Supporting Information for:

Characterization of the Genomically Encoded Fosfomycin Resistance Enzyme from *Mycobacterium abscessus*

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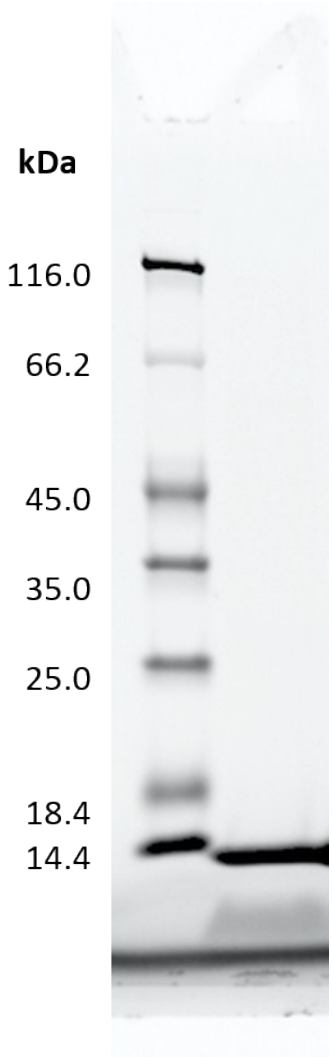

Figure S1. Purified FosM in the flow-through of the final hydroxyapatite column.

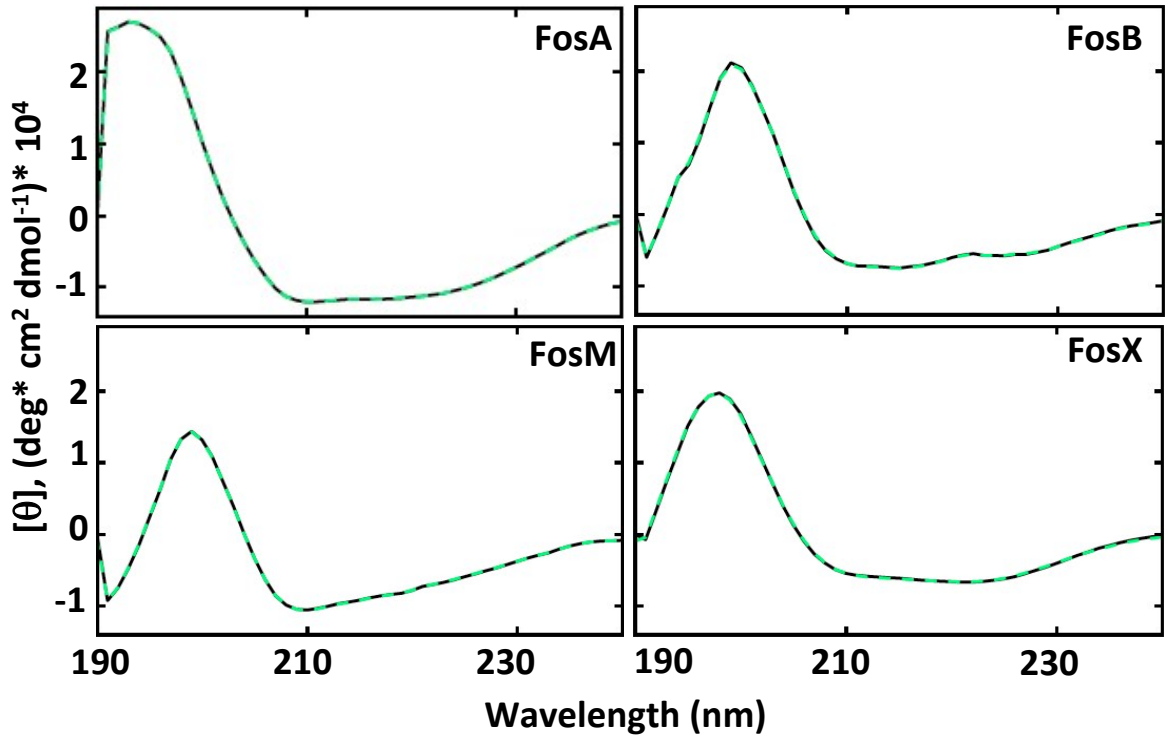

Figure S2. Experimentally determined circular dichroism (CD) spectra (black), and the calculated spectra generated by the estimated secondary structure composition (green). The estimated secondary structure compositions, and the respective spectra, were generated using the CDSSTR algorithm[1] through the online DichroWeb server.[2] The NRMSD values are 0.002, 0.019, 0.023, and 0.012 for the fits of FosA, FosB, FosM, and FosX, respectively.

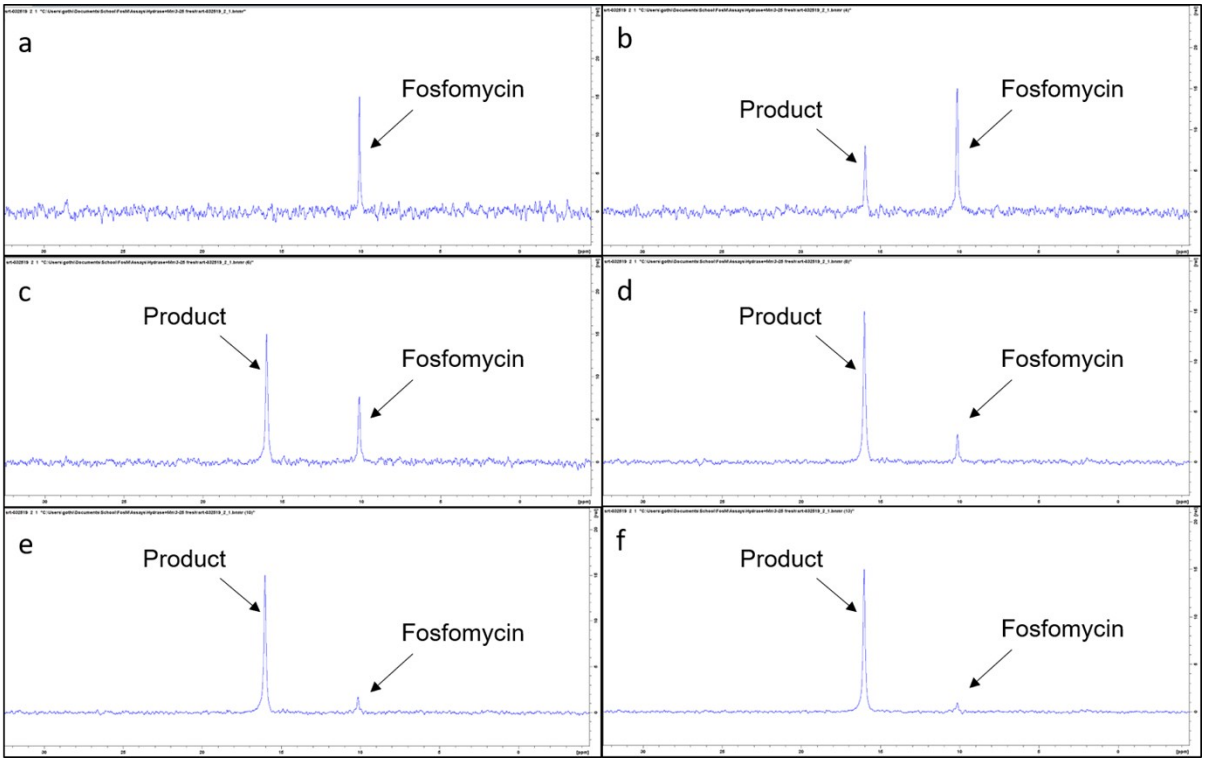

Figure S3. ^{31}P NMR data of a FosM kinetic assay. Sample was prepared with 8 mM fosfomycin, 20 mM HEPES (pH 7.5), and 0.6 µM FosM that had previously been loaded with Mn^{2+}. The reaction was allowed to continue at 25°C for (a) 1 minute, (b) 5 minutes, (c) 10 minutes, (d) 20 minutes, (e) 30 minutes, or (f) 60 minutes before collecting the spectrum using Bruker TopSpin software. The fosfomycin substrate peak is located near 10 ppm, while the thiol-fosfomycin product peak is near 16 ppm.

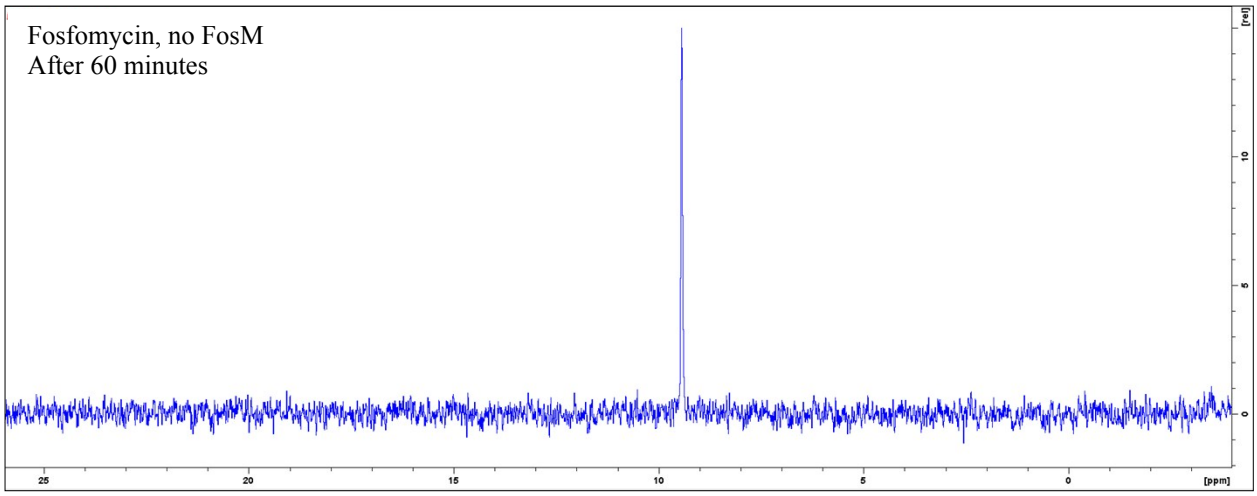

Figure S4. ^{31}P NMR spectrum of a control sample. Sample was prepared with 8 mM fosfomycin and 20 mM HEPES (pH 7.5) without enzyme. The sample was quenched with 6M urea after 60 minutes. The lack of a product peak shows that fosfomycin is stable in solution without the enzyme.

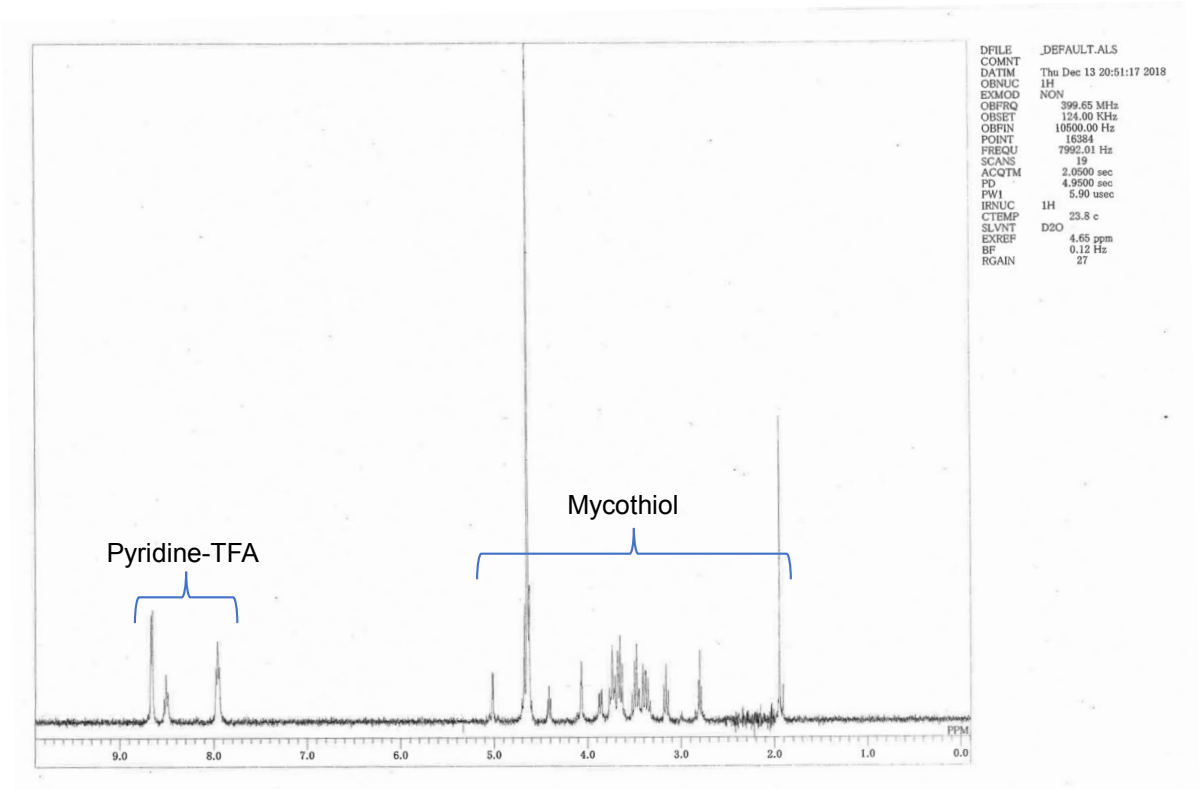

Figure S5. NMR spectrum of the MSH sample used in our kinetic experiments.

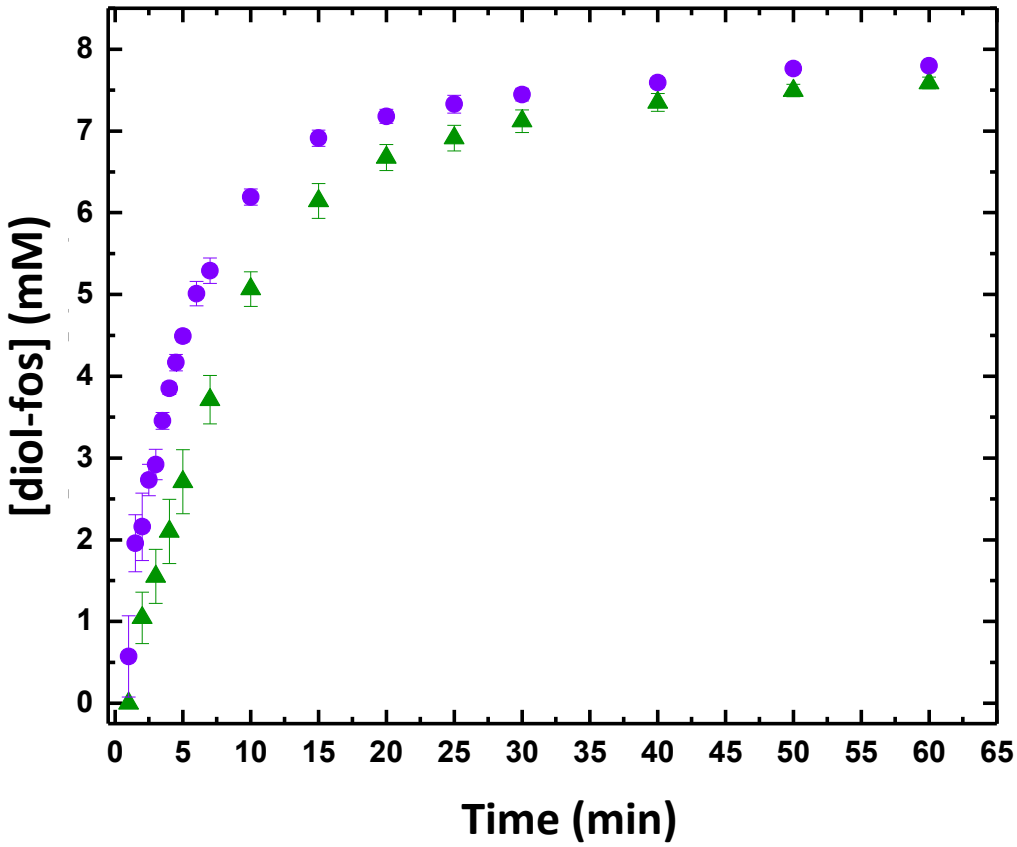

Figure S6. Time-trace kinetics for the FosM-catalyzed addition of water to fosfomycin in the presence (●) and absence (▲) of MSH. Neither the MSH not the impurities from the synthesis inhibited the hydrase reaction. Reactions were carried out at 25˚ C in 20 mM HEPES, pH 7.5 with 8 mM fosfomycin, 4 mM MSH (if applicable), and 0.6 μM enzyme.

References

1. N. Sreerama and R. W. Woody, *Anal. Biochem.*, 2000, **287**, 252-260.
2. L. Whitmore and B. A. Wallace, *Biopolymers*, 2008, **89**, 392-400.