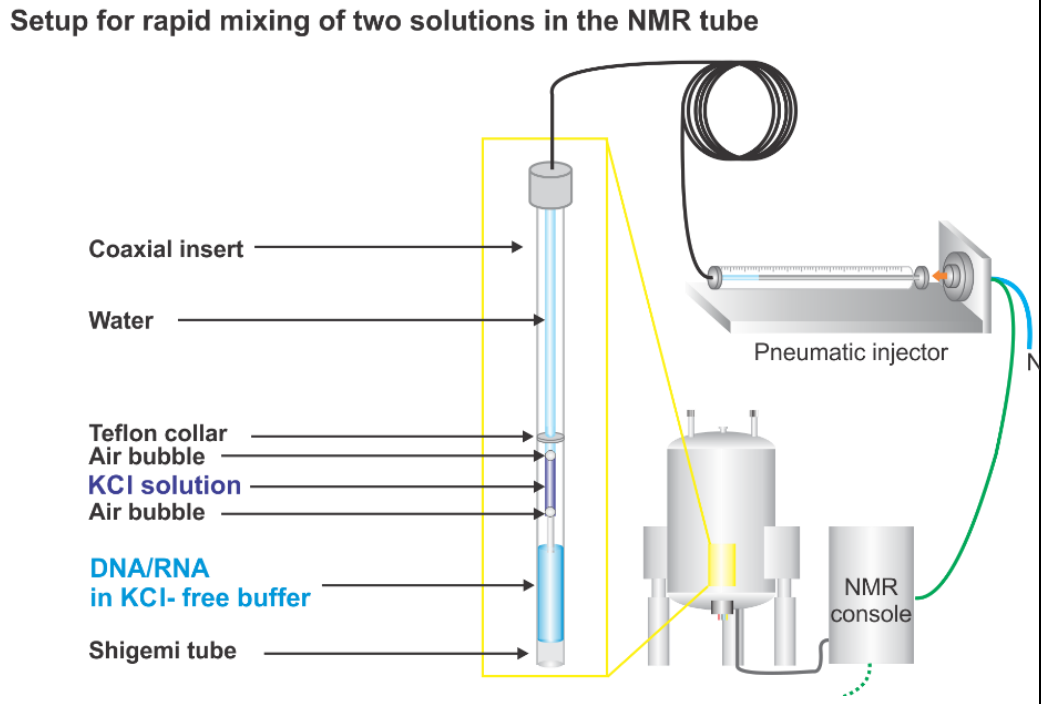
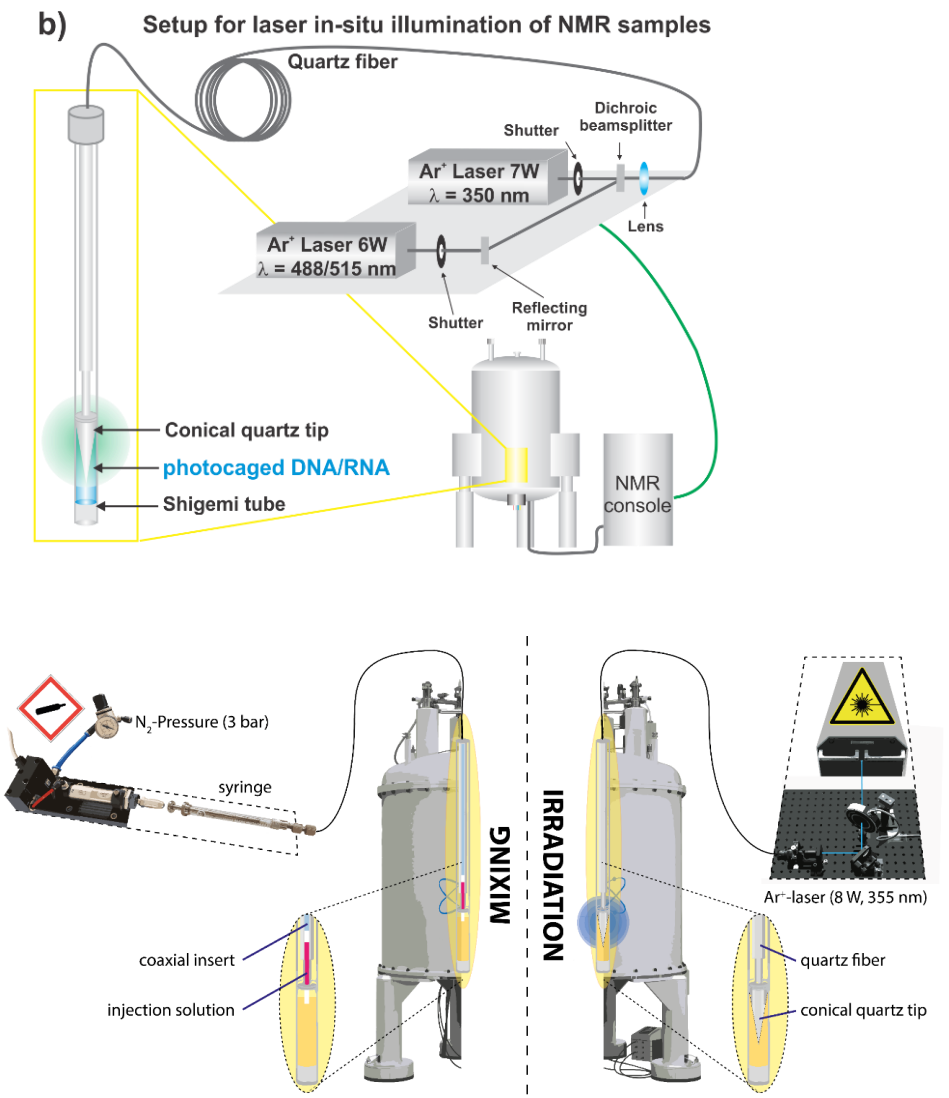
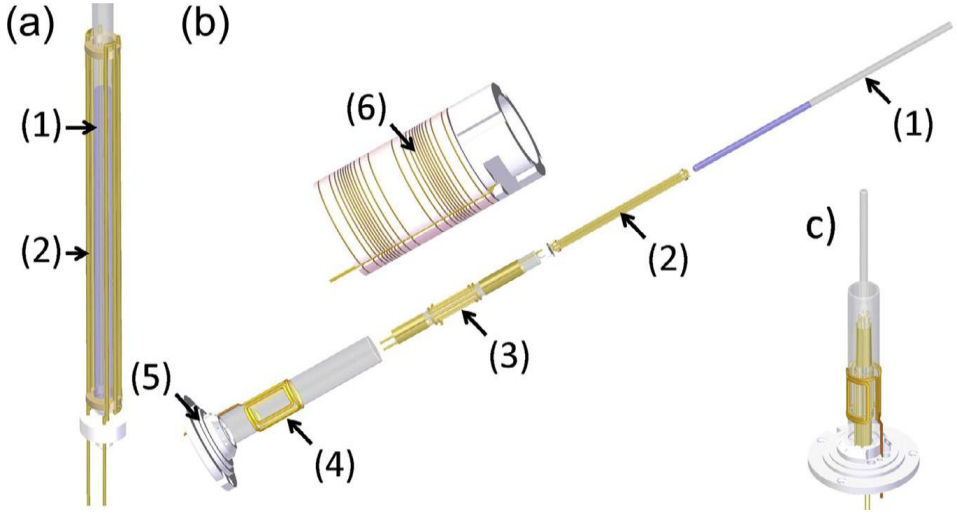
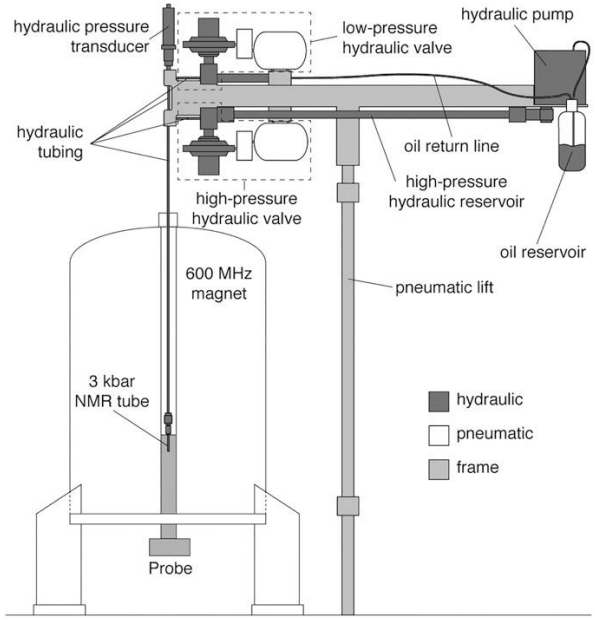
Method	Description of equipment and time resolution	Application	References
Rapid-Mixing			Mok KH, Nagashima T, Day IJ, Jones JA, Jones CJV, Dobson CM, Hore PJ. Rapid Sample-Mixing Technique for Transient NMR and Photo-CIDNP Spectroscopy: Applications to Real-Time Protein Folding. *J. Am. Chem. Soc.* 2003, 125 (41), 12484–12492. Bessi I, Jonker HRA, Richter C, Schwalbe H. Involvement of Long-Lived Intermediate States in the Complex Folding Pathway of the Human Telomeric G-Quadruplex. *Angew. Chemie - Int. Ed.* 2015, 54 (29), 8444–8448. Lieblein AL, Buck J, Schlepckow K, Fürtig B, Schwalbe, H. Time-Resolved NMR Spectroscopic Studies of DNA i-Motif Folding Reveal Kinetic Partitioning. *Angew. Chemie - Int. Ed.* 2012, 51 (1), 250–253.

Laser-induced			Wenter P, Fürtig B, Hainard A, Schwalbe H, Pitsch S. Kinetics of Photoinduced RNA Refolding by Real-Time NMR Spectroscopy. *Angew Chem Int Ed Engl* 2005, 44 (17), 2600–2603. Fürtig B, Wenter P, Reymond L, Richter C, Pitsch S, Schwalbe H. Conformational Dynamics of Bistable RNAs Studied by Time-Resolved NMR Spectroscopy. *J. Am. Chem. Soc.* 2007, 129 (51), 16222–16229. Buck J, Furtig B, Noeske J, Wohnert J, Schwalbe H. Time-Resolved NMR Methods Resolving Ligand-Induced RNA Folding at Atomic Resolution. *Proc. Natl. Acad. Sci*. 2007, 104 (40), 15699–15704.

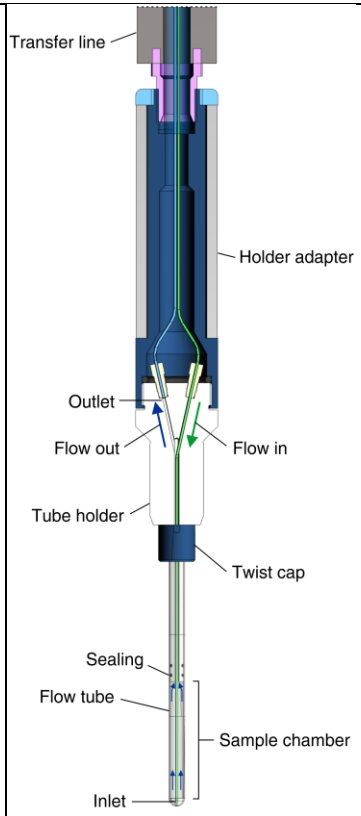
T-jump	The design of the probe consists of an integrated two coil assembly for NMR, generating, primarily, rf magnetic fields, and an additional ‘‘wire capacitor’’ with a shape similar to a saddle coil or cage coil, generating, primarily, an rf electric field, to allow a rapid rf heating of the sample. The inner wire capacitor has a special cage structure with an inner diameter of 2.5 mm. It is optimized to generate a homogeneous rf electric field with an oscillation frequency in the range from 190 MHz to 220 MHz. The wires of the cage act as ‘‘plates’’ of an rf capacitor. Therefore, they produce an rf electric field perpendicular to the main axis of the dielectric and/or conductive sample which causes rapid heating of the sample. The magnitude of the heating effect depends on various factors like the amplitude of the generated electric field, the length of the sample space exposed to it (filling height of the sample tube) as well as on the conductivity and on the mobility of the polar molecules/ions in solution. The temperature gradients vanish rapidly **within the first seconds after the T-jump**.  	Original paper describing the probehead.	Rinnenthal J, Wagner D, Marquardsen T, Krahn A, Engelke F, Schwalbe, H. *J. Magnetic Resonance* 2015, *251*, 84–93.
		Folding of cold denatured barstar, investigating stable intermediates.	Pintér G, Schwalbe H. (submitted)

P-jump	The apparatus controlling the hydrostatic pressure consists of a high-pressure reservoir of hydraulic fluid, either mineral oil or mineral spirit, that is connected through a spectrometer-controlled valve and stainless-steel tubing to the NMR sample cell (Fig. below). In the open state of the high-pressure valve, the aqueous protein solution inside the NMR sample cell rapidly equilibrates its pressure with the oil reservoir, which itself is pressurized by a pneumatically driven pump. The NMR sample cell, made of zirconium oxide (from Daedalus Innovations), has an inner diameter (i.d.) of 2.7 mm and an outer diameter of 5 mm, and is rated for pressures of up to 3 kbar. Operating pressures are typically somewhat lower (~2.5 kbar) to reduce wear on the overall system and fatigue of the sample cell. After the high-pressure valve has closed, the pressure in the NMR sample cell remains stable until a spectrometer-controlled low-pressure valve connects the transfer line to a vessel at atmospheric pressure. The aqueous protein solution is compressed by ~8% at 2.5 kbar, and the transducing fluid by ~12%. Upon switching back to low pressure, the hydraulic fluid expands into the atmospheric-pressure vessel and is recycled back by the high-pressure pump into the high-pressure reservoir. Work associated with pressurizing the water in the NMR sample cell results in an adiabatic temperature jump of ~3°C at 2.5 kbar; depressurization triggers a corresponding drop in temperature. The change in temperature of the more compressible and lower heat capacity transducing oil is considerably larger. Moreover, its high flow speed (up to 150 m/s) through the valve body and stainless-steel transfer tubing results in turbulent flow and significant frictional heating. Cooling of the pressurized fluid after the high-pressure valve has closed causes a modest drop of the pressure. Opening the high-pressure valve, a second time compensates for this drop. To minimize oxidative processes, the hydraulic fluid is kept under a N_2 atmosphere. The valve opening speeds are high, resulting in rapid pressure changes at the pressure sensor immediately adjacent to these valves (~1 ms for the 10–90% transition of the sigmoidal pressure profile). The actual rate of pressure change in the NMR sample, as measured by the resonance frequency of water under unlocked conditions, can either be comparable when using large diameter (2-mm i.d.) tubing, or substantially slower when using a small diameter (e.g. 0.5-mm i.d.). The use of small-diameter tubing reduces the volume of compressed transducing fluid, thereby extending longevity of the pump, but friction in the transfer	Hydrogen exchange monitored during refolding of ubiquitin initiated by pressure jump.	Alderson TR, Charlier C, Torchia DA, Anfinrud P, Bax A. *J. Am. Chem. Soc.* 2017, *139*, 11036–11039.
		Description of P-jump setup cycling folding study of ubiquitin.	Charlier C, Alderson TR, Courtney JM, Ying J, Anfinrud P, Bax A. *PNAS* 2018, *115*, E4169–E4178.

	line then limits the rate at which the NMR sample can be pressurized. When using large-diameter (2-mm) tubing for the transfer line, switching times for the low- to high-pressure transition approach 1 ms. However, with the ~8% compression of a 240-µL aqueous sample, the water-alkane interface will move by ~3.4 mm at a linear velocity of ~3.4 m/s. At 20 °C, this flow velocity corresponds to a Reynolds number of ~9,000, which is about 4.5 times the threshold where turbulence sets in. As a consequence, emulsification of the alkane-based hydraulic fluid into the protein sample can become visible after several hundred pressure cycles. For noncritical applications, where slower switching speeds suffice, we therefore opt to use narrower diameter (1-mm i.d.) tubing, which affords **switching times in the 3- to 5-ms range.**  *Major advantage is it can switch in both directions rapidly, allowing more sophisticated pulse sequences to be applied.*	Stroboscopic measurement of ^{15}N chemical shifts of ubiquitin during pressure induced folding to monitor intermediate states indirectly.	Charlier C, Courtney JM, Alderson TR, Anfinrud P, Bax A. *J. Am. Chem. Soc.* 2018, *140*, 8096–8099.
		Direct observation of on pathway folding intermediate of ubiquitin by interrupted folding, rapidly switching between high and low pressure states.	Charlier C, Courtney JM, Anfinrud P, Bax A. *J. Phys. Chem. B* 2018, *122*, 11792–11799.

Mixing combined with hypersense	**Dynamic Nuclear Polarization.** An Oxford Instrument Hypersense polarizer equipped with a 3.35T magnet was employed to hyperpolarize water. An EH-500 Edwards booster was added to the existing Oxford-supplied E2M80 vacuum pump, in order to further reduce operating pressures to below 1 torr. Thus, DNP was usually done at ~1.15 to 1.30 K, by irradiating a 10 mM 4-amino-tetramethylpiperidinyloxy nitroxide radical dissolved in ~150 µL of a solution consisting of 15% glycerol and 85% H_2O (vol/vol) at ~94.1 GHz. Microwave power levels and pumping times were set to 100 mW and 150 min, respectively. The ensuing hyperpolarized water pellets were dissolved with 2.6 mL of 99.9% D_2O with and without hypoxanthine; ~300 µL of the melted, hyperpolarized samples were then transferred using a preheated (50 °C) tubing line and injected into a medium wall 5-mm tube containing the RNA dissolved in buffered D_2O waiting in the NMR magnet. After the mixing, final H_2O:D_2O ratio was ~2%:98%. **Injection Setup.** An automated injection system was utilized to achieve robust, reproducible transfers, assuring minimum bubble and foam formation. The design of the injection system was previously described. In short, it relies on a two-state valve operation using both forward and backward gas pressures to regulate the volume of hyperpolarized sample reaching the NMR tube, and an Arduino-based software to control the injection setup. Previous optimization of the injection setup yielded driving and equilibration pressures of 17 and 3.5 bar.	Original paper describing combined DNP/ NMR setup.	Frydman L, Blazina D. *Nature Physics* 2007, *3*, 415–419.
		Investigating riboswitch conformational change upon ligand binding with DNP signal enhancement for low concentrated samples.	Novakovic M, Olsen GL, Pintér G, Hymon D, Fürtig B, Schwalbe H, Frydman L. *PNAS* 2020, *117*, 2449–2455.
		Metabolomic samples investigated by D-DNP to show the possibilities for low concentration samples.	Dumez JN, Milani J, Vuichoud B, Bornet A, Lalande-Martin J, Tea I, Yon M, Maucourt M, Deborde C, Moing A, Frydman L, Bodenhausen G, Jannin S, Giraudeau P. *Analyst* 2015, *140*, 5860–5863.

Light induced	Solid state/liquid state NMR applications, either in combination with or without DNP.	GPCR rhodopsin dynamics.	Kubatova N, Mao J, Eckert CE, Saxena K, Gande SL, Wachtveitl J, Glaubitz C, Schwalbe H. *Angewandte Chemie International Edition* 2020, DOI 10.1002/anie.202003671.
		Channelrhodopsin-2.	Becker-Baldus J, Bamann C, Saxena K, Gustmann H, Brown LJ, Brown RCD, Reiter C, Bamberg E, Wachtveitl J, Schwalbe H, Glaubitz C. *PNAS* 2015, *112*, 9896–9901.
		Rhodopsin decay kinetics of metastate II and III in solution state.	Stehle J, Silvers R, Werner K, Chatterjee D, Gande S, Scholz F, Dutta A, Wachtveitl J, Klein-Seetharaman J, Schwalbe H. *Angewandte Chemie International Edition* 2014, *53*, 2078–2084.
		Membrane protein enzyme kinetics by release of caged substrate.	De Mos J, Jakob A, Becker-Baldus J, Heckel A, Glaubitz C, *Chemistry – A European Journal* 2020, *26*, 6789–6792.

<table>
<tr>
<td>In-cell NMR Bioreactor</td>
<td>


The NMR bioreactor is a modified version of a commercial apparatus from Bruker (InsightMR). It consists of a flow NMR tube (o.d. = 5 mm, i.d. = 4.2 mm) connected to a watertight sealing that sets the total sample height to 38 mm, for a total sample volume of 526 µl. An inlet tubing provides fresh nutrients to the cells, which are confined in the flow NMR tube. The spent medium leaves the tube through an outlet located at the top (see Figure below). Inlet and outlet tubing pass through a 7-meter-long transfer line that is temperature-controlled through a water bath set at 37°C.

The flow unit is attached to a FPLC or a HPLC pump through PEEK tubing (o.d. = 1/16”, i.d. = 0.5 mm). During the in-cell NMR experiments, unlabelled DMEM (Sigma-Aldrich D5648, powder, reconstituted in sterile-filtered MilliQ H_2O and supplemented with 2% FBS, 10 mM $NaHCO_3$, antibiotics and 2% D_2O, pH 7.4) is supplied at a flow rate of 0.05 ml/min or 0.1 ml/min. The growth medium is contained in a 250 ml or 500 ml reservoir bottle kept at 37°C in the water bath. The bottle is sealed with a steel headpiece with two hose nozzles, one connected to the pump through a FEP tubing (o.d. = 1/8”, i.d. = 1.6 mm), the other connected to a 0.22 µm PTFE syringe filter for air intake.

The NMR bioreactor can be moved to different NMR spectrometers, and can be applied to analyse cells either embedded in hydrogel or suspended in the culture medium.

Hydrogel-embedded cells
Human cells are embedded in agarose threads (low-gelling agarose, Sigma-Aldrich, A4018) that are cast directly in the flow tube which contains a ~5 mm-high bottom plug of 1.5% agarose gel. The inlet consists of a PEEK capillary tubing (o.d. = 1/32”, i.d. = 0.5
</td>
<td>Original paper describing the microdialysis setup for suspended cells.

Optimization of hydrogel-embedded setup and application to protein-ligand interaction and protein chemical modification.</td>
<td>Cerofolini L, Giuntini S, Barbieri L, Pennestri M, Codina A, Fragai M, Banci L, Luchinat E, Ravera E. Biophysical journal 2019, 116, 239-247.

Luchinat E, Barbieri L, Campbell TF, Banci, L. Analytical chemistry 2020. DOI 10.1021/acs.analchem.0c01677.</td>
</tr>
</table>

	mm) inserted in the NMR tube down to ~6 mm from the bottom, while the outlet is a PTFE tubing (o.d. = 1/32", i.d. = 0.5 mm) attached at the top of the tube holder. **Suspended cells** Suspended cells are placed in the internal volume of flow tube, and the standard outlet is sealed. Human cells are kept in suspension in fresh Dulbecco's modified Eagle's medium (2% FBS) in the presence of 30% Percoll. The flow of nutrients occurs within a cylindrical membrane (CMA 20, 1 MDa MWCO; CMA Microdialysis, Solna, Sweden) in which inlet and outlet are coaxial. The microdialysis membrane is connected to two PTFE tubing (o.d. = 1/32", i.d. = 0.5 mm) serving as inlet and outlet for the bioreactor. The NMR bioreactor allows time-resolved multidimensional NMR experiments on high numbers of cells (3-6 x 10^7 human cells) which are kept viable and metabolically stable for up to 72 hours. Time resolution varies with the type of experiment and with the amount of signal. Ligand binding to an overexpressed intracellular protein target was observed by 1D ^{1}H NMR with a time resolution <7 minutes. Quantitative analysis of the time-resolved NMR data can be performed in a semi-automatic way to extract concentration profiles of different intracellular species, by multivariate curve resolution – least square fitting (MCR-ALS).		