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Supplemental Information

DNP-Enhanced MAS NMR: A Tool to Snapshot Conformational Ensembles of α-Synuclein in Different States

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Supporting Material

DNP-enhanced MAS NMR: a Tool to Snapshot Conformational Ensembles of α-Synuclein in Different States

Boran Uluca[1,2], Thibault Viennet[1,2], Dušan Petrović[1], Hamed Shaykhalishahi[1,2], Franziska Weirich[1,2], Ayşenur Gönülalan[1], Birgit Strodel[1,3], Manuel Etzkorn[1,2], Wolfgang Hoyer[1,2], Henrike Heise[*,1,2]

[1]Institute of Complex Systems, Structural Biochemistry (ICS-6), Research Center Jülich, 52425 Jülich, Germany

[2]Institute of Physical Biology, Heinrich-Heine-University Düsseldorf, Universitätsstraße 1, 40225 Düsseldorf, Germany

[3]Institute of Theoretical and Computational Chemistry, Heinrich-Heine-University Düsseldorf, Universitätsstraße 1, 40225 Düsseldorf, Germany

*corresponding author: h.heise@fz-juelich.de

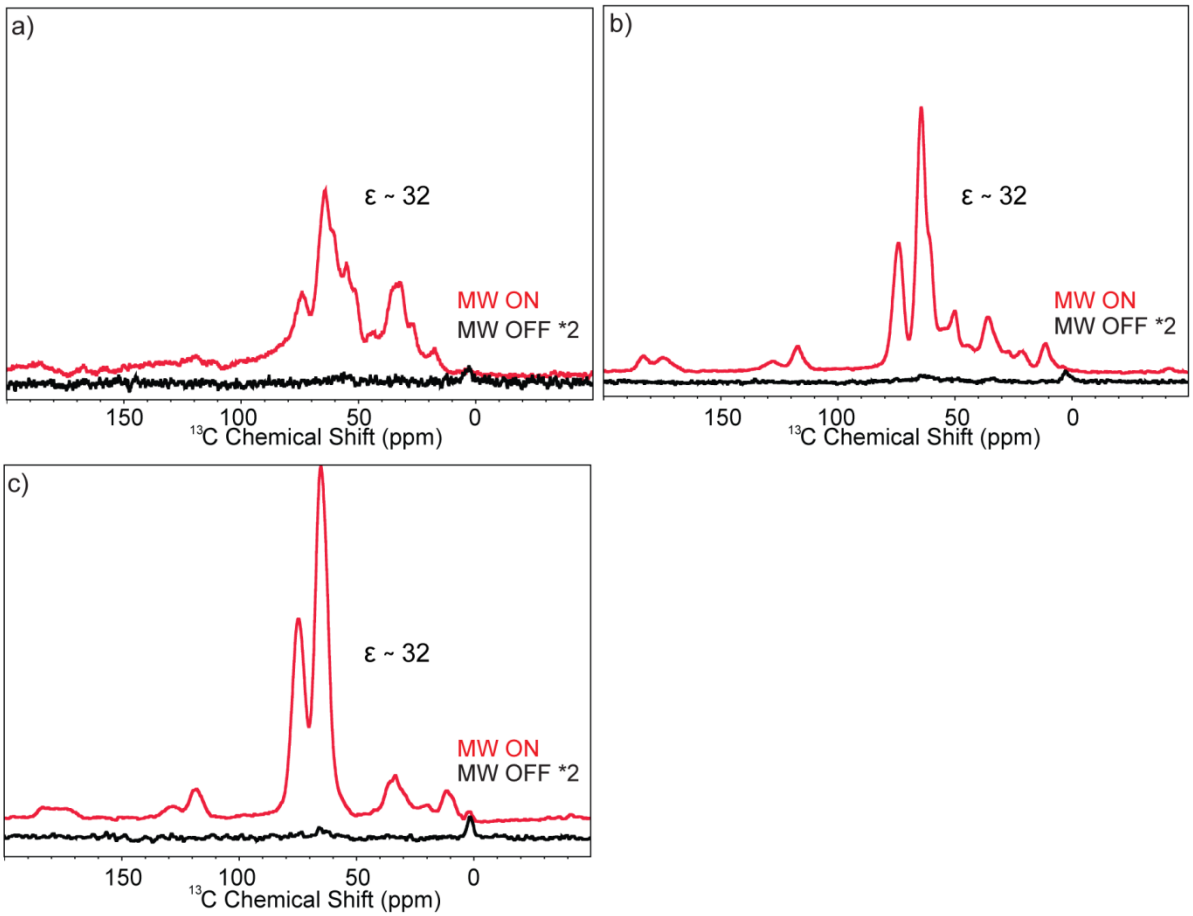

Figure S1: To determine the DNP enhancement factor, ^{13}C CP-MAS spectra were recorded with microwave irradiation (red) and compared to spectra recorded without microwave irradiation (black) under exactly the same experimental conditions. Enhancement factors of ~32 were obtained for the aliphatic region of the protein in all cases, i.e. for a) monomeric, b) fibrillary and c) α-syn monomers mixed with nanodiscs in a 2:1 protein to nanodisc molar ratio. All spectra were recorded at a nominal sample temperature of ~ 100 K, with a spinning speed of 8 kHz and 16 scans. Contact times for proton to carbon transfer via cross polarization were a) 100 µs, b) 400 µs, and c) 1150 µs.

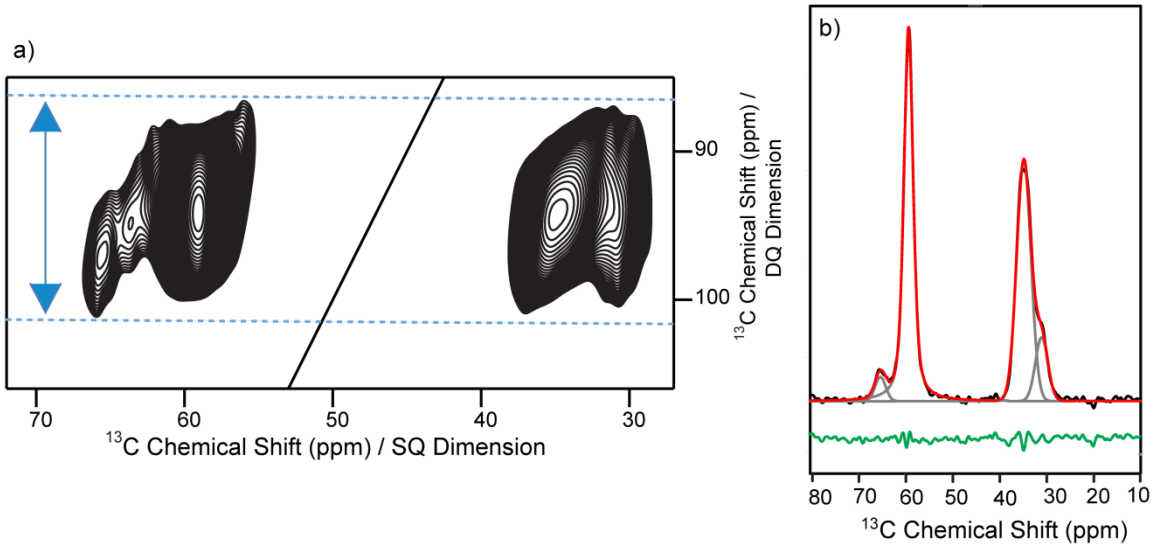

Figure S2: a) 2D ^{13}C-^{13}C correlation DQ/SQ spectrum of specifically ^{13}C labeled α-syn fibrils. 1D projections of 2D spectra were generated by summing up the Cα-Cβ cross-peak region for valines, which is indicated here by the blue dashed lines. b) The projections were deconvoluted using Gaussian line shapes with the help of the DMfit software. The projection is given in black and the single deconvoluted peaks in grey, the resulting fitting curve is shown in red, and the difference spectrum is given in green.

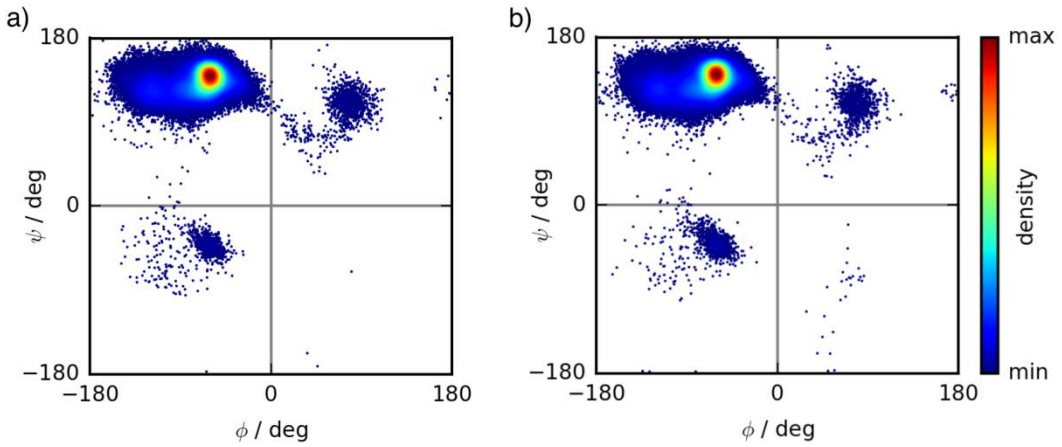

Figure S3: Ramachandran plots for the valine residue in the model peptides a) AGKTKEGVAGGA and b) GGVGG simulated with the CHARMM36m force field using the CHARMM modified TIP3P water model. The color bar shows the local data density.

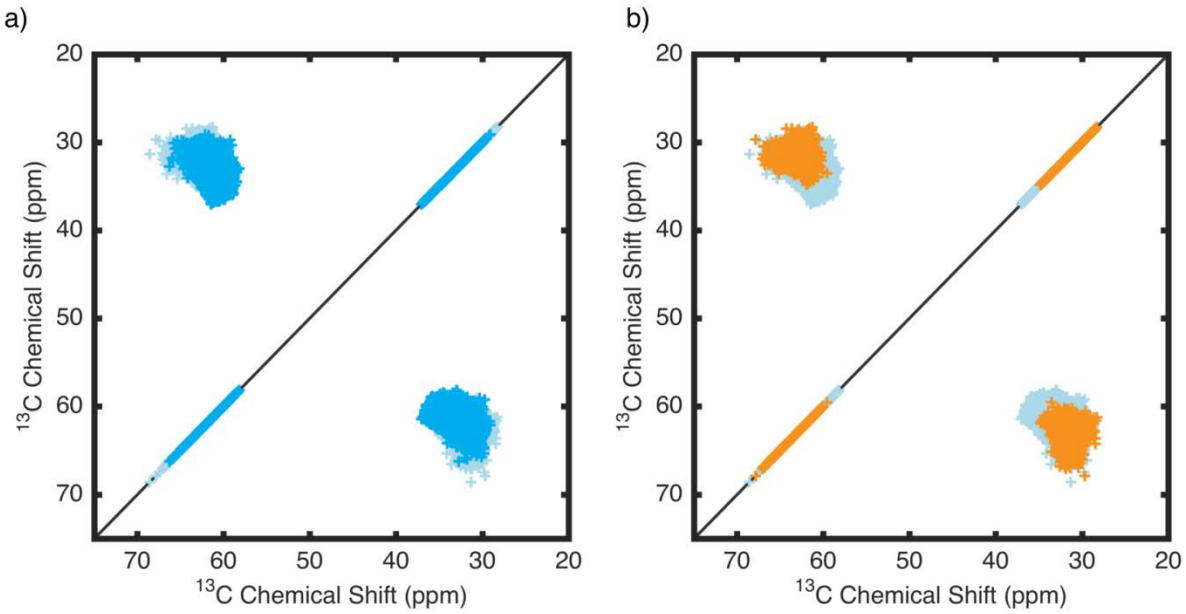

Figure S4: Conformational sampling and NMR shifts of the valine residue in the AGKTKEGVAGGA peptide from a MD simulation using the AMBER99SB*-ILDN force field in TIP3P explicit solvent. Chemical shifts of the full simulated ensemble are shown in light blue. The positions of specific regions are given as: a) β-sheet in blue, b) right handed α-helix in orange. A β-sheet like conformation is defined in the Ramachandran space as -180° < ϕ < -90° and -180° < ψ < -120° or 50° < ψ < 180°, and left handed α-helical conformation as -100° < ϕ < -30° and -67° < ψ < -7°.

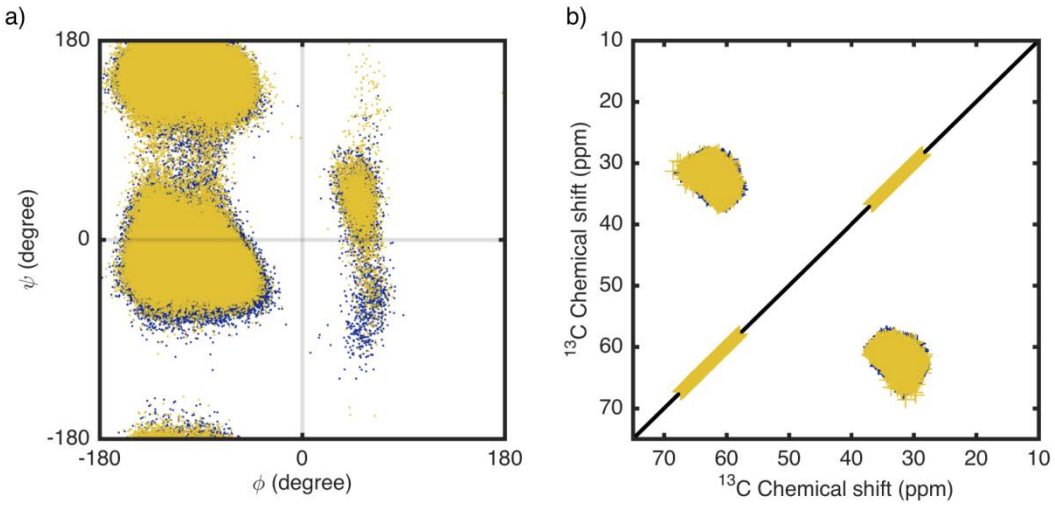

Figure S5: AMBER99SB*-ILDN force field used with generalized Born implicit solvent (blue) and TIP3P explicit solvent (orange) show great mutual resemblance in a) sampled conformational space and b) ^{13}C chemical shifts of the valine residue in the AGKTKEGVAGGA peptide. Figure S6 shows, however, that the relative ratio of the secondary structures is strongly dependent on the type of solvent used for MD simulations.

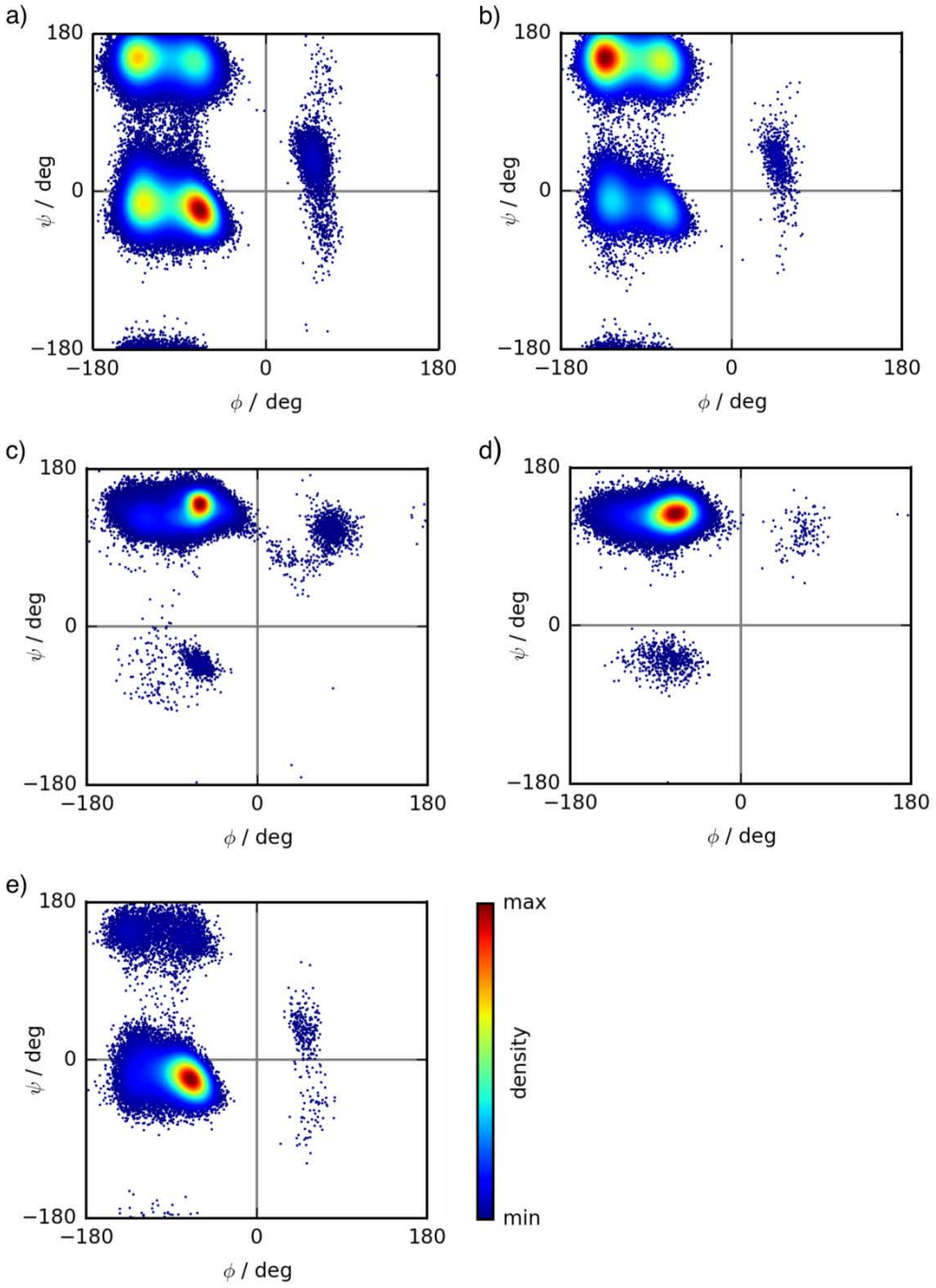

Figure S6: Force field comparison for conformational sampling of the valine residue in the AGKTKEGVAGGA peptide. The Ramachandran plots with data distribution are shown for a) AMBER99SB*-ILDN force field with TIP3P explicit solvent, b) AMBER99SB*-ILDN force field with TIP4P-D explicit solvent, c) CHARMM36m force field with CHARMM modified TIP3P solvent, d) CHARMM22* force field with TIP4P-Ew water, and e) AMBER99SB*-ILDN with implicit generalized Born solvent. The CMAP potential in the CHARMM simulations was not scaled. The color bar shows the local data density.

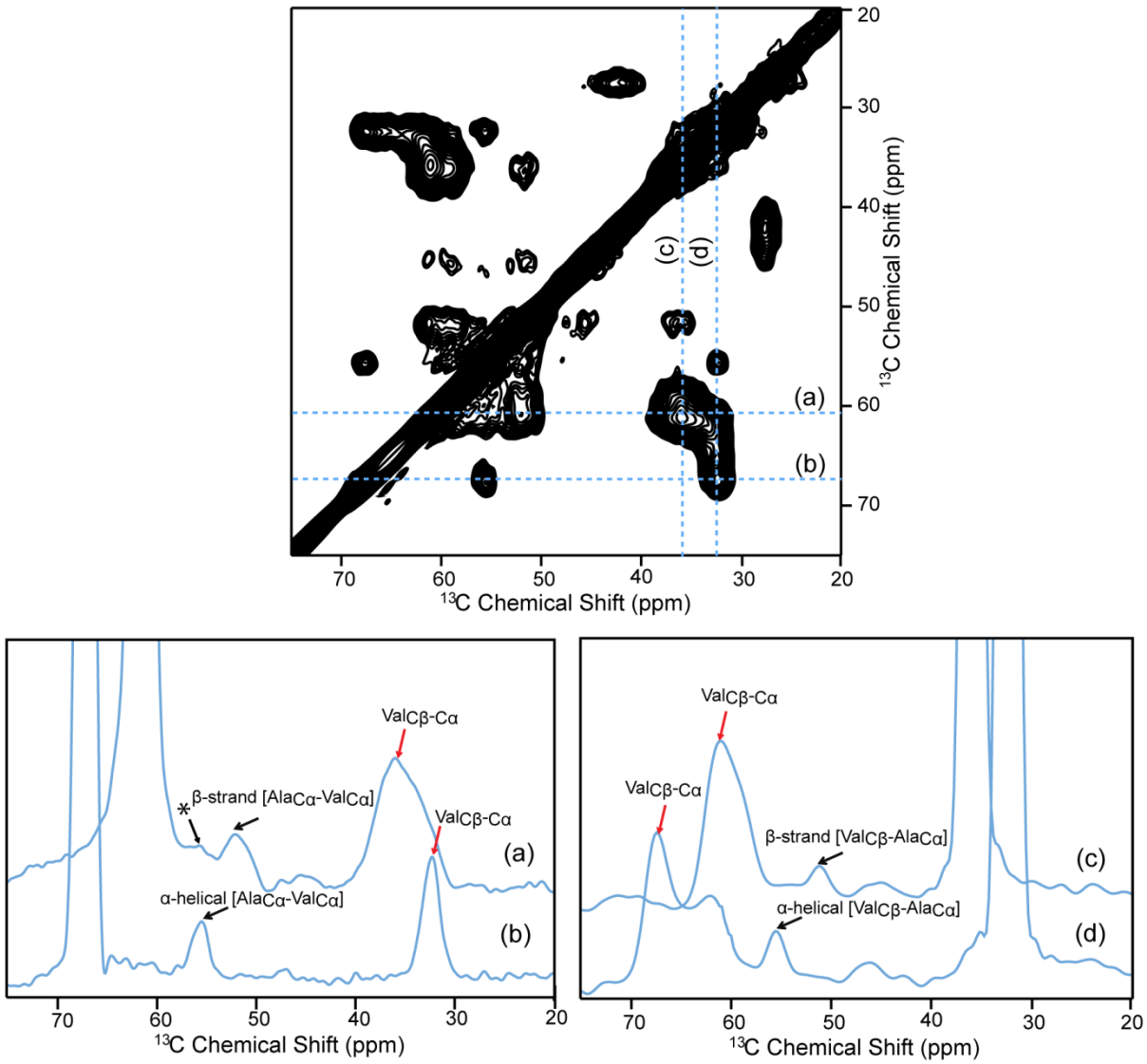

Figure S7: 2D ^{13}C-^{13}C PDSD DNP-NMR spectra of specifically ^{13}C labeled α-syn monomers recorded with a longitudinal mixing time of 1 s. From the 2D spectra, 1D slices were extracted for four cross sections at the positions labeled with (a), (b), (c) and (d). The black arrows indicate inter-residual valine-alanine correlations adopting the same secondary structure, and red arrows indicate intra-residual Cα-Cβ correlations of valines. A correlation of different local structural motifs, i.e., of β-strand Val(Cα) and α-helical Ala(Cα) might be contained in the peak marked with an asterisk. However, this peak is very close to the diagonal and therefore it is not possible to assess if it is a peak or a baseline distortion. Furthermore, there is no symmetry peak observed on the other side of the diagonal. Therefore, we conclude that there is no such correlation of neighboring α-helical and β-strand secondary structure motifs.