Advances in Oriented Sample Solid-State NMR of proteins

The preparation of perdeuterated (except for a few selected sites) proteins has made substantial contributions to NMR of large and challenging systems studied by solution NMR and magic angle spinning (MAS) solid-state NMR because of the beneficial effects of dilution of 'abundant' ^{1}H nuclei. We have extended the use of perdeuteration to stationary single crystal peptide and uniaxially aligned membrane and viral coat protein samples. In oriented-sample solid-state NMR perdeuteration extends the lifetime of dipolar oscillations in separated local field (SLF) experiments, which substantially narrows the linewidths in the ^{1}H-^{15}N heteronuclear dipolar coupling as demonstrated by the spectra from a single crystal of N-acetyl-leucine (NAL) (Fig. 1B) and the membrane-bound form of Pf1 coat protein in magnetically aligned bilayers (Fig. 1D). In contrast, the effects of ^{1}H homonuclear dipolar couplings are suppressed so efficiently by frequency switched Lee-Goldburg (FSLG) and multiple pulses cycles in PISEMA and SAMPI4 experiments that perdeuteration does not contribute to further narrowing of the resonances. Among other studies, the effects of perdeuteration on homonuclear spin-exchange experiments were examined; it did not influence the results of PDSD experiments, however, mismatched-Hartmann-Hahn experiments showed significant increases of cross-peak intensities.

Additionally, the two-dimensional PISEMO experiment has been augmented with a saturation pulse during the z-filter to effect water suppression in applications to stationary, aligned samples of uniformly ^{15}N labelled proteins in $^{1}H_2O$ solutions. The spectrum of Pf1 coat protein in magnetically aligned bacteriophage particles in Fig. 1E demonstrates that windowed ^{1}H-detection yields the same resolution as ^{15}N-detection on the same sample.

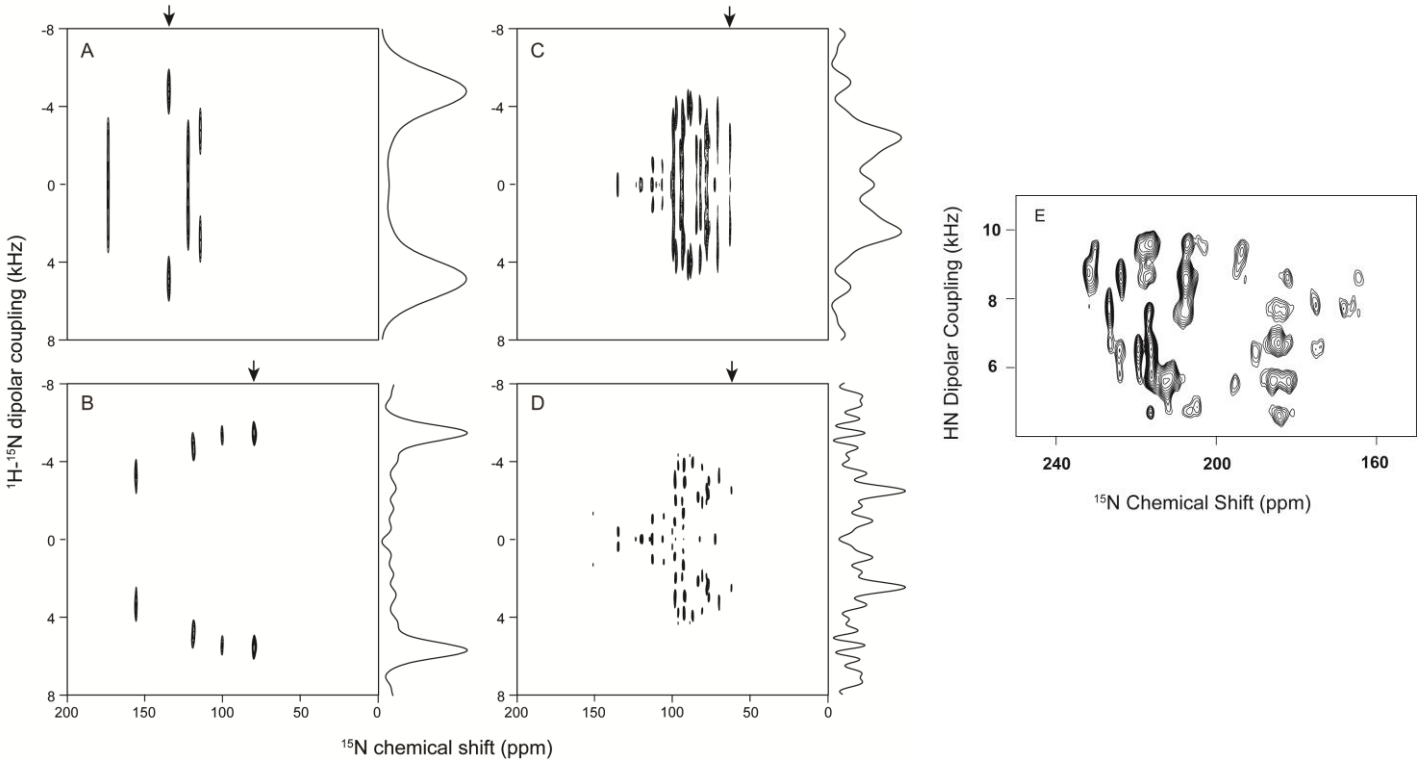

Fig. 1. A. – D. ^{1}H-^{15}N SLF spectra of ^{15}N labelled stationary samples. A. and B. are of NAL single crystals. C. and D. are of the membrane-bound form of Pf1 coat protein in aligned bilayers. The narrow linewidths in the dipolar coupling dimensions of B. and D. are a consequence of the perdeuteration of all sites except for the amide nitrogens. E. ^{1}H-detected PISEMO spectrum of the structural form of ^{15}N labelled coat protein in aligned bacteriophage particles in $^{1}H_2O$ solution.