Supporting Information

NMR-Based Mapping of Disulfide Bridges in Cysteine-Rich Peptides: Application to the μ-Conotoxin SxIIIA

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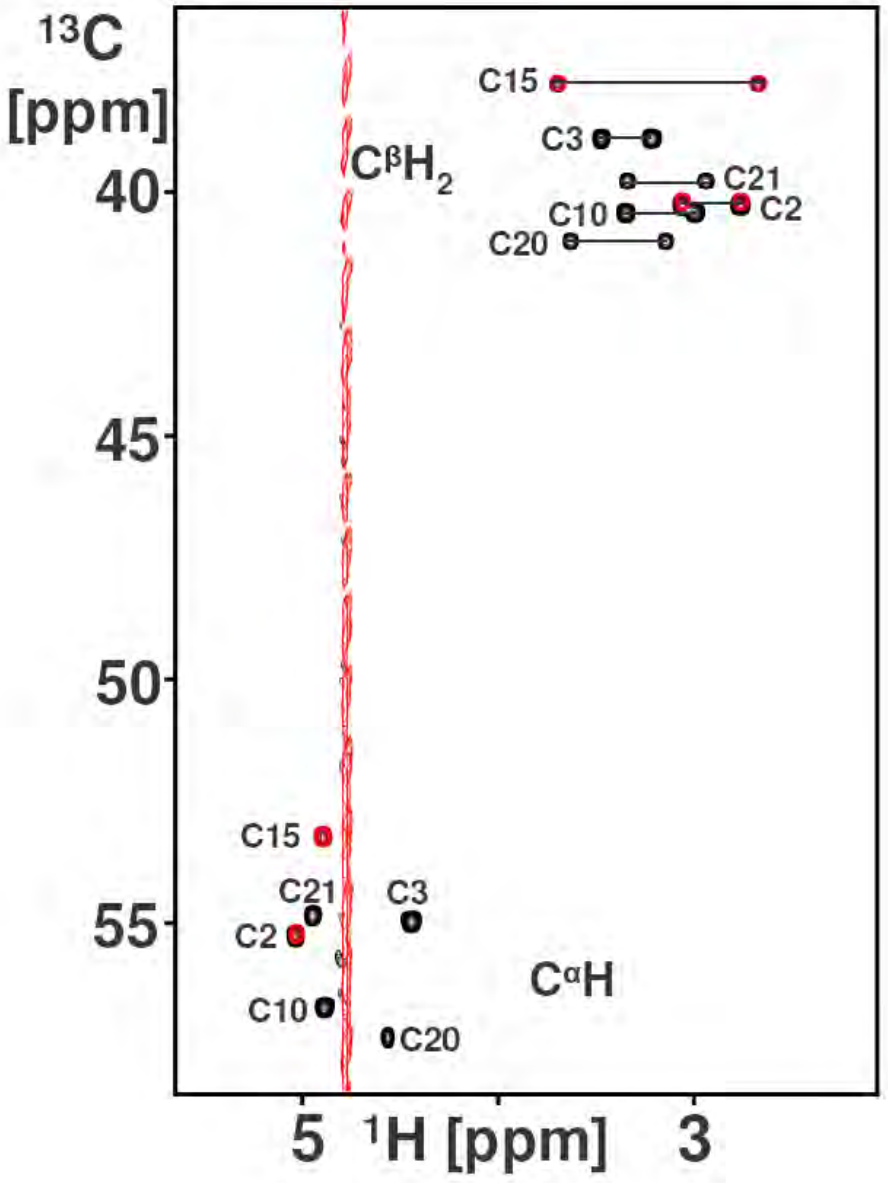
Table S1. Chemical Shifts for (^{15}N,^{13}C-Cysteine)$_6$ μ-SxIIIA.

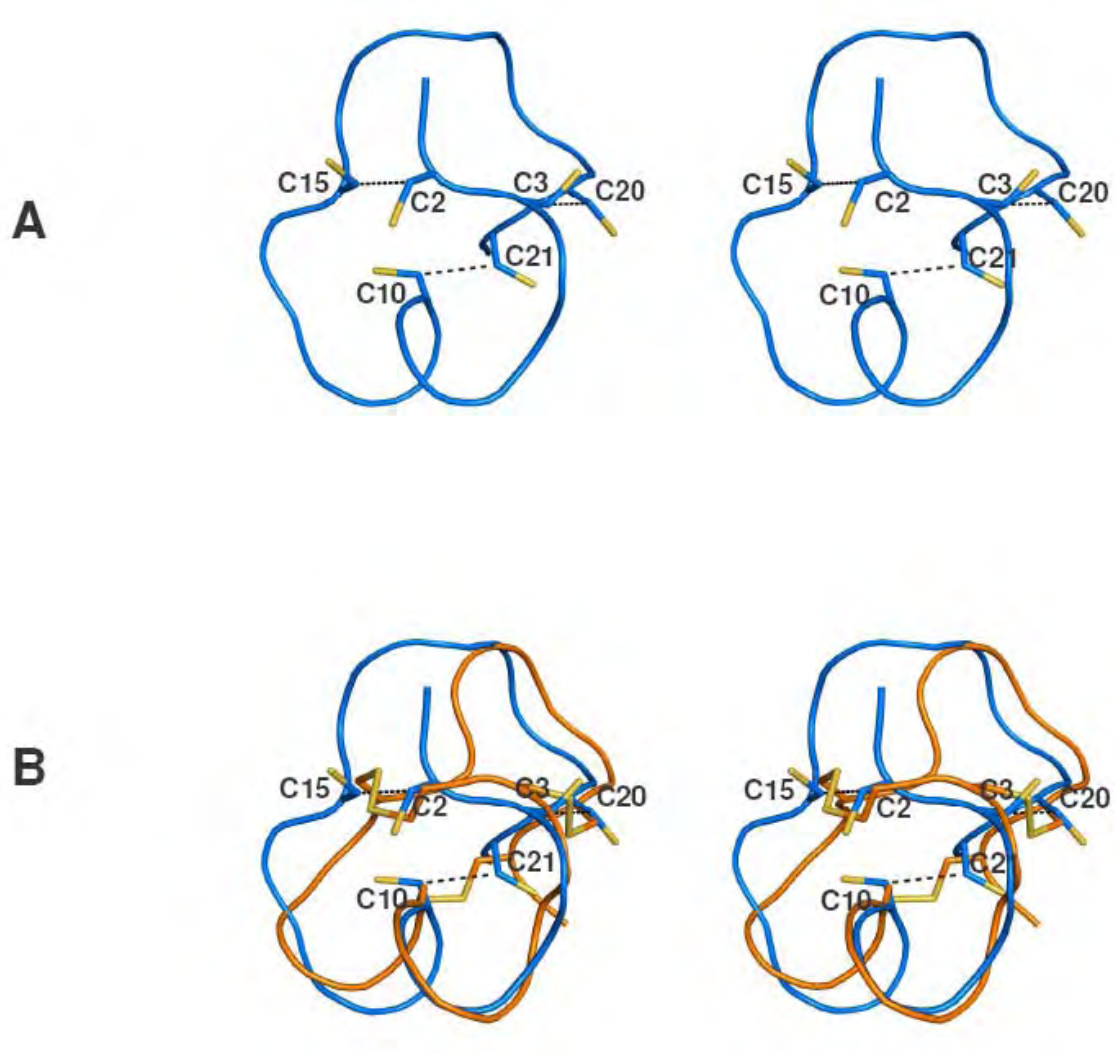
Residue	**N, H^N**	**C^α, H^α**	**C^β, H^β, H^β**
Cys 2		55.3, 5.04	40.2, 2.73, 3.04
Cys 3	114.4, 8.37	55.0, 4.45	38.9, 3.20, 3.45
Cys 10	119.1, 8.70	56.7, 4.88	40.4, 2.97, 3.34
Cys 15	113.8, 7.55	53.2, 4.89	37.8, 2.65, 3.69
Cys 20	113.8, 8.38	57.3, 4.58	41.0, 2.64, 3.61
Cys 21	119.8, 7.71	54.9, 5.20	39.7, 2.92, 3.33

Figure S1



Supplemental Figure 1. Overlaid 2D [^{13}C,^{1}H] HSQC spectra of C2,C3,C10,C15,C20,C21 ^{15}N-,^{13}C-enriched μ-SxIIIA (black) and C2,C15 μ-SxIIIA (red). Note the superposition of CH^{α} and CH^{β} signals for C2 and C15 and consistent resonance assignments.

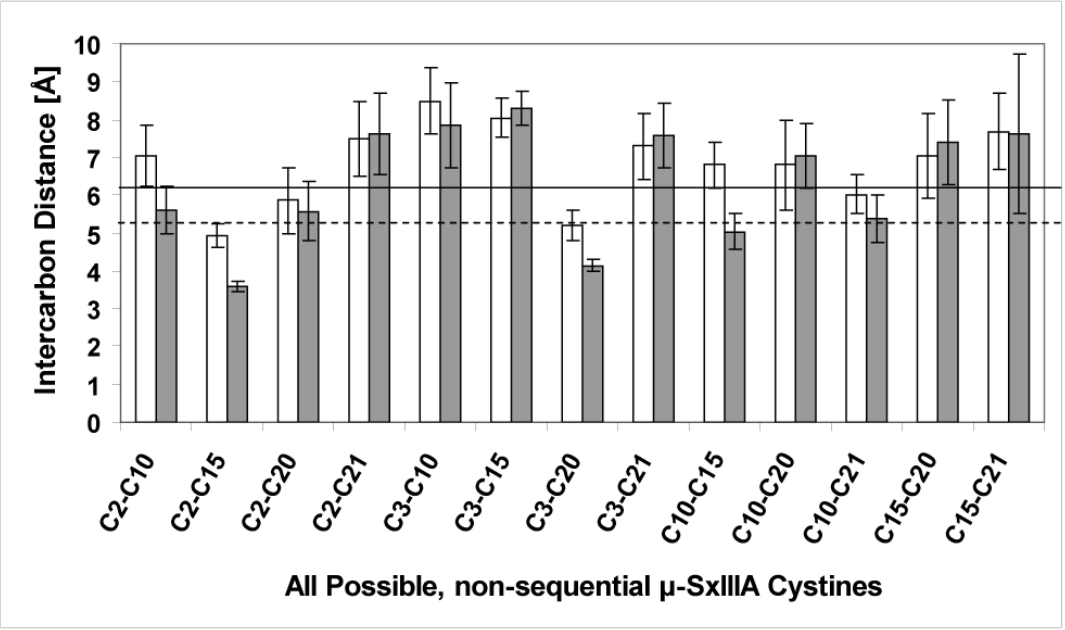
Figure S2



Supplemental Figure 2. Stereoview of a representative µ-SxIIIA structure calculated from the twelve NOEs listed in Figure 5 legend. The C2-C15, C3-C20, and C10-C21 cystines are defined with six, five, and one NOE(s), respectively. **Part A** is a stereo representation of the most abundant conformer from our fold calculations. Cysteines are shown with side chain heavy atoms and labeled with residue number. **Part B** shows the

same molecule superimposed on the cysteine heavy atoms of μ-SmIIIA coordinates (1Q2J.pdb). μ-SmIIIA is shown with an orange cartoon and the cysteine side chains are shown.

Figure S3



Supplemental Figure 3. Bar graph showing the average C^{α}-C^{α} (white) and C^{β}-C^{β} (grey) distances for all possible cystines in ten calculated μ-SxIIIA structures. Error bars show one standard deviation. Most cystines have a C^{α}-C^{α} distance of 5.2 Å (dashed line) for right-handed χ3 and 6.1 Å (solid line) for left-handed χ3 and a C^{β}-C^{β} distance of 2.9 – 4.6 Å with a most prevalent distance of 3.8 Å in proteins (Richardson DC, Richardson JS.

Principles and Patterns of Protein Conformation. In Prediction of Protein Structure and the Principles of Protein Conformation, G.D. Fasman, Ed., 1 ed. New York: Plenum Press (1989)1-98.). These criteria alone clearly identifies the C2-C15 and C3-C20 cystines. The remaining C10-C21 cystine has a C^{α}-C^{α} distance within the expected range however the C^{β}-C^{β} distance is too long; this can be explained by the absence of C21 $H^{\beta 2/3}$ constraints and by the fact that only one NOE defines the cystine. All other possible cystines have too large of C^{α}-C^{α} and/or C^{β}-C^{β} distances. From this structural exercise, we conclude that calculation of a family of NMR structures using this sparse set of NOEs is sufficient to unambiguously define the cystine pattern in µ-SxIIIA.