nature portfolio

https://doi.org/10.1038/s41596-022-00694-7

Supplementary information

A puromycin-dependent activity-based sensing probe for histochemical staining of hydrogen peroxide in cells and animal tissues

In the format provided by the authors and unedited

Supplementary Information

A Puromycin-Dependent Activity-Based Sensing Probe for Histochemical Staining of Hydrogen Peroxide in Cells and Animal Tissues

Kaede Hoshi,[1,†] Marco S. Messina,[1,†] Jun Ohata,[1] Clive Yik-Sham Chung,[1,4,5] Christopher J. Chang[1,2,3]*

[1]Department of Chemistry, [2]Department of Molecular and Cell Biology, [3]Helen Wills Neuroscience Institute, University of California, Berkeley, United States

[4]School of Biomedical Sciences and [5]Department of Pathology, The University of Hong Kong, Hong Kong, P.R. China

[†]Denotes equal contribution.

Data Analysis

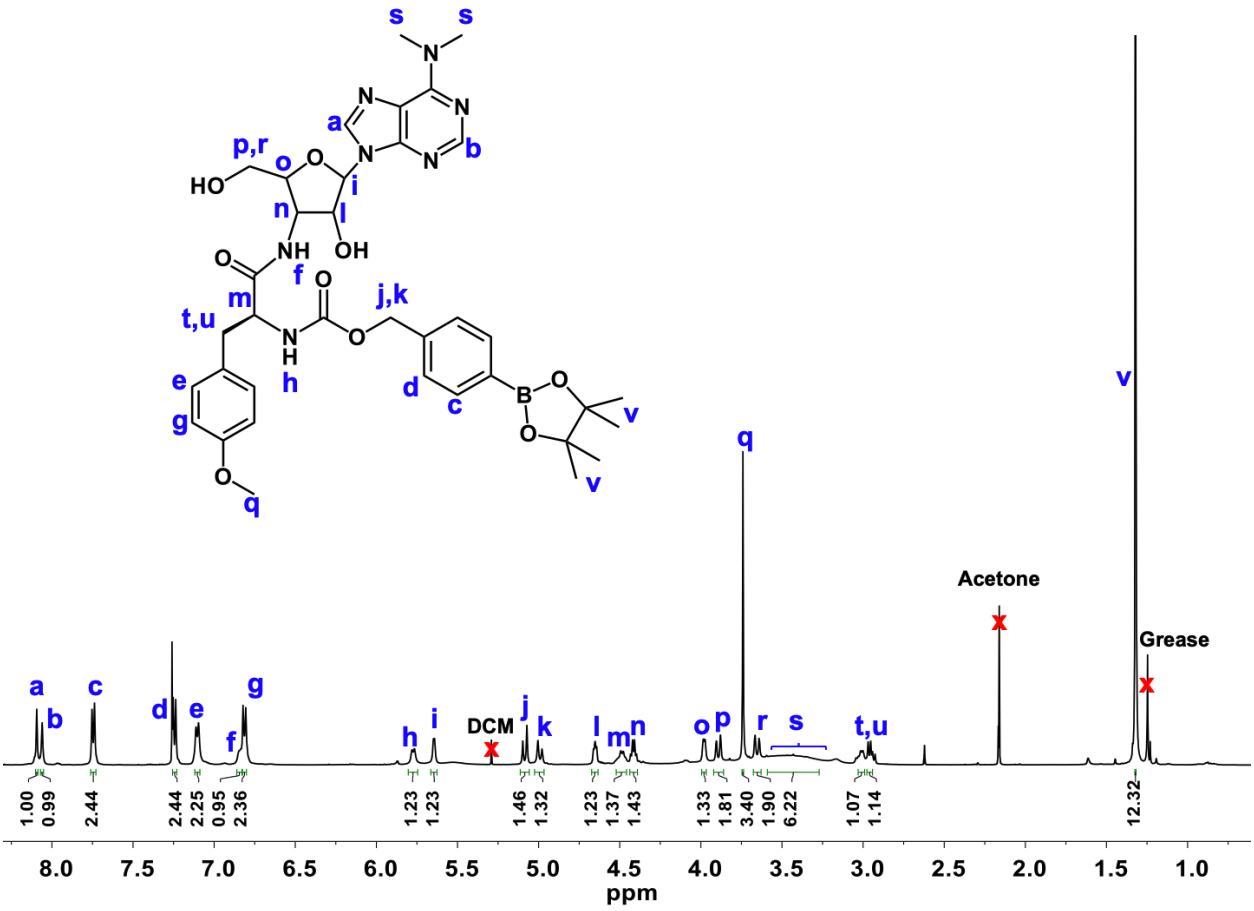
Quantification of the fluorescence intensity of confocal images.

Fluorescence intensity for endogenous H_2O_2 visualization experiments (Figure 4) was determined using Harmony High-Content Imaging and Analysis Software. Lower threshold values were set as background and kept constant throughout all measurements and the average fluorescence intensity of all cells in the whole image was measured. Mean fluorescence intensity from each technical replicate was measured from 2 fields (10X air objective, 0.3 NA) of view and three technical replicates were measured for each experiment. In order to remove a bias of the observed fluorescence signal by the difference of puromycin incorporation rate (i.e. protein synthesis rate) in samples, it is highly recommended to conduct additional control experiments with free puromycin (see *Anticipated results* section and steps 18–31) and normalize the signal for Peroxymycin-1-treated samples with the signal for puromycin-treated samples (i.e. divide the mean fluorescence intensity of Peroxymycin-1 treated samples by the mean fluorescence intensity of puromycin treated samples). Data used in Figure 4 was analyzed with GraphPad Prism 9.2 and expressed as mean ± SD (n = 3 technical replicates). Normalization for figure 4 was carried out through the Analyze->Data Processing->Normalize function and values for each Peroxymycin-1 treated replicate were normalized to the mean fluorescence intensity of Puromycin treated controls.

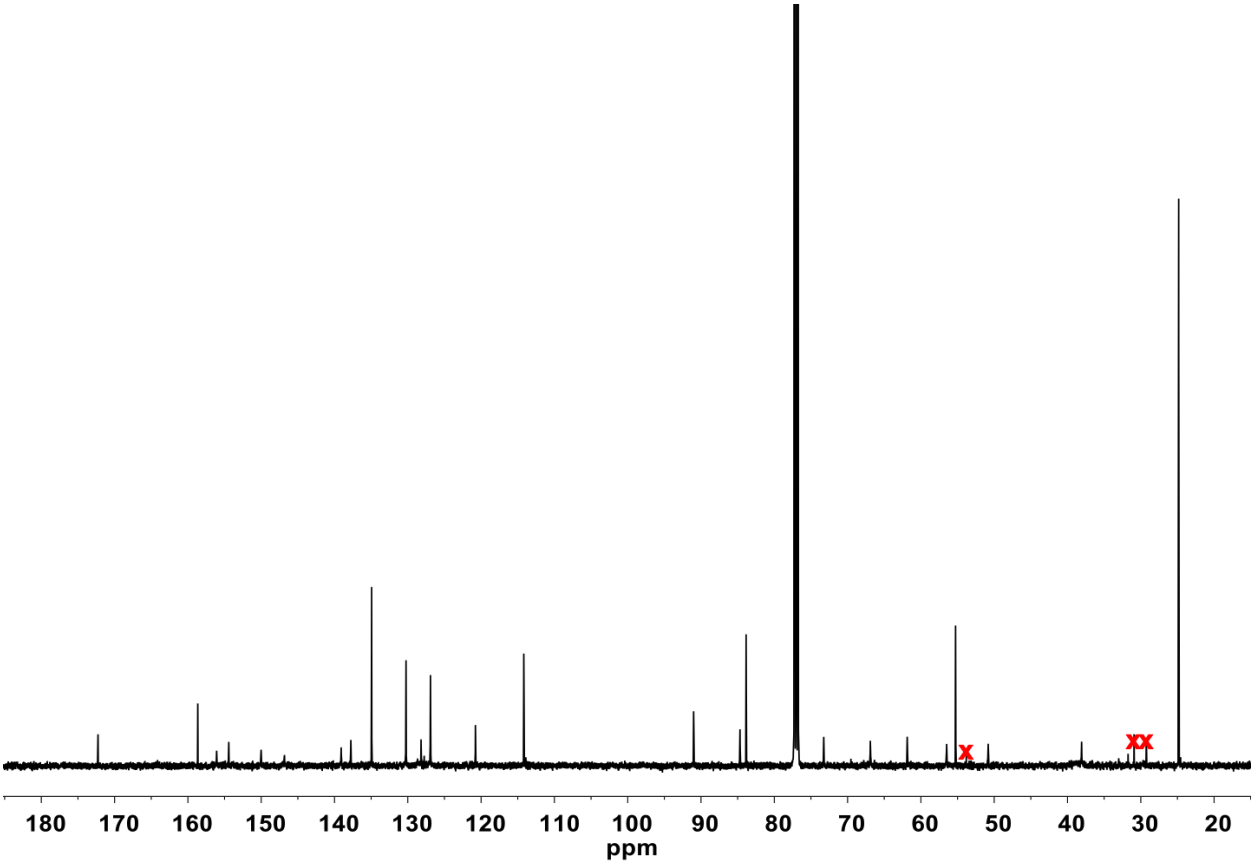
For liver tissues, a threshold value was set as background and the average fluorescence intensity of the whole image, F_{image}, was measured by ImageJ. The average fluorescence intensity of each sample group, F_{group}, was then calculated from F_{image} of five different images from triplicate experiments. F_{group} and SD were then used for graph plotting.

NMR Spectra

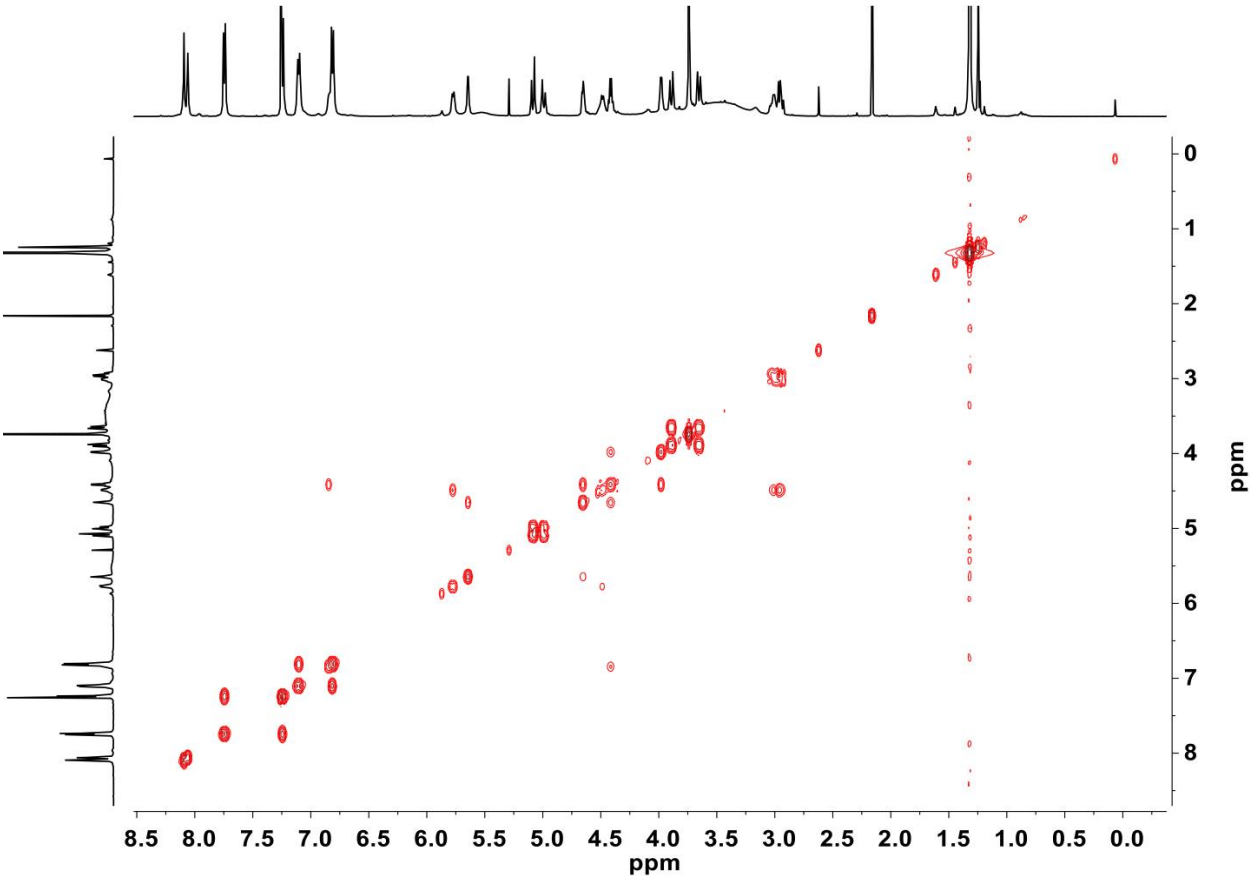
Peroxymycin-1



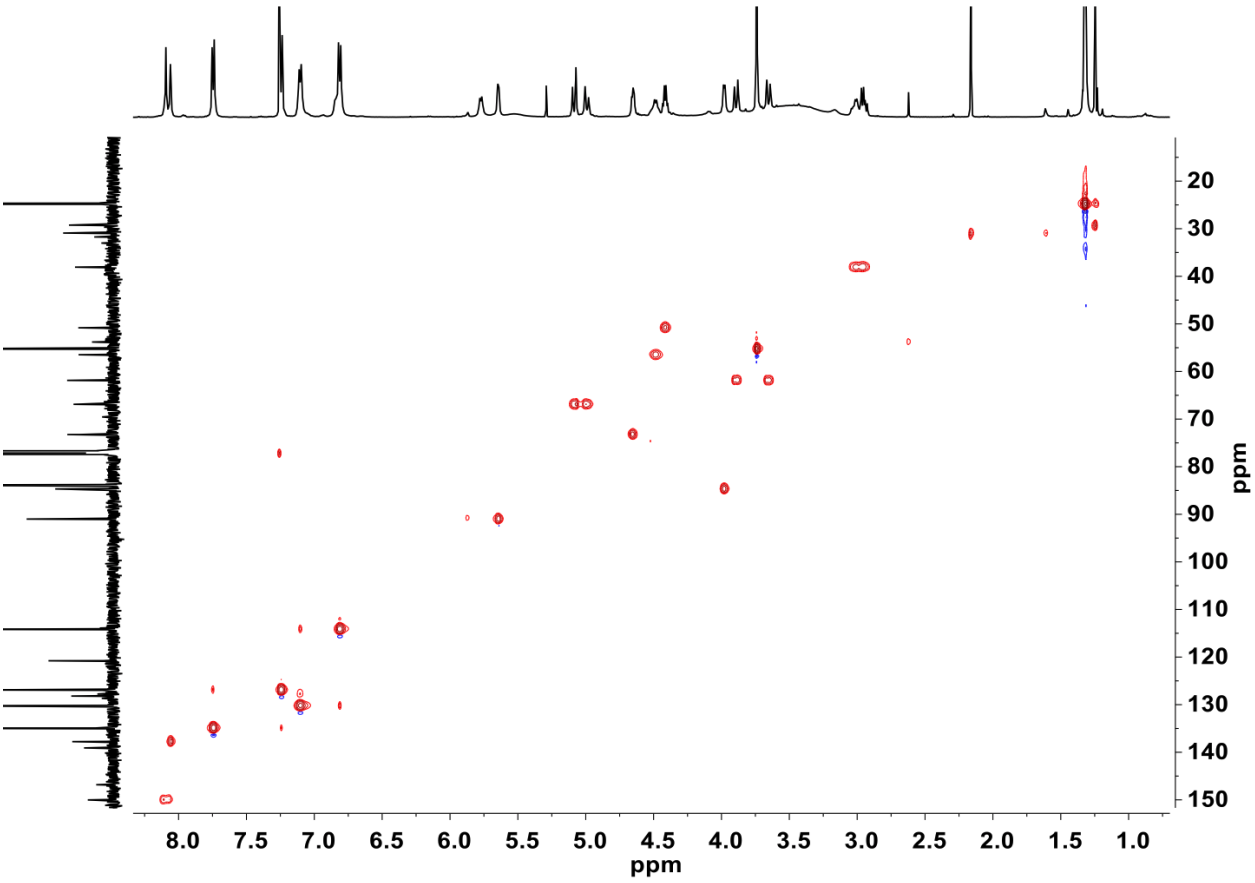
Supplementary Figure 1 | Annotated ^{1}H NMR spectrum of Peroxymycin-1 in $CDCl_3$.



Supplementary Figure 2 | ^{13}C NMR spectrum of Peroxymycin-1 in $CDCl_3$. “x” denotes silicon grease and solvent peaks (grease, acetone, CH_2Cl_2 from right to left).

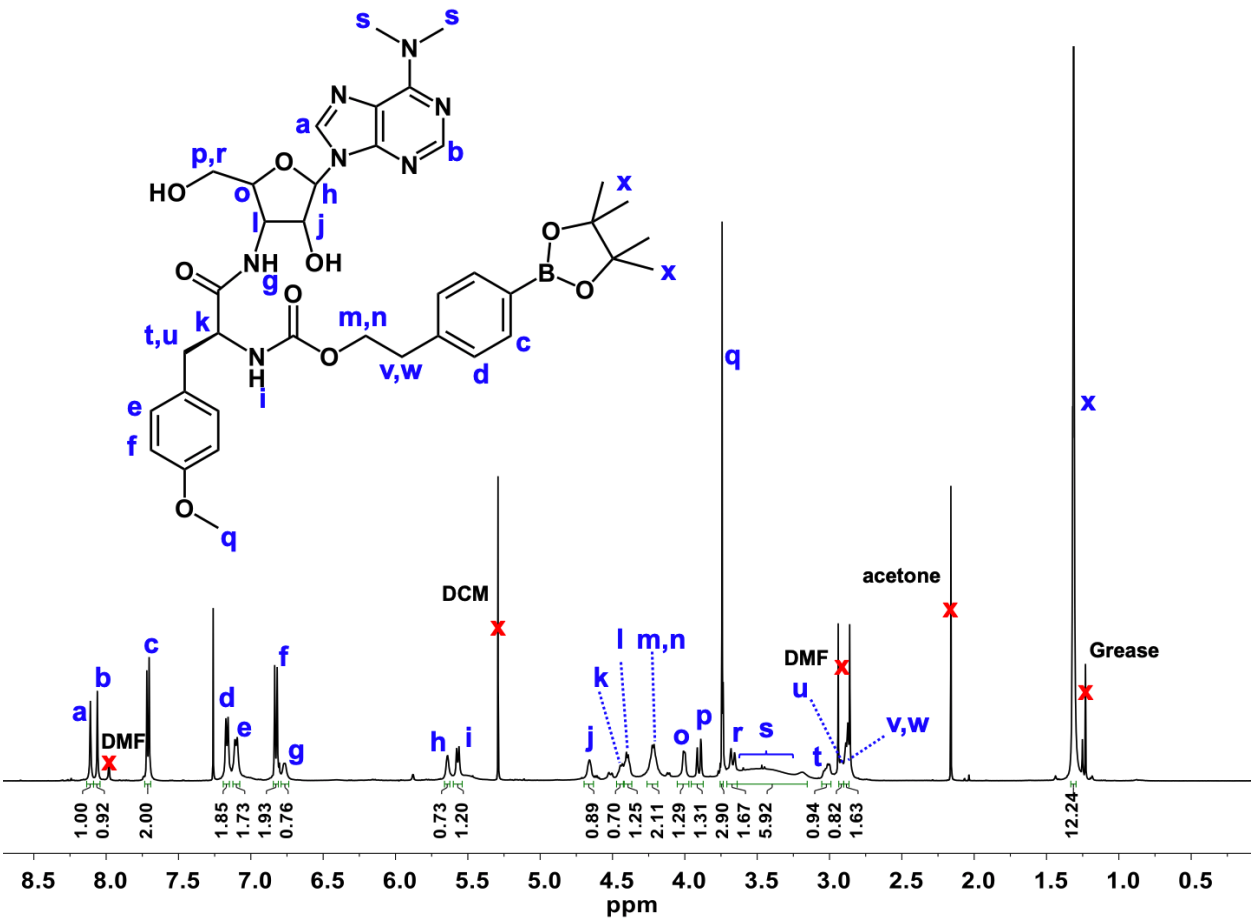


Supplementary Figure 3 | COSY NMR spectrum of Peroxymycin-1 in $CDCl_3$.

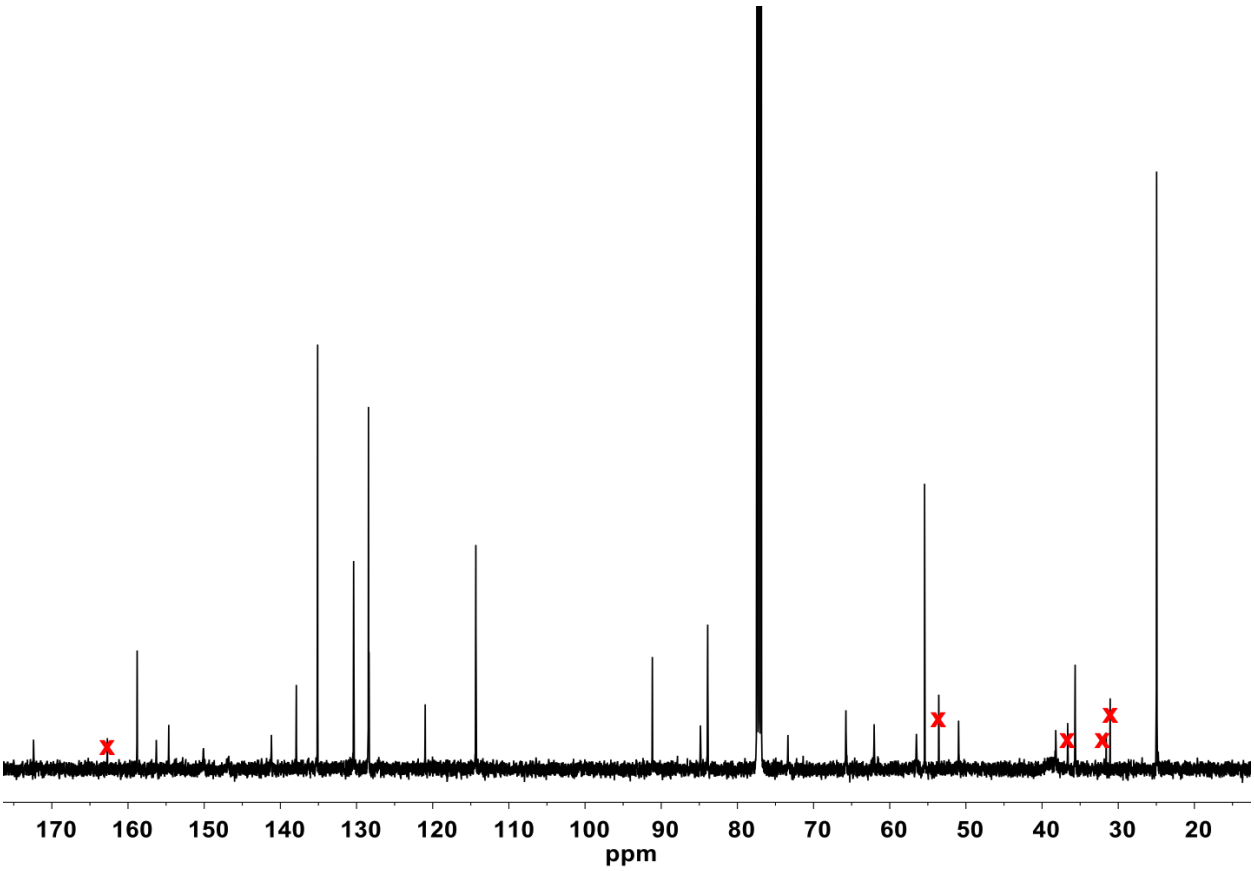


Supplementary Figure 4 | $^{1}H–^{13}C$ HSQC NMR spectrum of Peroxymycin-1 in $CDCl_3$.

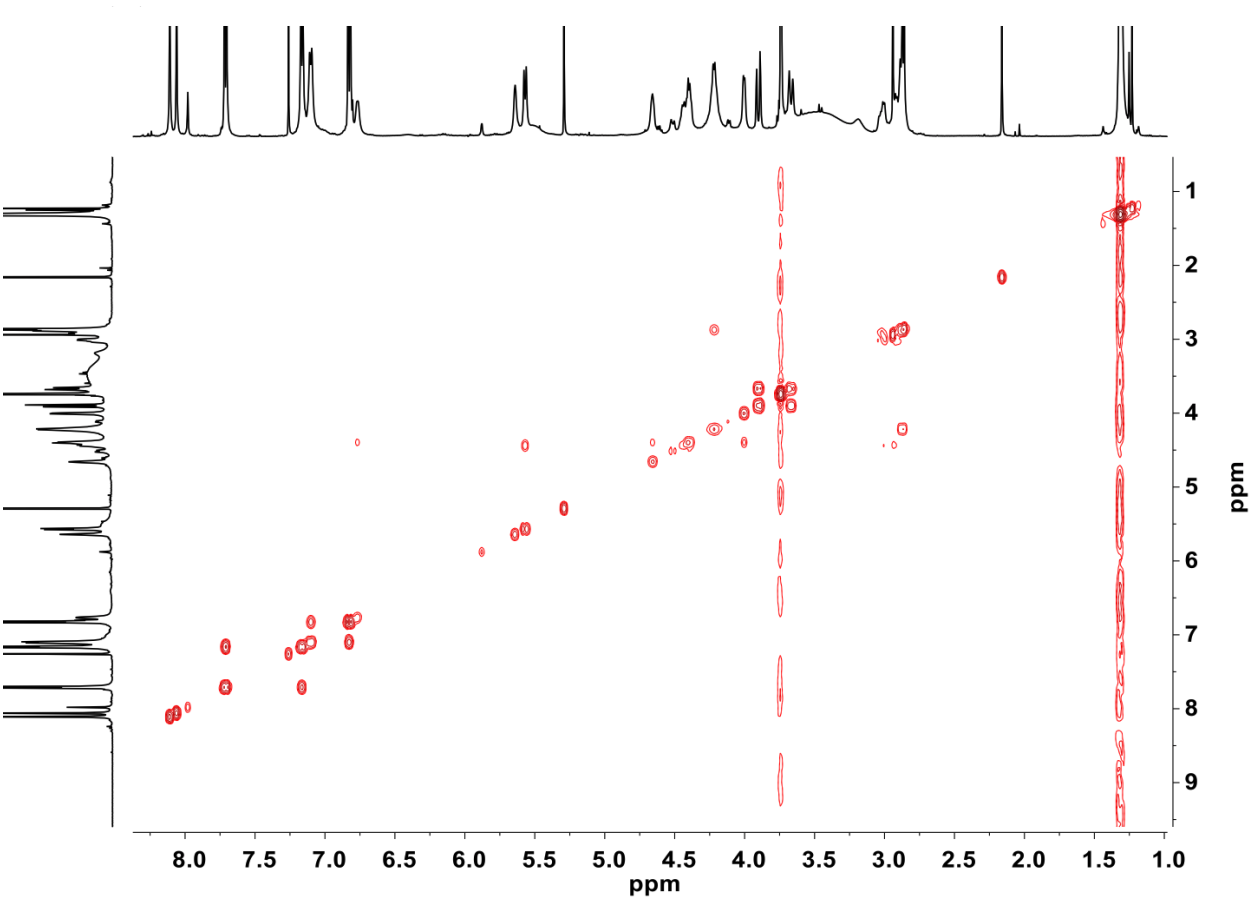
Ctrl-Peroxymycin-1



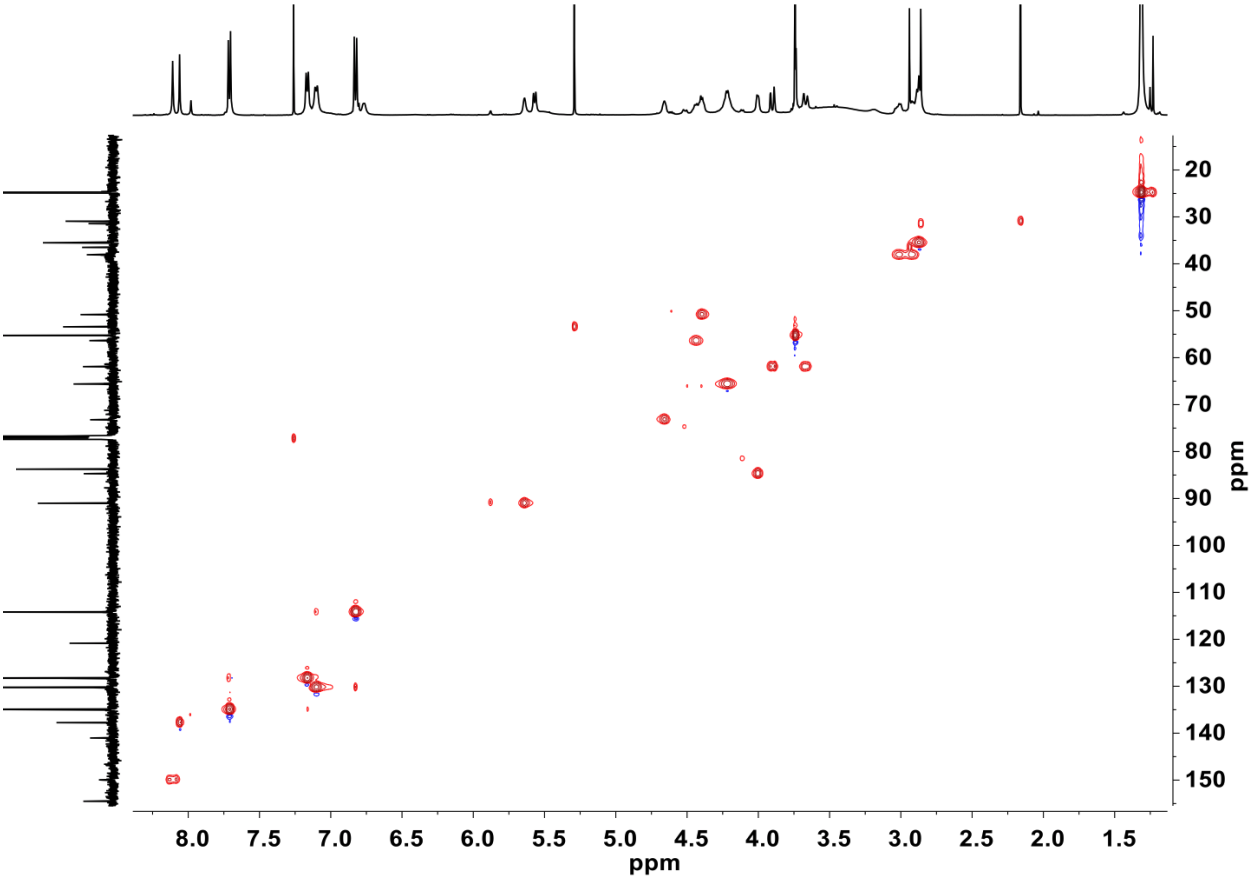
Supplementary Figure 5 | Annotated ^{1}H NMR spectrum of Ctrl-Peroxymycin-1 in $CDCl_3$.



Supplementary Figure 6 | ^{13}C NMR spectrum of Peroxymycin-1 in $CDCl_3$. “x” denotes silicon grease and solvent peaks (grease, acetone, CH_2Cl_2, DMF from right to left).



Supplementary Figure 7 | COSY NMR spectrum of Ctrl-Peroxymycin-1 in $CDCl_3$.



Supplementary Figure 8 | $^{1}H–^{13}C$ HSQC NMR spectrum of Ctrl-Peroxymycin-1 in $CDCl_3$.

References

1. Chung, C. Y.-S., Timblin, G. A., Saijo, K. & Chang, C. J. Versatile histochemical approach to detection of hydrogen peroxide in cells and tissues based on puromycin staining. *J. Am. Chem. Soc.* **140**, 6109–6121 (2018).