Analysis of Uncomplexed and Copper-complexed Methanobactin with UV/Visible Spectrophotometry, Mass Spectrometry and NMR Spectrometry

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Figures

Figure 1

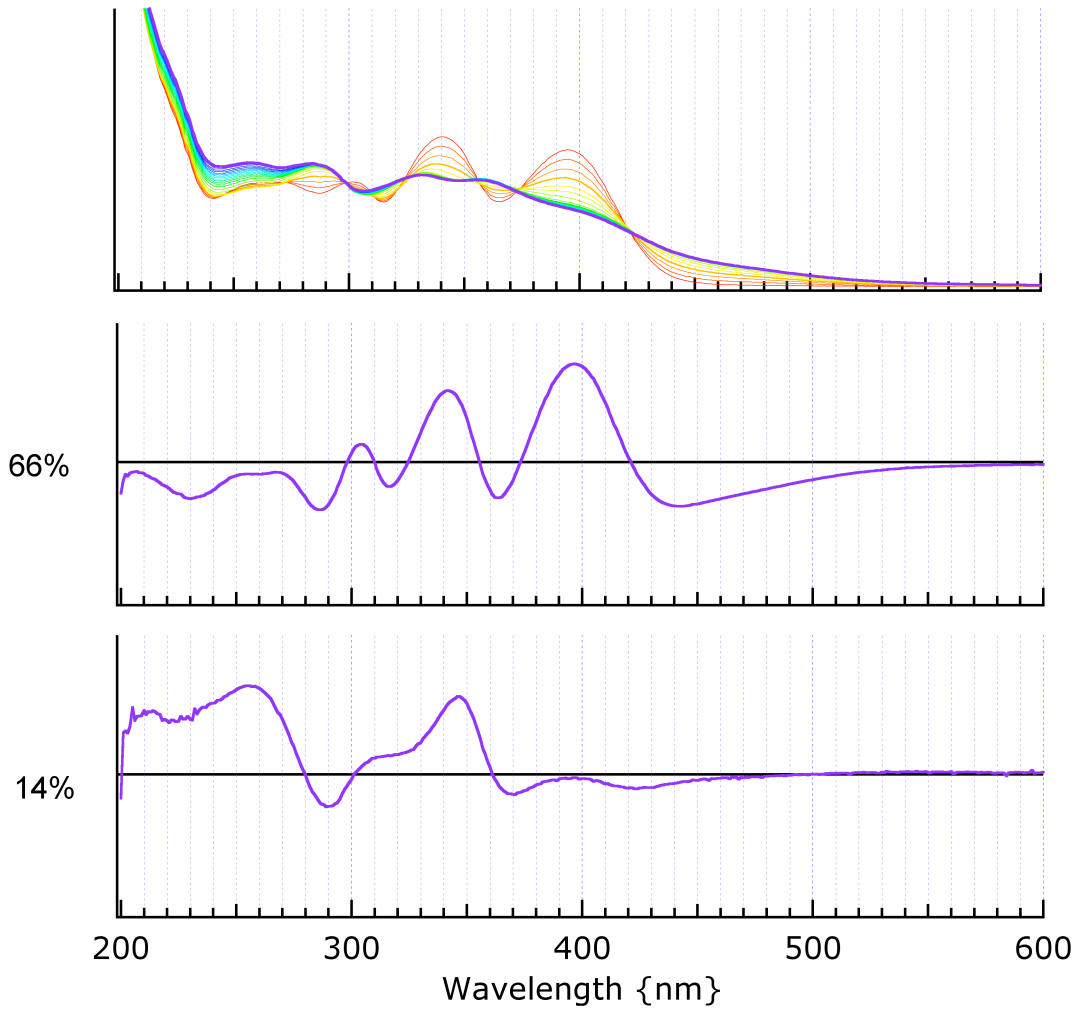


Figure 1: **Top**, the overlay of 21 UV/Vis spectra collected during a titration of methanobactin with Cu(II). The titration ranged from 0 to 2.0 Cu:mb. The methanobactin, Lot A, was dissolved to a concentration of 50 µM in 10 mM phosphate, *pH6.5,* The spectra are colored according to the rainbow from red (0.0 Cu:mb) to blue (2.0 Cu:mb). **Middle** and **bottom**, the first and second principle components obtained from a singular value decomposition of the 21 spectra. The average of the 21 spectra was subtracted from each prior to carrying out the decomposition. Together, the first and second principle components comprise 80% of the variations observed during the titration.

Figure 2

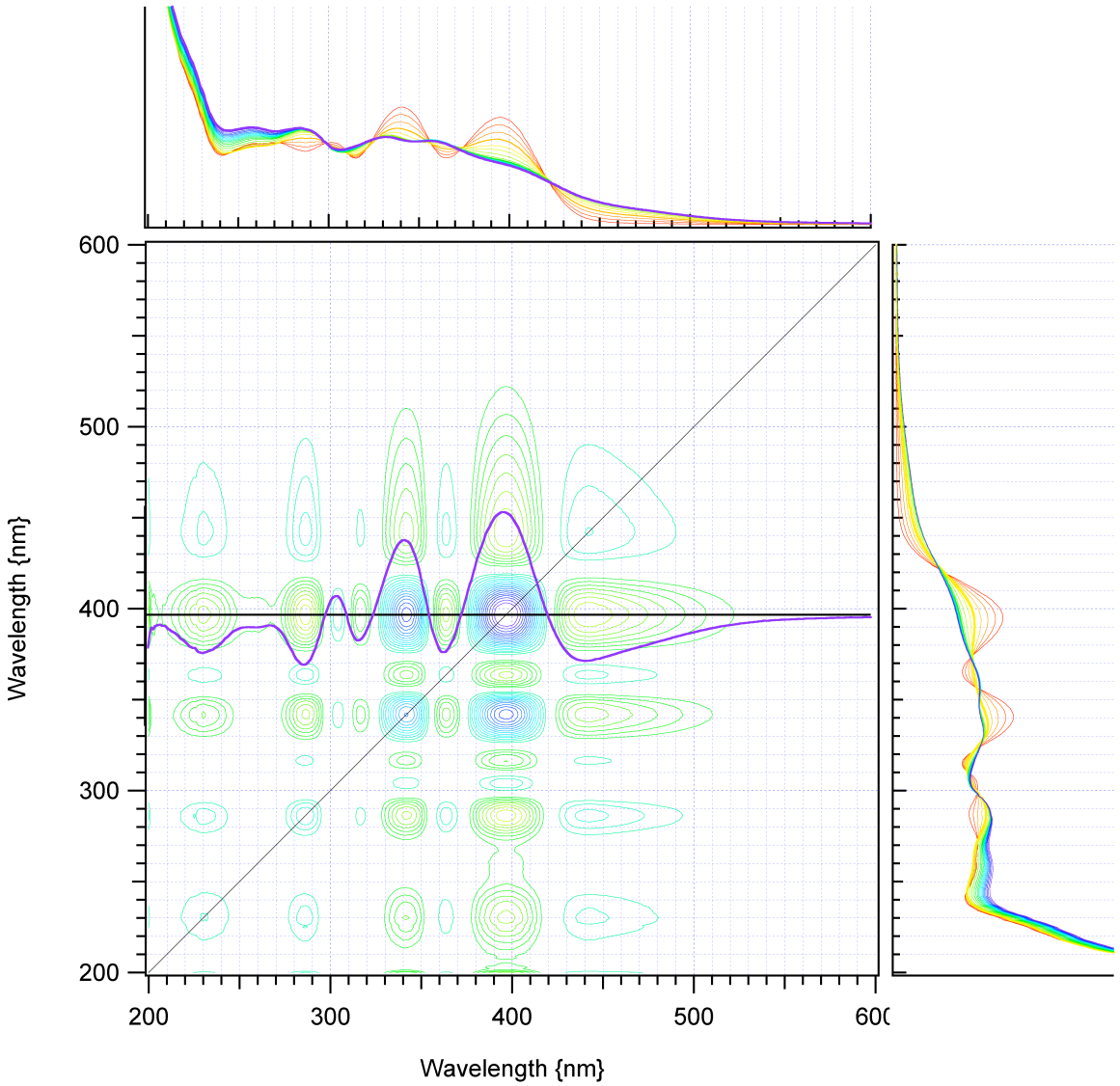


Figure 2: The synchronous component of a two-dimensional correlation analysis of the 21 UV/Vis spectra collected during a titration of methanobactin with Cu(II). The titration ranged from 0 to 2.0 Cu:mb. The first principle component, which accounts for 66% of the variation during the titration, has been overlaid along the line that shows the features in the spectra that change synchronously with changes occurring at 394 nm.

Figure 3

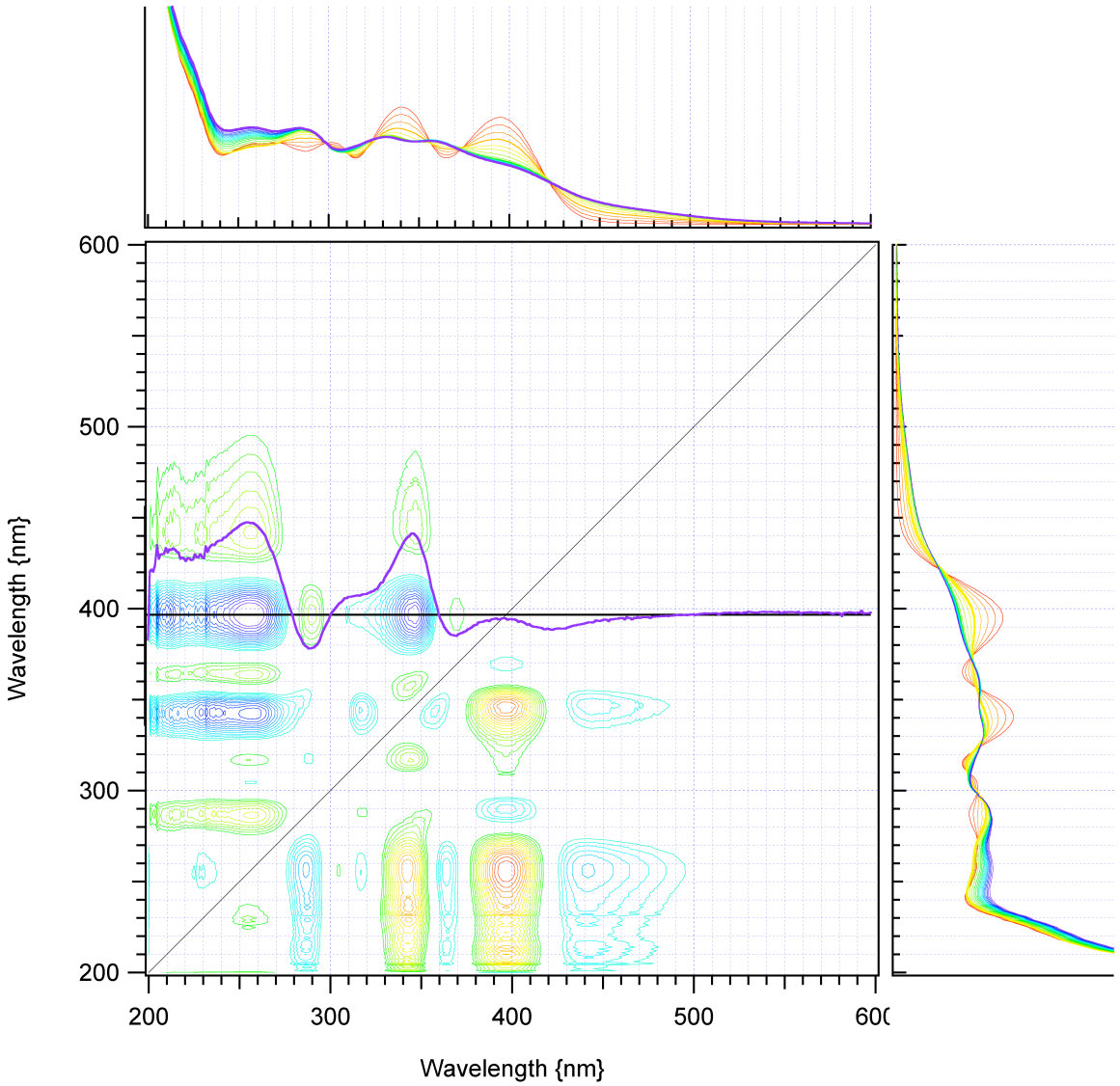
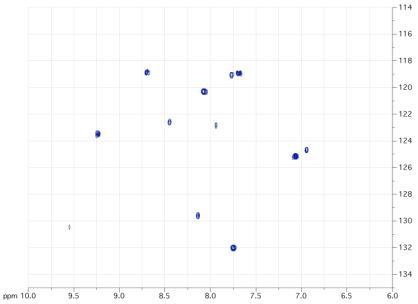


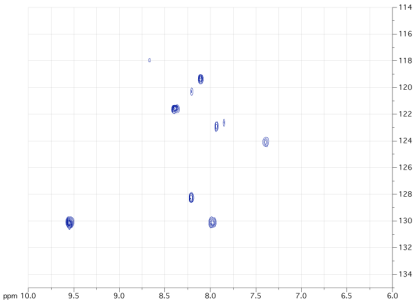
Figure 3: The asynchronous component of a two-dimensional correlation analysis of 21 UV/Vis spectra collected during a titration of methanobactin with Cu(II). The titration ranged from 0 to 2.0 Cu:mb. The second principle component, which accounts for 14% of the variation during the titration, has been overlaid along the line that shows the features in the spectra that change asynchronously with the changes that occur at 394 nm.

Figure 4

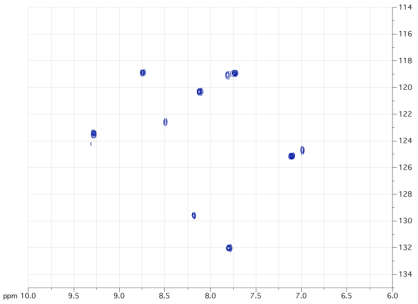
Figure 4: 600 MHz ^{15}N-HSQC spectra of 2 mM methanobactin Lot N, in 9 mM Phosphate, *pH*6.5, 25°C, at selected points during a titration with Cu(II). Shown are the ^{15}N-^{1}H cross peaks in the amide region of the ^{1}H spectrum. a) 0.0 Cu:mb, b) 0.1 Cu:mb, c) 0.2 Cu:mb, d) 0.4 Cu:mb, e) 0.6 Cu:mb, f) 0.9 Cu:mb, and g) 1.2 Cu:mb.



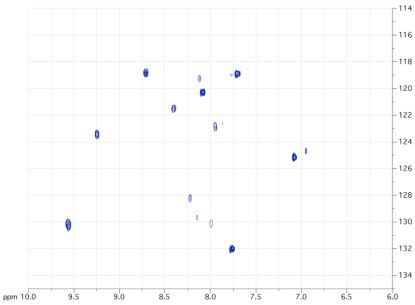
d) 0.4 Cu:mb



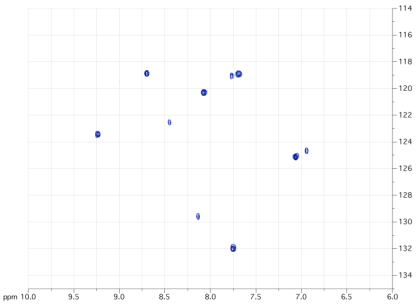
a) 0.0 Cu:mb



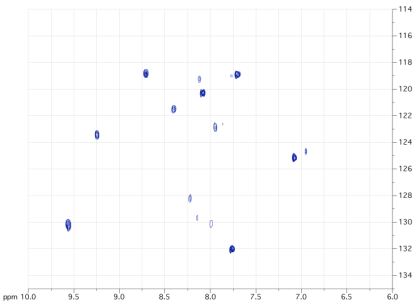
e) 0.6 Cu:mb



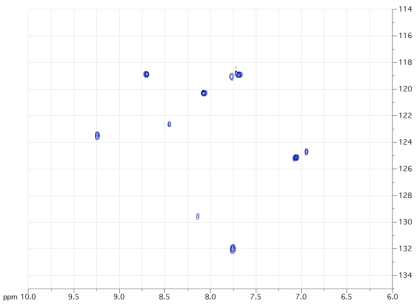
b) 0.1 Cu:mb



f) 0.9 Cu:mb

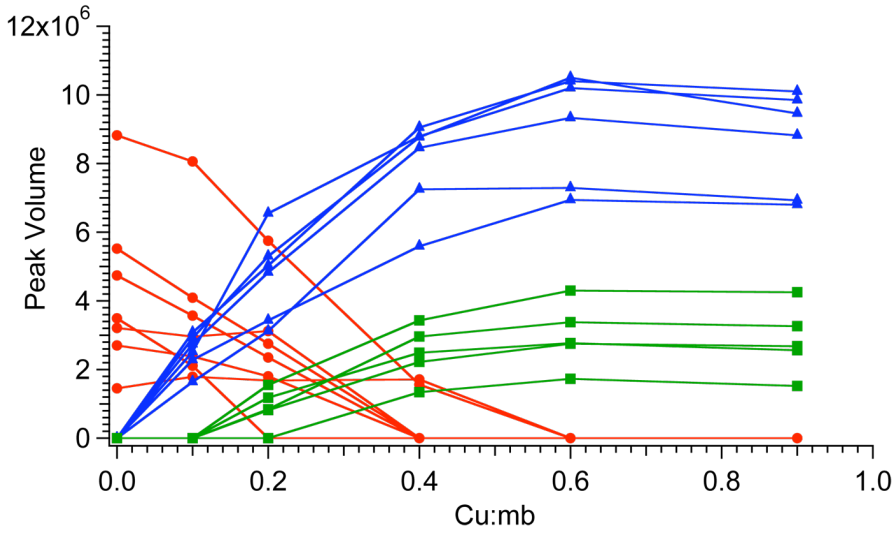


c) 0.2 Cu:mb

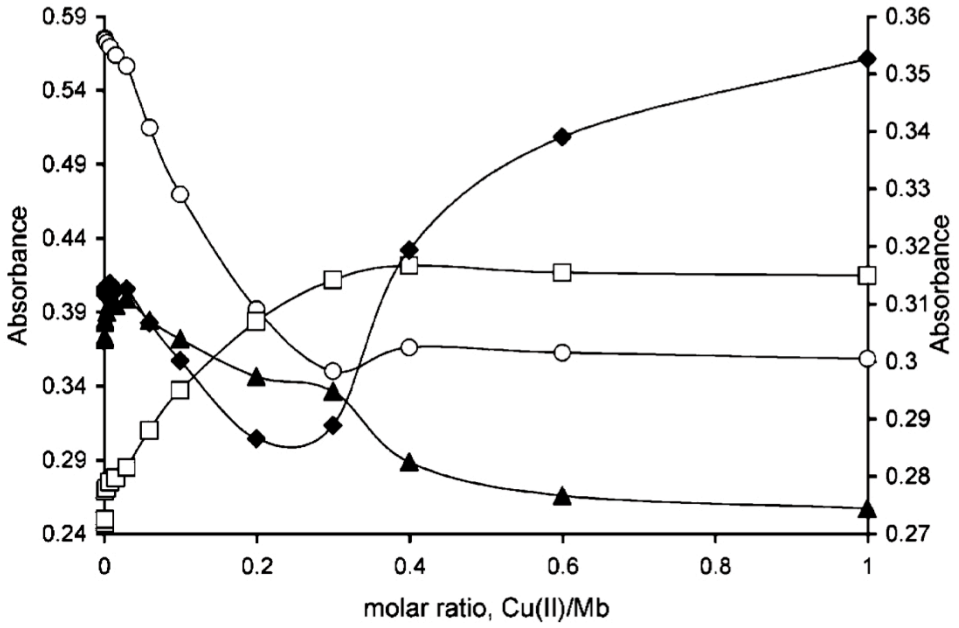


g) 1.2 Cu:mb

Figure 5



a)



b)

Figure 5: a) Peak volumes *vs.* the Cu:mb ratio for the ^{15}N-^{1}H crosspeaks shown in Figure 5. Red: The peaks that disappear upon addition of Cu(II), blue and green: the peaks that appear upon addition of Cu(II). b) Figure 5D from Choi *et al*., 2006{Choi, 2006 #1}, which shows the absorbances as selected wavelengths for a Cu(II) titration of methanobactin that was monitored by UV/Vis spectrophotometry.

Figure 6

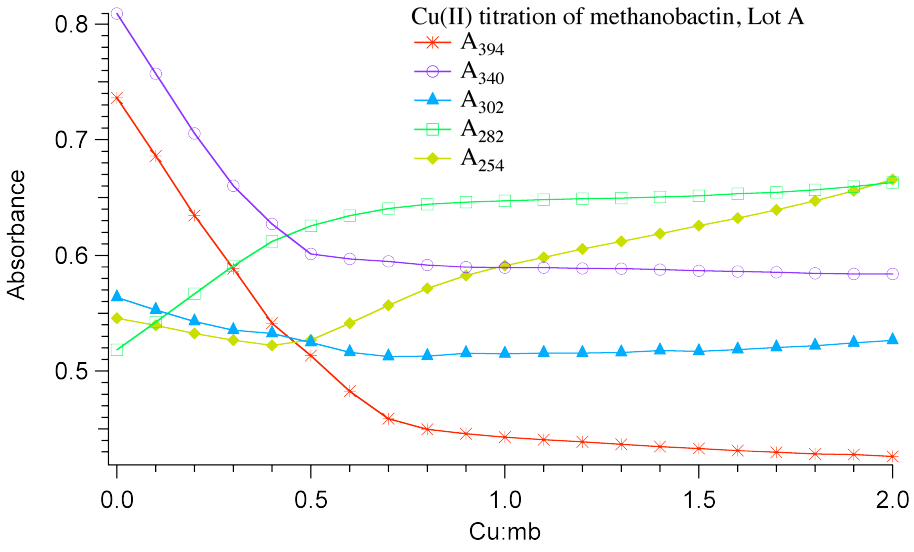


Figure 6: A plot of the UV/Vis absorbances at selected wavelengths for the data shown in Figures 1, 2 and 3. The wavelengths that were chosen are the same as those chosen by Choi *et al.,* 2006 in constructing the figure shown in Figure 5b. In this experiment, the spectral changes for all of the selected wavelengths except 254 nm, level off well before 1.0 Cu:mb.

Figure 7a

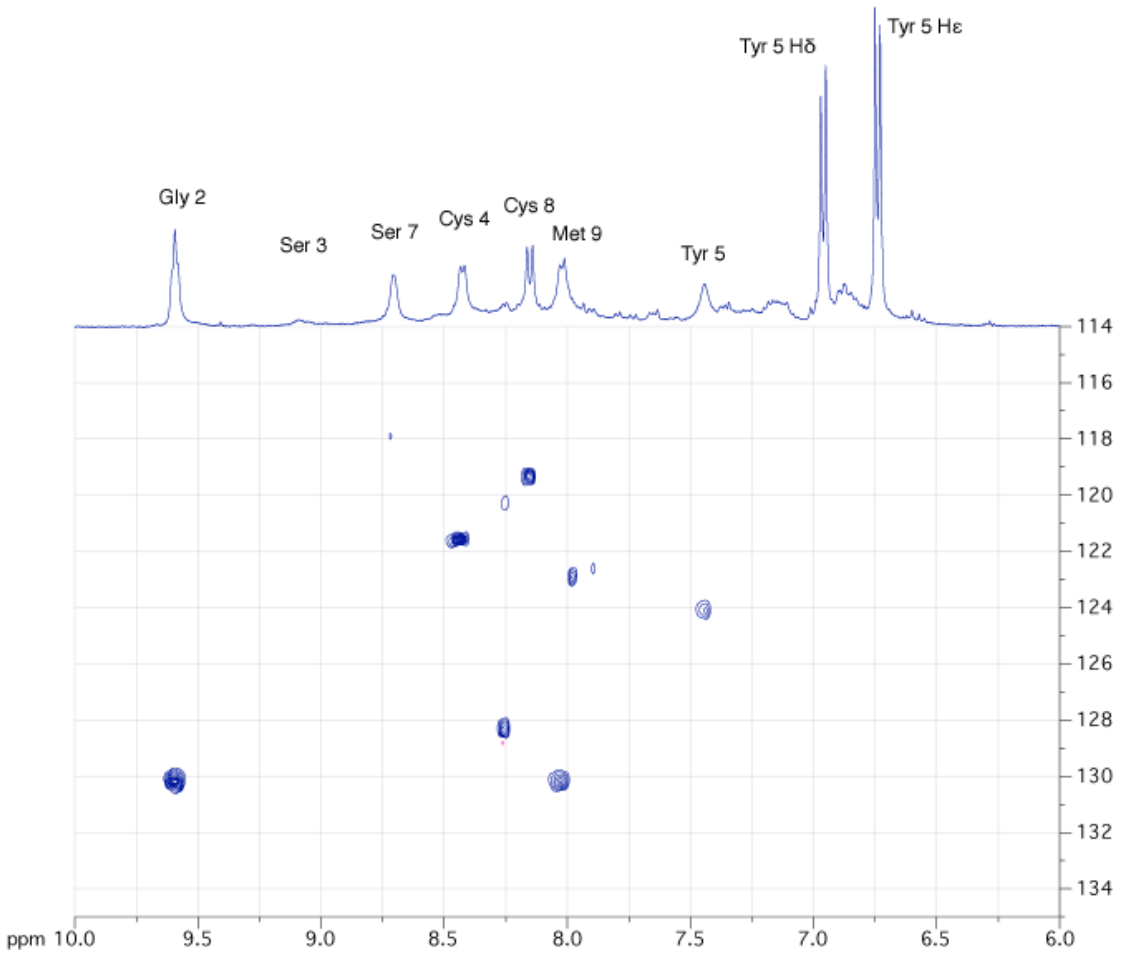


Figure 7a: The 600 MHz ^{15}N-HSQC spectra from Figure 5a, representing the starting point for the titration, along with a 1-dimensional ^{1}H spectra that was collected under similar conditions. The 1-dimensional spectrum was collected at 400 MHz on a 4 mM methanobactin Lot A sample dissolved in 9 mM Phosphate, *pH*6.5, 25°C with no Cu(II) added.

Figure 7a

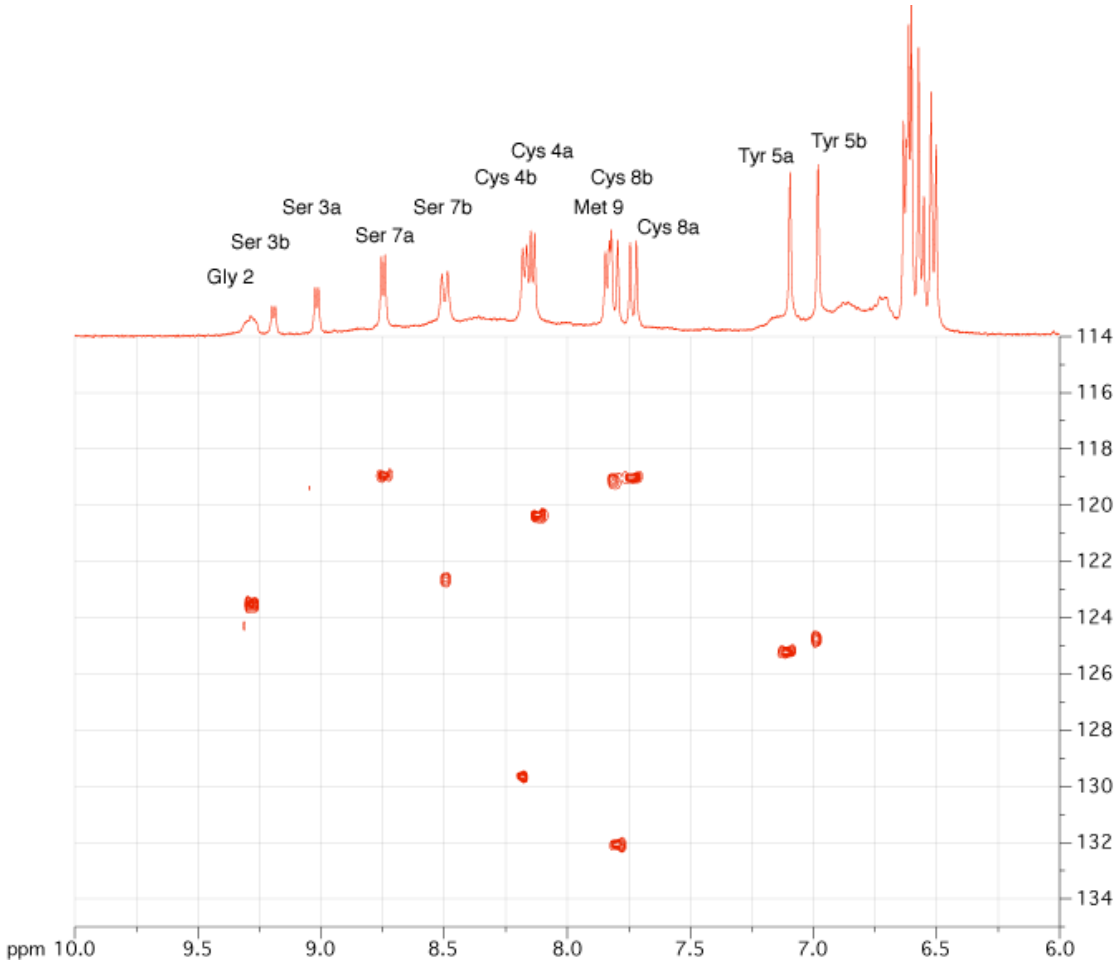


Figure 7b: The 600 MHz ^{15}N-HSQC spectra from Figure 5e, representing the endpoint for the titration, along with a 1-dimensional ^{1}H spectra that was collected under similar conditions. The 1-dimensional spectrum was collected at 400 MHz on a 2 mM methanobactin Lot B sample dissolved in 90%H_2O/10%D_2O, 25°C and titrated to 0.7 Cu:mb.

Figure 7c

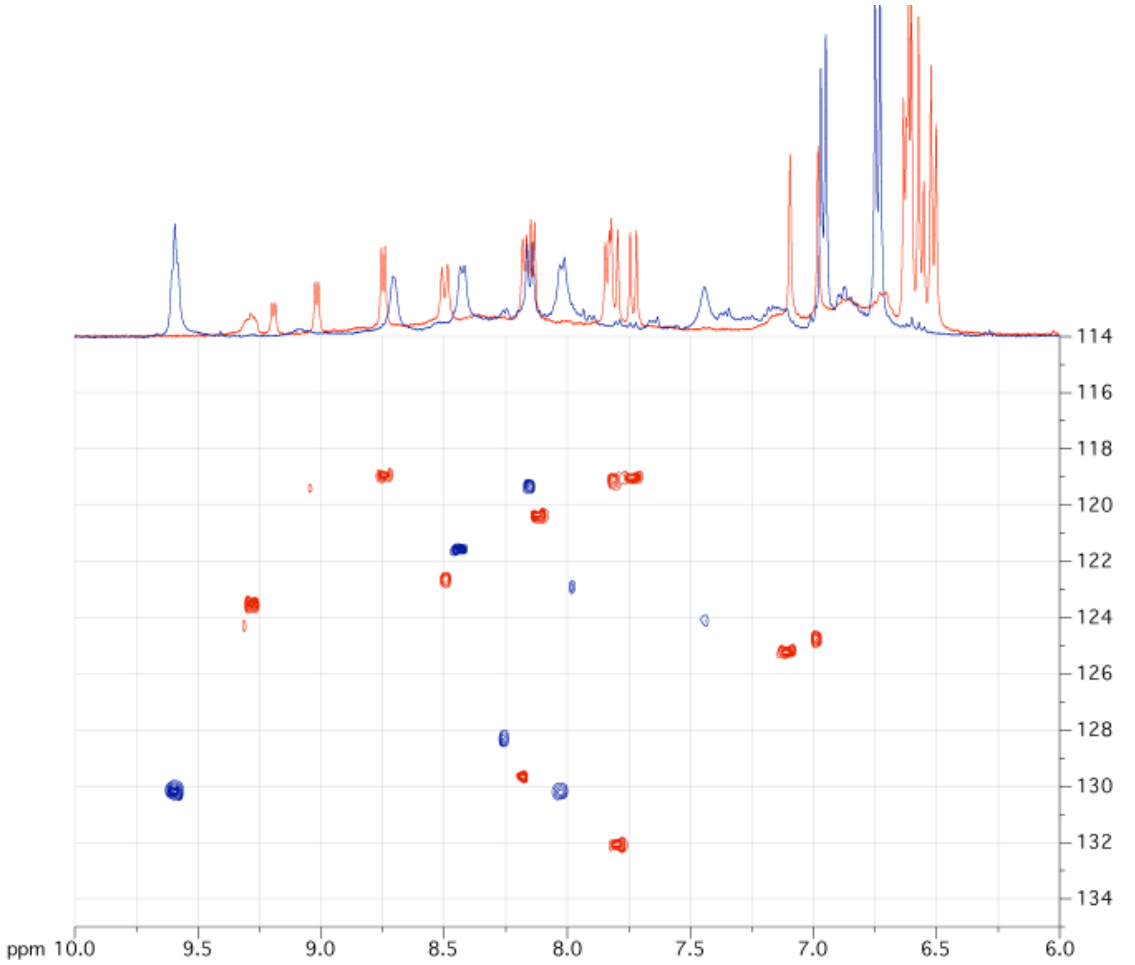


Figure 7c: An overlay of the spectra shown in Figures 6a and 6b.

Figure 8

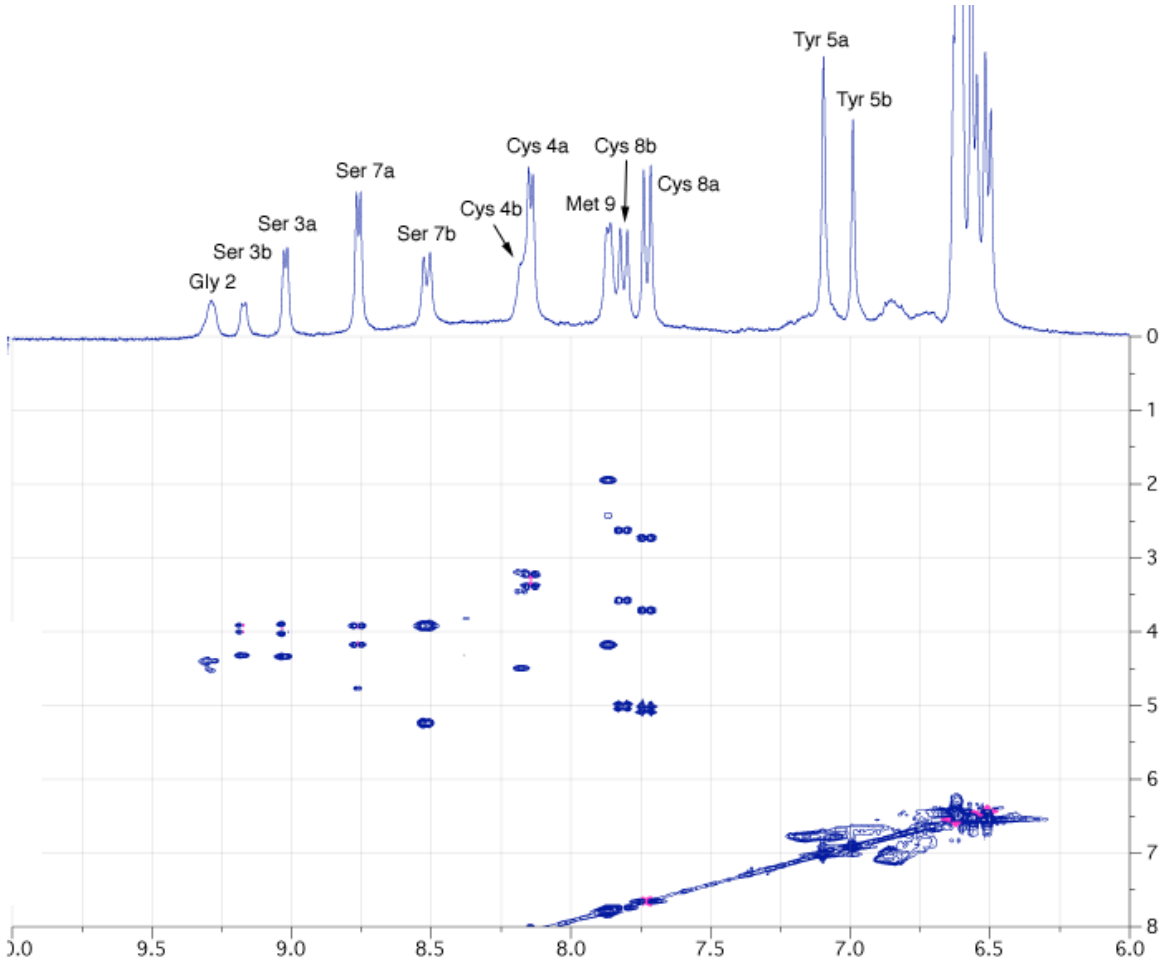


Figure 8: 400 MHz ^{1}H-TOCSY spectrum of a 2 mM methanobactin Lot B in 90% H_2O/10% D_2O, 25°C, complexed with Cu(II) at 0.9 Cu:mb.

Figure 9

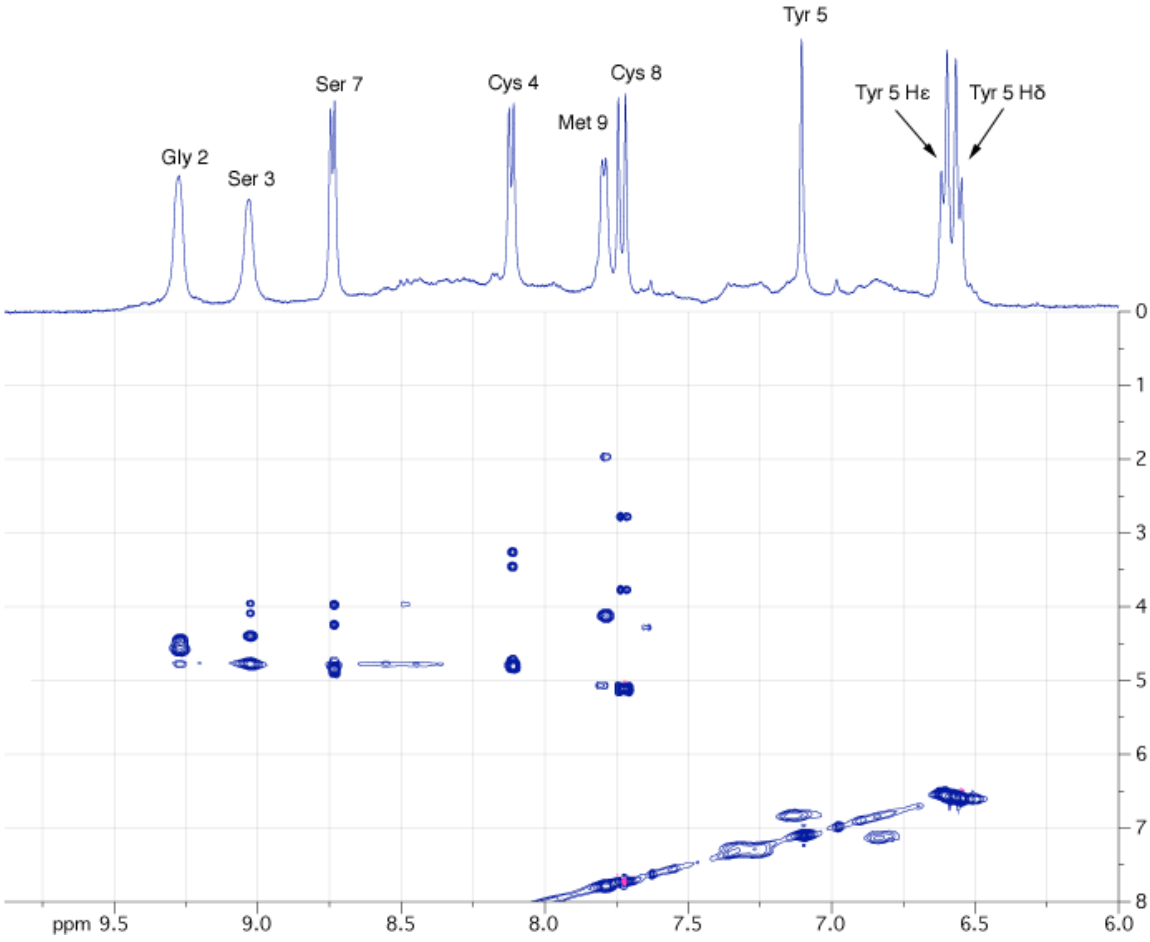


Figure 9: 400 MHz ^{1}H-TOCSY spectrum of methanobactin Lot A in 9 mM phosphate/10% D_2O, *pH*6.5, 25°C, complexed with Cu(II) at 0.55 Cu:mb.

Figure 10

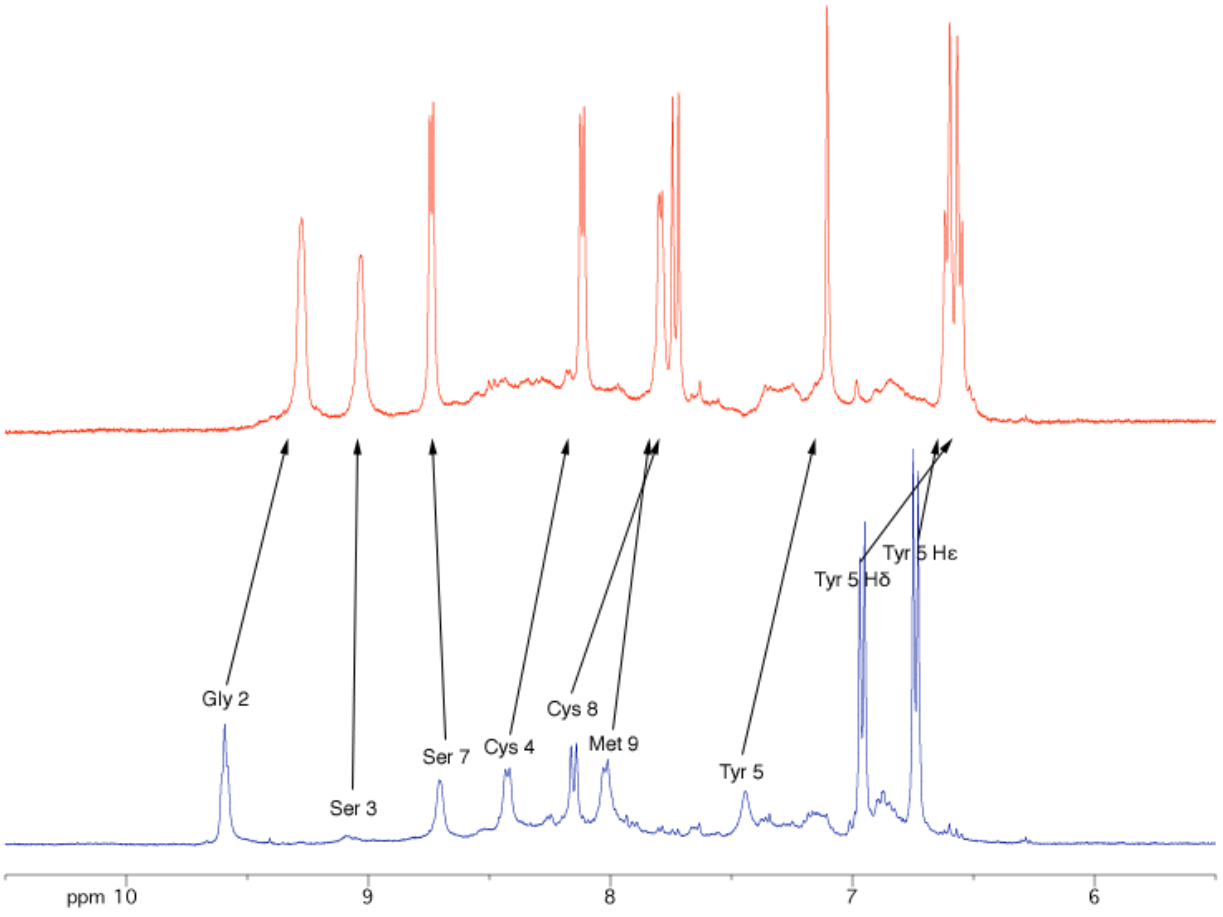
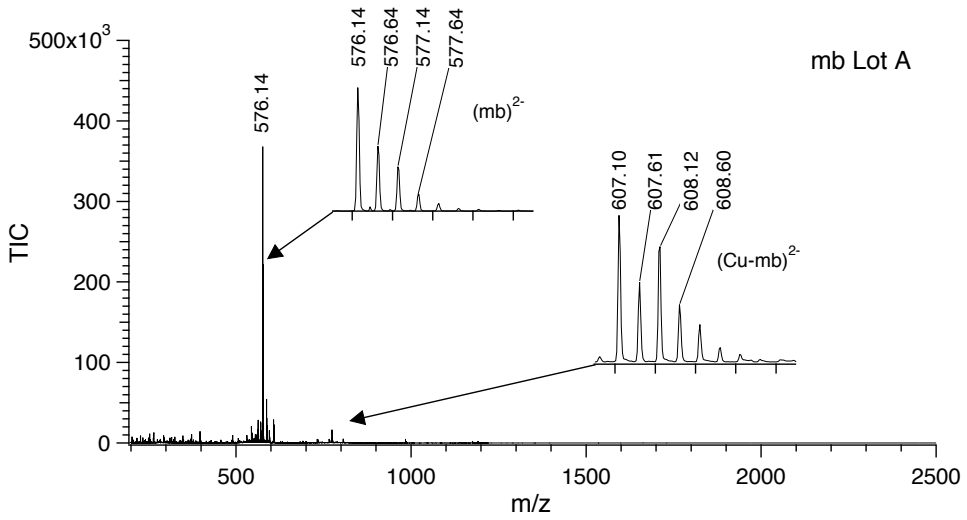
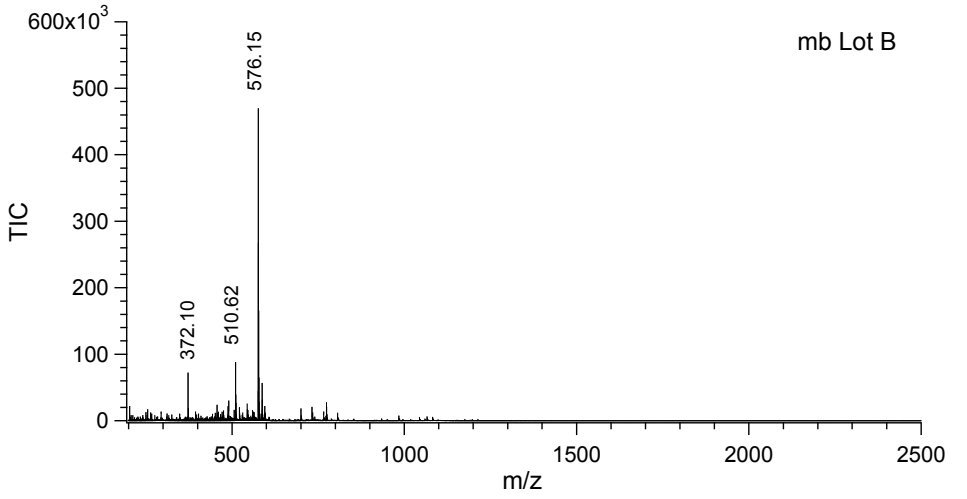


Figure 10: 400 MHz ^{1}H-spectrum of methanobactin Lot A in 9 mM phosphate/10% D_2O, *pH*6.5, 25°C. **Bottom**, uncomplexed. **Top**, complexed with Cu(II) at 0.5 Cu:mb.

Figure 11



a)



b)

Figure 11: ESI-TOF mass spectra in negative ion mode of methanobactin dissolved in H_2O. a) methanobactin Lot A, b) methanobactin Lot B.

Figure 12

Figure 12: Fractionation of the methanobactin Lot A sample by HPLC reverse phase chromatography. The sample was dissolved in 10 mM phosphate, *pH*6.5, loaded on the column, and eluted with a 1% to 99% methanol gradient containing 0.001% acetic acid. The **top panel** shows the elution profile and the remaining panels show the UV/Vis spectra for the labeled fractions.

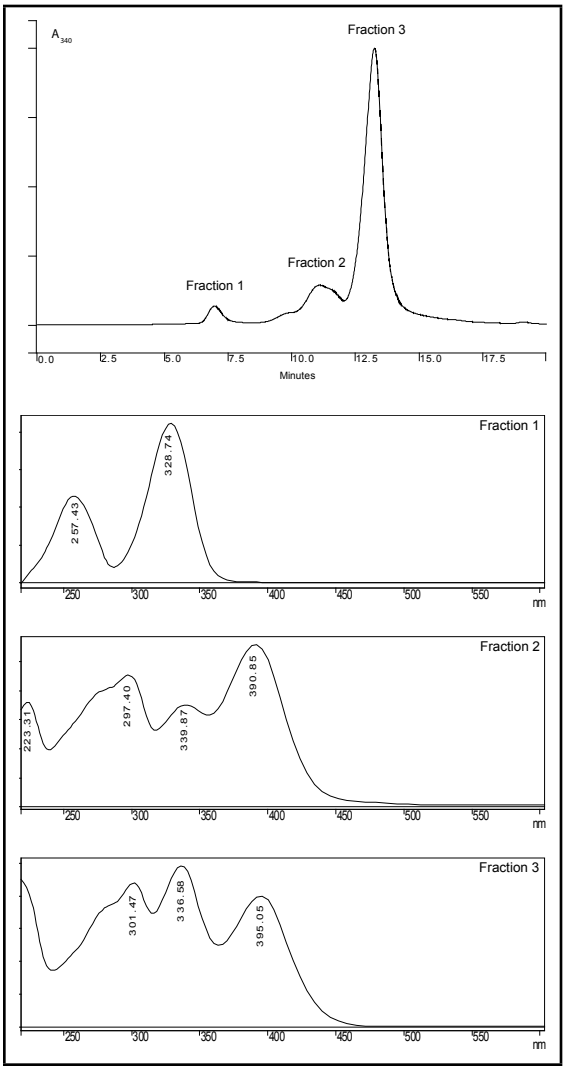


Figure 13

Figure 13: Fractionation of the methanobactin Lot B sample by HPLC reverse phase chromatography. The sample was dissolved in 10 mM phosphate, *pH*6.5, loaded on the column, and eluted with a 1% to 99% methanol gradient containing 0.001% acetic acid at a flow rate of 3 mL/min. The **top panel** shows the elution profile and the remaining panels show the UV/Vis spectra for the labeled fractions.

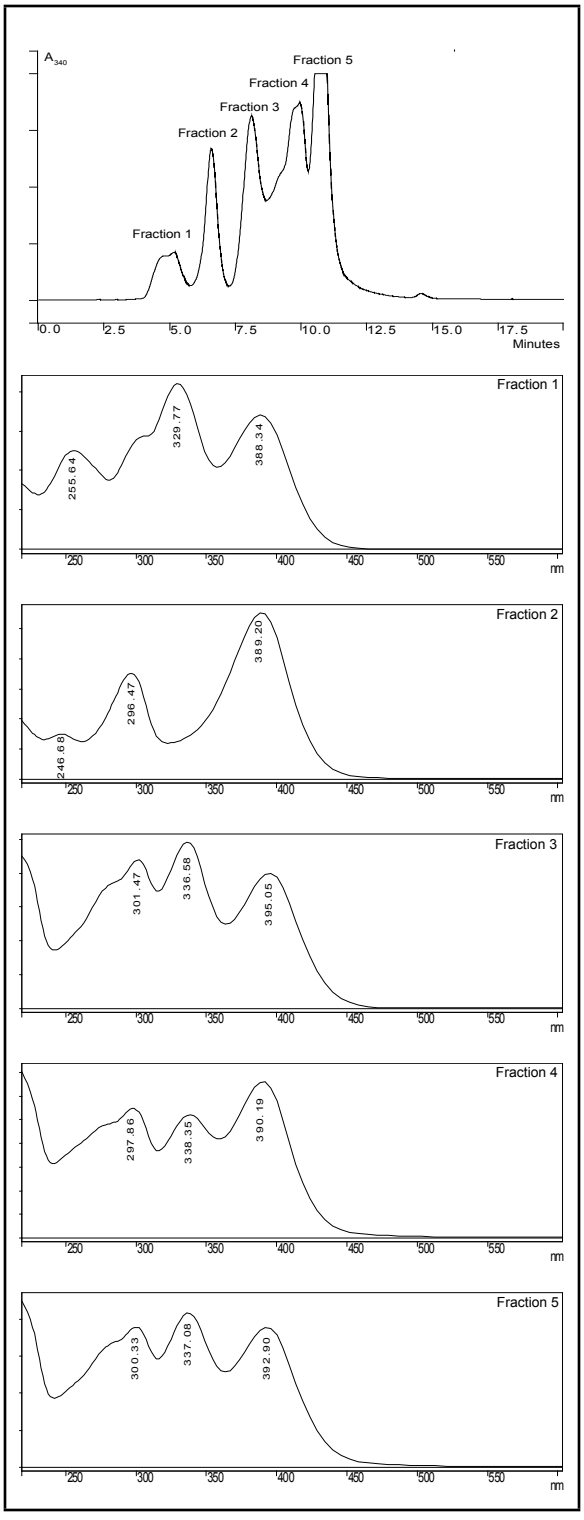
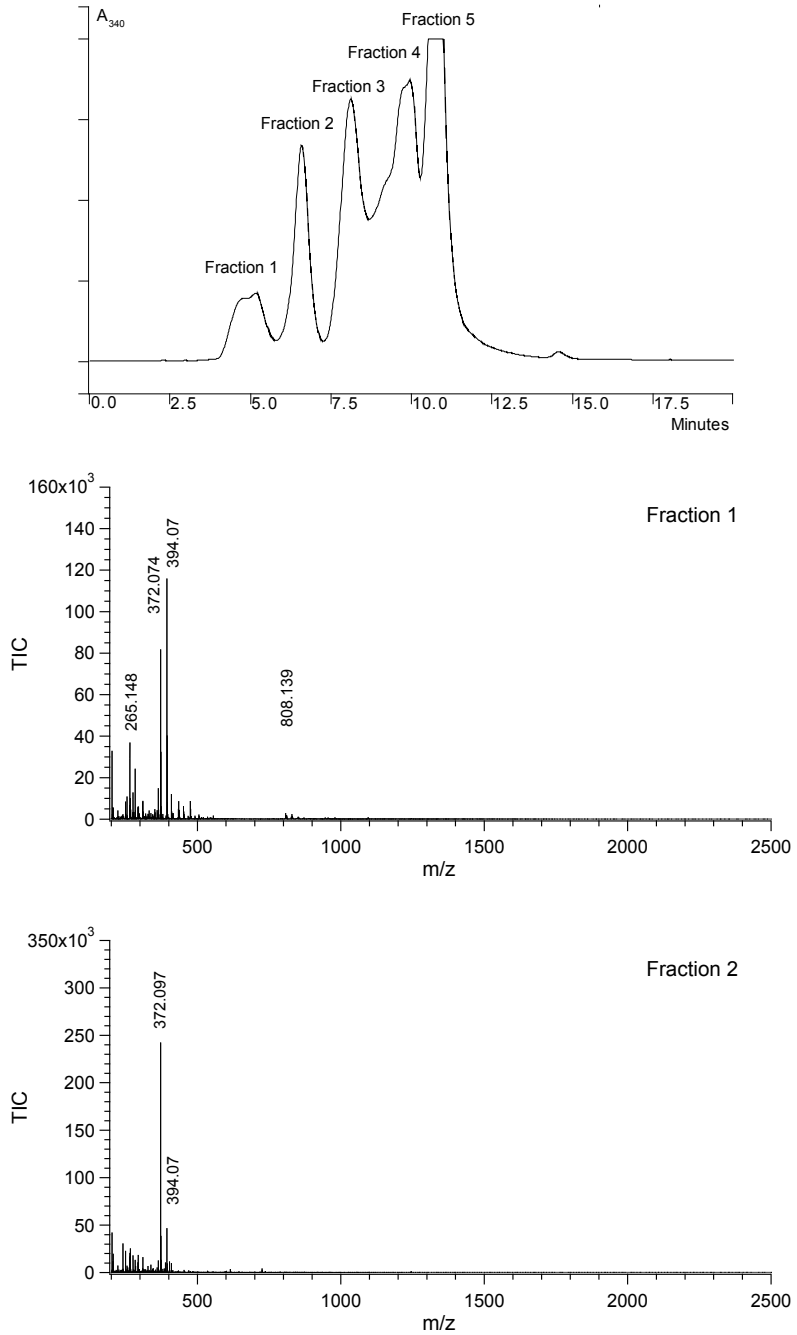
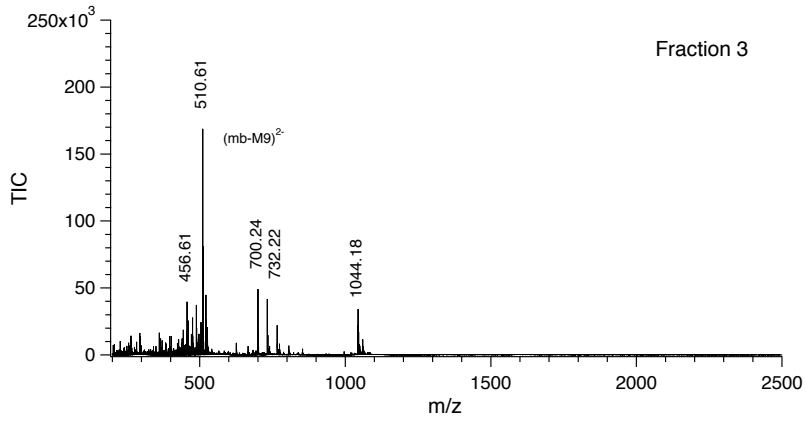
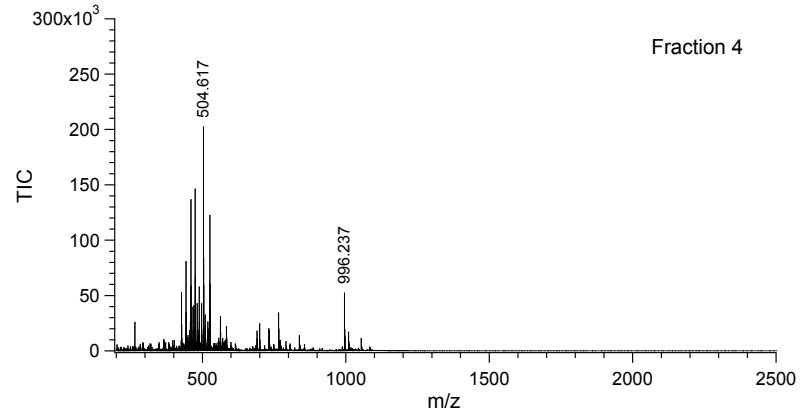


Figure 14

Figure 14: Fractionation of the methanobactin Lot B sample by HPLC reverse phase chromatography. The sample was dissolved in 10 mM phosphate, *pH*6.5, loaded on the column, and eluted with a 1% to 99% methanol gradient containing 0.001% acetic acid at a flow rate of 3 mL/min. The top panel shows the elution profile and the remaining panels show the UV/Vis spectra for the labeled fractions.




Fraction 3
250x10^3
200
150
100
50
0
TIC
510.61
(mb-M9)^2-
456.61
700.24
732.22
1044.18
500
1000
1500
2000
2500
m/z


Fraction 4
300x10^3
250
200
150
100
50
0
TIC
504.617
996.237
500
1000
1500
2000
2500
m/z

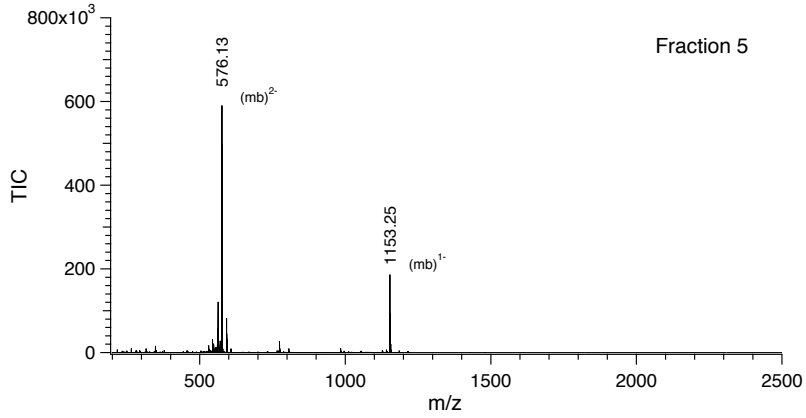
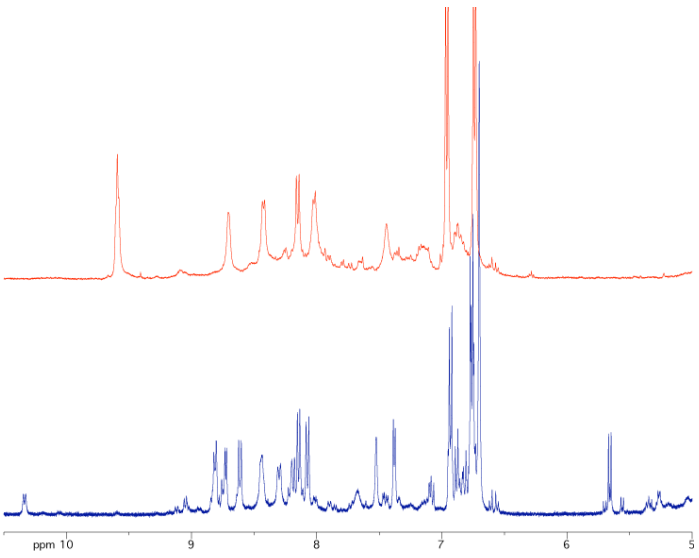
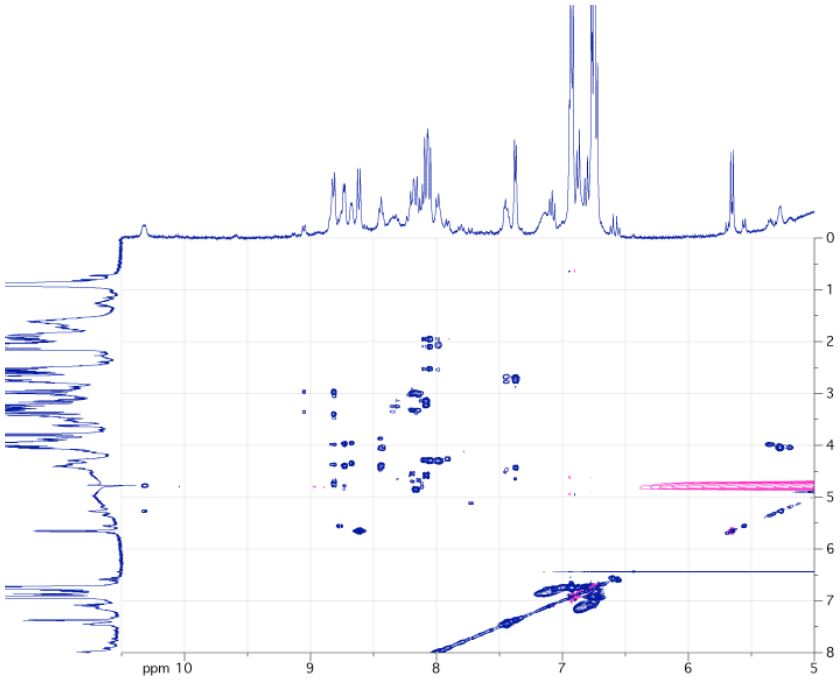

Fraction 5
800x10^3
600
400
200
0
TIC
576.13
(mb)^2-
1153.25
(mb)^1-
500
1000
1500
2000
2500
m/z

Figure 15



a) upper, b)lower



c)

Figure 15: NMR analysis of Fractions from HPLC fractionation of methanobactin Lots A and B (see Figures 12, 13 &14). Fractions were concentrated by rotavaporation, followed by lyophilization and dissolved in 9 mM phosphate, *pH*6.5, 10% D_2O. a) Upper spectrum in red: the 1-dimension ^1H spectrum for unfractionated mb-Lot A, shown for comparison; b lower spectrum in blue: the 1-dimension ^1H spectrum for Fraction 3 from mb-Lot A; b) TOCSY and ^1H-1D spectra of Fraction 5 from the Lot B run.

Figure 16

Figure 16: Fractionation of the methanobactin Lot B sample by HPLC reverse phase chromatography after exposure to Cu(II) at a ratio of 0.7 Cu:mb. The sample was dissolved in 10 mM phosphate, *pH*6.5, 100 mM $CuSO_4$ was added in increments, adjusting the *pH* back to *pH*6.5 after each addition using 100 mM NaOH. After reaching 0.7 Cu:mb, the sample was loaded on the column, and eluted with a 1% to 99% methanol gradient containing 0.001% acetic acid at a flow rate of 3 mL/min. The top panel shows the elution profile and the remaining panels show the UV/Vis spectra for the labeled fractions.

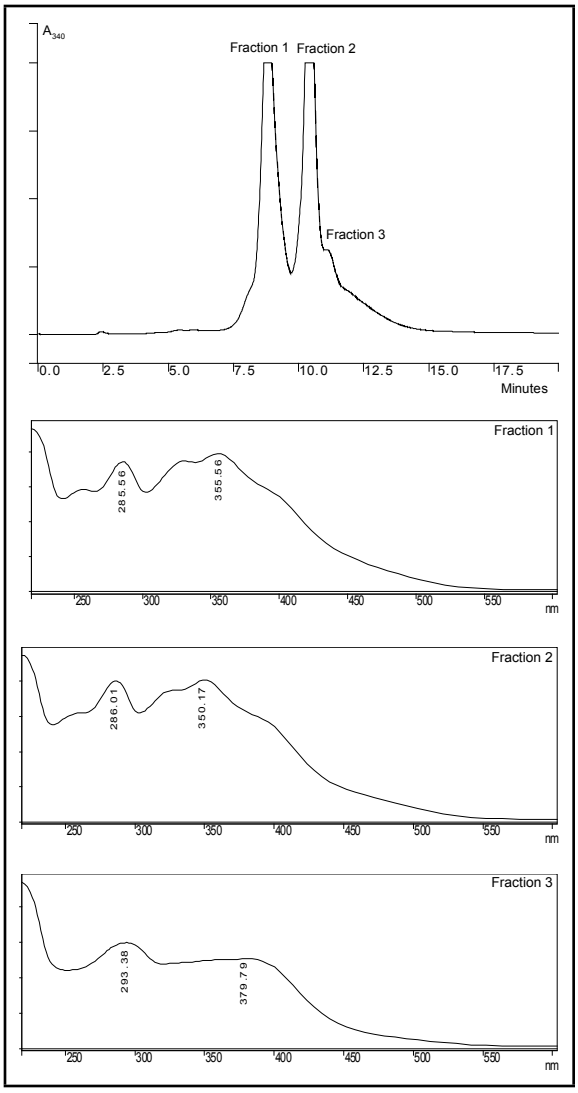


Figure 17

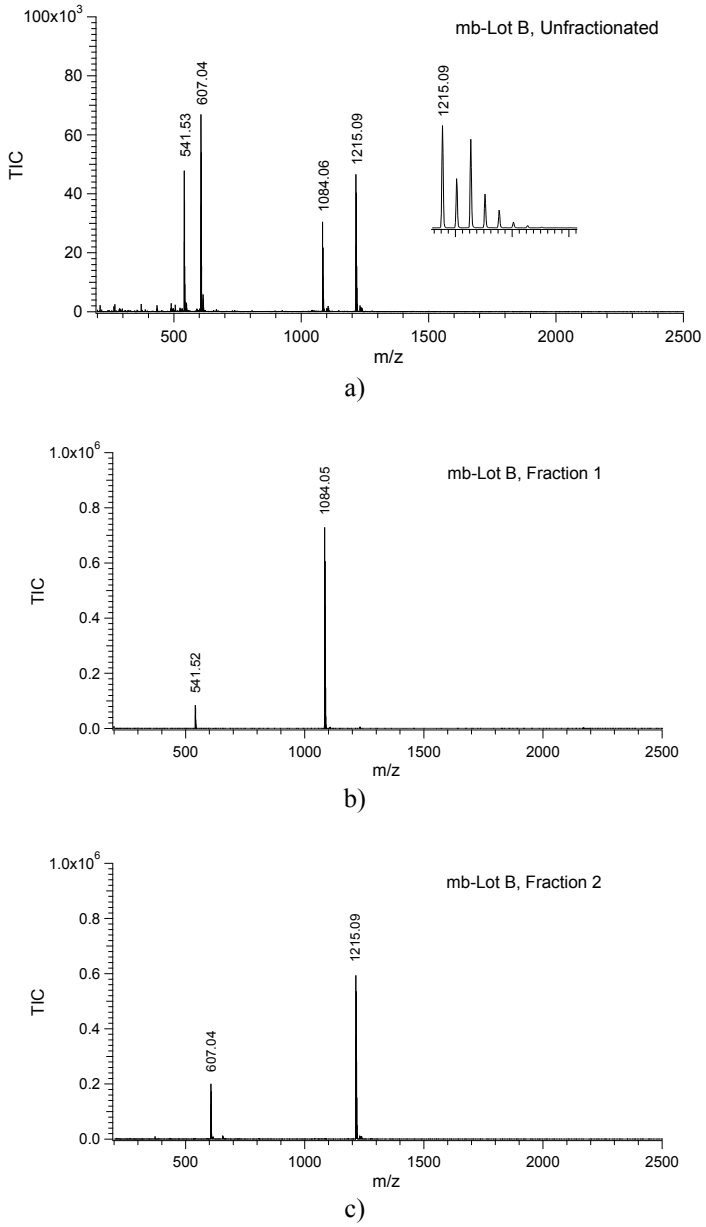


Figure 17: ESI-TOF mass spectra of HPLC fractions of methanobactin Lot B. The fractions were eluted from a Hamilton PRP-3 column using a 1% to 99% methanol gradient containing 0.001% acetic acid. The methanobactin samples was exposed to Cu(II) at a ratio of 0.7 Cu:mb prior to the HPLC run. **a**) The unfractionated sample, prior to the HPLC run, **b**) Fraction 1 from the HPLC run, and **c**) Fraction 2 from the HPLC run.

Figure 18

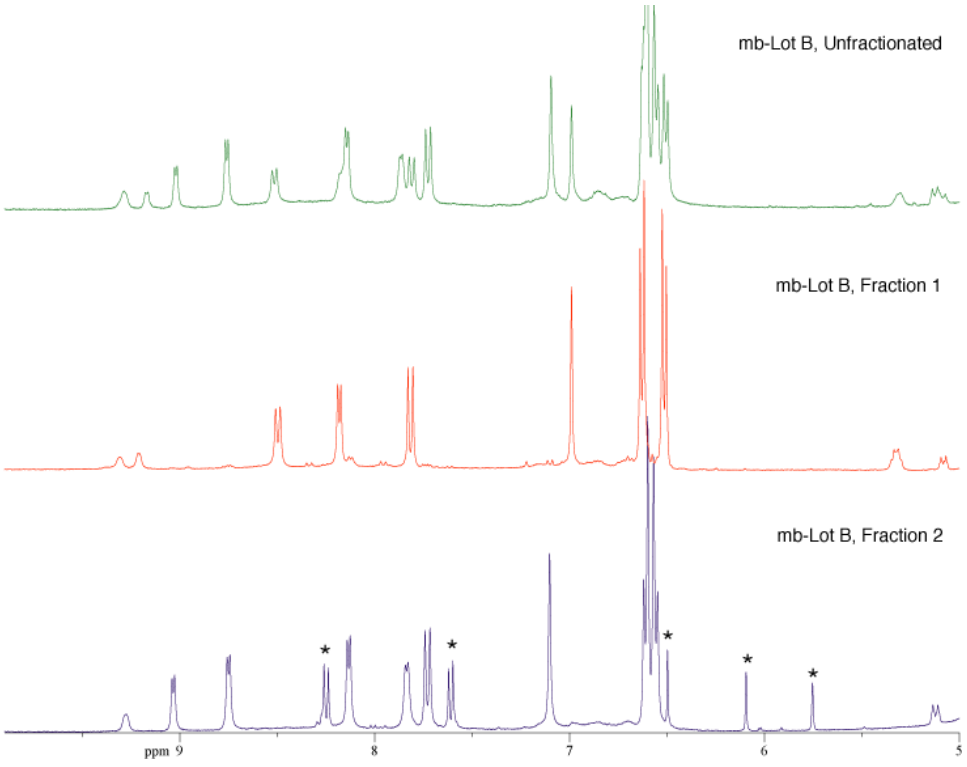


Figure 18: The ^{1}H spectra, focusing on the amide and aromatic region, of the HPLC fractions of methanobactin Lot B. The fractions are the same ones described in Figure 17 **Top**, the unfractionated sample, prior to the HPLC run, **Middle**, Fraction 1 from the HPLC run, and **Bottom**, Fraction 2 from the HPLC run. The resonances in Fraction 2 marked with stars arise from an identified contaminant that was picked up during rotavaporation.

Figure 19

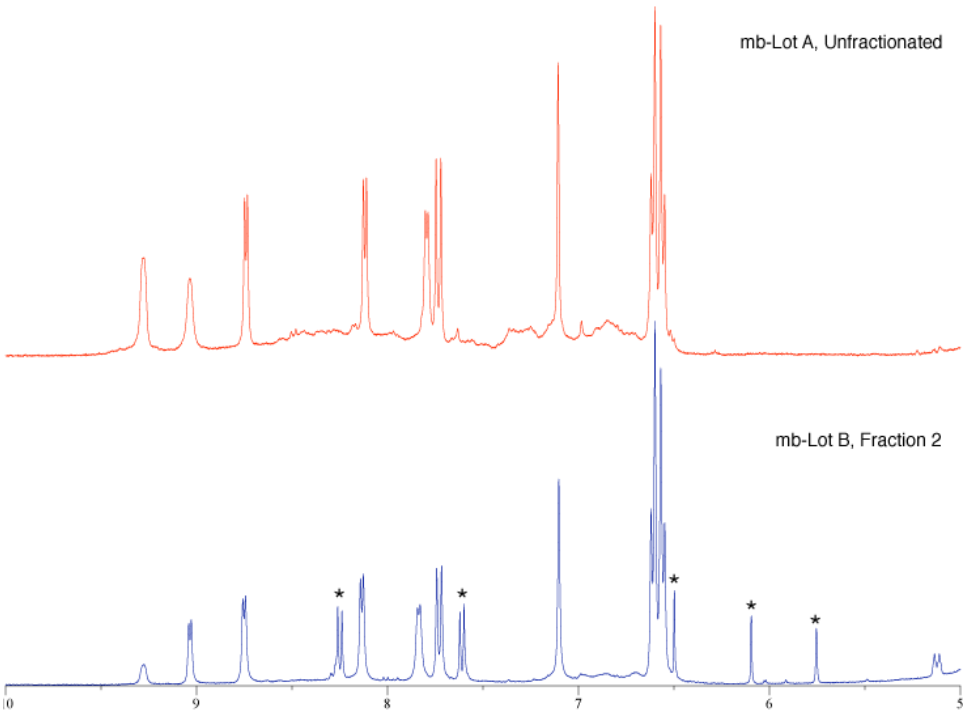


Figure 19: The ^{1}H spectra, focusing on the amide and aromatic region, Comparing mb-Fraction 2 from mb-Lot B with the unfractionated mb-Lot A. **Top**, The unfractionated mb-Lot A sample, **Bottom**, Fraction 2 from the HPLC run of mb-Lot B. The resonances marked with stars in the mb-Lot B Fraction 2 sample arise from an identified contaminant that was picked up during the rotavaporation.

Figure 20a

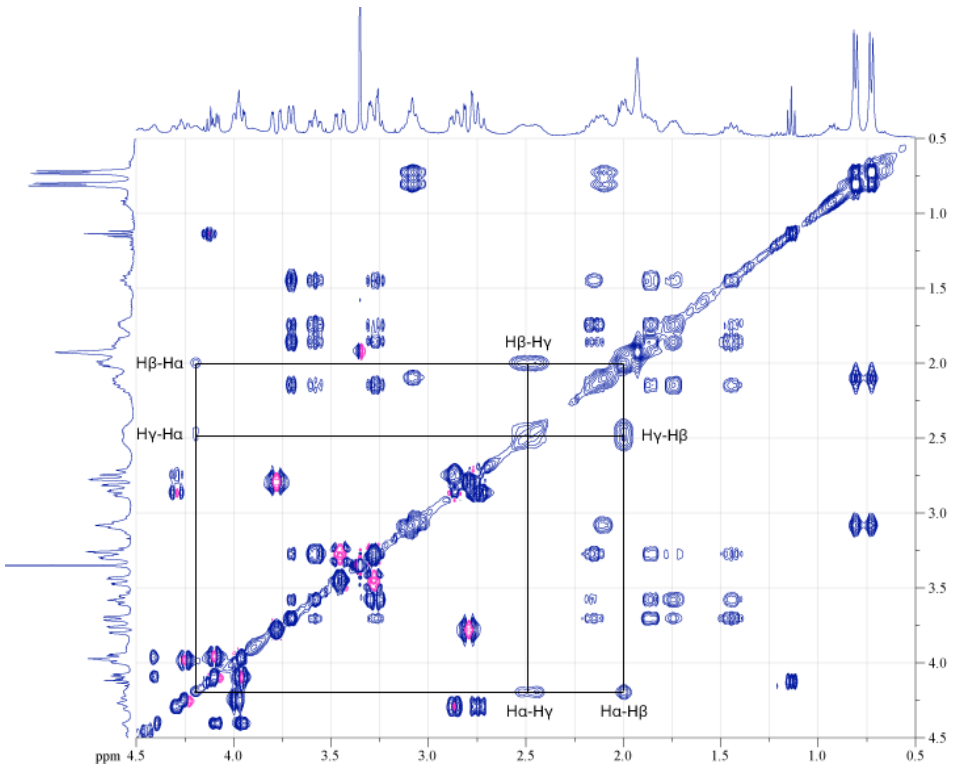


Figure 20a: The 400 MHz ^{1}H-TOCSY spectrum, focusing on the aliphatic region, for mb-Lot B, Fraction 2. The H_α, H_β, H_γ spin system for Met9 is labeled.

Figure 20b

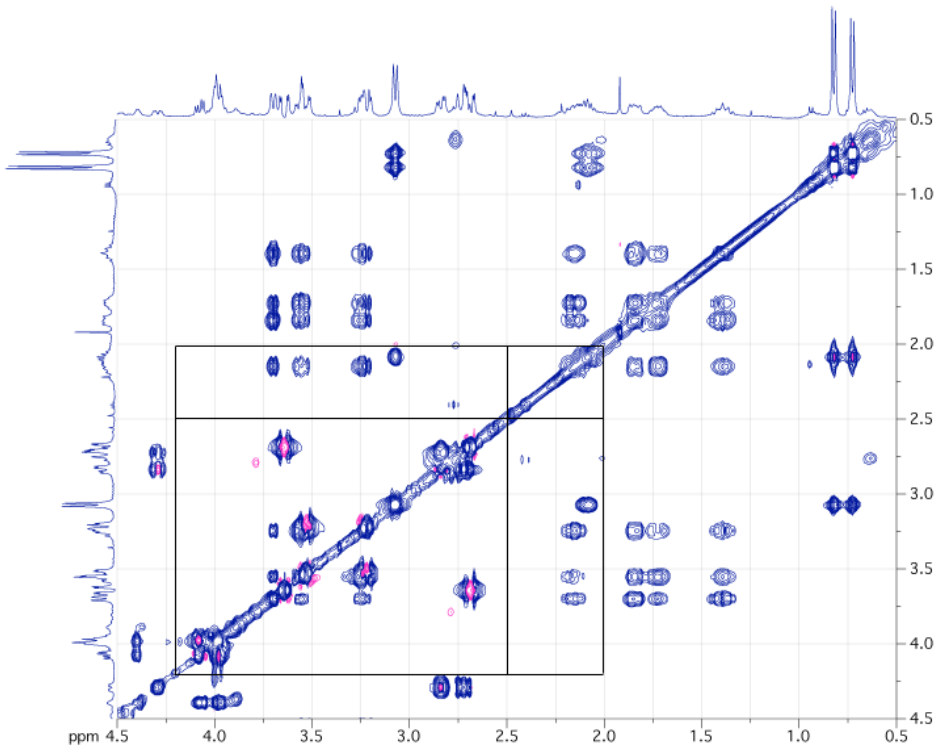


Figure 20b: The 400 MHz ^{1}H-TOCSY spectrum, focusing on the amide and aromatic region, for mb-Lot B, Fraction 1

Figure 21a

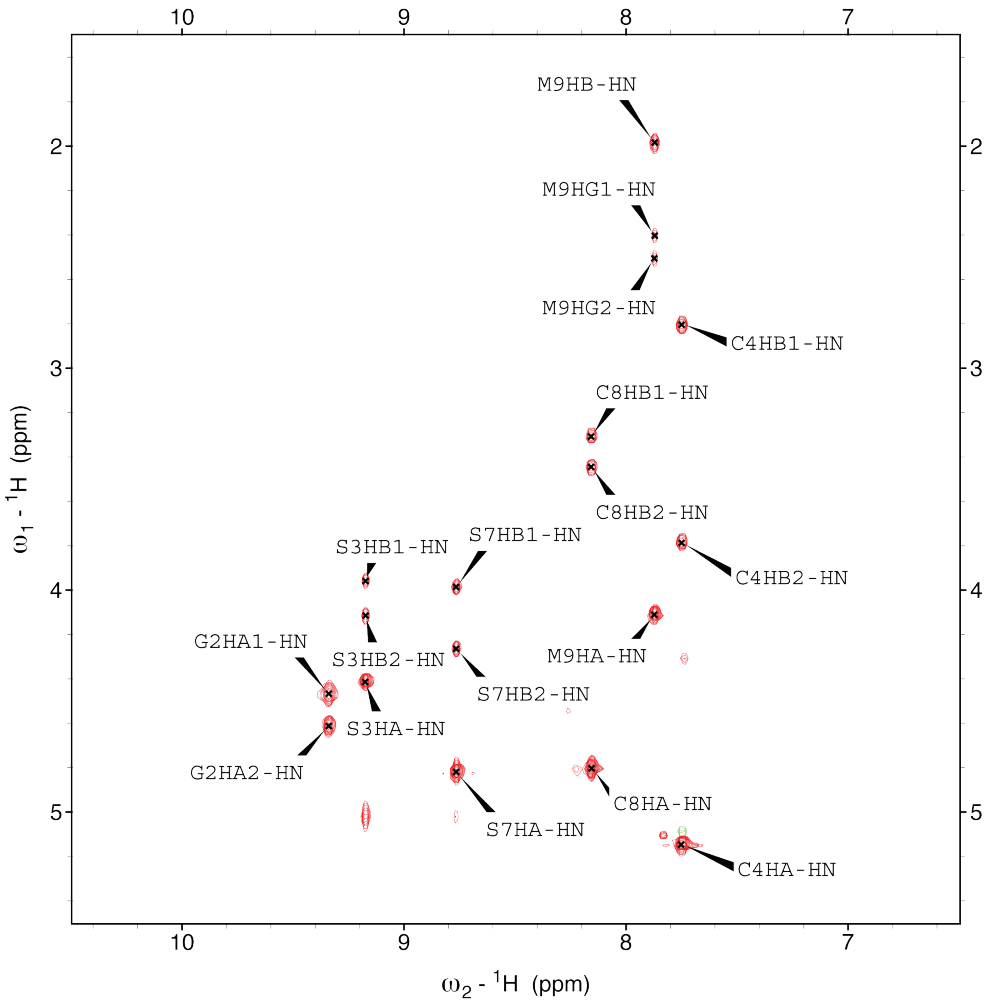


Figure 21a: The 600 MHz ^{1}H-TOCSY spectrum, focusing on the amide and aromatic region, for 6 mM mb-Lot A in 9 mM phosphate, 10% D_2O, *pH*6.5, 5°C. The sample was was titrated to 0.5 Cu:mb.

Figure 21b

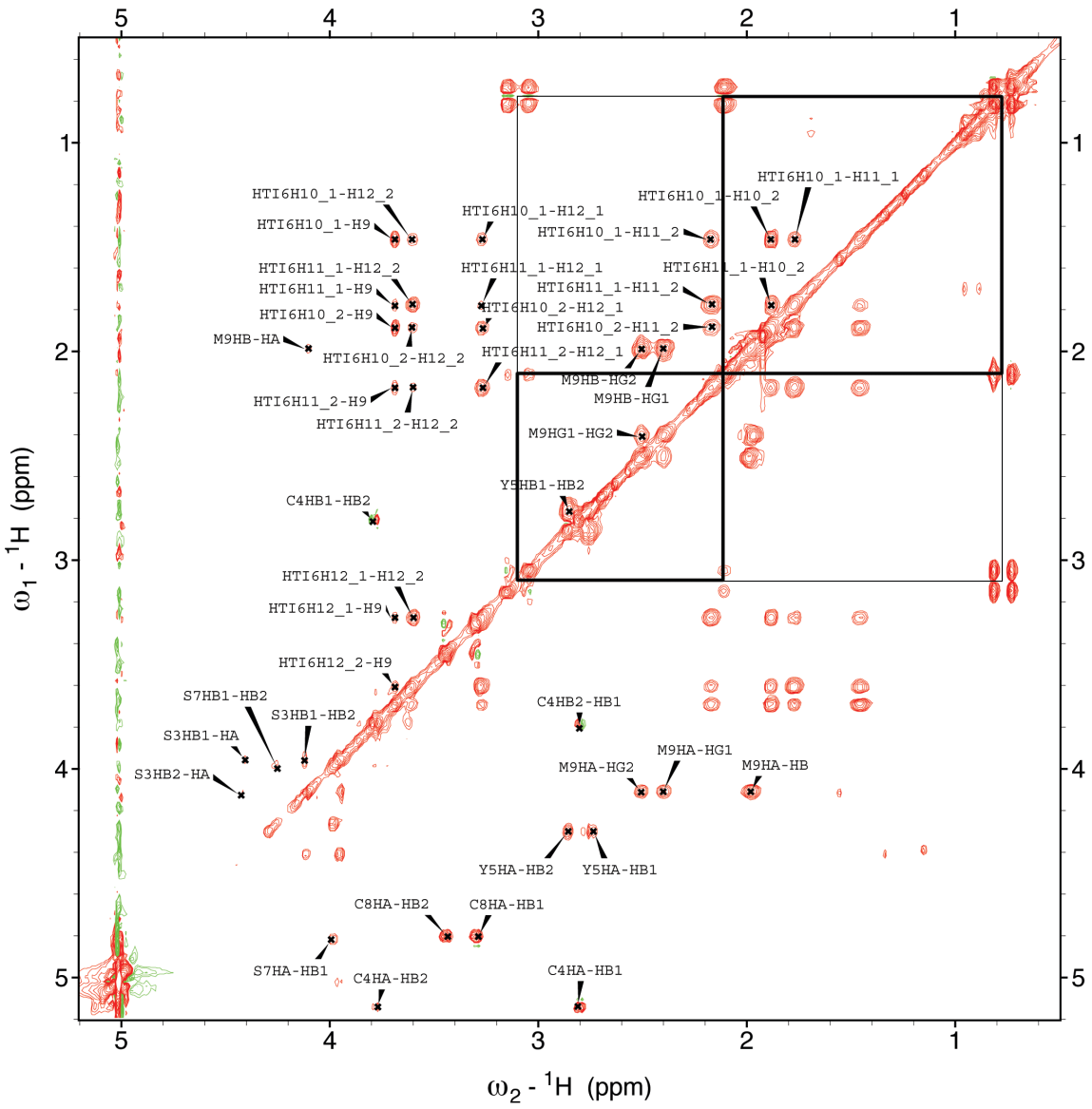


Figure 21b: The 600 MHz ^{1}H-TOCSY spectrum, focusing on the aliphatic region, for 6 mM mb-Lot A in 9 mM phosphate, 10% D_2O, *pH*6.5, 5°C. The sample was titrated to 0.5 Cu:mb. The boxes highlight an unassigned spin system that ostensibly should be arising from the isopropyl ester that is part of the THI1 residue.

Figure 22a

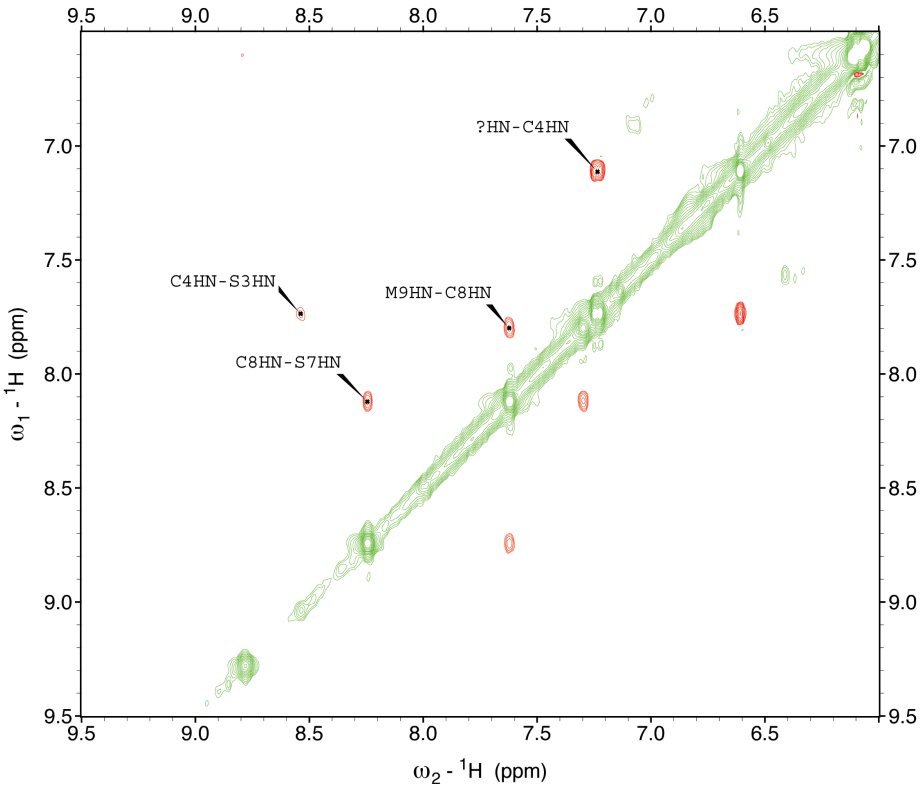


Figure 22a: The 400 MHz ^{1}H-ROESY spectrum, focusing on the amide/amide cross peak region, for 6 mM mb-Lot A in 9 mM phosphate, 10% D_2O, *pH*6.5, 27°C. The sample was titrated to 0.5 Cu:mb.

Figure 22b

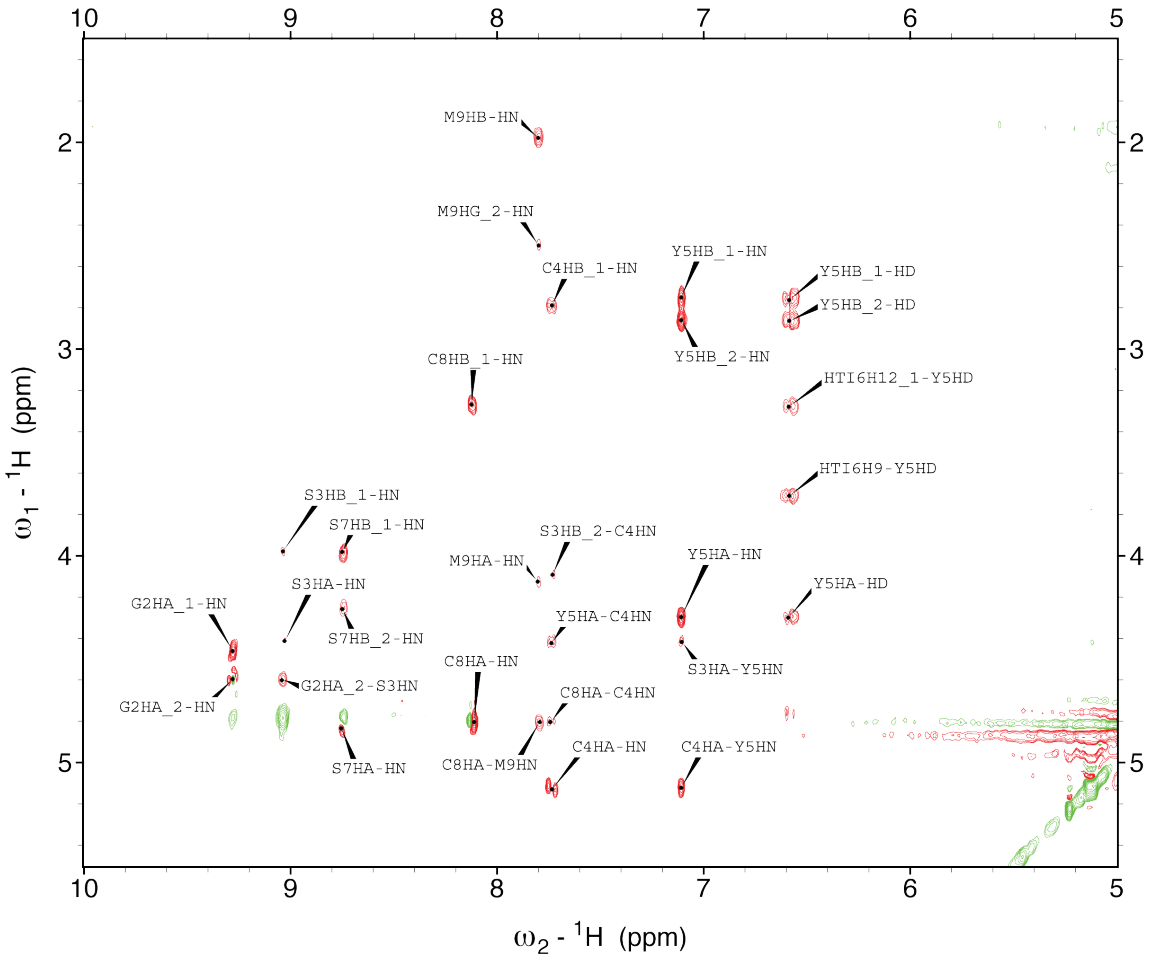


Figure 22a: The 400 MHz ^{1}H-ROESY spectrum, focusing on the amide and aromatic/aliphatic cross peak region, for 6 mM mb-Lot A in 9 mM phosphate, 10% D_2O, *pH*6.5, 27°C. The sample was titrated to 0.5 Cu:mb.

Table 1

Table 1: NOE's observed in the 400 MHz ^{1}H ROESY spectrum, focusing on the amide/aliphatic cross peak region, for 6 mM mb-Lot A in 9 mM phosphate, 10% D_2O, *pH*6.5, 27°C. The sample was titrated to 0.5 Cu:mb.

d(i,i+1)	***Tyr5 H_N***	***Hδ of Y5***
$d_{\alpha N}$*(G2,S3)*	$d_{\alpha N}$*(C4,Y5)*	$d_{\alpha\delta}$*(Y5,Y5)*
d_{NN}*(S3,C4)*	$d_{\alpha N}$*(S3,Y5)*	$d_{\beta\delta}$*(Y5,Y5)*
$d_{\beta N}$*(S3,C4)*	$d_{\alpha N}$*(Y5,Y5)*	$d_{9\delta}$*(HTI,Y5)*
$d_{\alpha N}$*(C4,Y5)*	$d_{\beta N}$*(Y5,Y5)*	$d_{12\delta}$*(HTI,Y5)*
d_{NN}*(S7,C8)*		
d_{NN}*(C8,M9)*		
$d_{\alpha N}$*(C8,M9)*		

Figure 23

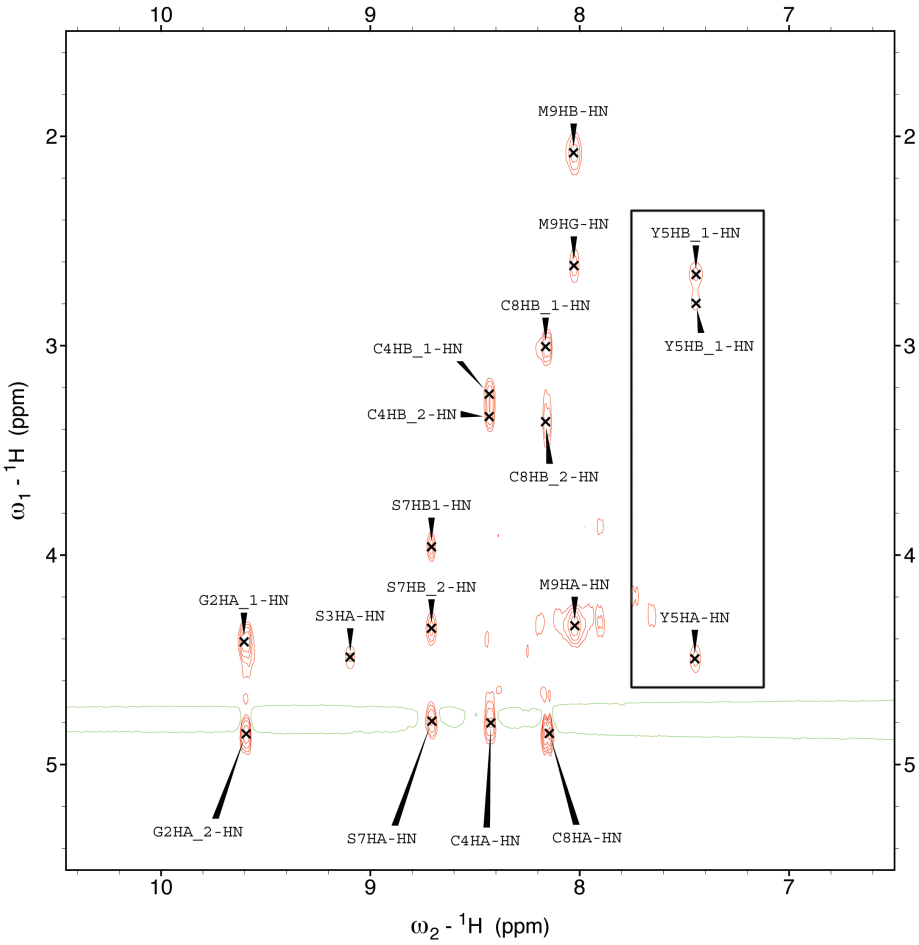


Figure 23: The 400 MHz ^{1}H-TOCSY spectrum, focusing on the amide and aromatic region, for 4 mM mb-Lot A in 9 mM phosphate, 10% D_2O, *pH*6.5, 25°C. The sample was not complexed with Cu(II).

Figure 24

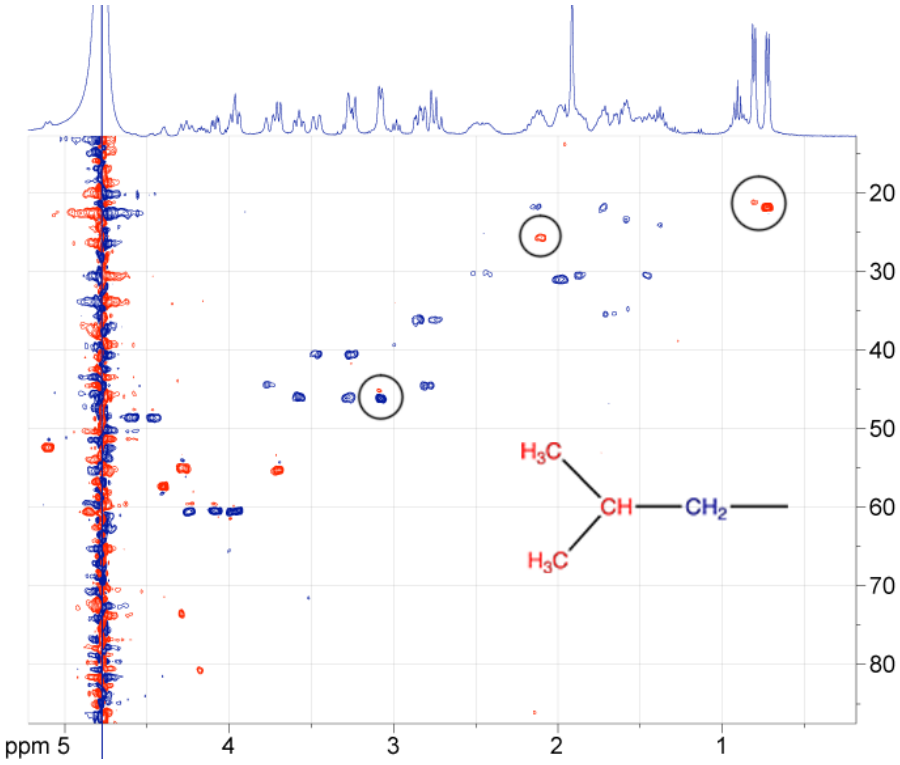


Figure 24: The 400 MHz multiplicity-edited ^{1}H-^{13}C-HSQC spectrum for 3 mM mb-Lot A in 9 mM phosphate, 10% D_2O, *pH*6.5, 25°C. The sample exposed to 1.6 Cu:mb prior to isolation by HPLC. The cross peaks for the proposed isobutyl group are circled.

Figure 24

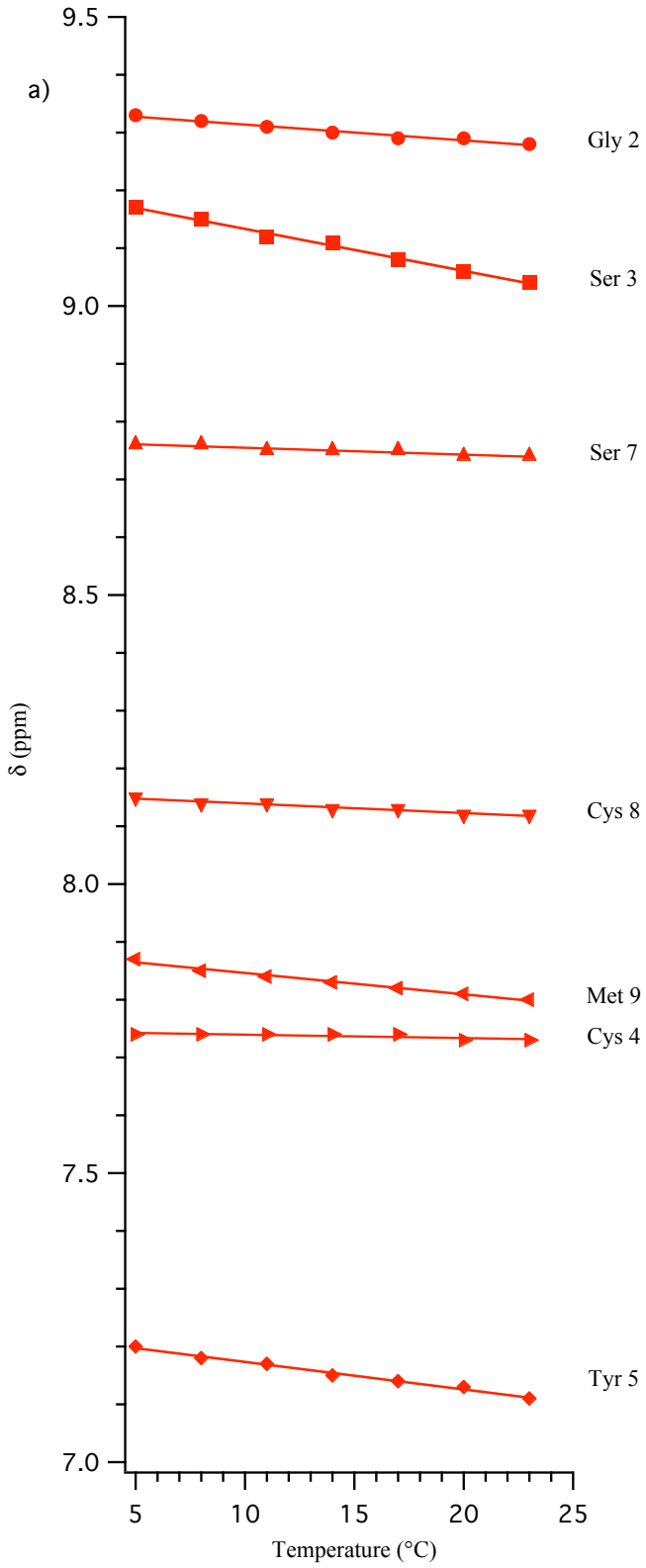


Figure 24: The temperature dependence of the chemical shifts for the peptide H_N's. Spectra were collected at 600 MHz from 5°C to 23°C. on a 6 mM sample of methanobactin Lot a dissolved in 9 mM phosophate, *pH*6.5. **a**) δ *vs T* plots. **b**, The Δδ/Δ*T* values for each residues peptide H_N. **c**) The J^3-value for each peptide H_N.

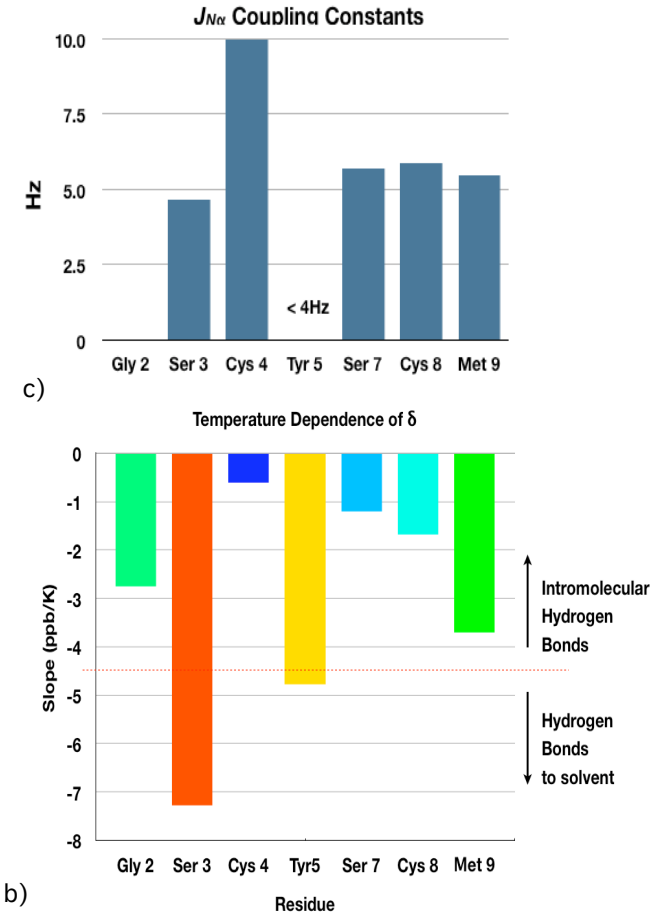


Table 2

Table 2: The backbone torsion angles for methanobactin based the crystal structure.{Kim, 2004 #2}The bonds that are in the 20 atom ring formed by the disulfide bond between the side chains of Cys4 and Cys8 are shaded.

	$^3J_{HN\text{-}H\alpha}$	ϕ	φ	ω
Gly2		69	-158.3	-173.3
Ser3	4.7	-82.4	9.4	167.4
Cys4	10	-119.3	25.4	-172.2
Tyr5		-65.2	132.5	-7.1
HTI6				-163.7
Ser7	5.7	-90.8	1.1	174.6
Cys8	5.9	-65.9	-16.3	175.8
Met9	5.5	-86.4		

Figure 25

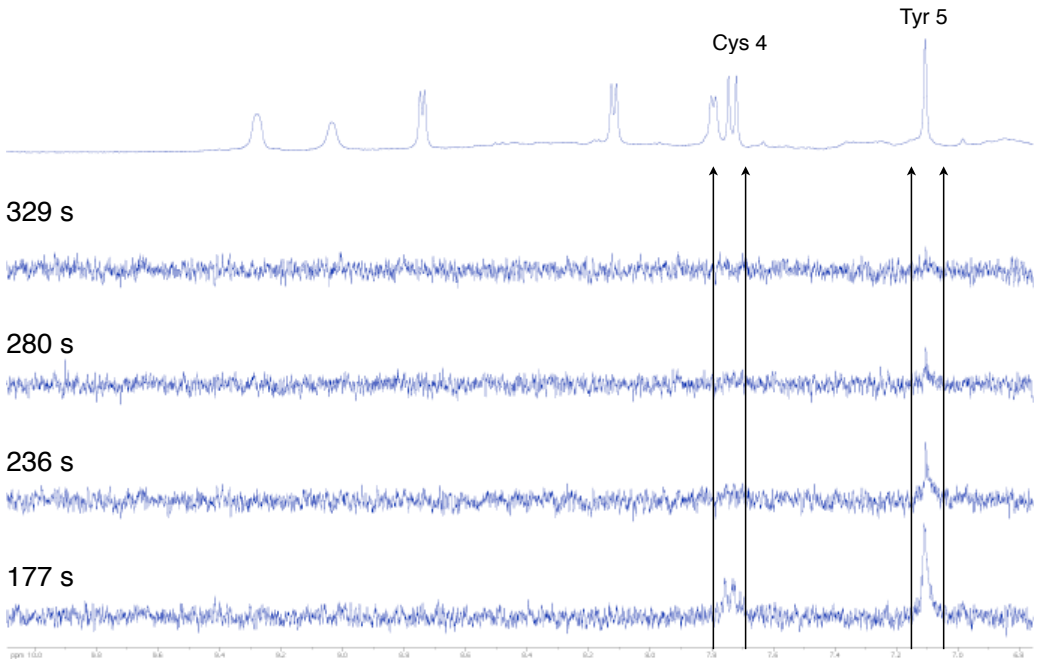


Figure 25: Hydrogen/deuterium exchange from methanobactin. A sample of mb-Lot A in 9 mM phosphate, *pH*6.5 was lyophilized and dissolved in D_2O. 400 MHz ^1H spectra were collected from 177 s to 329 s after the sample dissolved.

Figure 27

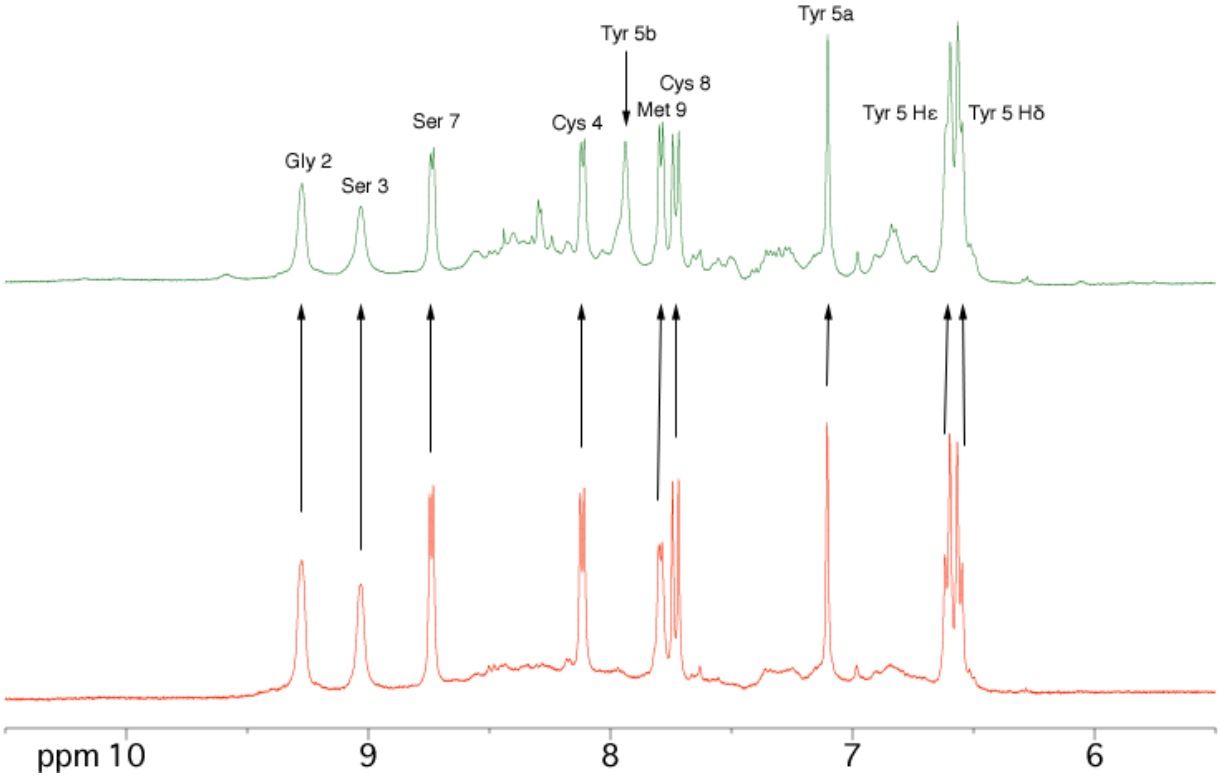
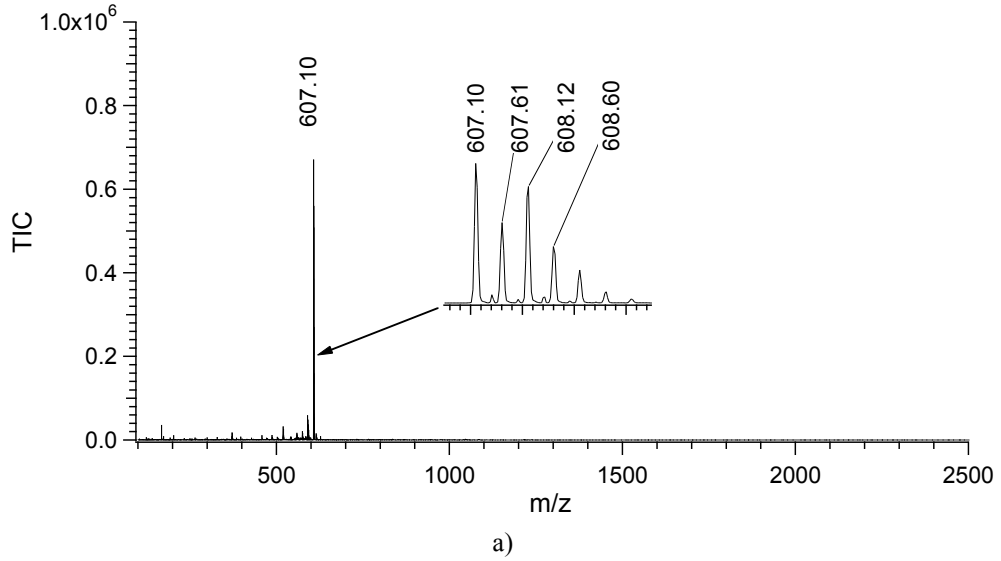


Figure 27: 400 MHz ^{1}H-spectra of methanobactin. **Bottom,** Lot A in 9 mM phosphate/10% D_2O, *pH*6.5, 25°C., complexed with Cu(II) at 0.5 Cu:mb (same spectrum as shown in Figure 10, top). **Top,** EDTA treated methanobactin.

Figure 28



a)

Figure 28: ESI-TOF mass spectra in negative ion mode of methanobactin after exposure to Cu(II) and treatment with EDTA to remove the copper.