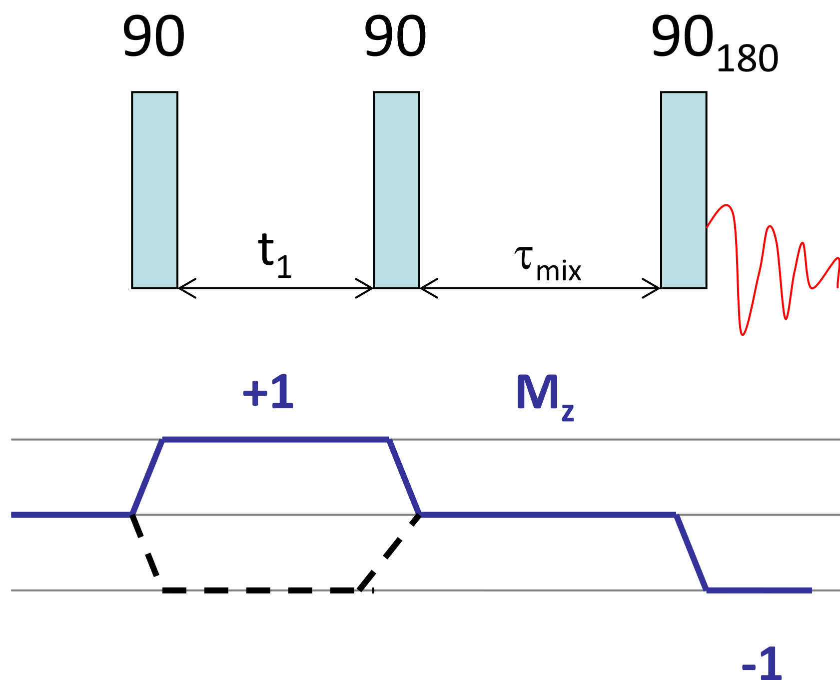
NOESY and EXSY Experiments

Correlation through incoherent magnetization transfer

Pulse Sequence for the NOESY and EXSY Experiment



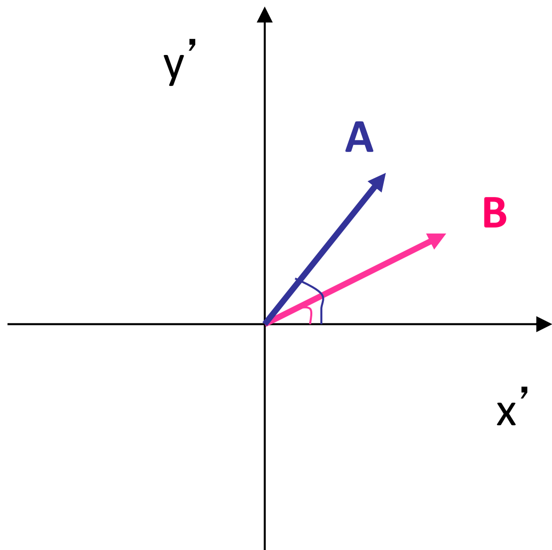
coherence path for absolute value spectra acquired with EXORCYCLE

there is a further 90 pulse with respect to COSY. It has a phase difference of 180 respect to the previous one

Assuming B_1 along y' :

the former pulse carries the magnetizatins of the A and B nuclei to the x' axis of the rotating frame

during t_1 the transverse magnetization vectors precess with angular frequency Ω_A and Ω_B



The components of the magnetization vector:

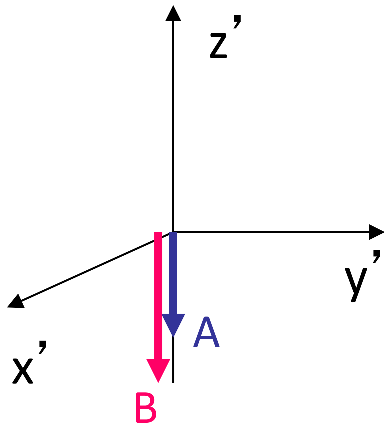
$M_{Ax'} = M_{A0}\cos(\Omega_A t_1)$

$M_{Ay'} = M_{A0}\sin(\Omega_A t_1)$

$M_{Bx'} = M_{B0}\cos(\Omega_B t_1)$

$M_{By'} = M_{B0}\sin(\Omega_B t_1)$

The latter pulse, again with B_1 along y', carries the components $M_{Ax'}$ and $M_{Bx'}$ up to –z, whereas it leaves unchanged the y' components. The latter components are deleted either by a phase cycle or by defoccussing by means of a pulsed gradient



During τ_{mix} the **exchange** of the nucleus from the A to the B site can occur (either intermolecular exchange or conformational conversion): EXSY

or magnetization **transfer** from B to A by (transient) **NOE**: NOESY

The last pulse, with B_1 along –y', carries the magnetization, which was along –z at the end of the mixing time, along x' thus becoming responsible for the signal, which is acquired by receiver.

The magnetization, which was precessing with Ω_A in t_1 and during τ_{mix} was transferred to B, precesses in t_2 with Ω_B, thus it originates a cross peak. The reciprocal holds for B→A

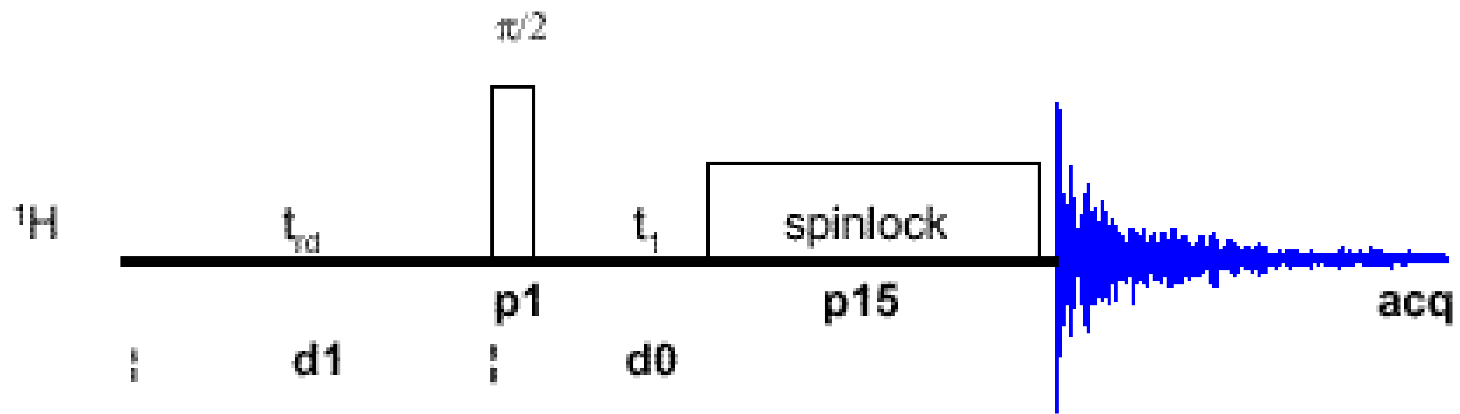
The NOESY phase sensitive spectra are advantageous because:

- ✓ the EXSY cross-peaks have the same sign of the diagonal peaks
- ✓ the NOESY cross peaks have opposite sign with respect to the diagonal peaks for positive NOE

- ✓ the NOESY cross peaks have the same sign of the diagonal peaks for a negative NOE
- ✓ the ROESY spectra are obtained applying a spin-lock during the mixing time
- ✓ the ROESY cross-peaks have always opposite sign with respect of diagonal peaks because ROE is always positive

ROESY

rotating frame Overhauser effect spectroscopy



Spinlock = mixing time, should not exceed 300 ms, best between $T_2/2$ and T_2

The strength of the ROE signal is proportional to the inverse sixth power of the distance between the atoms. The sensitivity is increased by choosing low-viscosity solvents and/or removing dissolved oxygen from the sample.

Prof. Jenny

Study of protein folding under native conditions by rapidly switching the hydrostatic pressure inside an NMR sample cell

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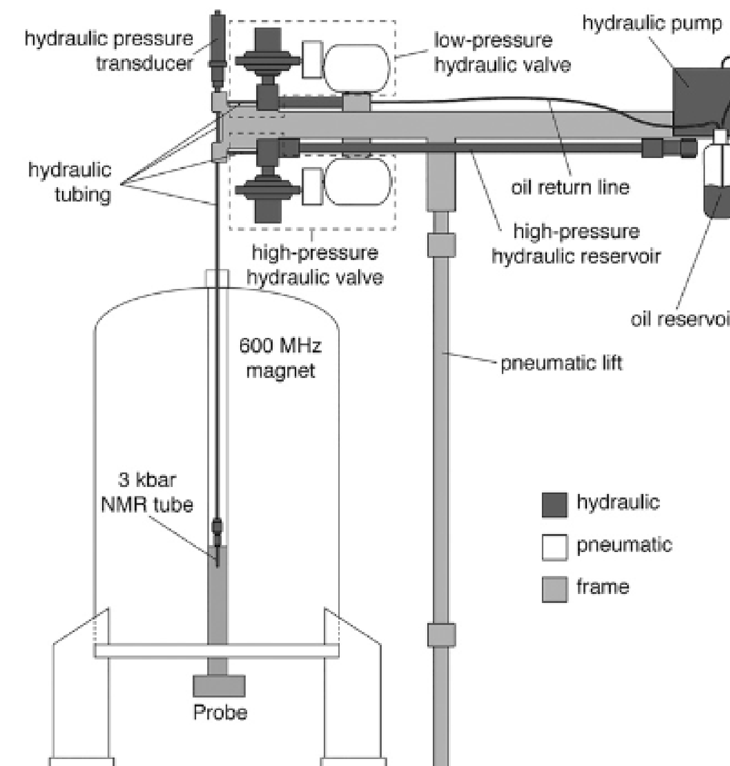
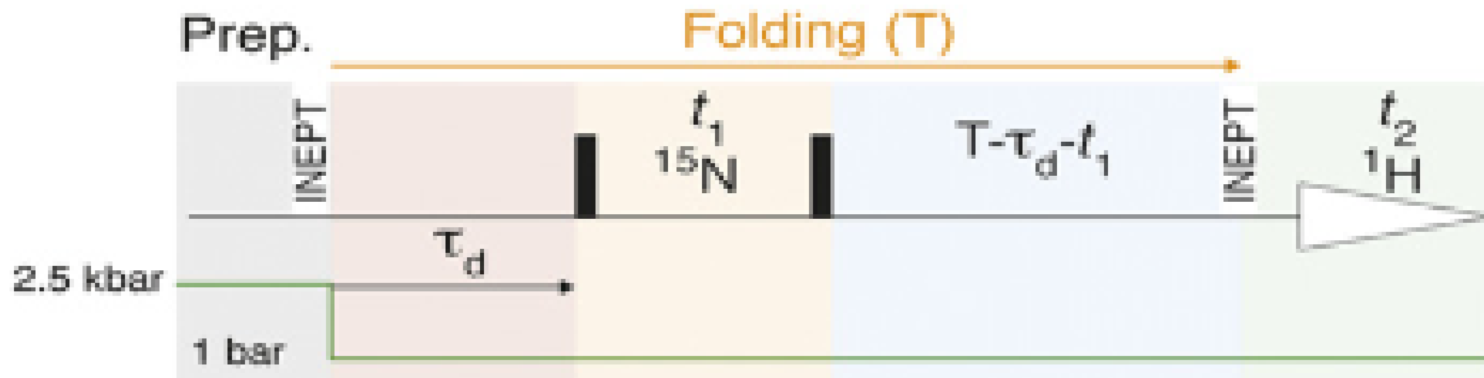


pressure jump apparatus

Observation of Ubiquitin Folding by zz-Exchange Spectroscopy

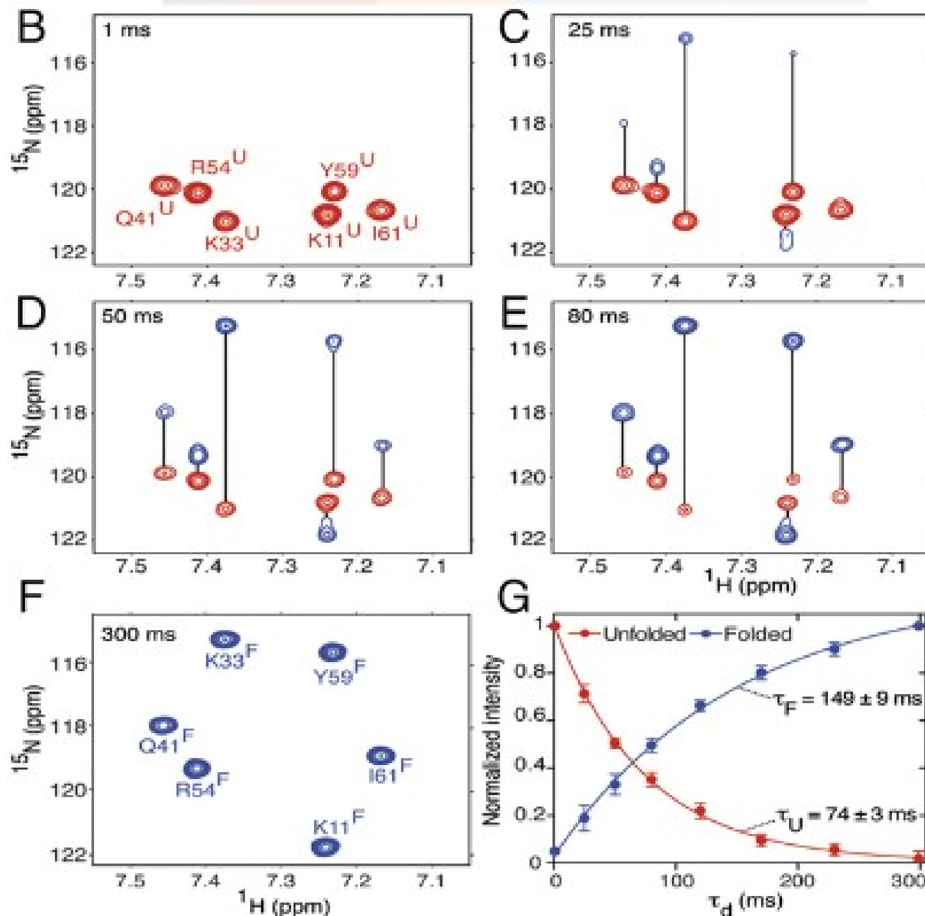


Schematic timing diagram of the pulse sequence.

At the end of a high-pressure equilibration period (12 s), the ^{1}H magnetization is transferred to ^{15}N z magnetization by a refocused **INEPT** pulse scheme.

Conversion to transverse ^{15}N magnetization and subsequent t_1 evolution, followed by a pulse that stores a $\cos(\omega_N t_1)$ fraction of this magnetization back to z, is initiated at time τd after the pressure drop, thereby encoding the ^{15}N frequencies present at τd.

At a fixed time, T, after the pressure drop, this encoded ^{15}N magnetization is transferred back to the amide proton for ^{1}H detection. T is chosen sufficiently long (330 ms) that most of the protein has folded at the time of detection



(B–F) Small regions of the HSQC spectra, recorded on a 280 μM $^{2}H/^{15}N/^{13}C$-enriched ubiquitin sample at indicated τd delay durations after the pressure drop.

All spectra correlate the frequency of the detected amide proton in the folded protein to the ^{15}N frequency of either the unfolded (red) or folded (blue) protein, with resonance intensities proportional to the fractions populating the unfolded and native states. Note that colors have been added manually. Residues are labeled by their one-letter residue code and number, with U and F denoting the unfolded and native state, respectively. (G) Time dependence of the resonance intensities observed in B–F. Fitted time constants show faster disappearance of resonances of the unfolded state than appearance of the folded final spectrum