Supporting Information for

The quorum-quenching metallo-γ-lactonase from *Bacillus thuringiensis* exhibits a leaving group thio effect[†]

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In order to rule out the possibility that the thio effect was due to product inhibition rather than due to a transient interaction that occurs during turnover, we synthesized the ring-opened products *N*-hexanoyl-homoserine (C6-Hse) and *N*-hexanoyl-homocysteine (C6-Hcy), determined their inhibition constants, and calculated the effect of product inhibition on initial hydrolysis rates. As described below, product inhibition is not responsible for the observed thio effect on k_{cat} values because its impact on the observed initial hydrolysis rates was minimal.

Synthesis of ring-opened products. To prepare *N*-hexanoyl-(*S*)-homoserine (C6-Hse), the starting lactone C6-HSL (118 μmol in 255 μL methanol) was treated with one equivalent of NaOH (in 235 μL water) and mixed briefly at room temperature. The pH was adjusted to 7.5 with measured additions of HCl (0.1 M) and NaOH (0.1 M) before use in kinetic experiments. Ring opening was confirmed by comparison to standards using TLC on silica (isopropanol: C6-HSL $R_f = 0.65$; C6-Hse $R_f = 0.50$) and by ^{13}C NMR (75 MHz, methanol-d_4) 14.31, 23.46, 26.68, 32.58, 37.03, 37.23, 53.73, 60.25, 175.74, 178.98. *N*-hexanoyl-(*R,S*)-homocysteine (C6-Hcy) was prepared in a procedure similar to that given by Duerre et al (*1*). Briefly, the starting

thiolactone C6-HCTL (50 μmol in 100 μL methanol) was treated with 7.5 equivalents of NaOH (in 75 μL water) and incubated for 5 min at 37° C. The reaction was stopped by addition of 6 equivalents of HCl (in 100 μL water) and the final pH was adjusted to 7.6 using NaOH (0.1 M) and HCl (0.1 M) and used immediately to avoid disulfide formation. Ring opening was confirmed by comparison to standards using TLC on silica (isopropanol: C6-HCTL $R_f = 0.83$; C6-Hcy $R_f = 0.57$) by ^{13}C NMR (75 MHz, 50% D_2O / 50% methanol-d_4) 14.21, 21.52, 22.99, 26.31, 31.94, 36.89, 37.57, 54.40, 176.65, 178.42; and also by thiol titration using 5,5'-dithiobis-(2-nitrobenzoic acid) (data not shown).

Determination of product inhibition constants. Using the phenol red based spectrophotometric assay described previously (*2*), initial rates for hydrolysis of C6-HSL (240 μM) by dicadmium AHL lactonase (18 nM) was determined at pH 7.4, 28° C, in the presence of increasing concentrations of either product, C6-Hse or C6-Hcy, and plotted below as a percent of the activity measured in absence of either product (Figure S1).

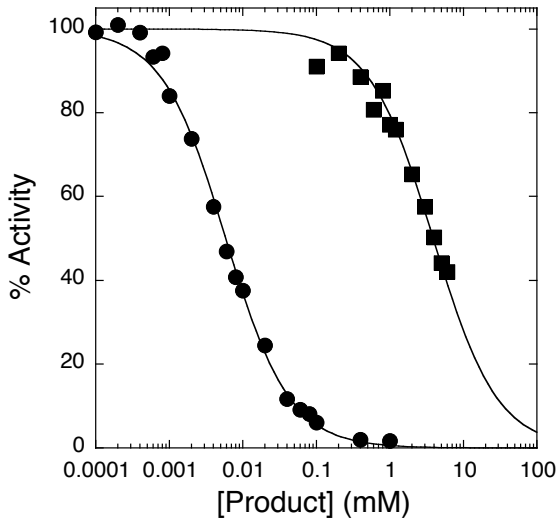

Figure S1. Product inhibition. IC_{50} values were determined for inhibition of C6-HSL hydrolysis by dicadmium AHL lactonase in the presence of either C6-Hcy (●) or C6-Hse (■).

Inhibition curves were fit using equation S1 and a hill coefficient (h) equal to one in order to determine the IC_{50} value for each product (*3*).

$$\%\,Activity = 100 - \left(\frac{100}{1 + (IC_{50} \div [I])^{h}} \right) \qquad \text{(S1)}$$

The IC_{50} values for inhibition by C6-Hcy and C6-Hse were determined to be 5.9 ± 0.2 μM and 3.9 ± 0.2 mM, respectively. Assuming competitive inhibition, the equation S2 was used to calculate K_i values for C6-Hcy and C6-Hse as 3.0 ± 0.1 μM and 2.0 ± 0.1 mM, respectively (*4*).

$$IC_{50} = K_i(1 + \frac{[S]}{K_M}) \qquad \text{(S2)}$$

Comparison of product inhibition with the thio effect. The thio effect is most significant with k_{cat} values measured using the thiophilic dicadmium AHL lactonase [$k_{cat}(O)/k_{cat}(S) = 100$] (Table 2). In these kinetic experiments, the highest concentrations of substrate used for C6-HSL (2 mM) and C6-HCTL (10 mM) gave linear absorbance decreases spanning at least the first 0.3 min, from which initial hydrolysis rates were calculated. During this initial 0.3 min, substrate hydrolysis produced 53 μM of C6-Hse and 16 μM of C6-Hcy products, respectively. Assuming that the back reaction (ring closure) is minimal, and substituting K_M for K_S and K_i for K_P, the contribution of product inhibition to these initial rates can be calculated using equation S3 (*5*).

$$\frac{v}{V_{\max}} = \frac{[S]}{K_S(1+\frac{[P]}{K_P})+[S]} \qquad \text{(S3)}$$

For both substrates, C6-HSL and C6-HCTL, product inhibition under these conditions was calculated to be less than 7% of the observed rate. This effect is less than the experimental error and is much less than the magnitude of the thio effect (100-fold). Therefore, product inhibition is not the cause of the observed thio effect on k_{cat} values.

(1) Duerre, J. A., and Miller, C. H. (1966) Preparation of L-homocysteine from L-homocysteine thiolactone. *Anal. Biochem. 17*, 310-315.

(2) Thomas, P. W., Stone, E. M., Costello, A. L., Tierney, D. L., and Fast, W. (2005) The Quorum-quenching lactonase from *Bacillus thuringiensis* is a metalloprotein. *Biochemistry 44*, 7559-7569.

(3) Copeland, R. A. (2005) *Evaluation of enzyme inhibitors in drug discovery : a guide for medicinal chemists and pharmacologists*, Wiley-Interscience, Hoboken, N.J.

(4) Cheng, Y., and Prusoff, W. H. (1973) Relationship between the inhibition constant (K_I) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol. 22*, 3099-108.

(5) Segel, I. H. (1975) *Enzyme kinetics : behavior and analysis of rapid equilibrium and steady state enzyme systems*, Wiley, New York.