Water-Exchanging Hydrogens' Positions in Biomolecules Detected via Long-Lived Coherences and Hyperpolarized 2D COSY

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Biomolecular folds depend on water-exchangeable protons and many cellular processes are rhythmed by hydrogen exchange. Amide hydrogens, in particular, regulate biomolecular interactions in concert with structural neighbors. Native structures of peptides and proteins can be solved by magnetic resonance spectroscopy in the liquid state, yet the positions of water-exchanging hydrogens often remain elusive due to sub-ms dynamics hampering the observation of their signals. When absent from spectra correlations, amide protons impede the assignment of entire networks of nuclei inter-connected by *J*-couplings, i.e., spin systems. Even when they can be observed, multi-dimensional spectral correlations involving these protons are slow to record due to their low intensity. Biomolecular interactions, especially if they are to be observed in real time in living cells, require fast 2D spectroscopy; when they occur outside equilibrium conditions, e.g., when a substrate is introduced at the start of the experiment, a limited time-window is available to the observer.

We report 1D and 2D fast spectroscopic methods to follow water-originating hydrogens in peptides and proteins. The 1D method relies on the effect of water-exchangeable protons on the high-field decay rate constants of long-lived coherences[1], the nuclear magnetic oscillations based on singlet-triplet transitions[2]. We show that the sign of these nuclear-permutation antisymmetric coherences can be switched by the experimenter[3]. Comparing the effect of water-exchanging hydrogens on long-lived coherences with different signs (noted herein LLC and LLC') in AlaGly dipeptide allows to position these hydrogen atoms with respect to Gly-$H^{\alpha 1}$ and Gly-$H^{\alpha 2}$ and, thereby, in the molecular framework.

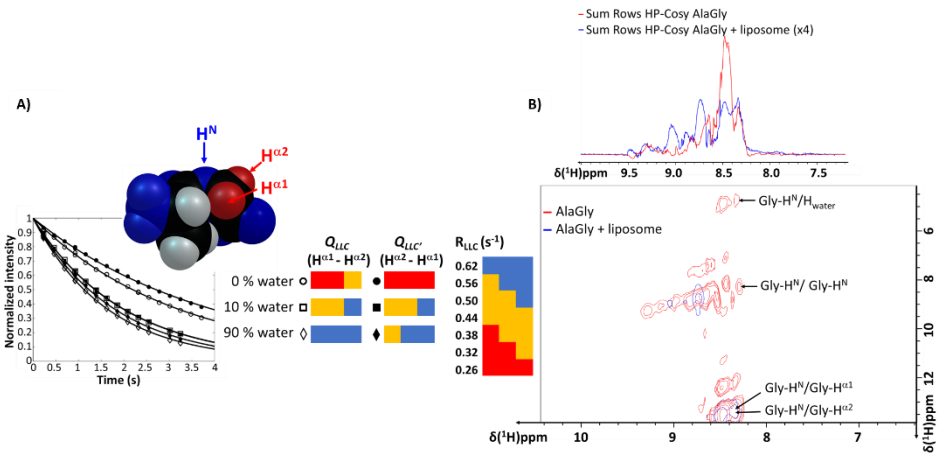

Figure 1: A) Observation of the effect of water-exchangeable protons on Gly-H^{α} long-lived coherences in the AlaGly dipeptide. The plot on the left shows the time dependence of LLC (full symbols) and LLC' (open symbols) intensities as the H_2O:D_2O ratio in the sample is increased. Intensity errors are of the size of the symbols. The LLC relaxation rate enhancement effect is shown in color coding on the right side: blue indicates high relaxation rate constants and red indicates low relaxation rate constants. B) Hyperpolarised water-based 2D COSY with folded indirect dimension for AlaGly recorded in t_{exp} < 1 minute (red) and changes in the spectrum appearing upon the introduction of liposomes in the AlaGly solution (blue).

In a new 2D approach, we show that the magnetisation of water protons can be enhanced by dissolution-Dynamic Nuclear Polarisation[4] by a factor ε ~ 400 compared to room-temperature equilibrium and transferred to labile -HN protons to record proton-proton correlation (COSY) maps[5,6]. Hyperpolarized water-based 2D COSY spectra were recorded for either free AlaGly or liposome-interacting AlaGly. Water-proton exchange-based correlations ('WAPOR') can identify solvent-exposed regions of peptides and derive their conformational propensities in the presence of liposomes[7]. The trajectory of hyperpolarized magnetisation reveals molecular networks connected by scalar couplings within a time-scale of one minute, compared to several hours with classical methods. These proton-only experiments are useful for structural biology, promising for improving contrast in biomedical imaging, as well as for pharmaceutical research, to probe the interaction of drug candidates with proteins at atomic resolution without recurring to expensive isotope enrichment.

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