# Solid State NMR Structural Characterization of MAX8 Peptide Nanofibers

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## Introduction

The peptide MAX8 (VKVKVKVKV$^{D}$PPTKVEVKVKV-$NH_2$) was designed by Schneider *et al.* to self-assemble controllably in order to form a 3-dimensional support structure for implanted stem cells and cell cultures. The peptide was designed to form a hairpin structure composed of 2 β-strand segments in the presence of salt. Self-assembly into a nanofiber network is hypothesized to be initiated by molecular hairpin formation.[1] In this project, we employed solid state nuclear magnetic resonance (NMR) spectroscopy on MAX8 nanofiber samples in order to produce a detailed molecular model.

## Experimental

Structural constraints determined by NMR were introduced into annealing molecular dynamics computer simulations to produce the molecular model. NMR constraints include site-specific $^{13}$C NMR peaks positions, which report on secondary structure, and inter-atomic distances between selectively $^{13}$C-labeled sites, which test predictions based on inter-molecular packing schemes. Site-specific knowledge of NMR peak positions is determined by 2D-exchange finite pulse radio frequency driven recoupling (fpRFDR) spectroscopy (**Figure 1a**).[2] Decays of NMR peak intensities under the PITHIRDS-CT $^{13}$C-$^{13}$C dipolar recoupling experiment[3] provide more quantitative information on inter-atomic proximities.

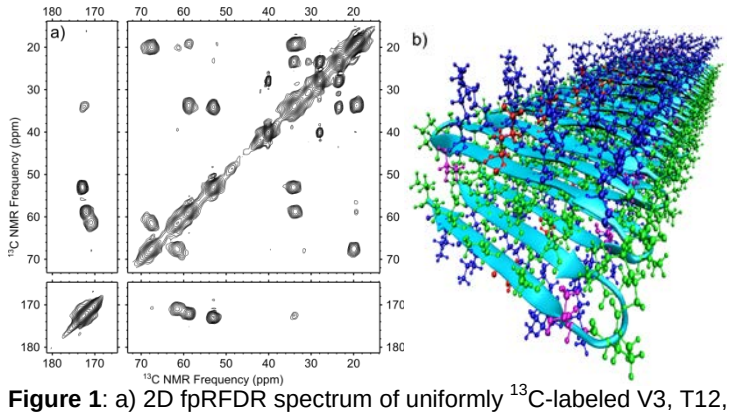


**Figure 1**: a) 2D fpRFDR spectrum of uniformly $^{13}$C-labeled V3, T12, and K17 MAX8, b) A possible all-atom molecular model for the MAX8 nanofiber.

## Results and Discussion

Characteristic secondary shifts of lysine and valine α- and β-carbons, as determined by fpRFDR spectroscopy, are consistent with linear β-strand secondary structure. Narrow linewidths indicate a well-ordered system though NMR spectra indicate that approximately 10% of the sample consists of a minority structure possessing different secondary structure. PITHIRDS-CT data for selectively $^{13}$C-labeled MAX8 nanofiber samples corroborate the formation of a closed hairpin, as reported by Schneider, *et al.*,[4] while also providing additional constraints which indicate further assembly into parallel β-sheets. Additional structural constraints are required to fully characterized the between sheet structure of MAX8 nanofibers though a possible molecular model is presented (**Figure 1b**).

## Conclusions

These results show that a simple repetitive amino acid sequence can produce nanofibers with well-defined molecular structure. However, the work also indicates that we presently have little predictive ability when it comes to several details of intermolecular packing. A known structure provides a basis for future work on assembly under different conditions and mechanistic studies of molecular structure formation.

## Acknowledgements

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## References

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