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UTOPIA NMR: Activating unexploited magnetization using interleaved low-gamma detection

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Abstract

A growing number of nuclear magnetic resonance (NMR) spectroscopic studies are impaired by the limited information content provided by the standard set of experiments conventionally recorded. This is particularly true for studies of challenging biological systems including large, unstructured, membrane-embedded and/or paramagnetic proteins. Here we introduce the concept of unified time-optimized interleaved acquisition NMR (UTOPIA-NMR) for the unified acquisition of standard high-γ (e.g. ^{1}H) and low-γ (e.g. ^{13}C) detected experiments using a single receiver. Our aim is to activate the high level of polarization and information content distributed on low-γ nuclei without disturbing conventional magnetization transfer pathways. We show that using UTOPIA-NMR we are able to recover nearly all of the normally non-used magnetization without disturbing the standard experiments. In other words, additional spectra, that can significantly increase the NMR insights, are obtained for free. While we anticipate a broad range of possible applications we demonstrate for the soluble protein Bcl-x_L (ca. 21 kDa) and for OmpX in nanodiscs (ca. 160 kDa) that UTOPIA-NMR is particularly useful for challenging protein systems including perdeuterated (membrane) proteins.

Keywords

Interleaved acquisition; low-γ nuclei; UTOPIA-NMR; ^{13}C-detection

Nuclear magnetic resonance (NMR) spectroscopy has the intrinsic capability to obtain/maintain atomic resolution in challenging biological systems in a native environment (Freedberg and Selenko 2014). However, as the complexity of the systems studied by NMR spectroscopy grows, the challenges to get the necessary information for accurate structural

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The pulse sequences used in this manuscript are beta-versions that require experienced users for their implementation. Until more user-friendly versions are released (on the corresponding authors homepage as well as in the Bruker library) please directly contact Dr. Manuel Etzkorn (manuel.etzkorn@hhu.de) to obtain the beta-versions as well as instructions/support for setting them up.

analysis also increase dramatically. Instrumental improvements, isotope labeling schemes (Lundstrom et al. 2012) and developments in pulse sequences (Diercks and Orekhov 2005; Farmer 1991; Frueh et al. 2009; Kay et al. 1990; Kay et al. 1992; Kern et al. 2008; Lescop et al. 2007; Parella and Nolis 2010; Perez-Trujillo et al. 2007; Sattler et al. 1995; Schanda and Brutscher 2005; Schanda et al. 2007; Szyperski et al. 1996) can shorten the time for data acquisition and improve the spectral quality, thus partially compensating the growing sample complexity. Several interesting concepts such as time-shared NMR (Farmer 1991; Kay et al. 1990; Kay et al. 1992; Parella and Nolis 2010; Perez-Trujillo et al. 2007; Sattler et al. 1995), BEST (Lescop et al. 2007) and SOFAST (Kern et al. 2008; Schanda and Brutscher 2005; Schanda et al. 2007) techniques, have been developed to use the available magnetization more effectively. In addition, the usage of a second receiver allows parallel signal acquisition of different nuclei (Chakraborty et al. 2012; Kupce et al. 2006; Kupce and Kay 2012; Kupce et al. 2010; Moore et al. 1991; Reddy and Hosur 2013). While these techniques can offer significant benefits for a magnitude of sample conditions, they often face limitations in challenging systems where fast relaxation processes, labeling patterns and/or low protein concentrations impede their use. This is particularly true for perdeuterated proteins, which do not allow the selective excitations of amide and aliphatic protons. In the following we present a complementary and generally applicable concept to convert normally not used magnetization into useful data. Our unified time-optimized interleaved acquisition (UTOPIA) setup optimizes polarization usage by the unified acquisition of high- and low-γ-detected experiments. Remarkably, UTOPIA-NMR is also very effective in challenging systems, i.e. the systems where the additionally accessible information is also needed the most.

In line with other techniques (Kupce et al. 2010; Parella and Nolis 2010; Perez-Trujillo et al. 2007; Salzmann et al. 2000; Wiedemann et al. 2014), our UTOPIA setup relies on the acquisition of several spectra using one single relaxation recycle period. Considering that in most NMR experiments the vast amount of time required to acquire a multidimensional spectrum is used up by this recycle period (i.e. waiting for the spins to relax back into a state that is close to the equilibrium), it is of fundamental importance that during each scan all of the available magnetization is used effectively (Fig. 1). In this respect, it is surprising that the magnetization residing on the low-γ nuclei, which in perdeuterated proteins can account for about half of the total accessible magnetization, is discarded by nearly all standard ^{1}H-detected NMR experiments. While in general the possibility exists to transfer the low-γ magnetization to suitable protons for detection, low-γ direct detection has several advantages: (i) complementary information content (due to e.g. improved resolution in the direct dimension, direct detection of deuterated side chains and appearance of proline signals), (ii) favorable relaxation properties in particular in large protein systems additionally increasing resolution and paired with fewer transfer steps also increased sensitivity. We therefore decided to activate non-used ^{13}C magnetization and read out the information using direct ^{13}C detection. State-of-the-art receivers allow the fast switching of the receiver channel, enabling the interleaved acquisition of e.g. a ^{1}H-detected and a ^{13}C-detected experiment using a single receiver system. Hence, using a joint recycle delay and the interleaved acquisition capability we can unify one ^{1}H-detected and one (or more) ^{13}C-detected experiment in a time-optimized way (Fig. 1b,c). In the following, the high-γ-

detected experiments will be referred to as parent experiment whereas the low-γ-detected experiment will be referred to as child. Note that this does not imply any hierarchy or polarization inheritance and is just used for simplicity and technical reasons (i.e. it is consistent with the terminology used by the Topspin software). This unified time-optimized interleaved acquisition offers a remarkable potential and flexibility for a multitude of applications. In general UTOPIA-NMR can be applied to all areas of NMR spectroscopy that involve at least two different nuclei species, however it will be most applicable in systems were the additionally activated magnetization source is significant in comparison to the conventionally used one, and/or the additional spectra should be acquired anyways. We therefore anticipate that four areas of research will particularly benefit from the UTOPIA setup:

1. NMR analytics/screening of (small) compounds. Here the acquisition of 1D or 2D spectra of high-γ (e.g. ^{1}H, ^{19}F) and low-γ (e.g. ^{13}C, ^{15}N, ^{31}P) nuclei can be carried out simultaneously, significantly reducing the total measurement time and increasing throughput.
2. The investigation of small soluble proteins at higher concentrations. Here excess in sensitivity can be split into additional experiments (using shared evolution periods).
3. The characterization of intrinsically disordered and/or paramagnetic proteins, which often focuses on the acquisition of a set of low-γ-detected experiments. If low-γ-detected experiments are the priority, UTOPIA-NMR can offer ^{1}H-detected experiments for free.
4. The investigation of perdeuterated biological systems including membrane proteins or large complexes.

While aspects 1. and 2. are in line with experiments that have been designed using parallel acquisition on multiple receiver setups (Kupce 2013; Kupce et al. 2006; Kupce et al. 2010), the UTOPIA approach has the advantage that no additional receiver upgrade has to be made. Our data (*vide infra*, and Figs. S1–S4) shows that the application of UTOPIA-NMR for these systems is straightforward and can significantly reduce measurement time. The application of UTOPIA-NMR with a focus on the low-γ detection (3.) is in many aspects similar to (and often simpler as) the application on larger, perdeuterated proteins with focus on the ^{1}H detection (4.). Notably, due to reduced decoupling requirements, UTOPIA-NMR can most easily be combined with TROSY-based polarization transfer mechanism, directly enabling the usage of all available spin ½ polarization sources of the protein.

In the following we will demonstrate the potential of the UTOPIA approach starting with the U-[^{2}H,^{13}C,^{15}N] labeled (fully ^{1}H back-exchanged) soluble Bcl-x_L protein (21 kDa). In general, the UTOPIA setup is most effective when spectra with similar total experimental time are combined. The unified acquisition of a ^{1}H,^{15}N-NOESY-TROSY (Zhu et al. 1999) with a ^{13}C,^{13}C-FLOPSY (Eletsky et al. 2003) experiment not only fulfills this criterion but also combines two very useful experiments (Fig. 2; also see Fig. S5 and Table S1 for more details on pulse sequence and experimental parameters). The comparison of the UTOPIA parent (Fig. 2b–e, red) with the same experiment recorded conventionally (Fig. 2b–e, black)

demonstrates that the unified acquisition of the child does not disturb the parent (see also Fig. S5b,c for comparison of 3D slices). Interestingly, also for the UTOPIA child experiment (Fig. 2f–g, red) only minor differences are visible in comparison with the reference data (Fig. 2f–g, black). After analyzing the intensities in the corresponding 1D slices, we verify that we were able to recover about 95% of the reference (previously not used) carbon magnetization. In other words, the low-γ-detected spectrum (Fig. 2f) can be recorded for free during the acquisition of a widely used standard experiment. The additional data can offer valuable information that can be used e.g. to easily cross validate Cα-Cβ assignments and/or to link Cα-Cβ to further carbons in the side chain and assign the full side chain in perdeuterated proteins. The latter is demonstrated for two selected residues of Bcl-x_L in Fig. 2f (Pro42 and Ile78).

The UTOPIA setup may be modified for a given labeling scheme. When e.g. used for perdeuterated proteins, like shown here, ^{1}H decoupling during ^{13}C detection is often not required and hence the proton magnetization will be completely unperturbed by the presence of the child experiment. Therefore, the length of the recycle delay can be shortened by the time for spin-state generation and acquisition of the child experiment without losing magnetization for the parent. Notably, the T_1 relaxation time of the ^{13}C magnetization is often several times larger than the ^{1}H T_1 (see Fig. S6 for ^{1}H and ^{13}C T_1 measurements in Bcl-x_L). The differences in T_1 relaxation times can be partly compensated by e.g. acquiring the child experiment only every second scan (as done for spectrum shown in Fig. 2f). If ^{1}H composite pulse decoupling is required during the ^{13}C-detection period, which will often be the case in non-deuterated proteins, e.g. for the study of intrinsically disordered proteins, the total acquisition time will be prolonged by the time of the child experiment (spin-state generation and acquisition). In this case the child cannot be recorded completely for free. The cost for acquiring the child experiment in these conditions is however just the net time for pulsing and detection. For the UTOPIA setup shown in Fig. 2a this only corresponds to ca. 5% of the total experimental time.

In general, the UTOPIA approach is very flexible, meaning that many different experiments can be designed, according to a particular sample or desired outcome. While the above shown setup combines two experiments with largely independent magnetization transfer pathways, the UTOPIA setup also has the potential to combine experiments with significant overlap in their transfer pathways. To demonstrate the flexibility of UTOPIA-NMR we developed a pulse sequence for the unified acquisition of a 3D ^{1}H,^{15}N-TROSY-HNCA experiment (Salzmann et al. 1998) with a 2D ^{13}C,^{15}N-CON experiment (Bermel et al. 2006) (Fig. 3b–f, see Fig. S7 and Table S1 for details on pulse sequence and acquisition parameters). Note that in this setup the disturbance of the ^{13}C magnetization of the child (CON) by the parent (TROSY-HNCA) can be minimized by introducing the joint recycle delay not at the end of the child (as it was the case for the UTOPIA setup shown in Fig. 2) but between the parent and the child. As shown in Fig. 3c–d, this setup, combined with suitable ^{1}H decoupling pulses in the child, also does not significantly disturb the ^{1}H excitation of the parent experiment (also see Fig. S8a and b, for comparison of the parent NH plane and 1D slices, respectively). The comparison of the UTOPIA setup with the respective reference spectra again shows that while no difference in the information content of the parent spectra is observed, the additionally obtained CON spectrum provides valuable

information such as a superior resolution in the CO dimension that can help to resolve ambiguities and correct peak positions in the conventional spectra (see Fig. S8 for comparison). In addition, in the case of Bcl-x_L, it directly enables the assignment of all proline residues, filling the gaps often found in chemical shift assignments (again this information comes for free during the acquisition of a standard experiment).

To demonstrate the usage of UTOPIA for even more challenging biological systems, we tested the approach on the membrane protein OmpX in (not NMR-optimized) nanodiscs (formed with MSP1D1 (Denisov et al. 2004; Hagn et al. 2013), 160 kDa - see supplementary methods for details on sample preparation). In previous studies of comparable samples we could e.g. not detect any Cα-Cβ connectivities in the membrane-embedded protein regions using conventional ^{1}H-detected experiments (Etzkorn et al. 2013; Hagn et al. 2013). Fig. 3d demonstrates (i) that UTOPIA-NMR works well in these systems and (ii) that due to the favorable relaxation properties of direct ^{13}C detection (that does not require long transfer steps from and to the amide proton) combined with TOCSY-type of magnetization transfer, Cα-Cβ connectivities can be obtained in these challenging systems. Notably, in these systems the additional information is very much needed for detailed NMR characterization and once more the UTOPIA setup provides this information for free during the acquisition of e.g. a NOESY-TROSY spectrum (Fig. S9).

Fig. 3e,f shows an example for the application of the UTOPIA setup to analyze small molecules. Here the acquisition of a 2D ^{1}H–^{1}H TOCSY spectrum with a direct 1D ^{13}C-detected spectrum were combined and tested with a sample of 2 mM unlabeled sucrose. Our data shows that it is also possible for this completely different class of samples to obtain useful information on the low-γ nucleus without disturbing the conventional ^{1}H-detected experiment (see supplementary information figures S3 and S4 for a more detailed discussion).

In conclusion, the UTOPIA-NMR approach provides a convenient way to exploit non-used magnetization. Here we focused on a setup that allows the acquisition of a low-γ-detected experiment without disturbing the ^{1}H-detected experiment. In principle the acquisition of more than one parent and one child experiment as well as the usage of more than two different channels should be feasible and may provide exciting opportunities for future developments of the UTOPIA setup. In addition, also joint evolution periods are possible as previously reported using parallel acquisition (see Fig. S3 and Fig. S4 for a setup mimicking (Kupce et al. 2010)). However, as compared to multi-receiver setups, small to considerable relaxation losses (depending on sample properties and experimental design) due to a delayed acquisition will be present in the interleaved setup. Moreover, one should consider that e.g. in protonated proteins, in contrast to perdeuterated proteins, the additional ^{13}C magnetization source is substantially weaker as compared to the ^{1}H source. If, in these cases ^{13}C detection is still desired, it may be beneficial to start an UTOPIA child experiment from the ^{1}H magnetization (e.g. by using selective pulses). In general, the UTOPIA setup is complementary to many previously developed acquisition techniques and therefore can also be directly combined with fitting approaches including the use of paramagnetic relaxation reagents (Theillet et al. 2011), non-uniform sampling (Frueh et al. 2013) and, in suitable samples, also with selective proton excitation techniques.

Due to the flexibility of the approach, the wide range of possible applications, and the fact that acquiring the additional information is very useful and can be obtained completely for free, we anticipate that this approach should become standard for all measurements that benefit from the additional information. The unified acquisition of two or more spectra has the additional advantage that the different spectra are obtained under exactly identical conditions facilitating peak alignment and providing unique possibilities in time-resolved spectroscopy. Noteworthy, many NMR analytics facilities regularly record a direct ^{13}C-detected spectrum of their compounds in addition to 2D $^{1}H,^{1}H$ correlated spectra. Since ^{13}C detection is mostly done at natural abundance, this spectrum often requires about half of the measurement time spend for each sample. As demonstrated in Fig. 3e–f, UTOPIA-NMR allows the detection of a ^{13}C spectrum during the acquisition of standard ^{1}H-detected experiments (also see Fig. S1 and S2). UTOPIA-NMR hence has the potential to nearly double the throughput of many NMR analytics facilities (the gain in efficiency may vary according to sample properties and the used setup, see Fig. S1 for a more detailed discussion). Depending on the perspective, UTOPIA-NMR can either reduce the experimental time by a factor of about two or it can deliver additional information for free during the acquisition of a conventional set of experiments. In particular the combination of already developed low-γ-detected experiments (including the protonless NMR approach (Bermel et al. 2005; Bermel et al. 2006)) with standard ^{1}H-detected experiments can directly provide valuable complementary information that may help to maintain reliable NMR-structural insights for increasingly complex samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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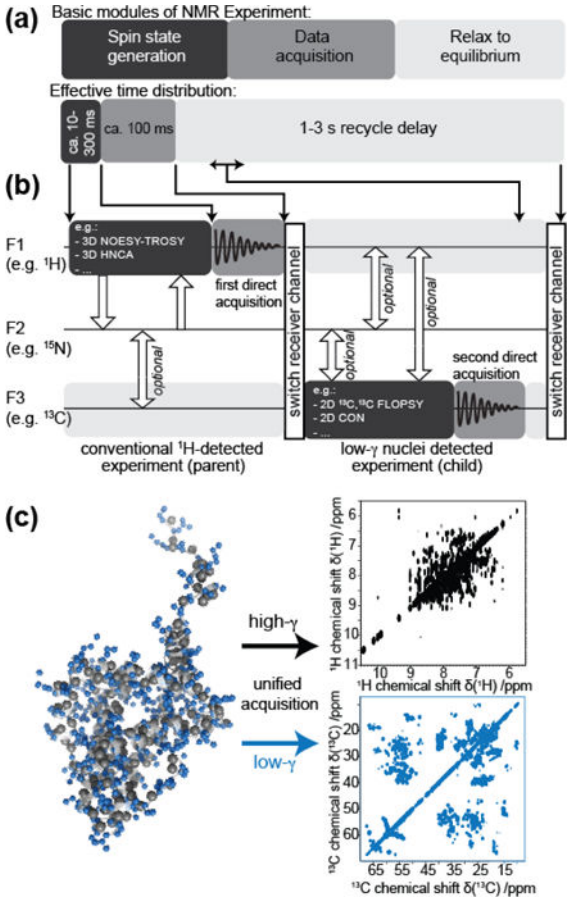

Fig. 1.

Schematic representation of the UTOPIA-NMR setup. **(a)** Modules of a standard NMR experiment and their conventional time distribution. **(b)** The UTOPIA-NMR concept relies on the acquisition of one (or more) low-γ nuclei detected experiment (child experiment) during the relaxation period of the conventional ^{1}H-detected experiment (parent). Switching the receiver channel twice during each scan allows for two direct acquisition periods on different channels (e.g. ^{1}H and ^{13}C) using only one recycle delay and a conventional single receiver system. The UTOPIA approach provides a large variety of possible combinations of magnetization pathways in parent and child that can be tailored for a broad range of applications. (c) Visualization of the different sources of polarization used for UTOPIA-NMR, exemplified for the perdeuterated protein Bcl-x_L. Grey and blue spheres represent amide protons and ^{13}C atoms, respectively; sphere volumes are scaled by the ratio of the γ-values of the nuclei. UTOPIA-NMR allows excitation and detection of the normally not used low-γ polarization (here ^{13}C) during the acquisition of the conventional ^{1}H-detected (high-γ) experiments

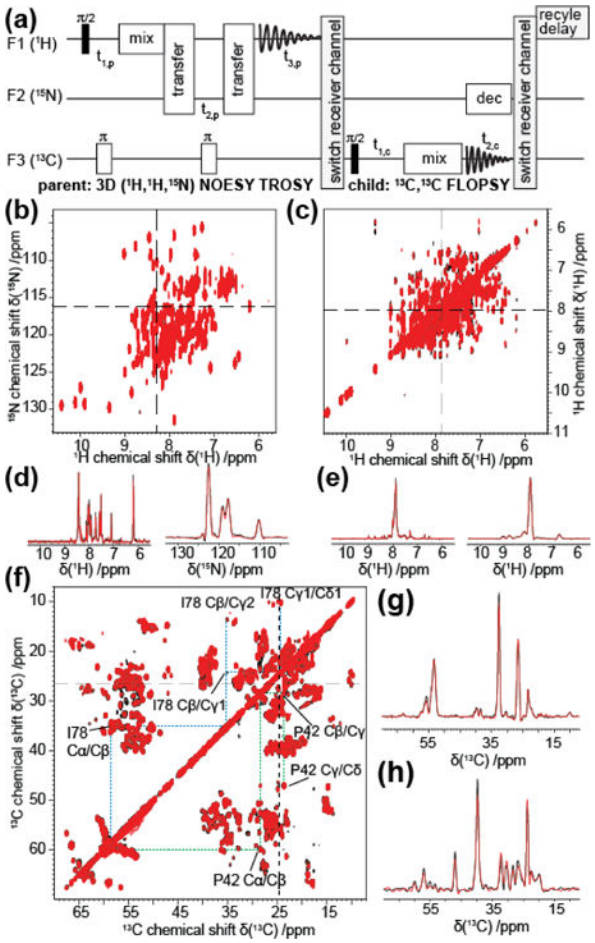

Fig. 2.

Application of UTOPIA-NMR on soluble perdeuterated Bcl-x_L. **(a)** Simplified pulse scheme for the unified acquisition of a ^{1}H-detected NOESY-TROSY (parent) and a ^{13}C-detected CC-FLOPSY (child). (See Fig. S5 for full pulse sequence.) **(b–h)** Superposition of spectra acquired conventionally (black) with data acquired in the UTOPIA setup (red). **(b,c)** First HN and HH plane of the 3D NOESY-TROSY; **(d,e)** 1D slices indicated in **(b,c)** respectively; **(f)** 2D CC-FLOPSY and (**g,h**) corresponding 1D slices. Note that the UTOPIA spectra (red) were recorded in exactly half of the time (i.e. 30 h) of the conventional ones (black, 2 × 30 h). Experimental conditions were 350 μM Bcl-x_L, 25°C, Bruker Avance III at 700 MHz (see supplementary methods for more details)

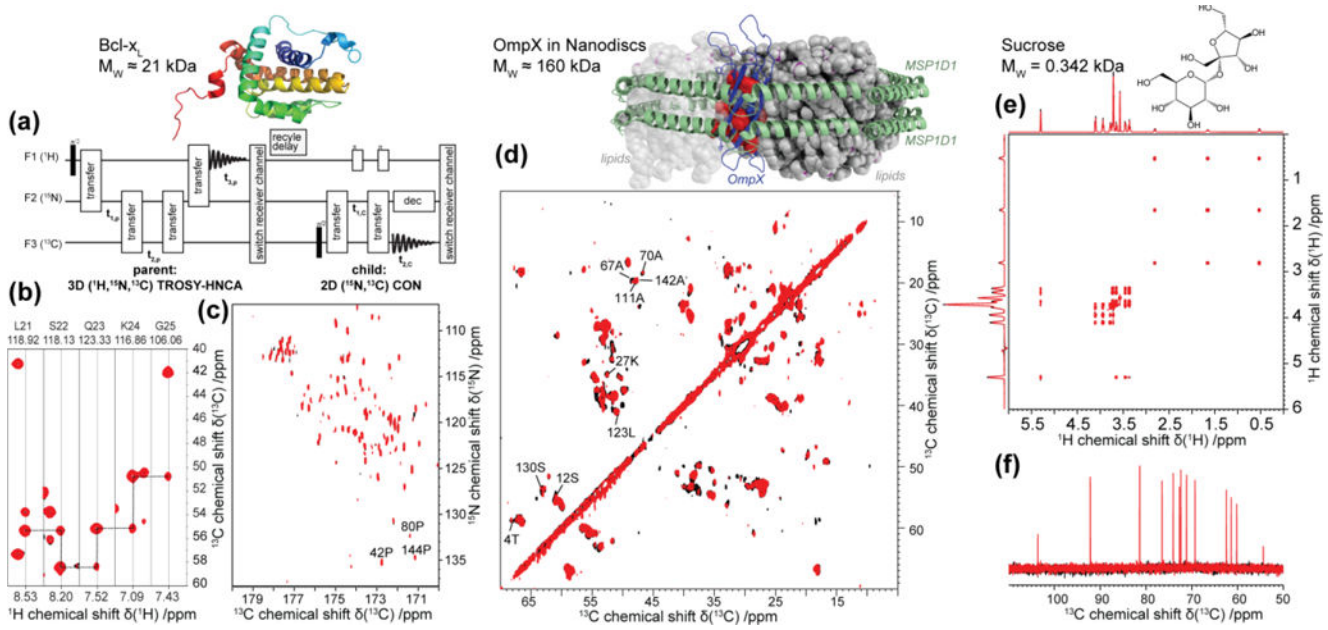

Fig. 3.

Selected applications of UTOPIA-NMR. **(a)** Simplified pulse scheme for the unified acquisition of a ^{1}H-detected TROSY-HNCA (parent) and a ^{13}C-detected CON (child) experiment (see Fig. S5 for full pulse sequence). **(b,c)** Superposition of spectra acquired conventionally (black) with data acquired in the UTOPIA setup (red). (**b**) Extract of the 3D TROSY-HNCA, (**c**) 2D CON. Total experimental time was 47 h for the conventional acquisition and 23,5 h for the UTOPIA data. Note that splittings in the CON spectrum due to CO-Cα J-couplings have been removed using maximum entropy deconvolution (Hoch et al. 2007) using nmrPipe (Delaglio et al. 1995), resulting in well-resolved signals for nearly all residues in Bcl-x$_L$ (including all prolines). **(d)** Application of UTOPIA-NMR to large biological systems. ^{13}C-detected FLOPSY spectrum recorded on OmpX in nanodiscs (200 μM perdeuterated OmpX at 700 MHz, total acquisition time was 60 h). Only the child experiment is shown (red) and compared to a conventional spectrum (black). Labels show easily identifiable Cα-Cβ correlations in the transmembrane region of OmpX (red residues in model above). **(e,f)** Typical NMR analytics spectra recorded on a sample of 2 mM unlabeled sucrose (2D ^{1}H,^{1}H TOCSY (e) and 1D ^{13}C (f)). In all cases the unified acquisition of parent and child allowed data acquisition in exactly half of the time as compared to the respective conventional setups