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COMMUNICATION

ADAPT-NMR 3.0: utilization of BEST-type triple-resonance NMR experiments to accelerate the process of data collection and assignment

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Abstract ADAPT-NMR (Assignment-directed Data collection Algorithm utilizing a Probabilistic Toolkit in NMR) is a software package whose Bayesian core uses on-the-fly chemical shift assignments to guide data acquisition by non-uniform sampling from a panel of through-bond NMR experiments. The new version of ADAPT-NMR (ADAPT-NMR v3.0) has the option of utilizing 2D tilted-plane versions of 3D fast spectral acquisition with BEST-type pulse sequences, while also retaining the capability of acquiring and processing data from tilted-plane versions of conventional sensitivity-enhanced experiments. The use of BEST experiments significantly reduces data collection times and leads to enhanced performance by ADAPT-NMR.

Keywords Automated chemical shift assignments · Bayesian NMR data acquisition · BEST-type experiments · Non-uniform sampling

In recent years, attempts to accelerate NMR data collection have resulted in several new approaches. These can be divided in two major categories: (a) NMR pulse programs that reduce the time complexity of NMR operations and allow faster data collection without compromising sensitivity (Atreya and Szyperski 2004; Bax and Grzesiek 1993; Brutscher 2013; Deschamps and Campbell 2006; Frydman 2006; Frydman et al. 2003; Kupce and Freeman 2003d; Lescop et al. 2010, 2007; Pervushin et al. 2002; Schanda and Brutscher 2005), and (b) methods that speed up data collection by minimizing the number of collected time points in indirect dimensions by invoking irregular sampling schemes (Barna et al. 1987; Hiller et al. 2005; Hoch et al. 2007, 2014; Hyberts et al. 2012; Kazimierczuk and Orekhov 2011; Kazimierczuk et al. 2010; Kim and Szyperski 2003; Kupce and Freeman 2003c; Maciejewski et al. 2006; Mobli and Hoch 2008; Orekhov et al. 2003; Qu et al. 2015; Szyperski et al. 2002).

The first group consists of those experiments that speed up data collection by significantly decreasing the recycle delay (d1). In order to prevent the loss of sensitivity, the longitudinal recovery of the magnetization for the protons being observed is accelerated through dipolar interaction with other protons in the molecule that are left unperturbed. This was first accomplished by the longitudinal ^{1}H optimized experiments (Deschamps and Campbell 2006; Pervushin et al. 2002) that use selective proton pulses in addition to the regular hard pulses to return the magnetization of unobserved protons back to equilibrium. More recently the band-Selective Optimized Flip-Angle Short-Transient (SO-FAST) HMQC experiments (Brutscher 2013; Schanda and Brutscher 2005), followed shortly by more generally applicable relatives the Band Selective Short Transient (BEST) experiments (Lescop et al. 2010, 2007) were introduced. These experiments use only selective pulses on the protons of interest throughout the pulse program to leave all other protons completely unperturbed. In all cases, the longitudinal recovery of the observed protons is accelerated through dipolar interaction

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with other protons in the molecule and experiments can be recorded faster with a shorter recycle delay without loss of sensitivity. In addition, for SOFAST HMQC experiments the rotational angles can be optimized for the shorter recycle delay to further enhance the sensitivity of the experiments. On the other hand, this optimization cannot be applied to the more complicated BEST pulse programs, which allow the concept of enhanced relaxation properties to be applied to a broader range of experiments (e.g. out-and-back experiments for backbone assignments of proteins).

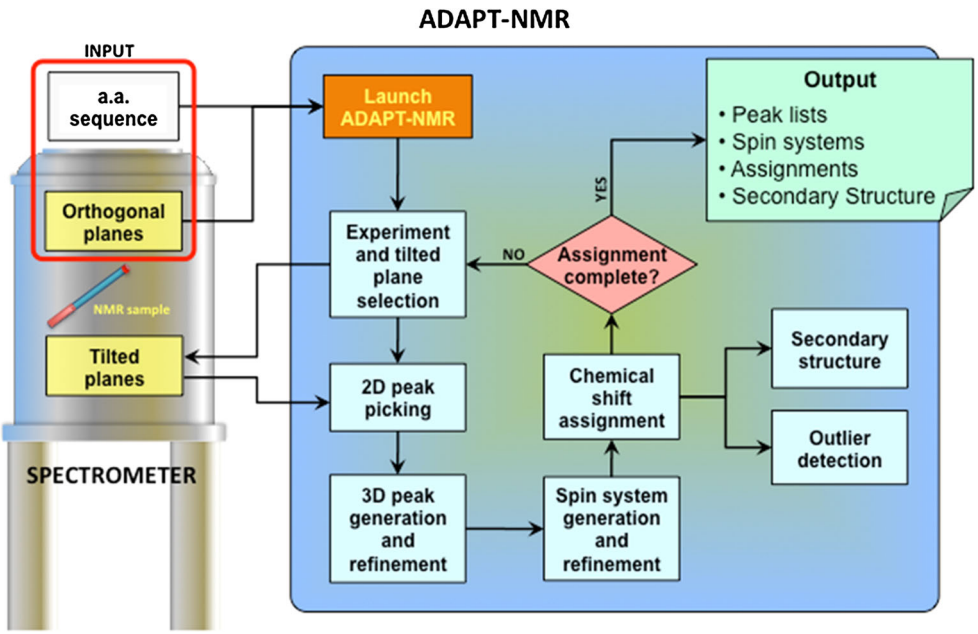
More recently, methods that afford time saving by reducing the number of acquired points in the indirect dimensions have attracted much attention. Among these methods are experiments in which the number of points acquired in the indirect dimensions is reduced in the frequency domain: Hadamard experiments (Bax and Grzesiek 1993; Kupce and Freeman 2003a, b, d) that utilize selective pulses to sample only certain frequencies, single scan experiments that sample different indirect evolution times in different slices of the sample (Frydman 2006; Frydman et al. 2003), and methods that sample sparse points directly in the time domain (Bahrami et al. 2012; Eghbalnia et al. 2005; Hiller et al. 2005; Hoch et al. 2007, 2014; Hyberts et al. 2012; Kim and Szyperski 2003; Kupce and Freeman 2003c; Maciejewski et al. 2006; Mobli and Hoch 2008; Orekhov et al. 2003; Szyperski et al. 2002). In the last group of methods, only a subset of the data is collected, and post-processing is then used to reconstruct the spectra (Hoch et al. 2007, 2014; Hyberts et al. 2012; Kupce and Freeman 2003c; Maciejewski et al. 2006; Orekhov et al. 2003) or to extract the signals without reconstruction of the spectra (Bahrami et al. 2012; Eghbalnia et al. 2005; Hiller et al. 2005). Thus, post-processing analysis plays a critical role in circumventing the lack of information caused by the irregular sampling, and separates this group from the aforementioned category of time complexity reduction by use of specific pulse programs.

One of the most common methods for irregular sampling uses a random distribution function to identify the time stencil of the data-collection (commonly known as non-uniform sampling). Different distribution functions have been examined to build the sparse sampling schedule (Hyberts et al. 2012; Orekhov et al. 2003; Orekhov and Jaravine 2011), and consequently different reconstruction/post-processing algorithms have been developed to improve the quality (sensitivity and resolution) of the final spectra.

Among non-uniform sampling methods, ADAPT-NMR (Bahrami et al. 2012) speeds up data collection by reducing multi-dimensional spectra to two-dimensional planes recorded at various projection angles (radial sampling). As a unique feature, ADAPT-NMR does not use a predefined data acquisition schedule; instead, it adaptively calculates the optimal schedule based on prior information on protein under study and the experiment to be acquired. The calculation in ADAPT-NMR is an iterative process that initiates with a fast analysis of the orthogonal planes, followed by a decision-making module that iterates between data collection and chemical shift assignments. At the end of each cycle ADAPT-NMR calculates a probability for the observed peaks and assigns chemical shifts based on a Bayesian probabilistic approach (Bahrami et al. 2009). For peaks with low probability, the decision-making module estimates which specific experiment and projection angle will provide the most information about the peaks. The requested 2D tilted plane is then collected and analyzed by the decision-making module in ADAPT-NMR for the next cycle of data collection. Thus, instead of blindly using a preset schedule, ADAPT-NMR uses the current knowledge (amino acid sequence and assigned chemical shifts) for the dynamic calculation of which tilted plane in which experiment is to be acquired. Furthermore, it is important to note that ADAPT-NMR does not attempt to reconstruct a final 3D spectrum; instead, each 2D projection plane is analyzed and the information combined to extract peak lists that are then used for chemical shift assignments. Figure 1 shows the overall workflow of the data collection and chemical shift assignments in ADAPT-NMR.

Because ADAPT-NMR utilizes modified versions of regular pulse programs for tilted plane acquisition, its data collection module can be improved by taking advantage of new experiments developed to boost sensitivity and accelerate data collection. The combination of irregular sampling schemes with fast data collection has been shown to be effective (Atreya and Szyperski 2004; Isaksson et al. 2013), and we introduce here a new version of ADAPT-NMR (v3.0) that gives the user the option to use BEST-type data collection for backbone experiments. As explained above, BEST experiments speed up data acquisition by shortening the recovery delay between transients while retaining sensitivity (Brutscher 2013; Dingley and Pascal 2011; Schanda and Brutscher 2005; Schanda et al. 2007). For out-and-back backbone experiments this is accomplished by using selective pulses on amide protons throughout the pulse program, such that the longitudinal recovery of amide proton magnetization is accelerated through dipolar interaction with unperturbed protons in the protein. The experiments can then be recorded faster by using a shorted recycle delay without compromising their sensitivity.

To examine effect of using BEST type experiments, we compared the running-time of ADAPT-NMR when using regular pulse programs against that of using BEST pulse programs. For these comparisons, we collected data on a



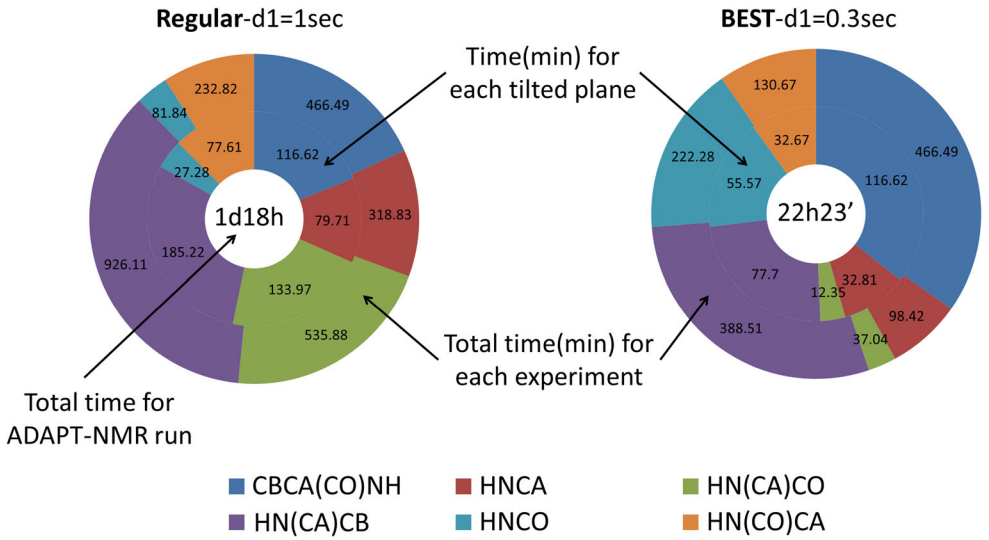


Fig. 1 Workflow of ADAPT-NMR showing the alternation between data acquisition and chemical shift assignment steps

Fig. 2 Running time complexities of ADAPT-NMR using regular (*left*) and BEST (*right*) pulse programs. The six experiments used are *color coded*. BEST versions of all of these with the exception of CBCA(CO)NH were used in generating the plot at the right. The *center circle* shows the total elapsed time for both data collection and chemical shifts assignment (regular 42 h and BEST-type experiments 22 h 23 min). The *inner ring* shows the time required for collecting and analyzing each tilted plane of the corresponding experiment, while the *outer ring* indicates the total running times of each experiment. Because both trials used regular CBCA(CO)NH pulse program, its running time is equivalent. The recovery delay between transients was 1.0 s for regular experiments and 0.3 s for BEST experiments

sample of [U-^{13}C, U-^{15}N]-chlorella ubiquitin (76 amino acids) on a Varian 600 MHz spectrometer equipped with a cryogenic probe. The use of BEST experiments reduced the total time for data-collection and assignment of the backbone chemical shifts by a factor of more than 1.9 (Fig. 2).

We also compared the assignment probabilities generated by ADAPT-NMR (Fig. 3a,b) and found that the higher sensitivity gained by using the BEST experiments improved the probability of the chemical shift assignments for TYR59, while keeping the probabilities of the other residues in the acceptable range of (0.5, 1).

After testing ADAPT-NMR 3.0 on ubiquitin, we examined the method on several other proteins. Figure 4 shows the running time complexities for six proteins that were used to further evaluate the performance and accuracy of ADAPT-NMR 3.0. Table S1 shows the sample

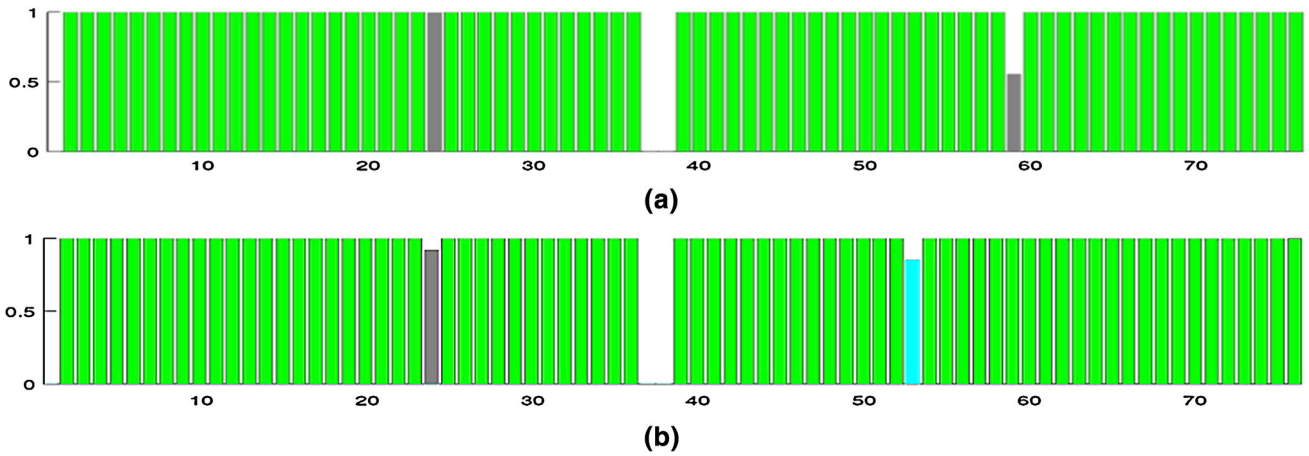


Fig. 3 **a** The probabilities of assignments being correct for ubiquitin achieved by ADAPT-NMR using regular data collection. **b** These probabilities for ubiquitin achieved by ADAPT-NMR using BEST experiments. Using BEST experiments yielded assignments with better probabilities, indicating that the Bayesian core of ADAPT-NMR assigned the chemical shifts with more confidence. Color codes: *green* the assignment probability of a residue is higher than 99 %, *cyan* the assignment probability of a residue is between 85–99 %, *gray* indicates a residue with insufficient evidence (peak information) to be precisely assigned by ADAPT-NMR

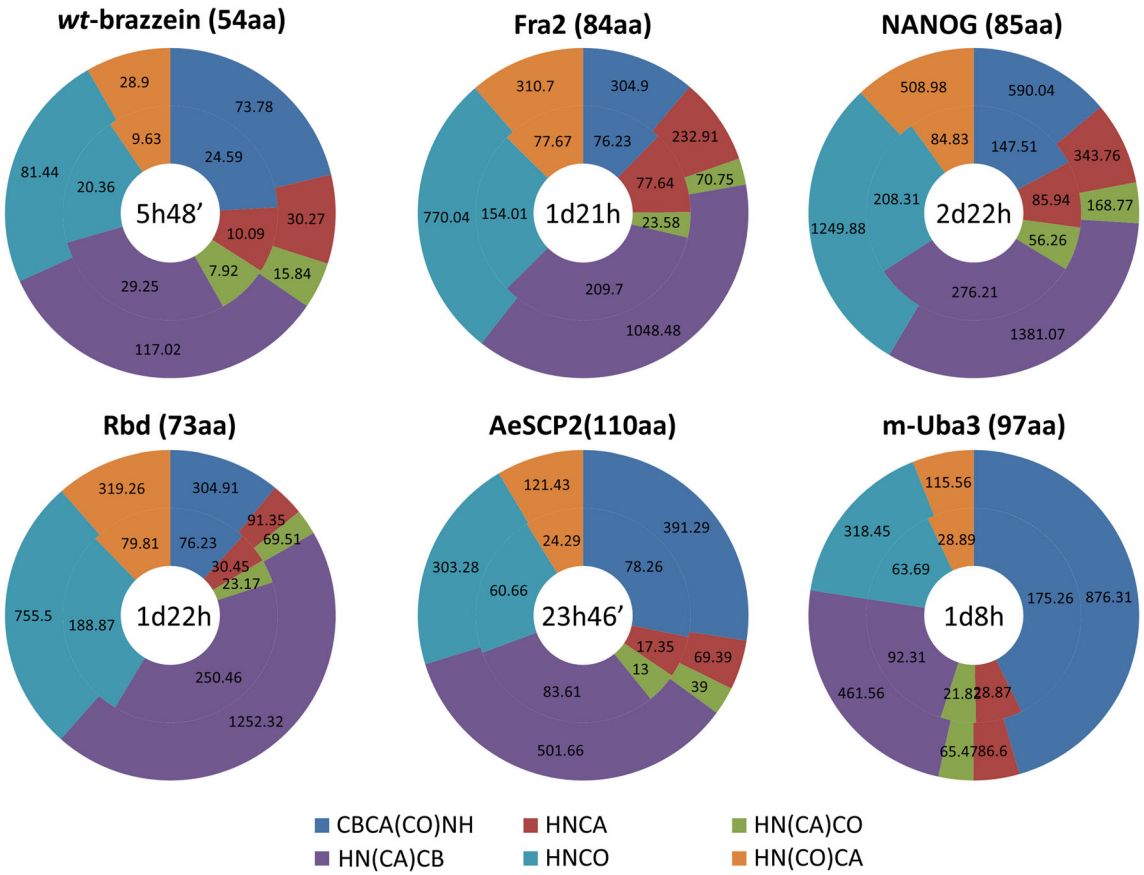


Fig. 4 Individual panels report the running time complexity for ADAPT-NMR v3.0 using BEST experiments for six proteins ranging in size from 54 to 110 amino acid residues. The experiments are color coded according to the key at the *bottom* of the figure. The total running time for data collection and assignment is shown at the *center* of each circle. The *inner ring* indicates the running times in minutes for collecting and processing each tilted plane, while the outer ring shows the total time in minutes used for each experiment

Table 1 Assignment results for six proteins achieved by ADAPT-NMR 3.0 using BEST experiments

Protein name	Num. residues	Num. manually assigned residues	Correct assignment (%)
Brazzein	53	50	94.0
Rbd	73	70	97.1
Fra2	85	77	100
NANOG	85	73	86.3
Uba3	97	81	97.5
AeSCP2	110	106	88.7

The 'correct assignment percentage' was calculated by dividing the number of correctly assigned amide nitrogen and proton by ADAPT-NMR by the number of manually assigned atoms

conditions and Tables S2-S8 show the data collection parameters. In these tables we report the elapsed time for data acquisition using the BEST experiments and the theoretical time improvement of using BEST-type versus the regular experiments recorded with a recovery delay of 1 s. Furthermore, in order to assess the accuracy of the new package, we compared backbone assignments for the six proteins achieved by ADAPT-NMR 3.0 against manual assignments (Table 1). In all cases, the accuracy of assignments was acceptable, considering the fully automated nature of the data collection, analysis and assignment process in ADAPT-NMR.

In conclusion, the new ADAPT-NMR software package takes advantage of BEST-type experiments to accelerate data collection and increase sensitivity of the spectra.

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