

## Supplemental material

TABLE S1: Summary of data collection and structure refinement for the dead-end complex of *E. coli* MurA with fosfomycin.\*

E. coli MurA-fosfomycin	
<b>Data collection</b>	
Space group	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
Unit cell dimensions (Å)	a=64.4 b=61.8 c=134.4 α=β=γ=90°
Resolution range (Å)	20-1.7 (1.8-1.7)
Unique reflections	59154 (8874)
Completeness (%)	99.0 (95.9)
I/σI	27.7 (15.2)
R <sub>merge</sub> <sup>b</sup> (%)	3.3 (9.2)
<b>Structure refinement</b>	
Protein atoms	3,151
Average B-factor (Å <sup>2</sup> )	15.7
Inhibitor atoms	8 at Cys115 (8.4)
Average B-factor (Å <sup>2</sup> )	10.1
UNAG atoms	39
Average B-factor (Å <sup>2</sup> )	10.1
Solvent molecules	444
average B-factor (Å <sup>2</sup> )	27.7
rmsd <sup>a</sup> bonds (Å)	0.011
rmsd angles (°)	1.5
R <sub>cryst</sub> <sup>c</sup> (%)	15.9
R <sub>free</sub> <sup>d</sup> (%)	18.7
Cross-validated estimated coordinate error:	
From Luzzati plot (Å)	0.18
From SigmaA (Å)	0.08

\* Values in parentheses refer to the highest resolution shell.

<sup>a</sup>  $R_{\text{merge}} = 100 \times \sum_h \sum_i |I_{hi} - \bar{I}_h| / \sum_h I_{hi}$  where h are unique reflection indices.

<sup>b</sup> r.m.s.d. = root mean square deviation from ideal values.

<sup>c</sup>  $R_{\text{cryst}} = 100 \times \sum |F_{\text{obs}} - F_{\text{model}}| / \sum F_{\text{obs}}$  where  $F_{\text{obs}}$  and  $F_{\text{model}}$  are observed and calculated structure factor amplitudes, respectively.

<sup>d</sup>  $R_{\text{free}}$  is  $R_{\text{cryst}}$  calculated for 1185 randomly chosen unique reflections, which were excluded from the refinement.

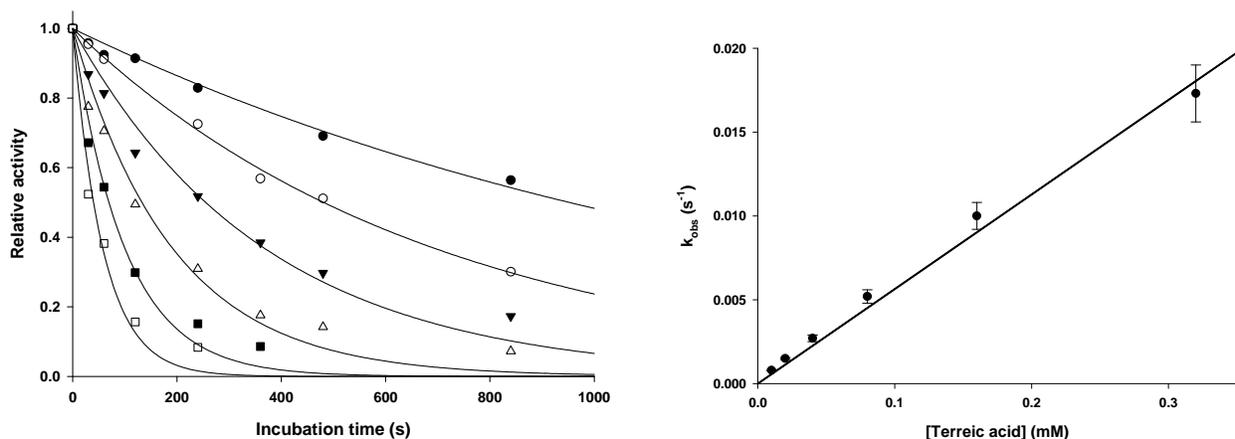


FIGURE S1. Inactivation of *E. cloacae* MurA by terreic acid in the presence of 0.005 mM UNAG. The terreic acid concentration was 0.01 mM (●), 0.02 mM (○), 0.04 mM (▲), 0.08 mM, (△), 0.16 mM (■), and 0.32 mM (□). Data were fit to equation 1. Right: replot of the observed first-order rate constant of inactivation vs terreic acid. Data were fit to equation 2 yielding  $k_{\text{inact}} = 56.4 \pm 1.9 \text{ M}^{-1} \text{ s}^{-1}$ .

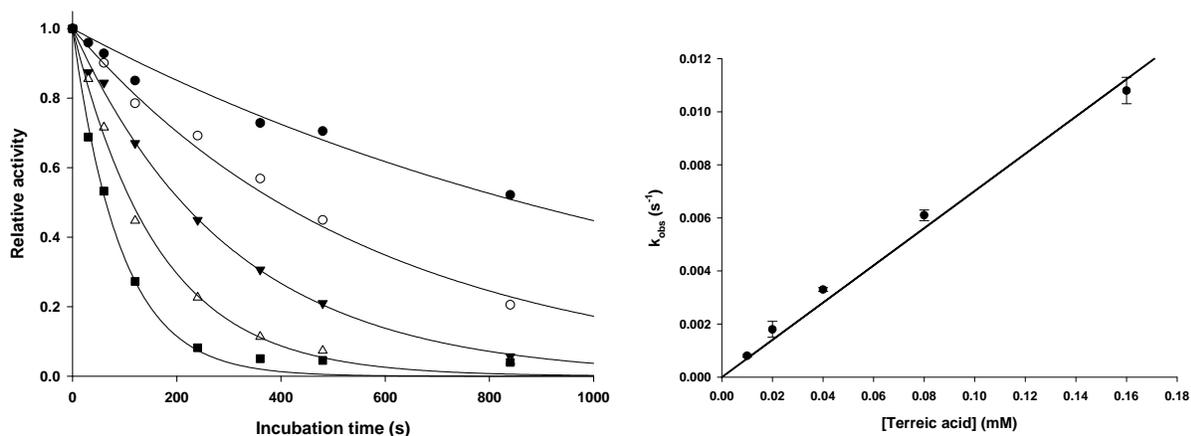


FIGURE S2. Inactivation of *E. cloacae* MurA by terreic acid in the presence of 0.010 mM UNAG. The terreic acid concentration was 0.01 mM (●), 0.02 mM (○), 0.04 mM (▲), 0.08 mM, (△), and 0.16 mM (■). Data were fit to equation 1. Right: replot of the observed first-order rate constant of inactivation vs terreic acid. Data were fit to equation 2 yielding  $k_{\text{inact}} = 70.1 \pm 2.5 \text{ M}^{-1} \text{ s}^{-1}$ .

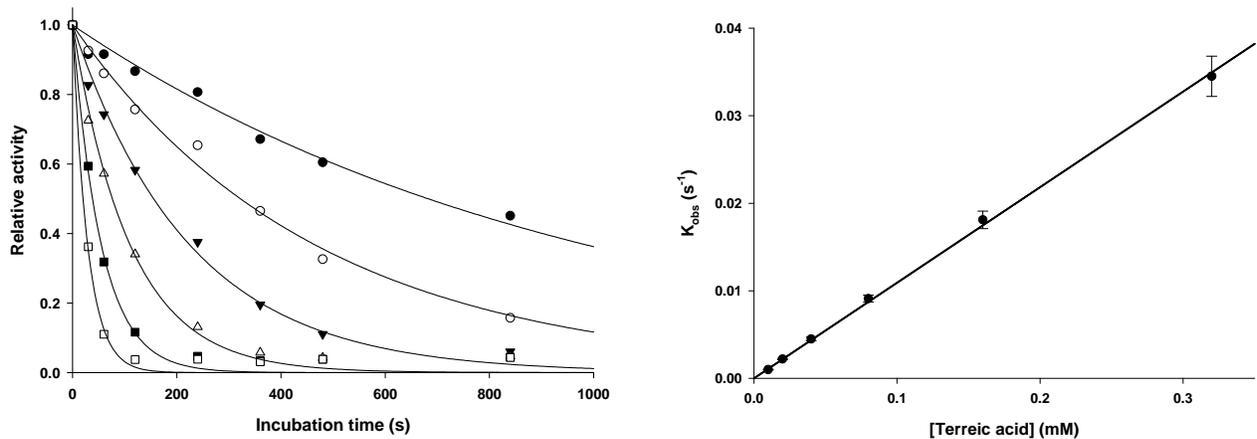


FIGURE S3. Inactivation of *E. cloacae* MurA by terreic acid in the presence of 0.025 mM UNAG. The terreic acid concentration was 0.01 mM (●), 0.02 mM (○), 0.04 mM (▲), 0.08 mM (△), 0.16 mM (■), and 0.32 mM (□). Data were fit to equation 1. Right: replot of the observed first-order rate constant of inactivation vs terreic acid. Data were fit to equation 2 yielding  $k_{inact} = 109.1 \pm 1.0 M^{-1} s^{-1}$ .

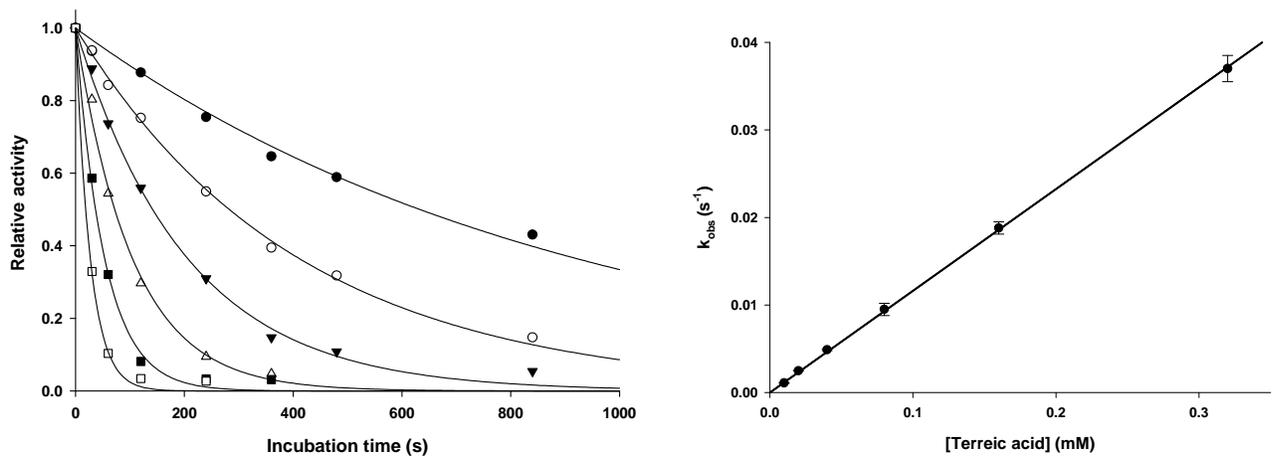


FIGURE S4. Inactivation of *E. cloacae* MurA by terreic acid in the presence of 0.05 mM UNAG. The terreic acid concentration was 0.01 mM (●), 0.02 mM (○), 0.04 mM (▲), 0.08 mM (△), 0.16 mM (■), and 0.32 mM (□). Data were fit to equation 1. Inset: replot of the observed first-order rate constant of inactivation vs terreic acid. Data were fit to equation 2 yielding  $k_{inact} = 116.2 \pm 0.6 M^{-1} s^{-1}$ .

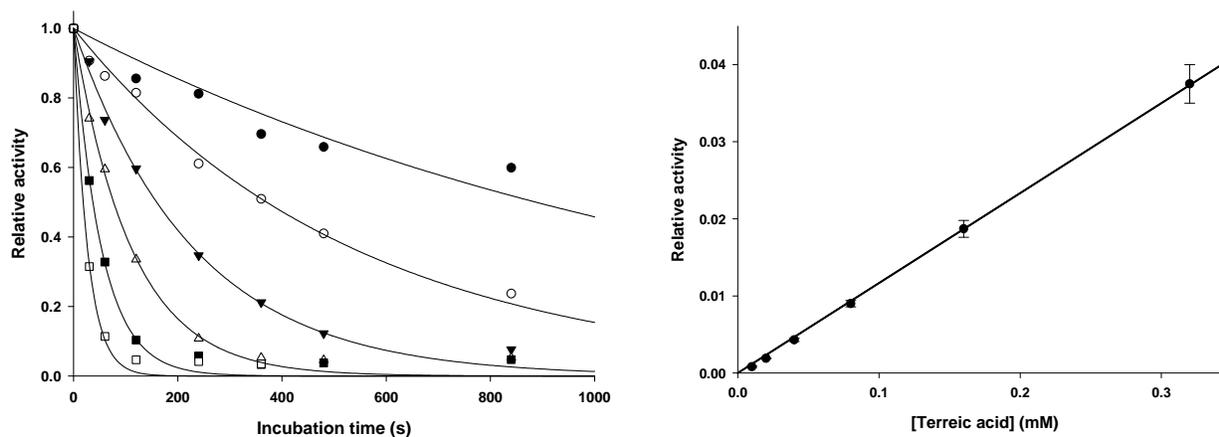
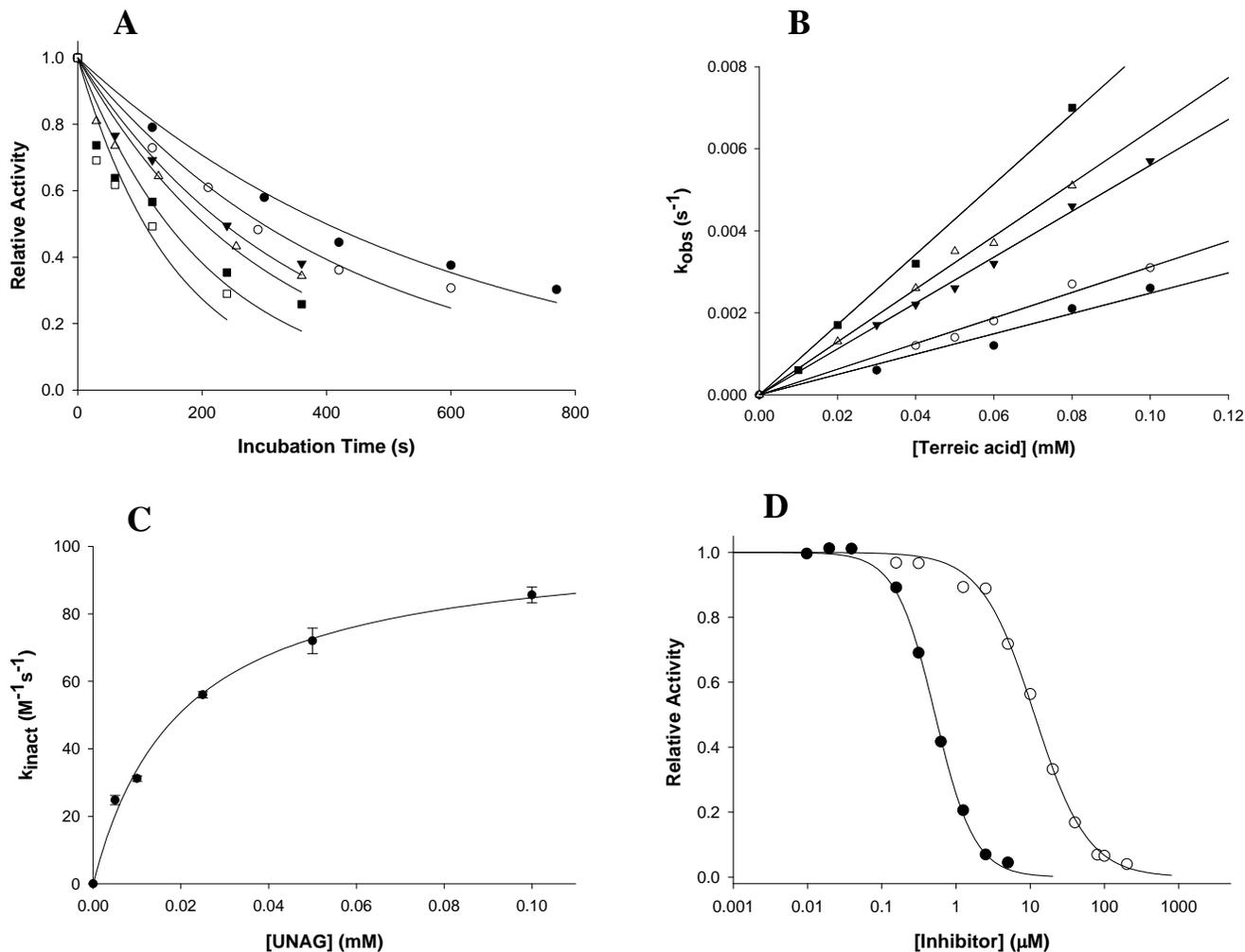


FIGURE S5. Inactivation of *E. cloacae* MurA by terreic acid in the presence of 0.1 mM UNAG. The terreic acid concentration was 0.01 mM (●), 0.02 mM (○), 0.04 mM (▲), 0.08 mM, (Δ), 0.16 mM (■), and 0.32 mM (□). Data were fit to equation 1. Inset: replot of the observed first-order rate constant of inactivation vs terreic acid. Data were fit to equation 2 yielding  $k_{\text{inact}} = 116.7 \pm 0.9 \text{ M}^{-1} \text{ s}^{-1}$ .



**FIGURE S6: Inactivation of *E. coli* MurA by terreic acid using the MurA-MurB coupled assay.**

(A) Time-dependent loss of MurA activity in the presence of 0.025 mM UNAG. The terreic acid concentration was 0.03 mM (●), 0.04 mM (○), 0.05 mM (▼), 0.06 mM (△), 0.08 mM (■), 0.10 mM (□). Data were fit to equation 1.

(B) Replot of the observed first-order rate constants of inactivation ( $k_{obs}$ ) vs. terreic acid at varying UNAG concentrations [0.005 mM (●), 0.01 mM (○), 0.025 mM (▼), 0.05 mM (△), 0.10 mM (■)]. Data were fit to equation 2.

(C) Replot of the second-order inactivation rate constants ( $k_{inact}$ ) vs. UNAG concentration. Data were fit to equation 3, yielding  $k_{inact}^* = 102 \pm 1.8 \text{ M}^{-1}\text{s}^{-1}$  and  $K_{d(\text{UNAG})} = 0.02 \pm 0.001 \text{ mM}$ .

(D) Dose-response curves for inhibition of MurA by fosfomycin (●) and terreic acid (○). The enzyme was preincubated with inhibitor and 1mM UNAG for five minutes before the reaction was started by addition of 1mM PEP. Data were fit to equation 4, yielding  $\text{IC}_{50}$  values of  $0.52 \pm 0.01 \text{ }\mu\text{M}$  for fosfomycin and  $11.4 \pm 0.5 \text{ }\mu\text{M}$  for terreic acid.

The assay mixture (1 mL) contained 50 mM HEPES (pH 7.5), 50 mM KCl, 0.59  $\mu\text{M}$  MurB, 0.25 mM NADPH, 0.32  $\mu\text{M}$  MurA, 1 mM PEP and 1 mM UNAG. MurA was pre-incubated with UNAG and terreic acid (or fosfomycin) in 975  $\mu\text{l}$  of 50 mM HEPES buffer (pH 7.5); at various time intervals, the reaction was initiated by addition of 1 mM PEP, 1 mM UNAG and MurB. The decrease in NADPH absorbance was recorded at 340 nm. *E. coli* MurB was cloned and purified as described (Schonbrunn et al. (2000) Biochemistry 39, 2164-2173).