Encounters with the SOFAST-HMQC

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Introduction

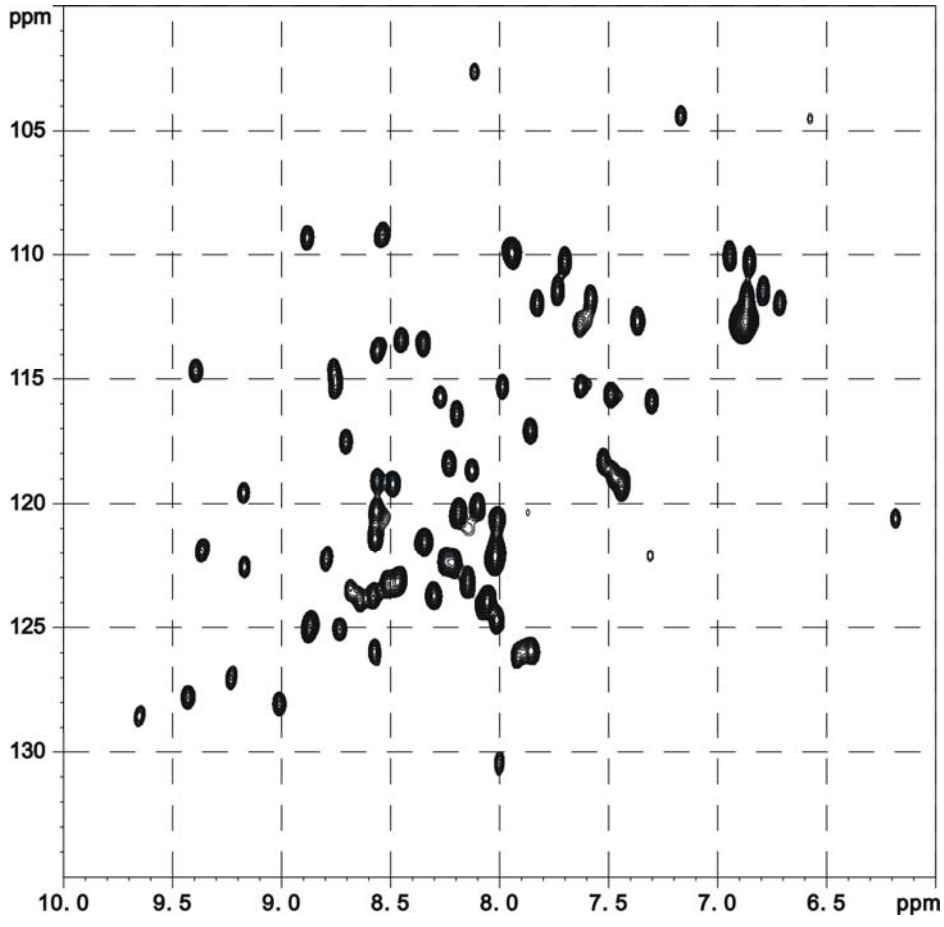
The Ernst angle

Longitudinal Relaxation

SOFAST-HMQC

- Ubiquitin
- „Nascent chain“

^{15}N-HSQC of Ubiquitin



An ^{1}H,^{15}N-correlation is of central importance in protein NMR

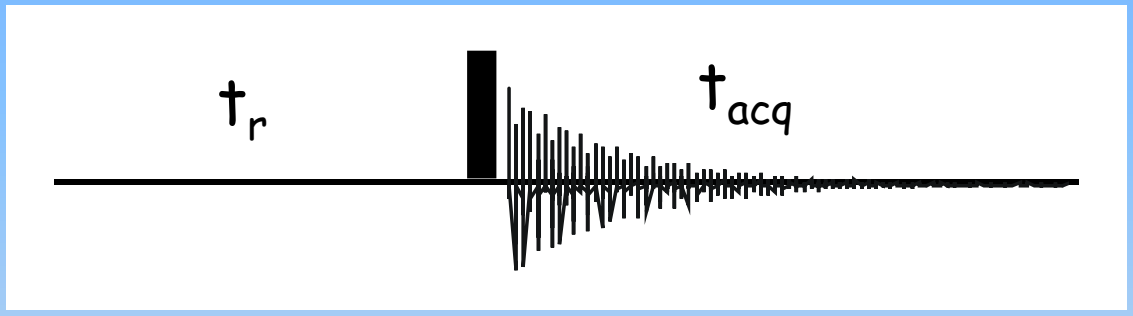
An $^{1}H,^{15}N$-correlation

- is a „fingerprint" of the protein
- is a good test whether the protein is folded or not
- is the seed spectrum for sequence specific assignment
- is used for relaxation measurements
- is used for detection of interactions
- is used for folding experiments
- is a very sensitive experiment (given ^{15}N-labeling)

it would be desirable to be able

to record it fast

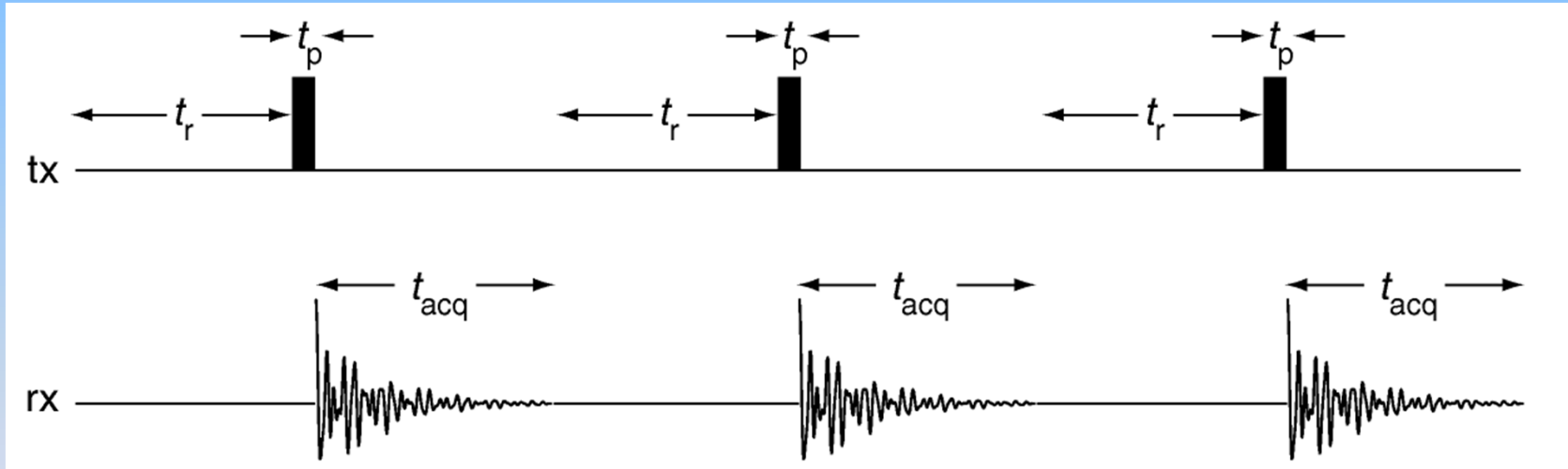
This poses the question how we can achiev a maximum signal-to-noise in a minimum time



For a simple one-dimensional spectrum this question has been answered by Ernst

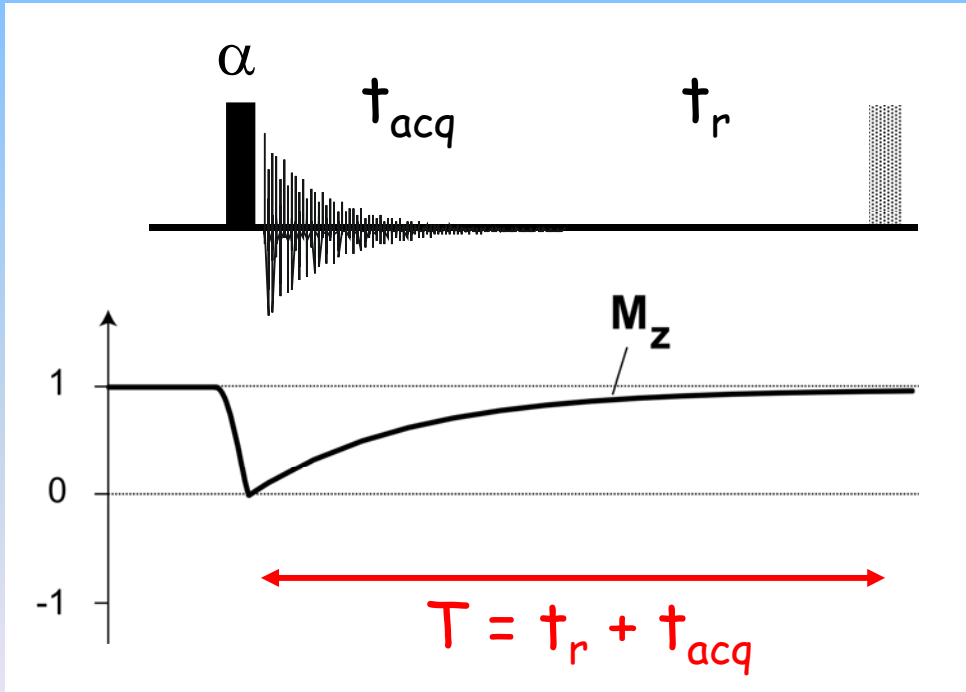
If only one pulse would be applied, the best experiment would use a 90° pulse. With that a maximum of magnetisation would be transferred in the x,y-plane.

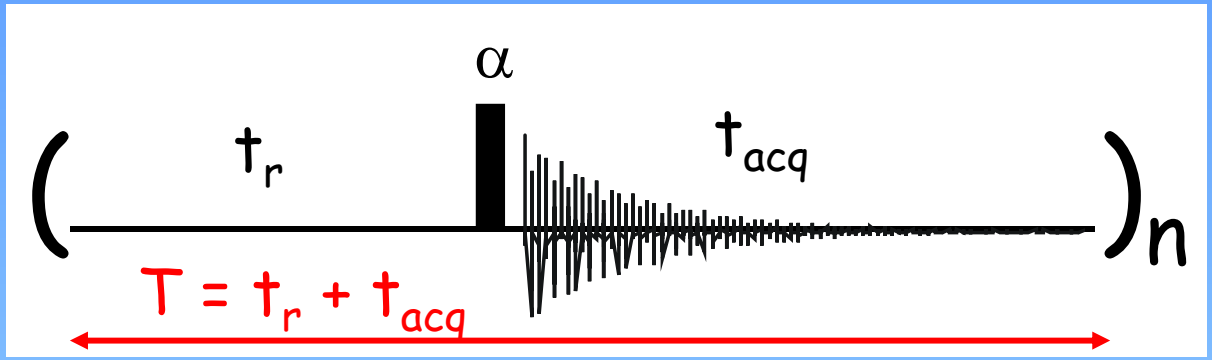
But because of S/N usually more than one scan is performed

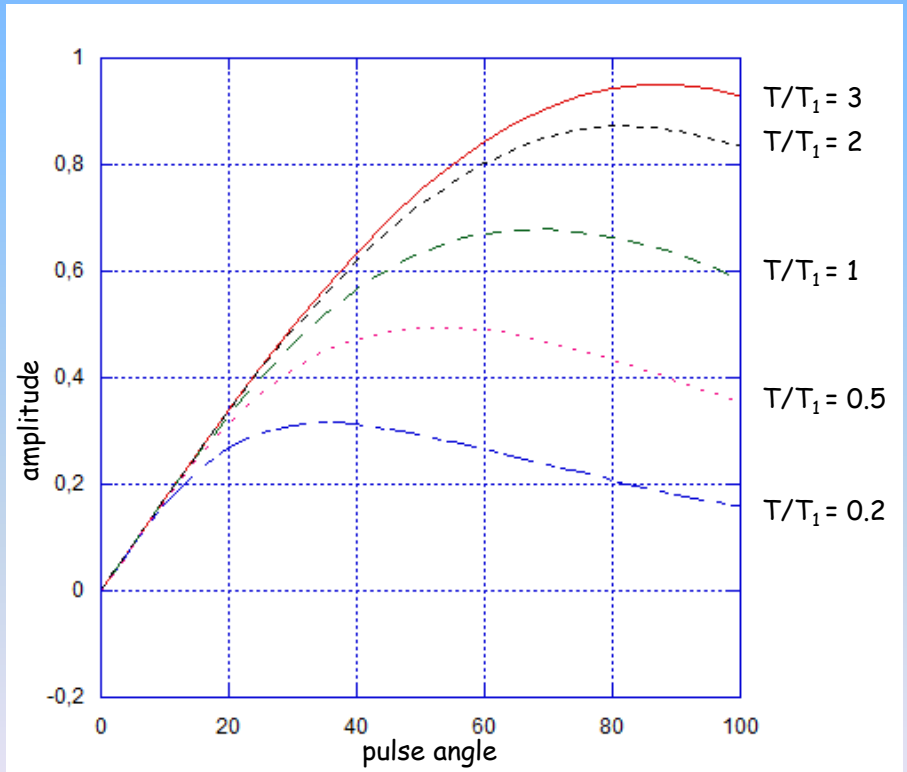


$$T = t_r + t_{acq}$$

After the pulse z-magnetization is recovering with a typical rate, the T_1-relaxation time. This magnetization is used for the next pulse and the relation between the pulse angle α and the ratio T/T_1 is important





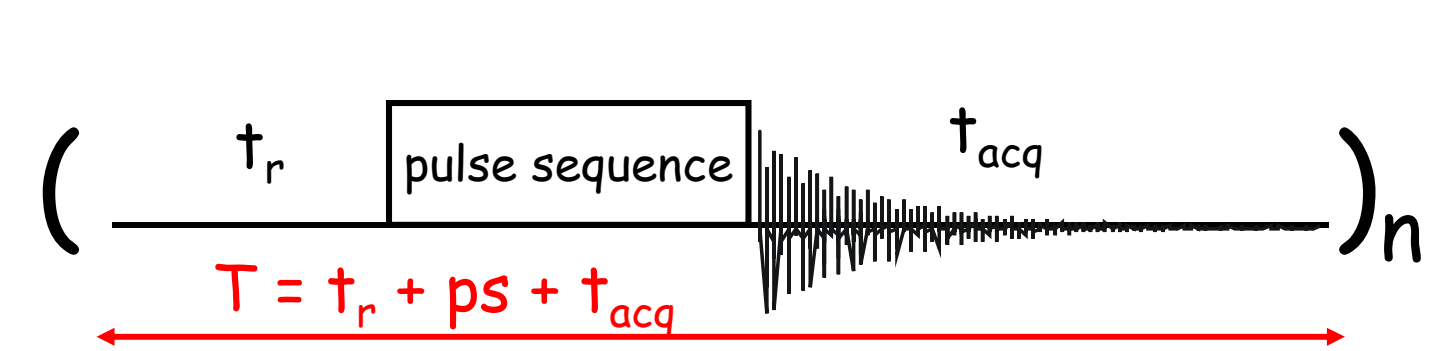


The result is the „Ernst angle“:

$\cos \alpha = \exp(-T/T_1)$

that gives best S/N per time (but not necessarily realistic intergrals)

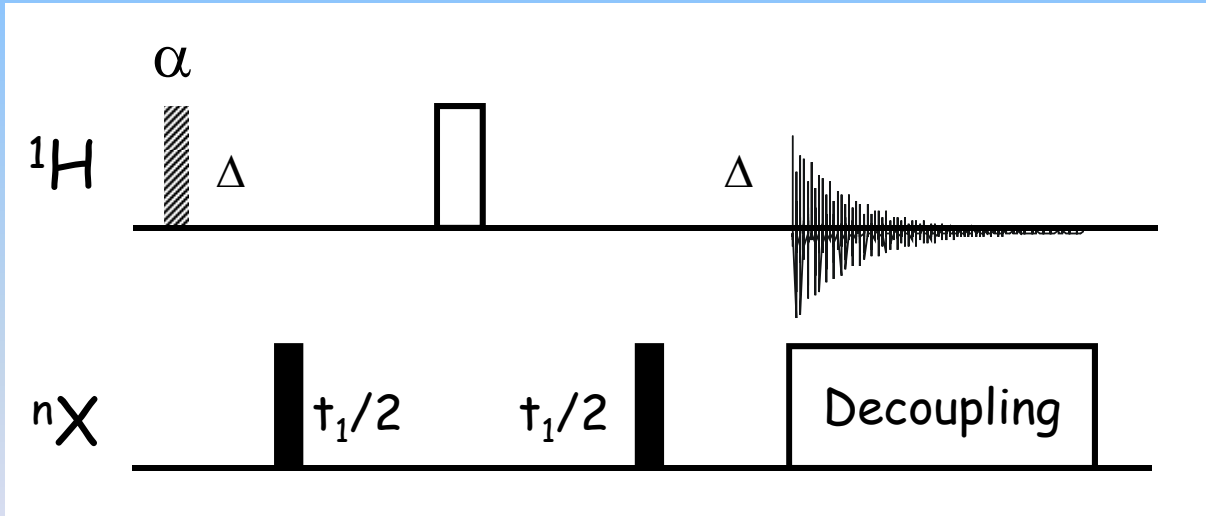
For multidimensional experiments the situation is more complicated since the pulse is now replaced by a complex pulse sequence



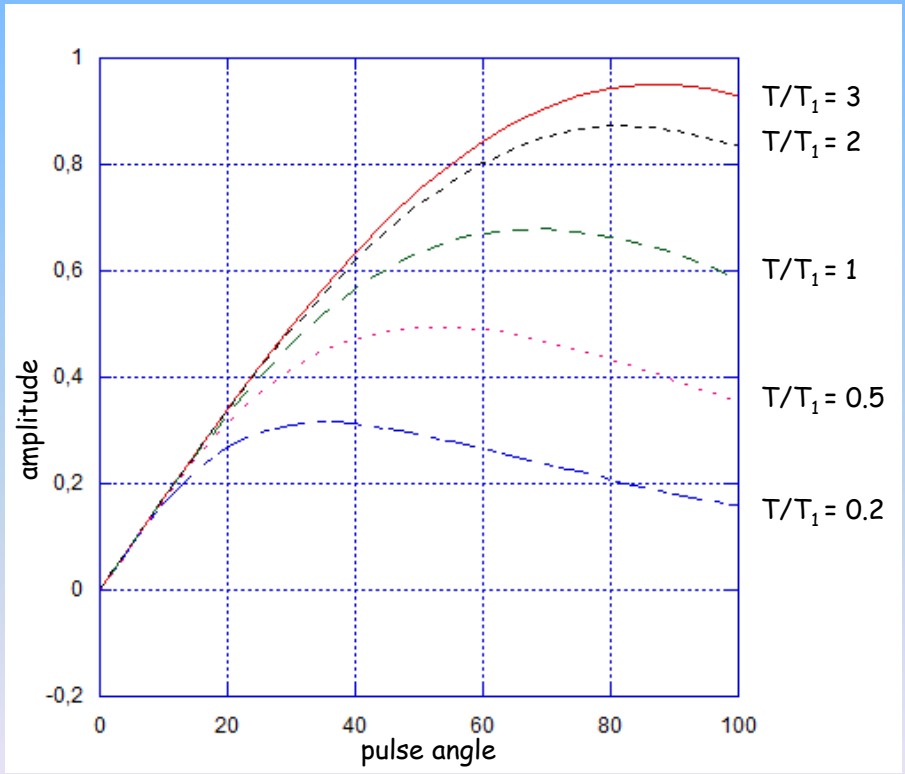
This has been analyzed in detail by

A.Ross, M. Salzmann, H. Senn *J. Biomol. NMR* **10**, 389-396 (1997)

They finde that for most sequences the 90° pulse is the best option, but for an HMQC they derive an initial pulse of 120° to allow for fast pulsing with 200 msec recovery delay: The Fast-HMQC



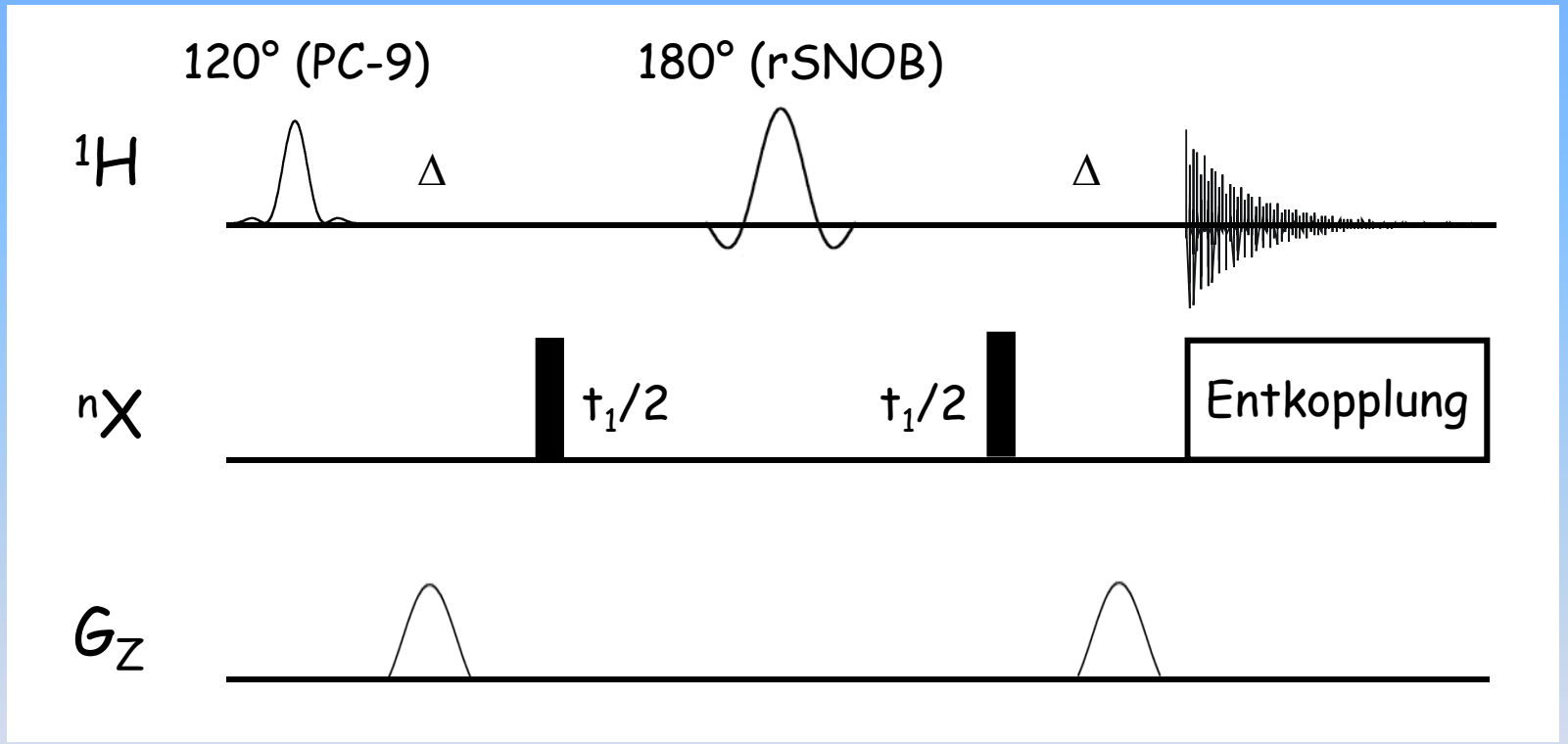
Obviously the value for the relaxation time T_1 has an influence on the repetition rate. For a given pulse angle a shorter T_1 makes a shorter T possible



There are several examples of shortening the T_1 relaxation time, e.g. in the vicinity of paramagnetic centers. This is, however, not generally applicable. But it was shown that an excitation of the amide protons without exciting the other protons in a protein can considerably shorten the relaxation as compared to an unselective excitation

K. Pervushin, B. Vögeli, A. Eletsky *J.Am.Chem.Soc.* **124**, 12898-12902 (2002)

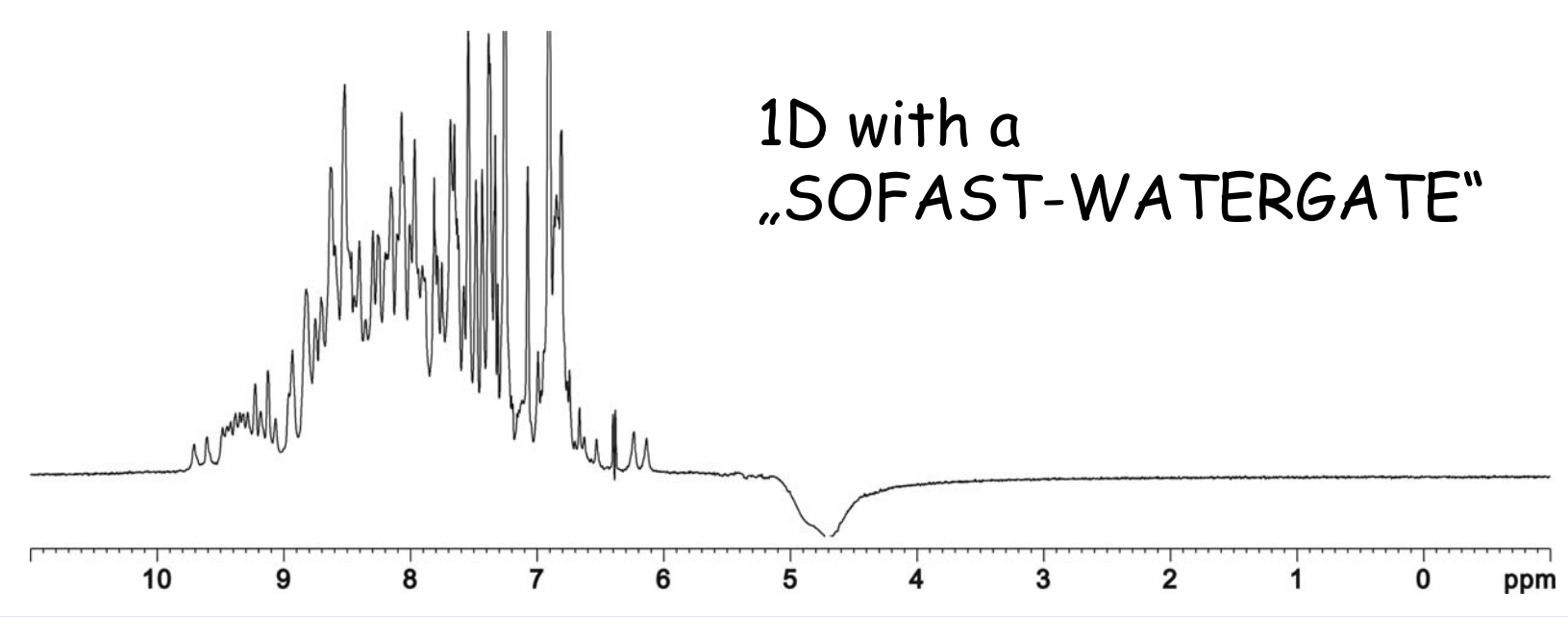
The SOFAST-HMQC takes up both tricks: the Ernst-angle and the reduction of T_1



P. Schanda, B. Brutscher *J.Am.Chem.Soc.* **127**, 8014-8015 (2005)
P. Schanda, E. Kupce, B. Brutscher *J. Biomol. NMR* **33**, 199-211 (2005)

The selective pulses are adjusted so that only the region of amino protons is excited

PC-9 2000 usec
rSNOB 667 usec
} @ 900 MHz



The selective pulses depend on the field strength

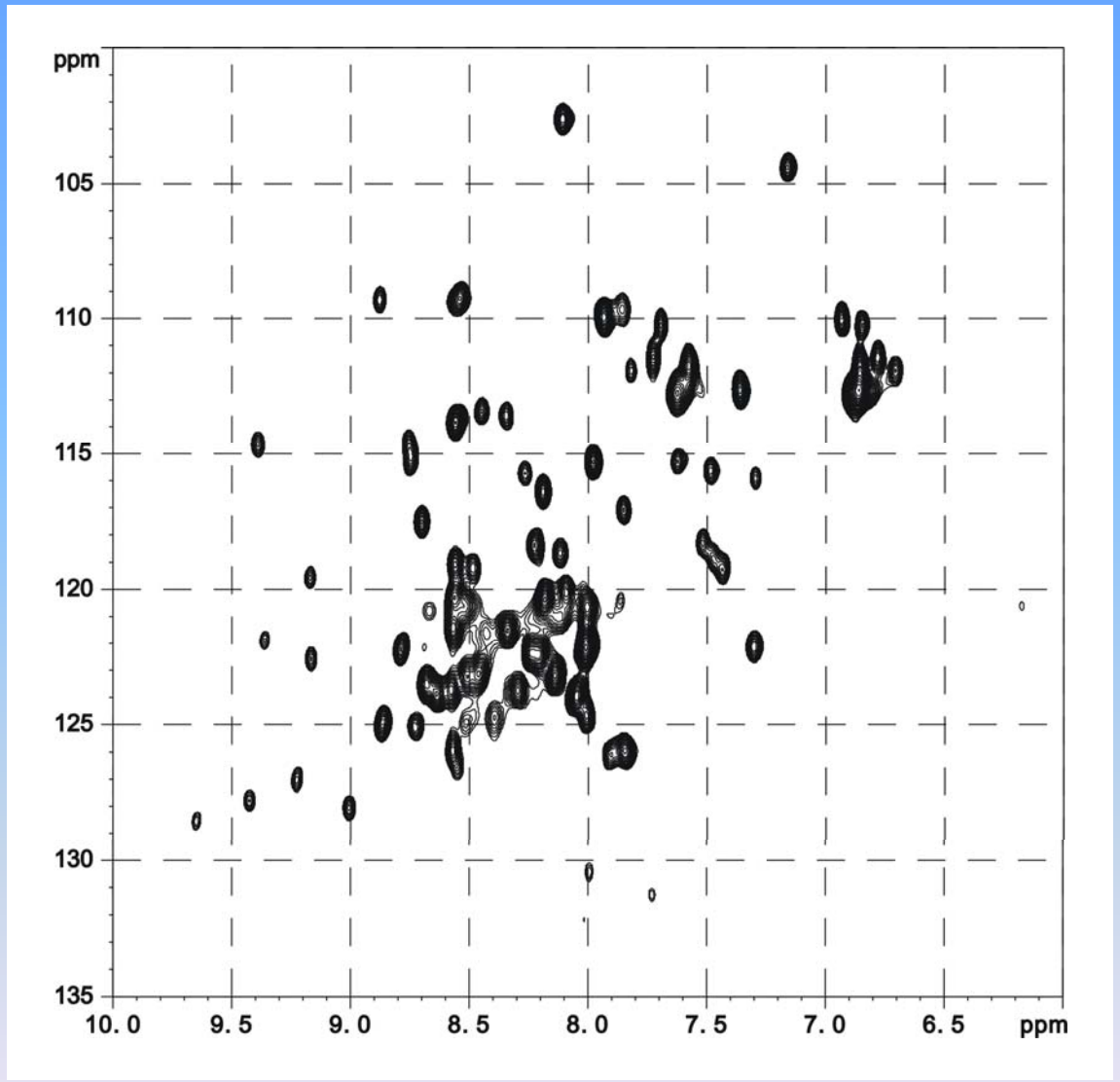
	600 MHz	900 MHz
PC-9	3000 μsec	2000 μsec
rect.	*281.3 μsec*	*187.5 μsec*
rSNOB	1000 μsec	667 μsec
rect.	*106.8 μsec*	*71.2 μsec*

With a linear amplifier the power for the pulse can thus be easily calculated

Potential problems are probe heating because of the high repetition rate and the fact that the lock does not really have time to stabilize the field.

The probe heating might be especially problematic when using cryoprobes.

Bruker recommends to shorten the relaxation delay until the heating of the cryoprobe is getting close to zero



SOFAST-HMQC

of ^{15}N-Ubiquitin

900 MHz

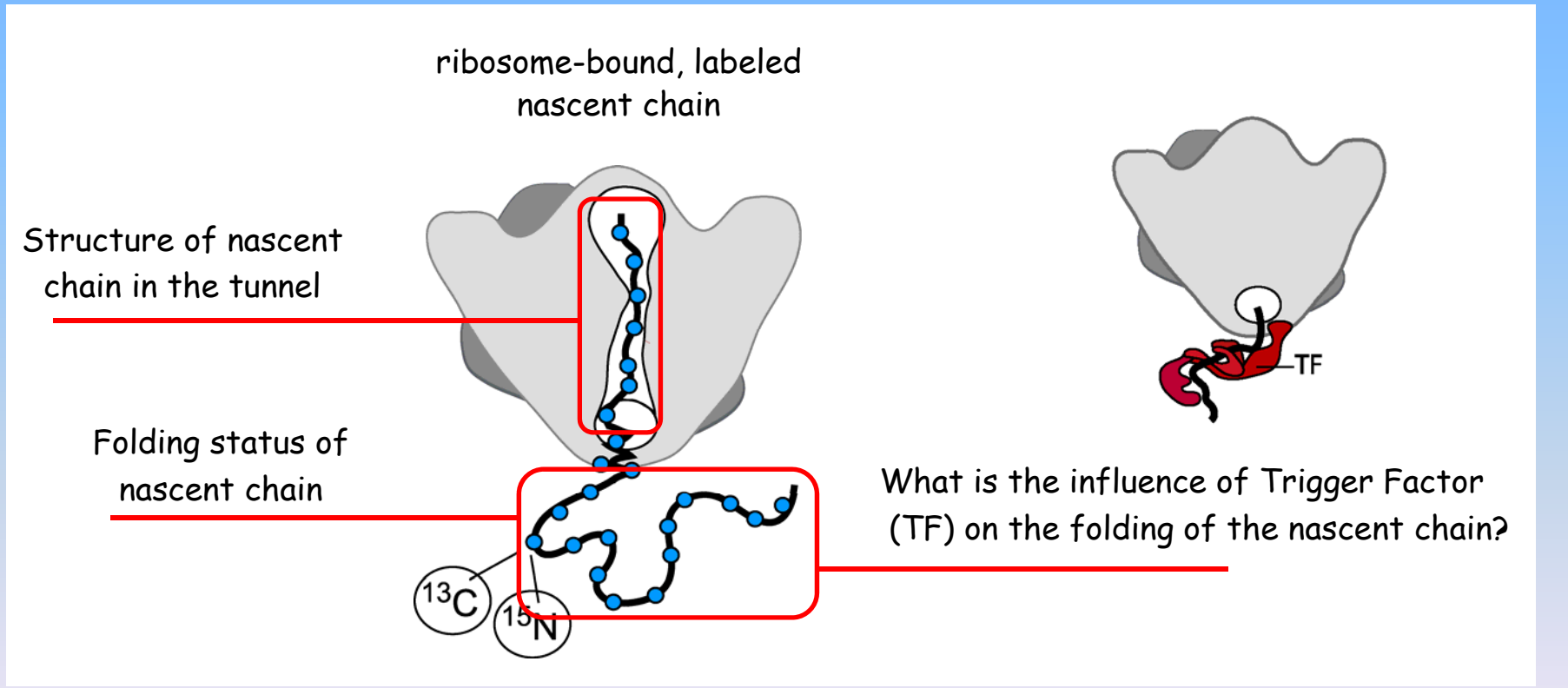
cryo-probe

2 scans, 128 FIDs

T=97 msec

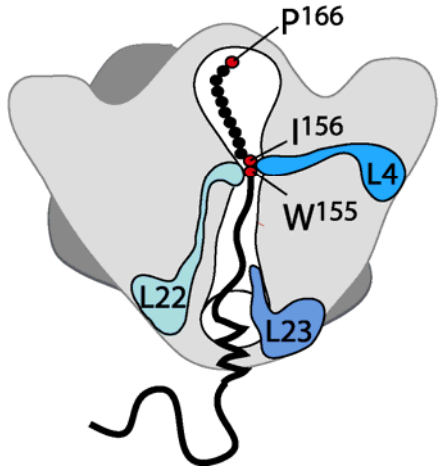
Messzeit 25 sec

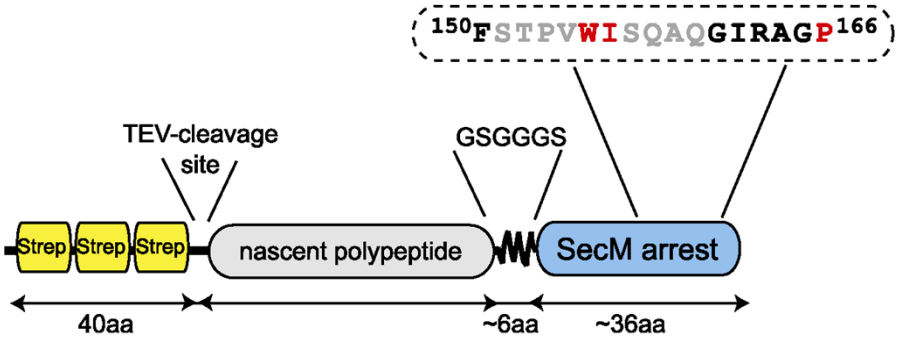
An alternative to shortening the measurement time is to improve the S/N in a given time for very demanding samples: the nascent chain bound to the ribosome



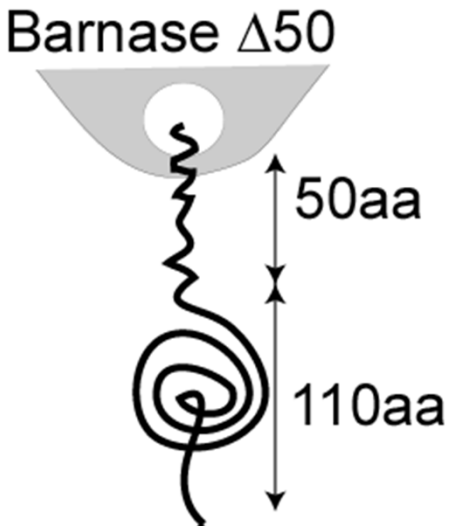
Samples have been prepared and the preparation optimized in the group of B. Buckau (Heidelberg)

SecM ^{150}FSTPVWISQAQGIRAGP^{166}

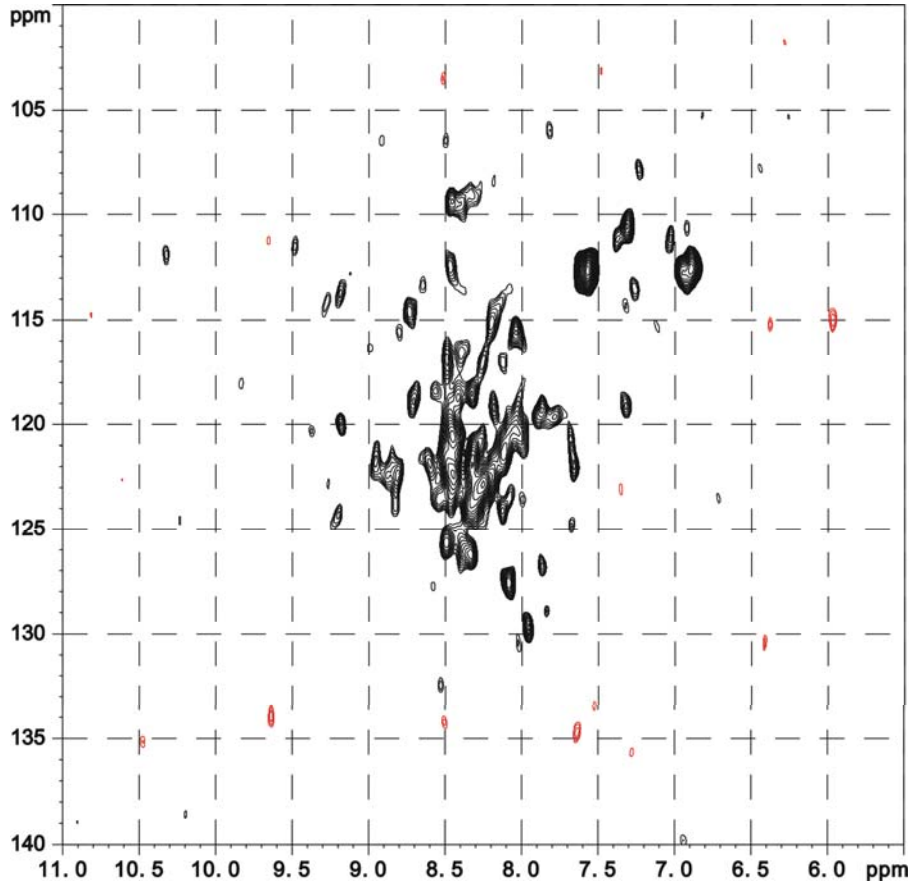




We tried it with Barnase, that is known to give a folded protein when expressed alone



10 μM/l
2000 scans
128 FIDs
20 hours



acknowledgement

FMP

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