^{1}H longitudinal relaxation enhancement in ^{13}C detected NMR experiments on IDPs

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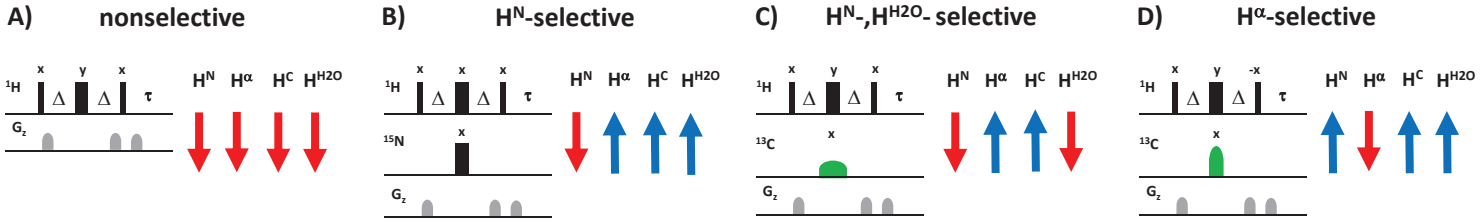
Exclusively heteronuclear NMR experiments based on ^{13}C direct detection[1-3] now provide a valuable tool to study intrinsically disordered proteins. ^{1}H is often used as a starting polarization source to increase the sensitivity of the experiments offering the possibility of easily manipulating different sets of ^{1}H spins (H^N, H^α, H^C, H^{H2O}) to achieve longitudinal relaxation enhancement[2,3]. We would like here to evaluate the various contributions to longitudinal relaxation enhancement in the case of IDPs, using as a model protein a paradigmatic one, α-synuclein. The results provide useful information to design more complex multidimensional NMR experiments as well as to characterize the compactness and/or solvent accessibility of different parts of the polypeptide chain

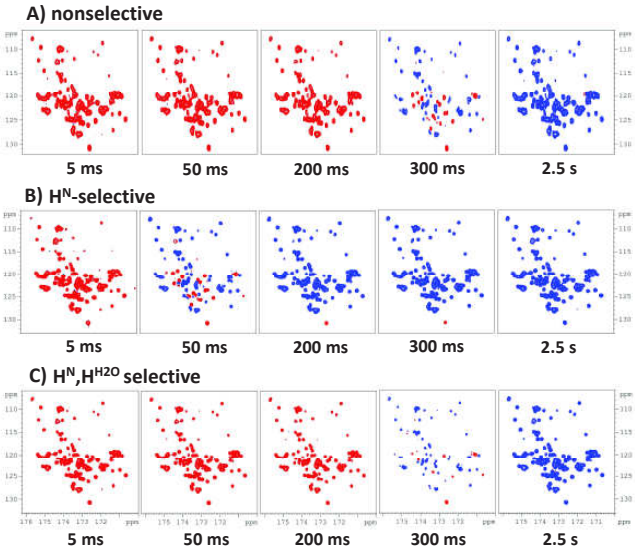
^{1}H-flip variants to invert different sets of ^{1}H spins (H^N, H^α, H^C, H^{H2O})



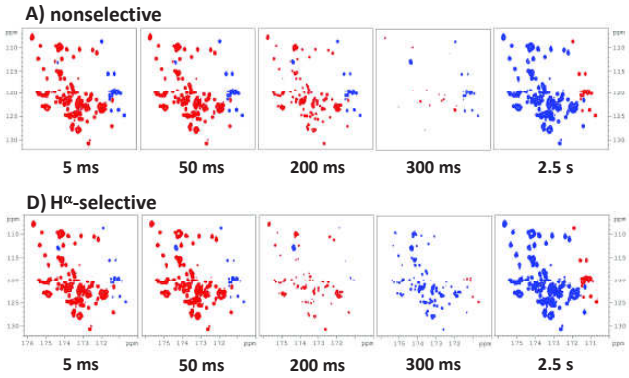
The ^{1}H-flip approach[2,3] can be easily used to invert different pools of spins, exploiting the large one-bon proton-heteronucleus cuplings (2Δ=1/JHX). The different ^{1}H-flip variants illustrated above can be exploited to control the recovery of H^N and H^α polarization to the equilibrium value under different initial conditions.

2D CON maps to monitor ^{1}H inversion recovery-profiles

The different ^{1}H-flip variants were included in the beginning of H^N-flip CON[3] and H^α-flip CON[2] 2D experiments. These are well suited as ^{1}H are only used as an initial polarization source. The experiments are demonstrated on α-synuclein (1 mM, pH 7.4, 297 K, [Pi] 20 mM, [NaCl] 200 mM). The 2D maps acquired with the different ^{1}H-flip variants are shown as a function of the inversion recovery delay, τ. It can be easily recognized the T_{null} for the different ^{1}H-flip variants.

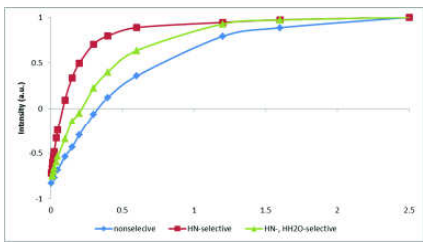


(A) nonselective, (B) H^N- and (C) H^N-,H^{H2O}- selective ^{13}C-detected 2D ^{1}H-flip HNCO experiments of α-synuclein with different inversion recovery delays τ.

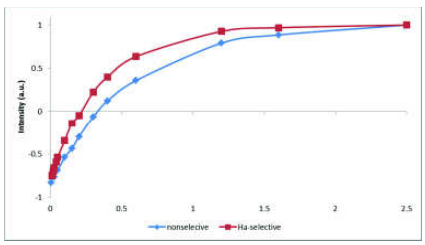


(A) nonselective and (D) H^α-selective ^{13}C-detected 2D ^{1}H-flip HCACON experiments of α-synuclein with different inversion recovery delays τ.

Different contributions to ^{1}H longitudinal relaxation enhancement on α-synuclein



Representative inversion recovery profiles for H^N proton spins under different initial conditions.



Representative inversion recovery profiles for H^α proton spins under different initial conditions.

As an example the different inversion recovery profiles of 118 Val are shown for H^N and H^α proton spins.

Inversion recovery profiles were analysed with Dynamics Center 2.0

References: 1. Bermel W., Felli I.C., Kümmerle R., and Pierattelli R., *Concepts Magn. Reson* .32 A, 183–200 (2008); 2. Bermel W., Bertini I., Felli I.C., and Pierattelli R., *J. Am. Chem. Soc.*, 131, 15339-15345 (2009); 3. Bertini I., Felli I.C., Gonnelli L., Kumar V. M. V., and Pierattelli R., *ChemBioChem*, 12, 2347– 2352 (2011)

Acknowledgments: FP7-PEOPLE , IDPbyNMR: High resolution tools to understand the functional role of protein intrinsic disorder- Project n. 264257)