

Supplementary Information

Modeling of Cu(II)-based Protein Labels Using Rotamer Libraries

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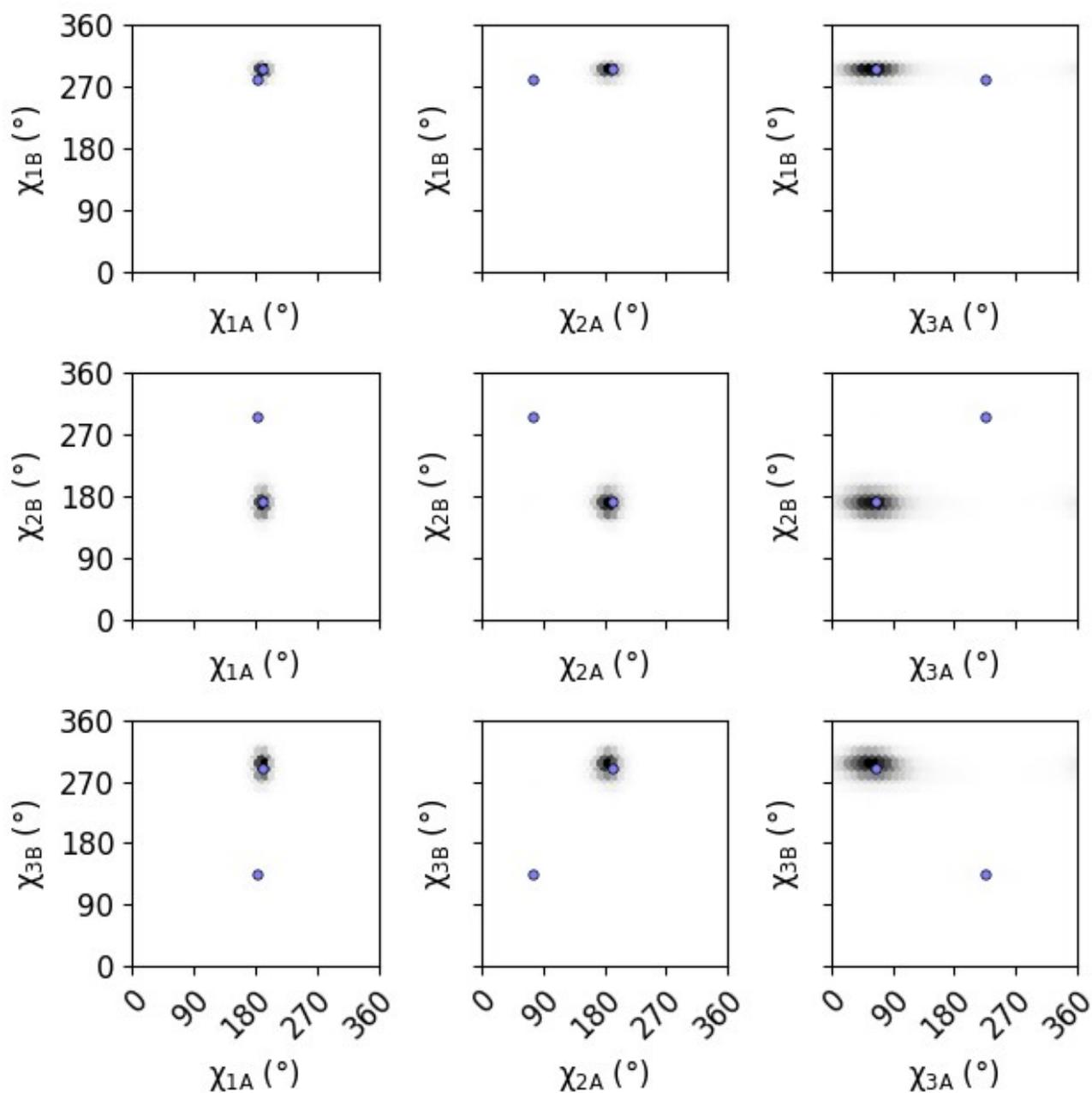


Figure S1. Side chain dihedral plots for α -helix context MD simulations and rotamer libraries. MD simulation data are plotted as hex-bin histograms over all frames in the helical context. Rotamer library elements are plotted as blue circles. Dihedral definitions are the same as those defined in Figure 1.

We first consider the dihedral angles at the three α -helical sites. Figure S1 plots the distributions of dihedral angles from the MD simulations. The plot shows that the MD simulations for the α -helix have the mean and standard deviations of $\chi_{1A} = 181^\circ \pm 9^\circ$,

$\chi_{2A} = 182^\circ \pm 24^\circ$, and $\chi_{3A} = 63^\circ \pm 35^\circ$ for the first histidine residue, while the second residue conforms to $\chi_{1B} = 293^\circ \pm 9^\circ$, $\chi_{2B} = 187^\circ \pm 28^\circ$, and $\chi_{3B} = 295^\circ \pm 29^\circ$. These angles are within expectation based on the energetically favorable rotamers of trans (t, $\sim 180^\circ$), gauche⁺ (g⁺, $\sim 60^\circ$), and gauche⁻ (g⁻, $\sim 300^\circ$). Furthermore, the standard deviations in χ_1 and χ_2 are consistent with the deviations observed in natural side chains and nitroxide labels¹⁻⁴. This observation indicates that the rigidity of dHis-Cu(II) does not come from the deviations of the dihedral angles. Instead, the rigid conformational space originates from the fact that histidine residues have a smaller number of dihedral angles than common nitroxide labels. Furthermore, the accessible χ_1 and χ_2 for the histidine residues in the dHis-motif are further limited by the bifunctionality of the dHis-Cu(II) label. Figure 2 primarily shows one conformation for each of χ_1 and χ_2 in dHis-Cu(II), whereas a canonical histidine side chain can access all three conformations of t, g⁻, and g^{+1,2}. Overall, the dihedral angles of dHis-Cu(II) show homogeneous conformations for the different α -helix dHis-Cu(II) MD rotamers.

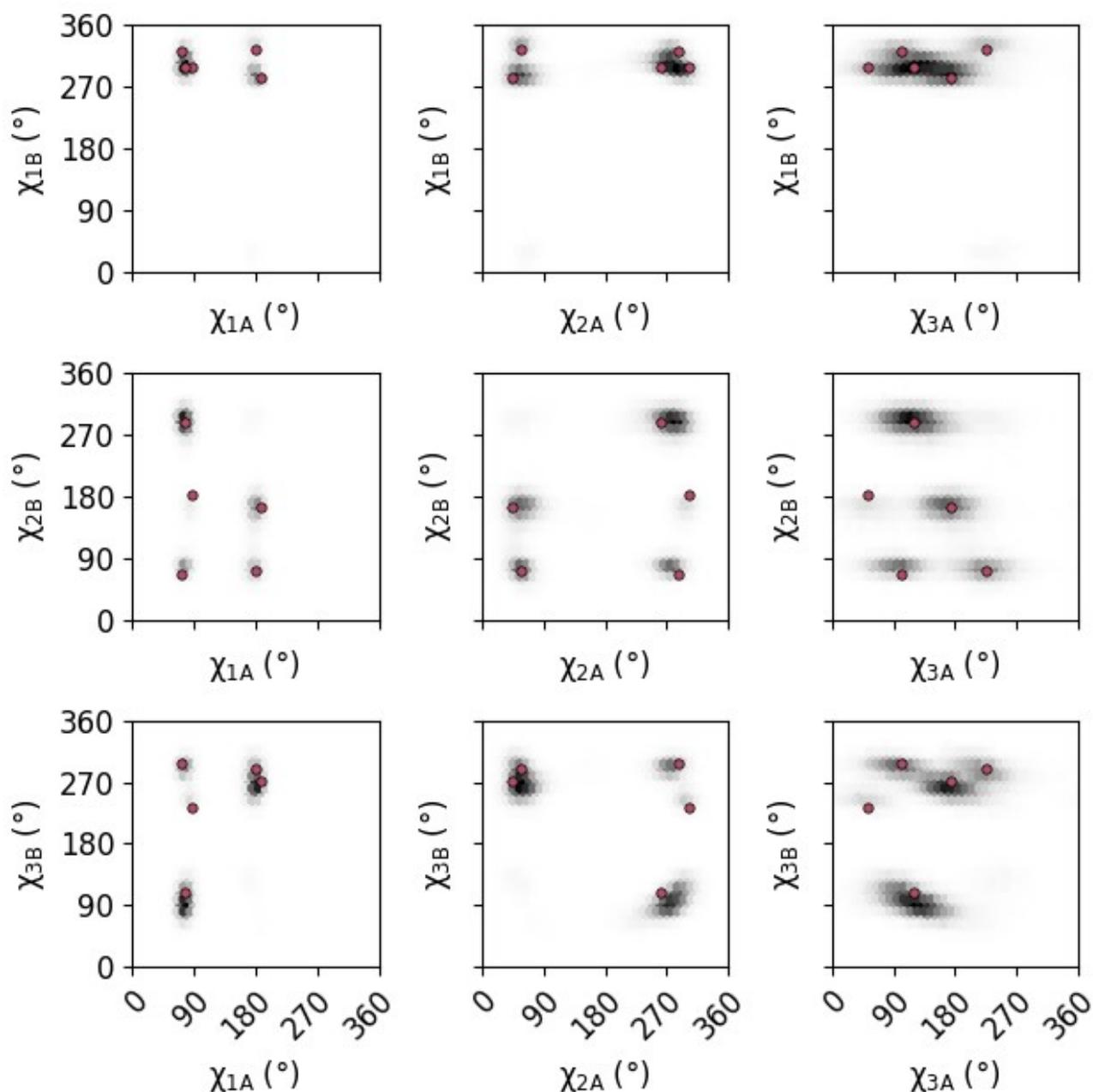


Figure S2. Side chain dihedral plots for β -strand context MD simulations and rotamer libraries. MD simulation data are plotted as hex-bin histograms over all frames in the strand context. Rotamer library elements are plotted as red circles. Dihedral definitions are the same as those defined in Figure 1.

In contrast to α -helix, dHis-Cu(II) on β -sheet sites primarily show more variations in the conformation of the label. Figure S2 plots the distributions of dihedral angles from the MD simulations of β -sheet dHis-Cu(II). For the first histidine residue, Figure S2

primarily shows two conformations [χ_{1A} , χ_{2A}] of [g+, g-] and [t, g+] with χ_{3A} covering a large range between 60° to 250°. The large range of χ_{3A} seems to reflect a highly flexible coordination environment of Cu(II) for dHis-Cu(II)-NTA in the context of β -sheet sites. On the other hand, the second histidine conforms to three different conformations [χ_{1B} , χ_{2B}] of [g-,t], [g-,g-], and [g-,g+] with χ_{3B} being either g- or g+. However, despite the large variations in the dihedrals of dHis-Cu(II)-NTA on β -sheets, the different rotamers lead to a similar Cu(II) ion positioning, depicted in Figure 2.

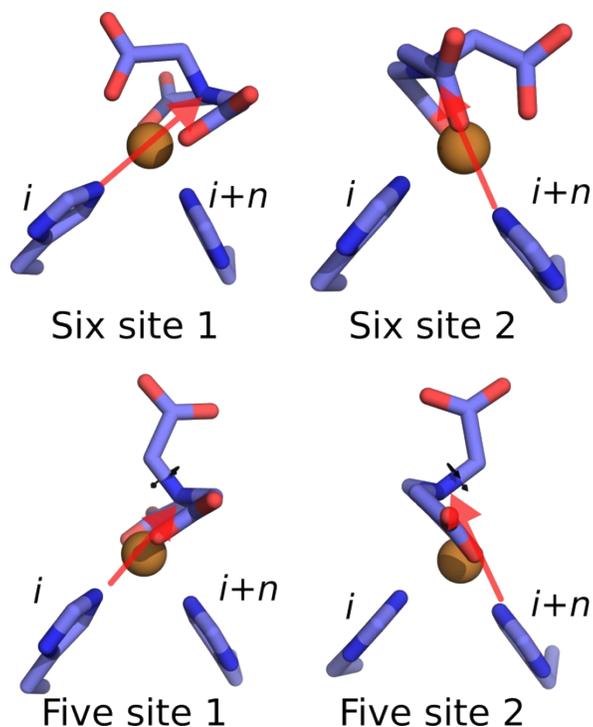


Figure S3. CREST coordination geometry starting structures.

Movie S1: Movie of the modeling of dHis-Cu(II) label using the bifunctional label modeling procedure in chiLife, available at <https://youtu.be/so6YIsietO8>

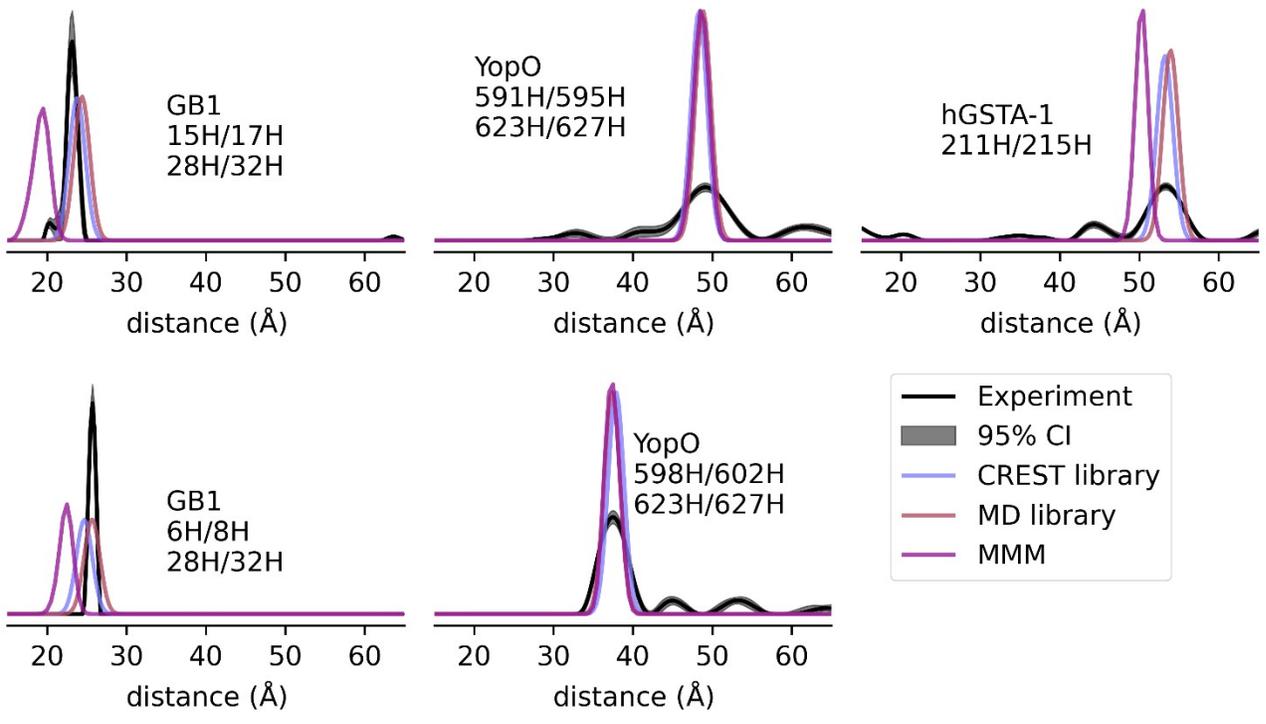


Figure S4. Comparison of predicted and experimental distance distributions.

Bibliography

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