

## Principle

The chemical and physical principles underlying protein function can only be unraveled by gaining insight into both structural and dynamic features.

Relaxation measured at various lower field strengths and detected at high field with high spectral resolution provides dynamic information linked to structure.

Relaxometry with a sample shuttle:

- Polarization at high field.
- Relaxation at a wide range of low fields.
- Detection at high field.

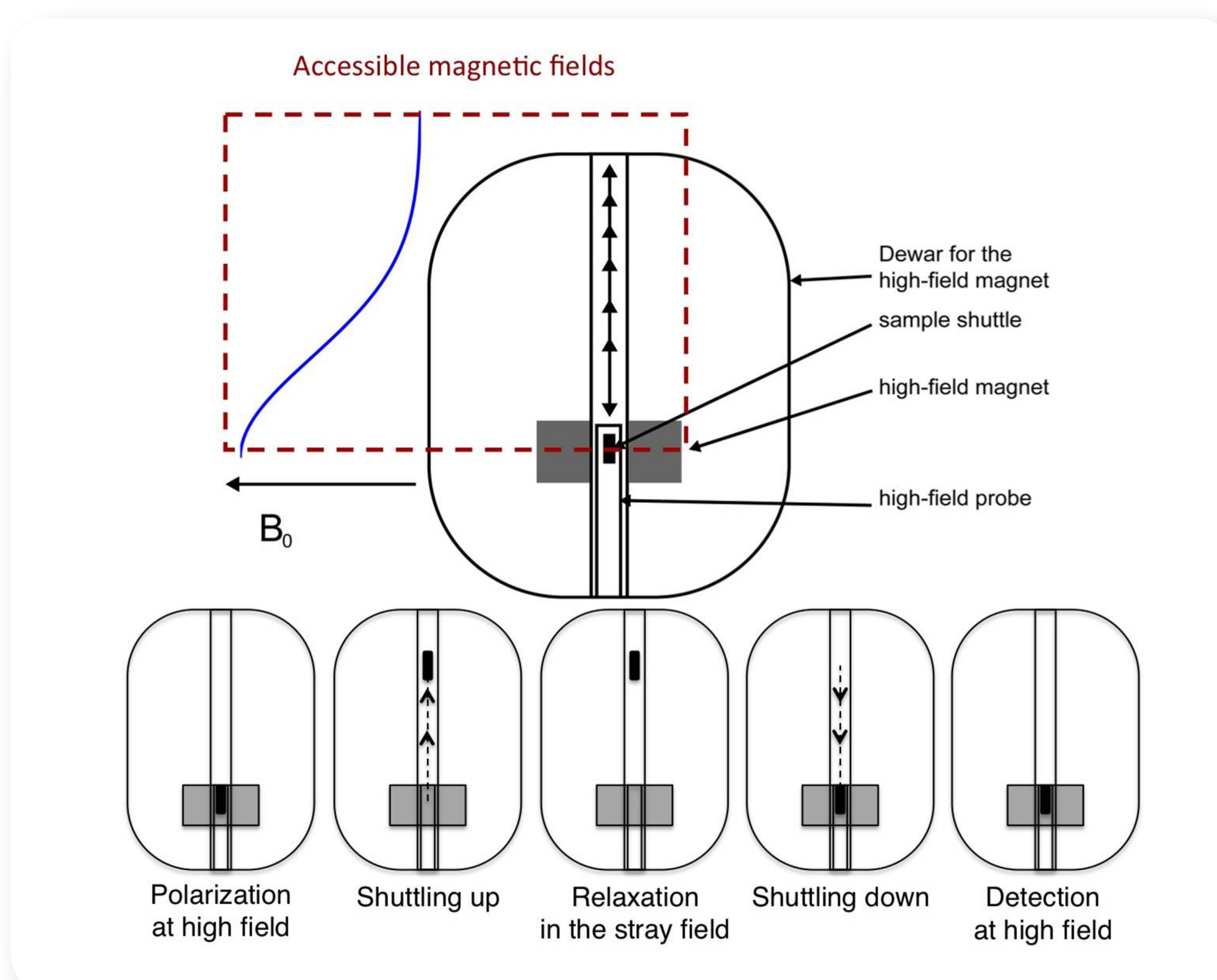


Fig. 1 Principle of high-resolution relaxometry.

## Hardware

The shuttle consists of a dedicated probe (Figure 2a,b), a shuttle transfer system, and a pneumatic control unit for the shuttle motion driven by pressurized air.

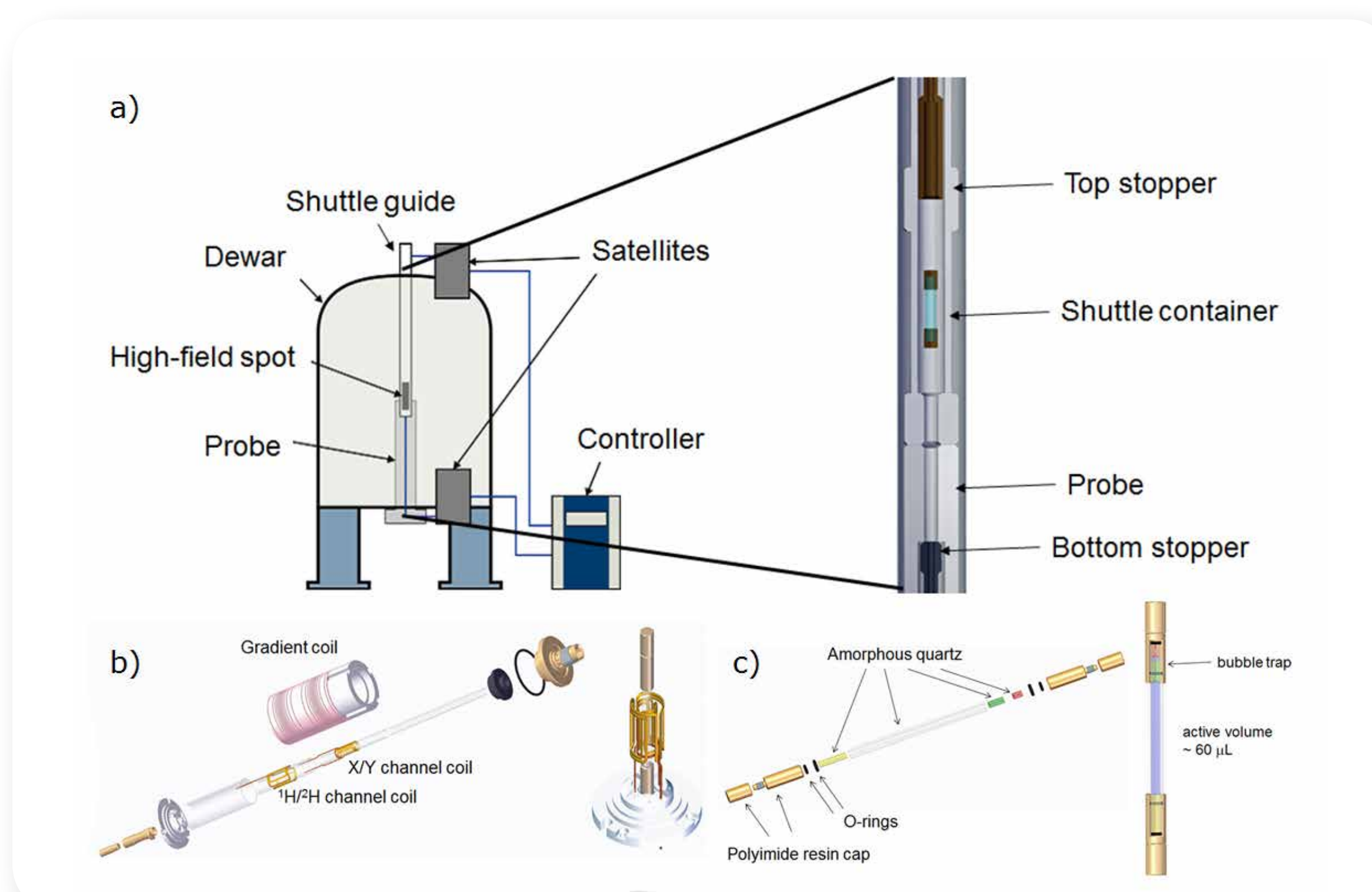


Fig. 2 Shuttle setup a) Pneumatic sample shuttling, b) Triple-resonance probe, c) Shuttle container

The probe is tuned  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^1\text{H}$  and  $^2\text{H}$  (lock) in TXO configuration. A special feature of the probe is its vibration damping system to minimize the impact of the fast shuttle motion thus achieving a spectral resolution and line shapes necessary for liquids high-resolution NMR.

A special quartz material is used as a sample container for the protein samples (Figure 2c).

## Results

First results of longitudinal relaxation rates with 3mM  $^{15}\text{N}$ -labeled ubiquitin were recorded at 7 different low magnetic fields <sup>(1)</sup> shown in Figure 3.

In addition, a full set of conventional  $^{15}\text{N}$  relaxation experiments was recorded without shuttling at 14.1, 18.8, and 22.3 T using state-of-the-art methods to identify and cancel cross-correlation effects.

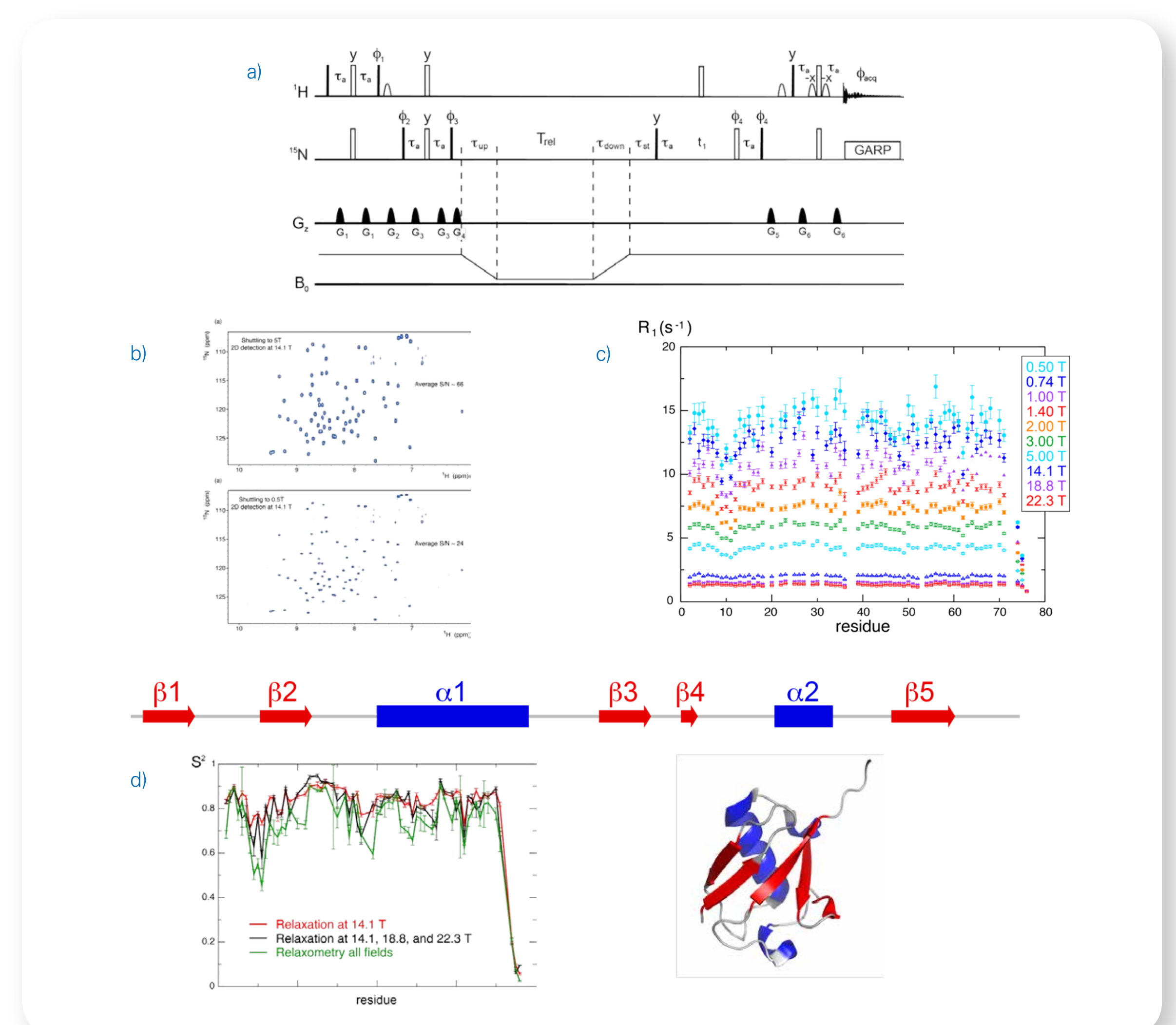


Fig. 3 a) Pulse sequence for  $^{15}\text{N}$  longitudinal relaxation, b)  $^{15}\text{N}$  longitudinal relaxation spectra, c)  $^{15}\text{N}$  longitudinal relaxation rates at 10 different magnetic fields, d) Order parameters in Ubiquitin.

1) C. Charlier, S. N. Khan, T. Marquardsen, P. Pelupessy, V. Reiss, D. Sakellariou, G. Bodenhausen, F. Engelke, F. Ferrage, *J. Am. Chem. Soc.* 2013, 135, 18665.

## Summary

- Combining relaxation data at various fields with high-resolution NMR data provides information on protein dynamics associated with structural information.
- The high-resolution shuttle NMR relaxometer as an accessory to high-resolution NMR spectrometers enables the study of protein structure and dynamics.

