# Characterizing Structural Heterogeneity in Computationally Designed Miniproteins

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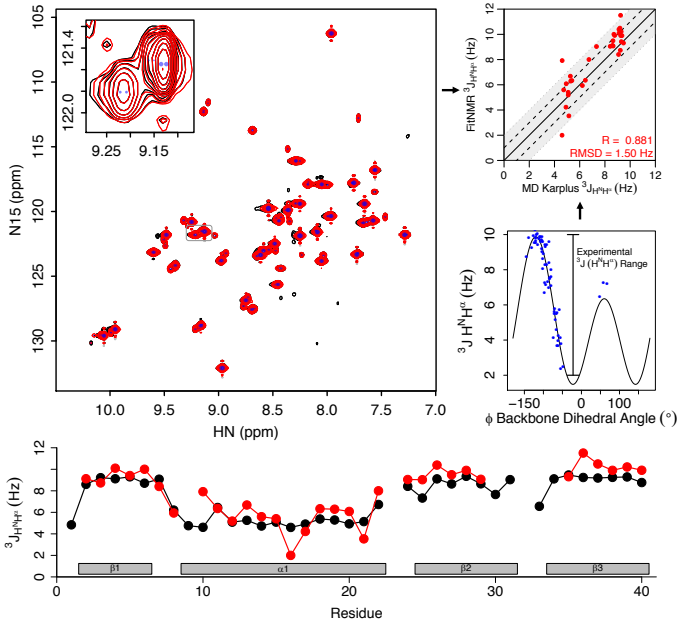
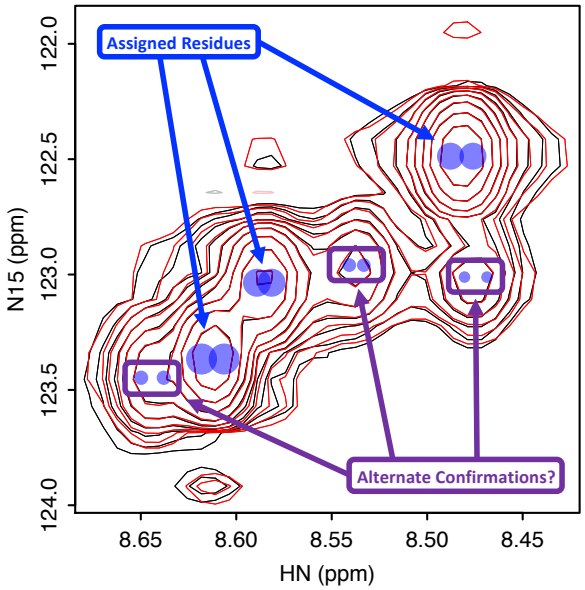
## Introduction

Compared to small molecules, *de novo* Rosetta-designed miniproteins have the potential to dock to the surface of other proteins with higher target specificity. Previous work has shown success of such designs, delivered by nasal spray in mice, in treating botulism and influenza, though a more thorough understanding of their structure and dynamics is needed. Here we use Nuclear magnetic resonance (NMR) techniques to investigate one example, EHEE_rd2_0005. A new method, FitNMR, was used to fit a $^{15}$N relaxation series obtained for this miniprotein and has shown the ability of the algorithm to resolve underlying scalar couplings which were overlooked by other methods. The scalar couplings agree with the designed secondary structure but peak doubling, also observed in these spectra, may be suggestive of structural heterogeneity, dynamic exchange, or a combination of each. Variable temperature HSQC studies have suggested the protein undergoes a global unfolding event upon heating, and a molecular dynamics simulation shows a tryptophan flip, either of which may cause the peak doubling. Current work is aimed at trapping specific states through heat shocking and separation via Size Exclusion Chromatography (SEC). The species present in the chromatogram are being studied by HSQC to map changes in peak intensities and positions to further explain the features present in the NMR spectra. We hope this work will insight the design of miniproteins as therapeutics to ensure the intended functions are carried out in the cell.

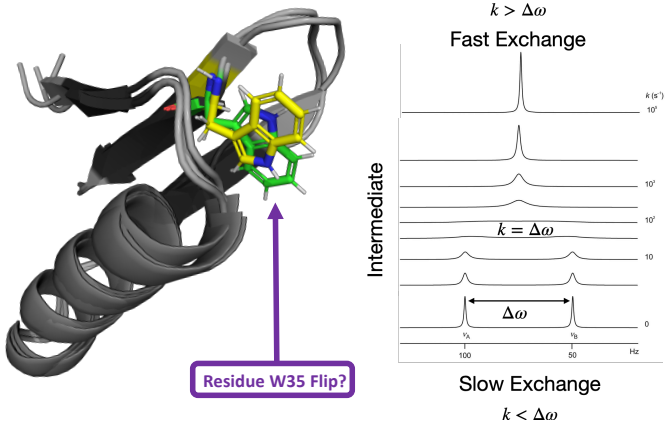
## Application I: Extracting Dihedral Angles from Ordinary HSQCs



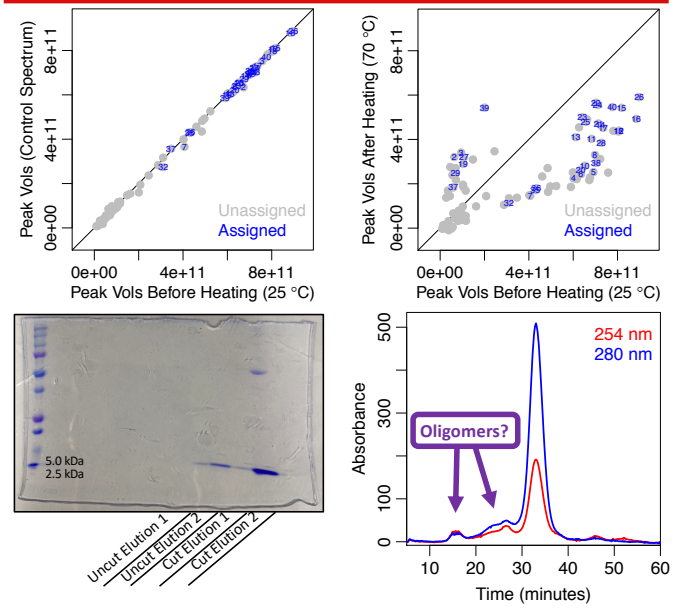

## Peak Doubling Suggests Alternate Confirmations




## Alternate Confirmation Hypothesis I: Exchanging States?




## Alternate Confirmation Hypothesis II: Oligomerization?




## Conclusion

The current implementation of FitNMR supports a wide range of applications in part due to this description of non-ideal data. Users may tailor their peak fitting to take advantage of properties shared by peaks (chemical shifts, line shapes, etc.) both within and between spectra with simultaneous peak fitting. The algorithm identified satellite peaks in the relaxation data set which may be suggestive of dynamics, namely the exchanging of states due to the W35 residue flip or potential oligomerization occurring in solution. The contribution of each event has yet to be attributed to the peak doubling observed in each increment of the relaxation series.

## Future Directions

Dynamics within the miniprotein will be probed on the slow timescale by ZZ-Exchange Spectroscopy at an elevated temperature. CPMG spectroscopy will investigate fast timescale motions. The SEC method will be further optimized to separate oligomeric states in solution. After separation, HSQC spectra will be collected for each species observed in the chromatogram to monitor changes in peak volumes and positions.

## Acknowledgments

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