Supporting information

MhuD from *Mycobacterium tuberculosis* - probing a dual role in heme storage and degradation

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Figures S1 – S7

Figure S1. SDS-PAGE gel from a MhuD purification.
Samples were run on a 4–20% SDS PAGE gel. Lane 1: protein ladder; lanes 2 & 3: MhuD following overnight incubation with TEV protease; lane 4: MhuD following reverse nickel column elution; lane 5: MhuD following gel filtration.

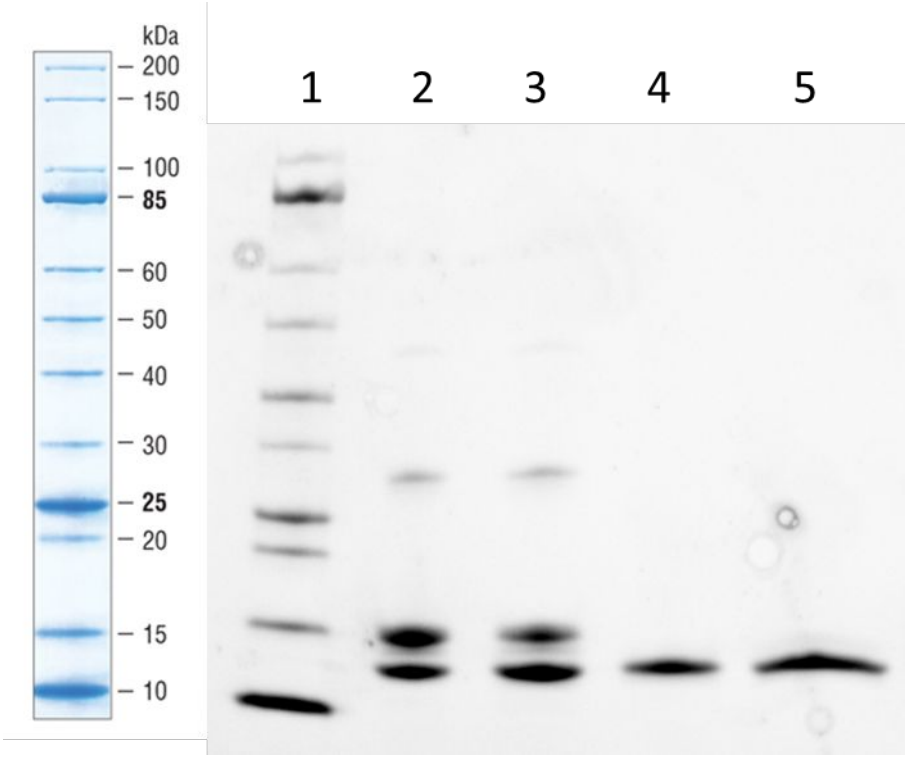


Figure S2. Analysis of MhuD dimeric state using analytical ultracentrifugation (AUC).
AUC experiments provided a sedimentation coefficient of ~2.08S and frictional ratio of f/f0 1.39 for MhuD, which correlates with an estimated molecular mass of 21 kDa and indicates that MhuD is dimeric in solution.

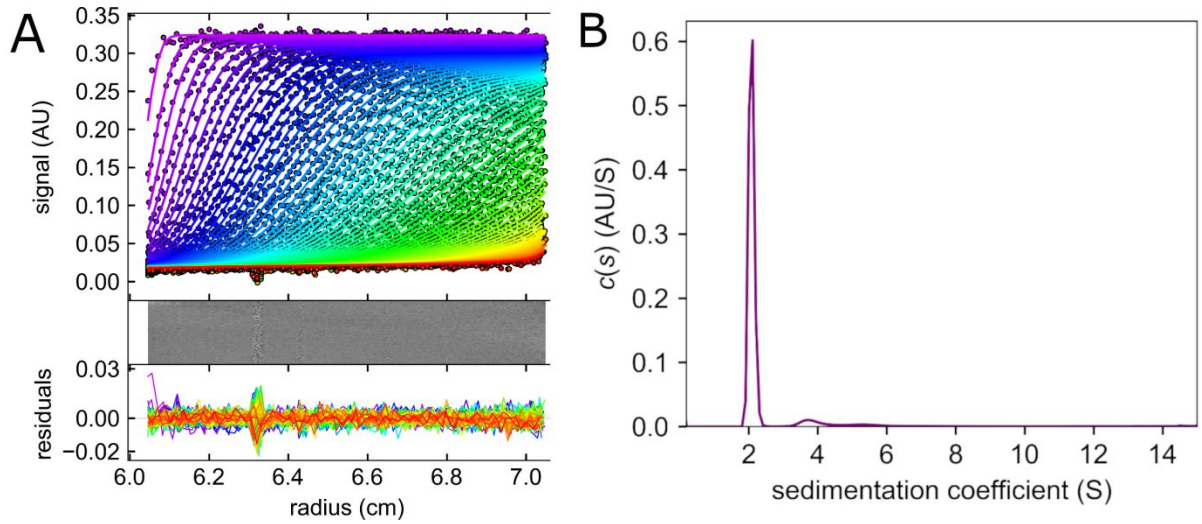
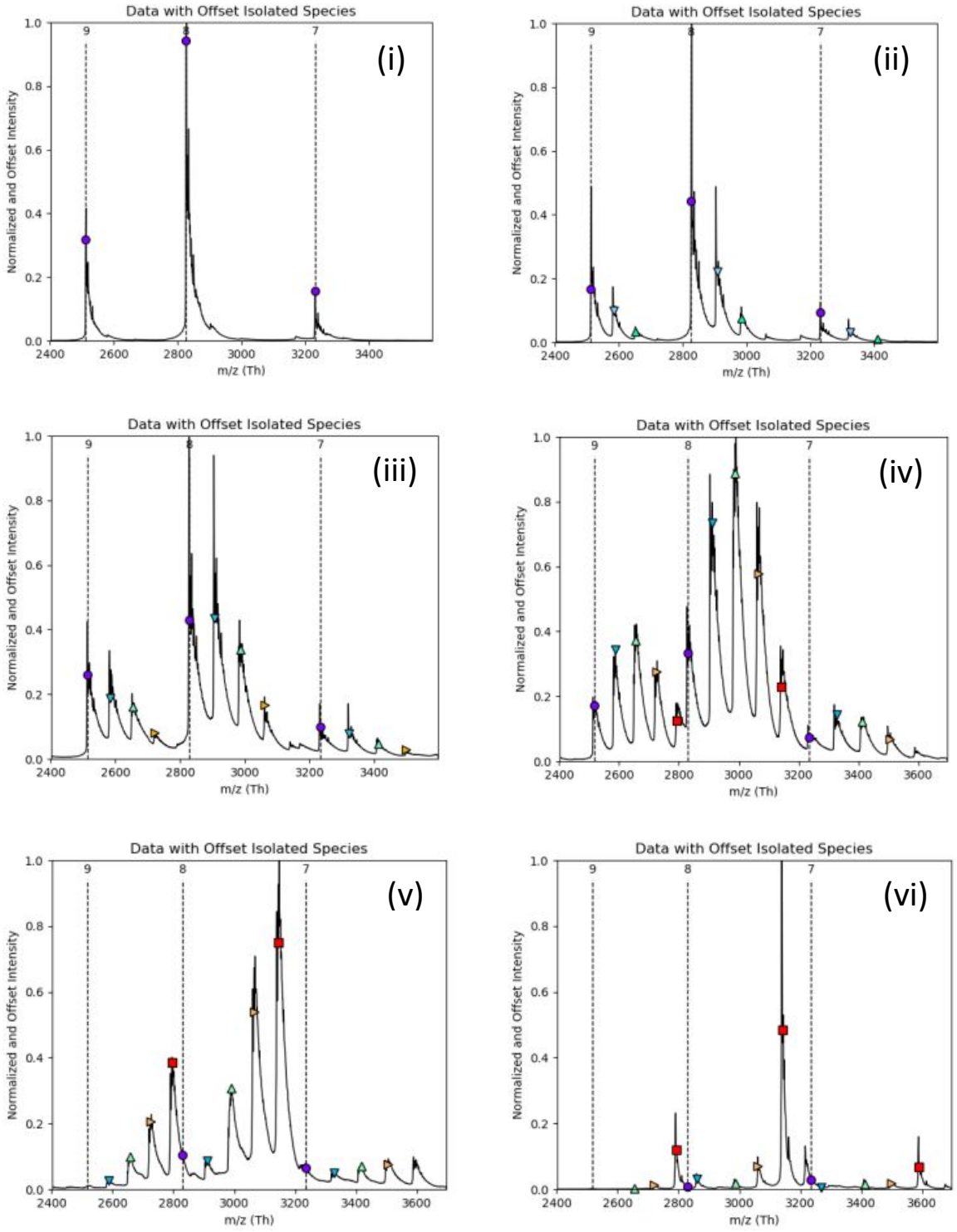


Figure S3. Absolute and deconvoluted nESI-MS spectra over various hemin concentrations. MhuD:heme stoichiometries are shown through **A)** absolute nESI-MS spectra (peaks labelled with respective charge states) and **B)** deconvoluted spectra. Samples contained 5 µM MhuD in 100 mM ammonium acetate + 0.5% DMSO + 0, 2.5, 5, 10, 20, and 30 µM hemin (panels (i), (ii), (iii), (iv), (v) and (vi), respectively). Apo-, 1-, 2-, 3- and 4-heme bound forms of MhuD are represented by purple circles, blue triangles, cyan triangles, orange triangles and red squares, respectively.

A



B

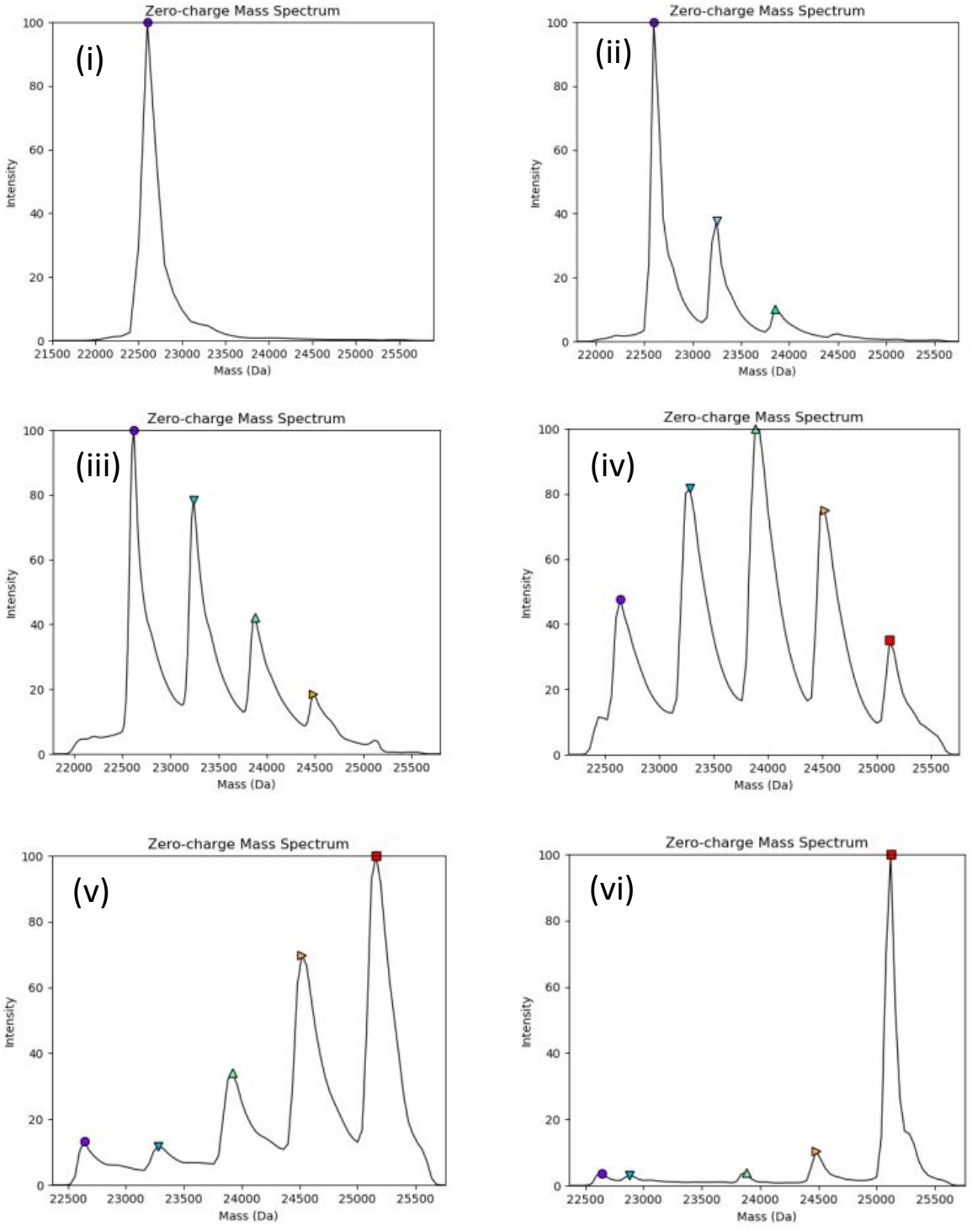

Zero-charge Mass Spectrum
(i)
Intensity
Mass (Da)
Zero-charge Mass Spectrum
(ii)
Intensity
Mass (Da)
Zero-charge Mass Spectrum
(iii)
Intensity
Mass (Da)
Zero-charge Mass Spectrum
(iv)
Intensity
Mass (Da)
Zero-charge Mass Spectrum
(v)
Intensity
Mass (Da)
Zero-charge Mass Spectrum
(vi)
Intensity
Mass (Da)

Figure S4. Collision cross section distributions ($^{TW}CCSD_{N2}$) for apo- and heme-bound MhuD [M + 8H]$^{8+}$ charge states.

The figure shows minor, but consistent, increases in the $^{TW}CCSD_{N2}$ of MhuD as the number of bound heme molecules increases.

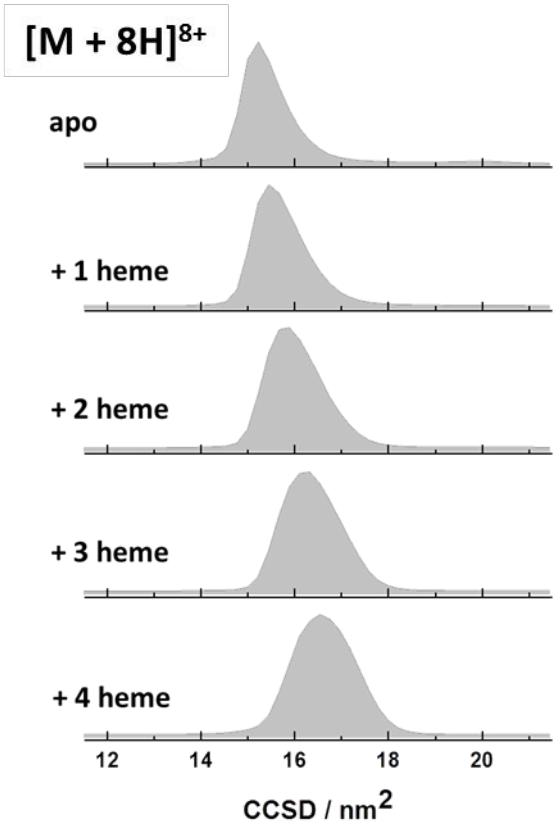


Figure S5. Absolute IM-MS spectrum of 10 µM MhuD + 10 µM deuteroheme (+0.5% DMSO). This spectrum shows $[M + 8H]^{8+}$ and $[M + 9H]^{9+}$ charge species for apo, 1-, 2-, 3- and 4-deuteroheme bound MhuD (purple circles, blue triangles, cyan triangles, orange triangles and red squares, respectively).

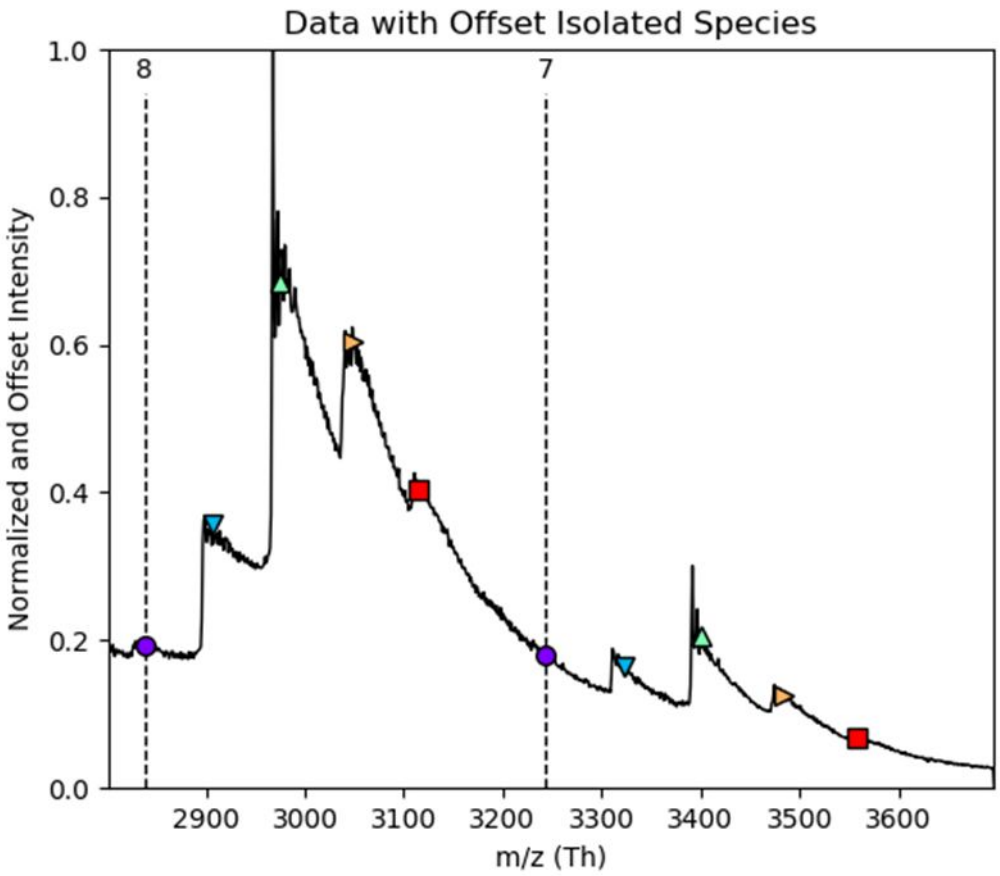


Figure S6. Absolute IM-MS spectrum showing heme species formed through aIM-MS analysis of 2-heme bound MhuD (*m/z* 2982)

The spectrum shows monoheme (*m/z* 616), diheme (*m/z* 1232) and diheme + water (m/*z* 1250) species that are formed during aIM-MS analysis of 2-, 3- and 4-heme bound forms of MhuD.

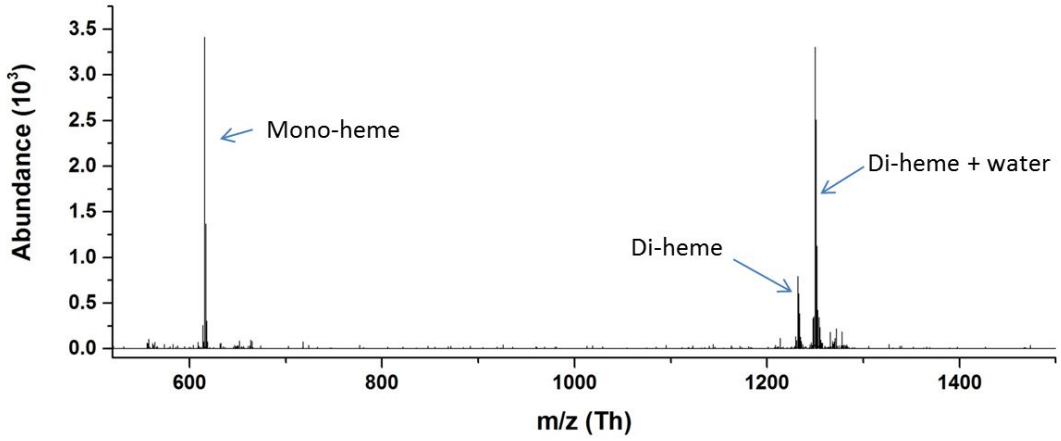


Figure S7. Effect of heme-MhuD incubation times on nESI-MS spectra.
Comparison of nESI-MS spectra when 5 µM MhuD + 10 µM heme (0.5% DMSO) are incubated at A) room temperature for 1 hour and B) 4°C overnight shows negligible differences in heme binding stoichiometry.