

Looped, PROjected Concatenated Cross-Polarization (L-PRO CCP): An improved transfer scheme for tackling $H \rightarrow N \rightarrow C$ transfers in solvent-exchanging systems

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INEPT- and HMQC-based pulse sequences¹ are widely used to transfer polarization between heteronuclei, particularly in biomolecular spectroscopy: they are easy to setup and involve low power deposition. Still, these J-based polarization transfers are limited by the occurrence of fast solvent chemical exchanges.^{2,3} An established alternative to improve these heteronuclear transfers employs J-driven cross polarization (J-CP),⁴ which transfers spin-order by spin-locking the heteronuclear reservoirs under Hartmann-Hahn conditions.⁵ While sensitive to the settings of this matching and while imposing higher RF loadings on the probe, J-CP's spin-locking imparts a certain immunity against chemical exchange and relaxation effects –making it a potential method of choice to tackle disordered proteins or fast exchanging nucleic acids.^{3,6} Further, while Zuiderweg and co-workers⁷ showed that a combination of triple- and double-resonance cross polarization (Concatenated CP, CCP) can better $H \rightarrow N \rightarrow C$ transfers comparing to INEPT-based counterparts, rapid exchanges with the solvent will also conspire against this transfer –eventually rendering inefficient. We have recently shown that certain inefficient polarization transfer processes of this kind can be improved by repeated projective operations,⁸ in an anti-Zeno-like process that accelerates transfers by exploiting their initial rapid buildup stages.⁹ Figure 1a present an adaptation of these concepts to a CCP aimed at improving the efficiency of $H \rightarrow N \rightarrow C$ transfers, in the presence of fast chemical exchanges with the solvent. The resulting Looped, PROjected (L-PRO) CCP experiment involves an initial triple-resonance CP (highlighted in yellow) that puts in contact all three spin reservoirs; during this period 1H polarization is depleted, ^{15}N magnetization grows up to a level allowed by solvent exchange and RF mismatches, and the ^{13}C get somewhat polarized. Post-contact 90° pulses project all these states along the z-axis; this is followed by a second double-resonance CP period (highlighted in magenta), where ^{15}Ns pass their polarization to ^{13}Cs while 1Hs recover their original thermal values thanks to fast exchanges with the solvent. To facilitate the latter recovery, the water magnetization is effectively spin-locked together with exchangeable NH protons³. The behavior is of limited efficiency owing to the exchange-driven averaging of the 1H - ^{15}N J-transfer; L-PRO compensates this by repeatedly looping the buildup while relying on the ^{13}C 's longer lifetimes. Figure 1b illustrates the behavior expected from this procedure on a three-spin system throughout multiple loops, in a setting that assumes both RF inhomogeneity and rapid 1H recovery owing to the chemical exchanges. Notice how the multiple repetitions eventually polarize fully, by repeatedly drawing spin order from the surrounding solvent. Figure 1c shows a validation of such simulations, based on detections of urea's carbon signal at pH=3 and at three different temperatures –i.e., at three different exchange rates with water. In all these cases, fast exchanges average out 90 Hz J_{HN} coupling depriving INEPT-based transfers from much usefulness; the L-PRO CCP scheme, by contrast, remains efficient and delivers ^{13}C enhancements that are very close to the theoretical maximum for all cases. Preliminary results demonstrate the feasibility of extending these principles further and using them for the simultaneous polarization of both carbonyl and alpha carbons, facilitating the execution of HNCO and HNCA 3D acquisitions in a single experiment. Potential applications of these triple resonance concepts on IDPs involving labile, chemically exchanging protons, will be presented.

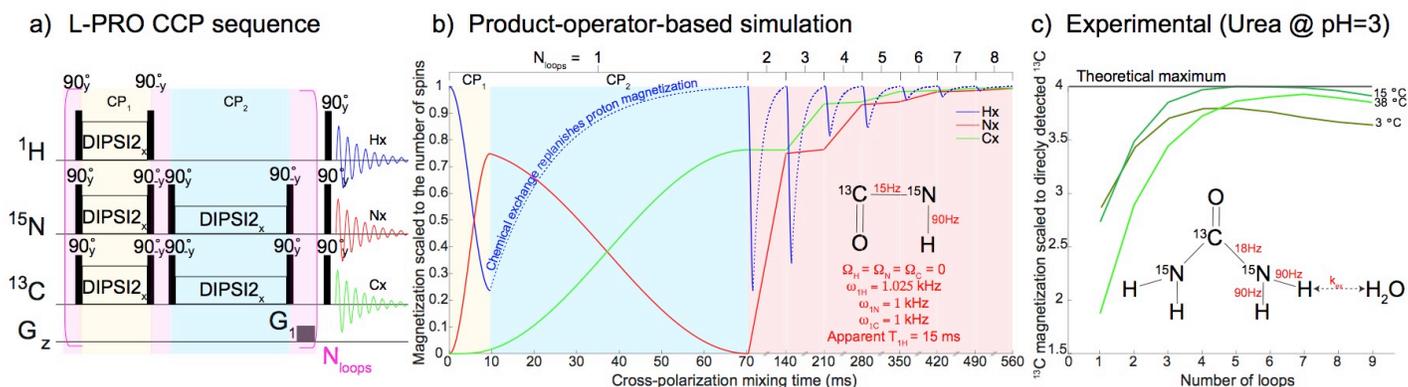


Figure 1. (a) Pulse sequence of the L-PRO CCP sequence; operators were propagated based on spin density matrices while solvent exchange was accounted for by repolarizing the H_z components. (b) Behavior simulated for the indicated three-spin system throughout multiple loops of the pulse sequence; operators were propagated based on spin density matrices while solvent exchange was accounted for by repolarizing the H_z components. (c) Experimental L-PRO CCP results measured for urea's carbonyl ^{13}C in H_2O at three different temperatures as a function of CCP looping

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