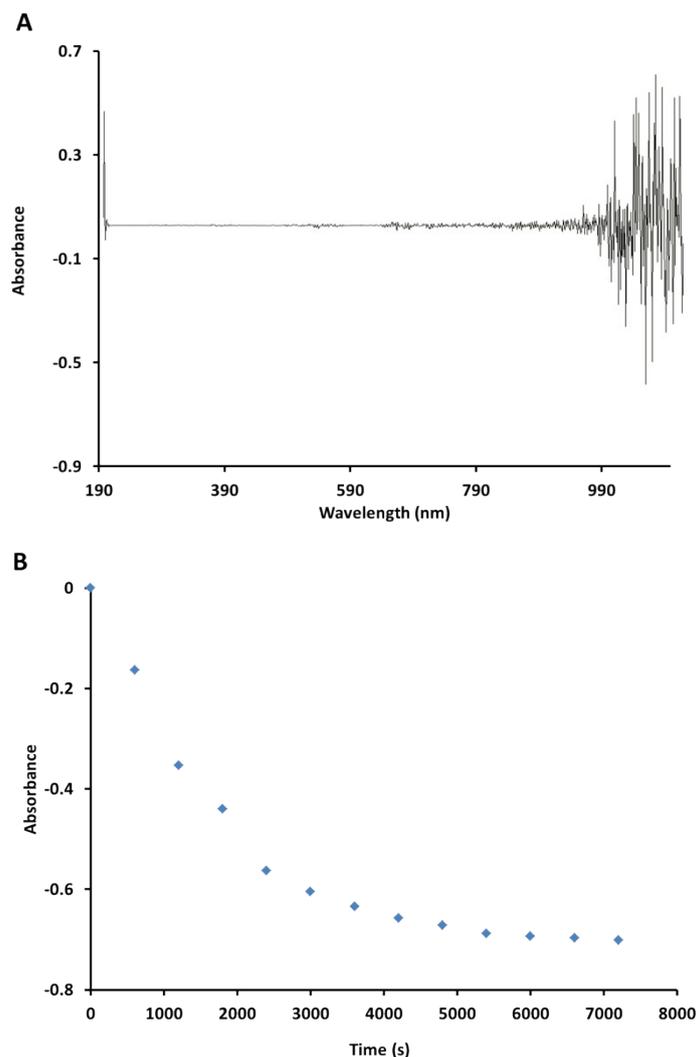


Fig. S1 UV-Vis spectrum of 80 μM cefazolin in the presence of L1 *E. coli* cell suspension ($\text{OD}_{600} = 0.15$) in the mode of kinetics. Setting the background signals from the cells and sample preparation as blank (A); the cefazolin was being hydrolyzed (B)

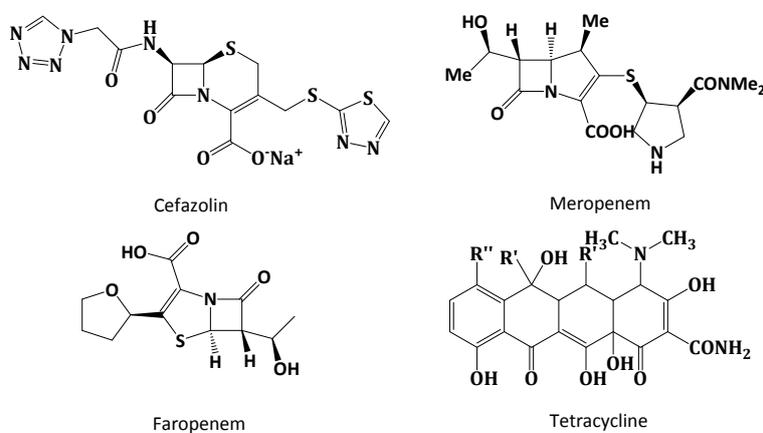


Fig. S2 Structures of antibiotics cefazolin, meropenem, faropenem and tetracycline

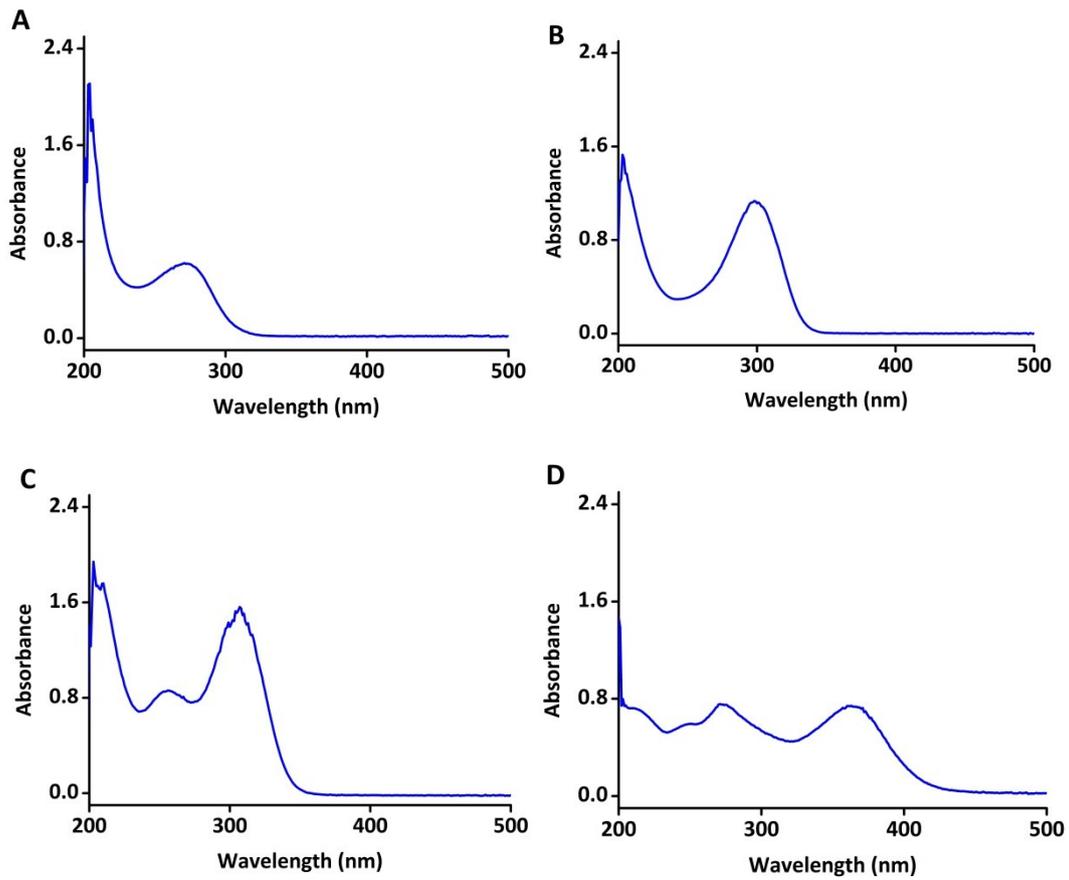


Fig. S3 UV-Vis spectra of cefazolin (A), meropenem (B), faropenem (C) and tetracycline (D)

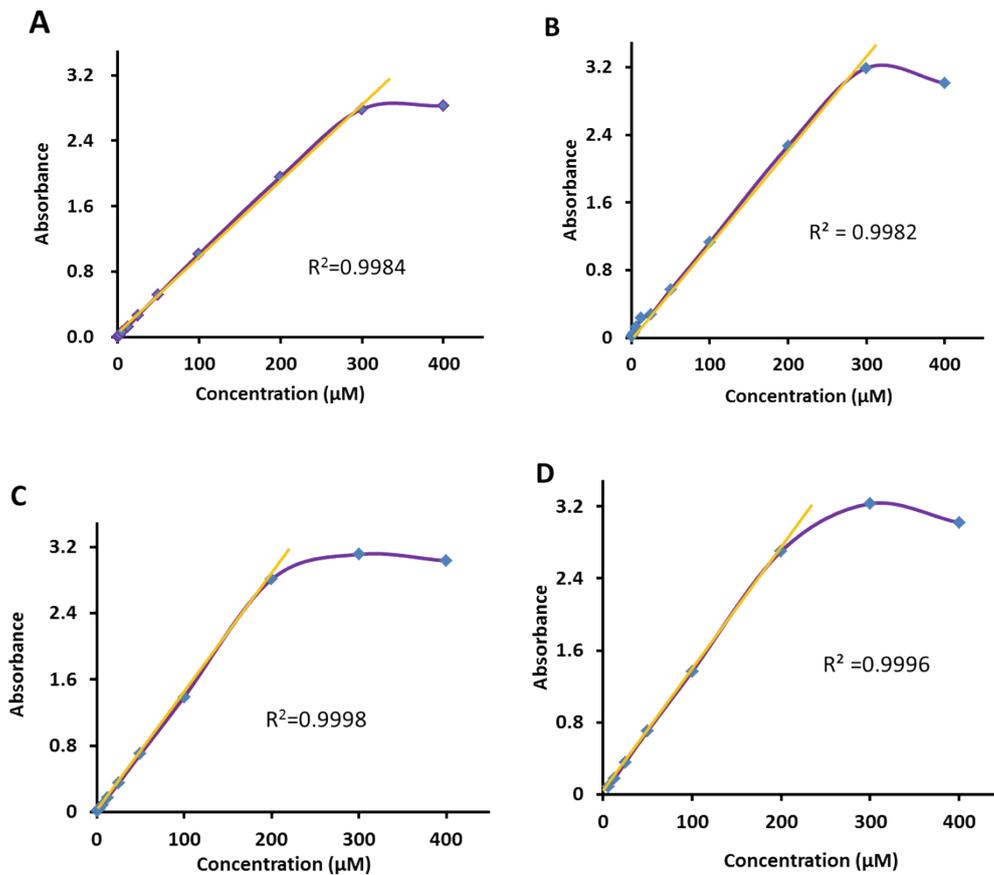


Fig. S4 The absorbance changes with concentrations of the antibiotics tested. The calibration curves showing the linearity relation for the antibiotics cefazolin (A), meropenem (B), faropenem (C), and tetracycline (D). All samples were prepared with 50 mM phosphate buffer, pH 7.0.

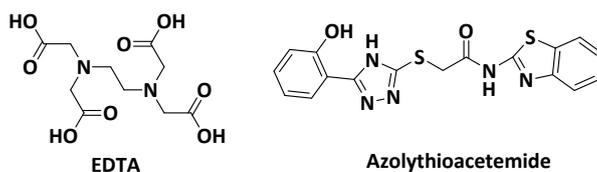


Fig. S5 Structures of EDTA and azolythioacetamide (ATAA)

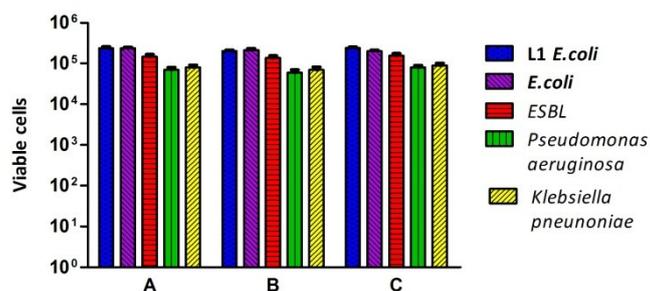


Fig. S6 Plating colony tests to examine the viability of all the cells before and after UV-Vis experiments and gained the average number of colonies in triplicate. All cells in these tests were from the same batch. The fold of dilution is labeled on each section of the LB (with and without kanamycin) plates. The cells without the treatment of cefazolin were plated on plate A. Cells with the addition of 80 μ M cefazolin before and after three-hour UV-Vis experiments were plated on plate B and C, respectively. The section labeled with 10^5 fold dilution is used for the study of cell viability. This data demonstrate that the cells were alive during the UV-Vis experiments and there is little difference of cell viability before and after the experiments. ($P > 0.2$. No significant difference).

References

- [1] Y. L. Zhang, K. W. Yang, Y. J. Zhou, E. L. Alexander, O. Peter and M. W. Crowder, *Chem. Med. Chem.*, 2014, **9**, 2445-2448.
- [2] M. W. Crowder, T. R. Walsh, L. Banovic, M. Pettit and J. Spencer, *Antimicrob Agents Chemother*, 1998, **42**, 921-926.
- [3] C. Dalvit, E. Ardini, G. P. Fogliatto, N. Mongelli and M. Veronesi, *Drug Discov. Today*, 2004, **9**, 595-602.