



Dirk Labudde, Dietmar Leitner, Rüdiger Winter, Peter Schmieder,
Mario Schubert and Hartmut Oschkinat

Automated Setup of NMR Experiments

Abstract

The high-resolution structure determination of proteins by nuclear magnetic resonance (NMR) requires the set-up of numerous NMR experiments and adjustment of their acquisition and processing parameters. The manual setting of acquisition parameters therefore depends on expert knowledge and is time consuming and error prone. In order to automate this task to achieve high throughput in a structural genomics context, the software tools PASTE (Protein Structure factory acquisition setup tool for NMR Experiments) and PAPST (Processing and Acquisition Parameter Setup Tool) were written in Tcl/Tk language and directly incorporated into the software package XWINNMR (Bruker GmbH, Rheinstetten, Germany), which runs on an NMR spectrometer. As a pre-requisite for the use of PASTE and PAPST, NMR data sets and pulse programs were standardised. The individual experiments are contained in these standard data sets and identified by a numbering scheme. At present, there are 265 NMR experiments in what is known as the B-library. PASTE and PAPST, together with the B-library, enable novice users to set up extended sets of single-, double- and triple-resonance NMR experiments in a minimal amount of time.

Key Words

NMR spectroscopy, protein structure determination, automation, PASTE, PAPST

Structural genomics is a rising discipline in the field of biotechnology that involves the high-throughput structural determination of proteins.^{1–3} As with the Human Genome Project, which unravelled the DNA sequence of the human genome by replacing tedious and time-consuming tasks with automated procedures, parallelisation and robotics are key issues that will be addressed.

Several private and governmental initiatives have been instituted over the years with the aim of determining protein structures in a high-throughput manner.^{4–6} One of these initiatives is the Protein Structure Factory (PSF) located in Berlin, Germany. It is an integrated approach to protein-structure determination,⁷ combining the computer-based analysis of genes by bioinformatics

techniques, automated gene expression and purification of gene products, generation of a biophysical fingerprint of the proteins and determination of their three-dimensional structures either in solution by NMR spectroscopy or in the crystalline state by X-ray diffraction.

Generating a high-resolution 3-D structure with the NMR of a protein requires the preparation of protein samples with different labelling patterns. Depending on the labelling scheme, various sets of 3-D and 2-D NMR spectra have to be recorded and analysed. The acquisition of up to 50 spectra per protein project can easily be reached.

The PASTE and PAPST programs were created to simplify the acquisition and processing of NMR data. The concept includes the use of a library of standardised NMR experiments, which includes appropriate parameter

The authors are with Forschungsinstitut für Molekulare Pharmakologie, RobertRössle-Str. 10, D-13125 Berlin, Germany; tel: +49 30 94793 160; fax: +49 30 94793 169; e-mail: labudde@fmp-berlin.de. The authors thank Ms. Andrea Steuer for secretarial assistance, and are grateful for the financial support of this work by the Federal Ministry for Education and Research. Dr Dirk Labudde received his Ph.D from the Universität Rostock under the supervision of Prof. Röpke in the area of computer simulations. His main scientific interests focus on the development of computer algorithms for protein structure determination. Dr Dietmar Leitner is currently working as a Post-Doc. He received his Ph.D. in chemistry from the Freie Universität Berlin; his topic was the characterisation of unusual nucleic acid structures. His main scientific interests are the high-throughput structure determination of proteins by NMR spectroscopy. Dr Rüdiger Winter (software development), Dr Peter Schmieder (NMR spectroscopy of proteins) and Mr Mario Schubert (Ph.D student, development of novel NMR pulse programs) are working in Prof. Oschkinat's group. Prof. Dr Hartmut Oschkinat is the head of the department structural biology at the Forschungsinstitut für Molekulare Pharmakologie (FMP) and Professor of Structural Chemistry at the Freie Universität Berlin. His main research interests focus on the determination of proteins structures in solution and the study of structure and function of membrane-bound proteins and signal transduction protein domains.

settings for protein projects. Each NMR experiment is defined by its experiment number (Expno), which is identified by PASTE together with the pulse program name. The acquisition and processing parameters can be set and updated using PAPST.

" THE PASTE AND PAPST PROGRAMS WERE CREATED TO SIMPLIFY THE ACQUISITION AND PROCESSING OF NMR DATA. "

Results

B-library

PAPST and PASTE act on a set of pre-defined NMR data sets with well-adjusted parameters (the B-library). This NMR experiment library contains all conceivable NMR experiments that may be of use in a protein-structure determination project. The library can constantly grow, depending on the needs of the user and new scientific developments. The data sets to be executed are defined either individually in an interactive manner or by macros containing pre-selected experiment combinations using PASTE. The maintenance of the B-library is performed by PAPST, which allows the adjustment of pulse lengths, power levels and offsets, which change their values within an intermediate time range.

PASTE

PASTE can copy NMR experiments from the B-library into one data set, which then consists of the selected experiments. This is done by a graphical interface. Acquisition parameters that need to be altered in each experimental session, such as the offset of the proton transmitter frequency (O1), the receiver gain (RG), the proton 90° pulse length (P1), the number of scans (NS) or proton frequency lists used in some triple-resonance experiments, can be interactively adjusted by PASTE, either in selected data sets or all at once. PASTE also executes the copied NMR experiments in any desired order. Another key feature of PASTE is the possibility to execute pre-defined sets of NMR experiments (canons). The different canons may be chosen by the user, depending on the size of the protein and the type of labelling applied.

Graphical interface and handling

PASTE, which is written in the programming language Tcl/Tk, can be started from XWINNMR spectrometer software. In the graphical user interface (GUI) of PASTE that subsequently appears, the name of the protein project must be entered and the labelling state selected. Depending on the labelling scheme chosen (first window), the next window offers a group of NMR experiments from which relevant ones can be selected by clicking the mouse (*Figure 1*). At this stage, the pulse program and most of the acquisition and processing parameters are defined. The selected experiments occur in the status window, which informs the user of the successful selection and the order in which the experiments will be executed (*Figure 2*). After finishing the experiment selection, the button

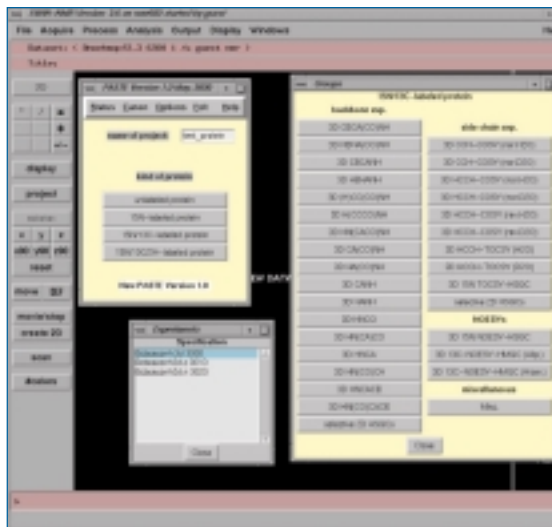


Figure 1 GUI of PASTE (upper left) opened from the XWINNMR software environment. After selecting the protein labelling pattern, a menu of NMR experiments is provided (right). At the lower left, a particular NMR experiment is chosen indicated by the blue bar.

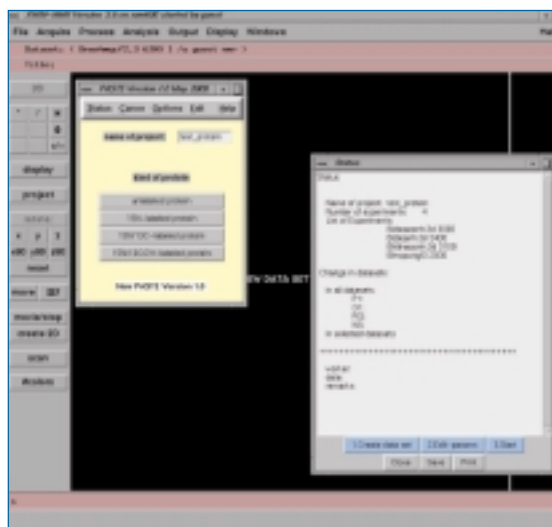


Figure 2 A set of NMR experiments is copied from the B-library by PASTE after executing "1. Create data set," and is reported in the Status window (right).

"Create data set" copies the listed NMR experiments into the user-defined work directory. Several acquisition parameters do change their value at each spectrometer session (see above). With the help of "edit-params," acquisition parameters can be altered uniformly in all data sets or selectively (*Figure 3*). The parameters P1 and O1, for example, have the same value in all data sets, whereas RG and NS might be different in some data sets. Proton frequency lists that depend on the O1 value are automatically changed. The input of the parameters discussed is shown in *Figure 3*. All edited parameters are reported in the Status window (*Figure 4*).

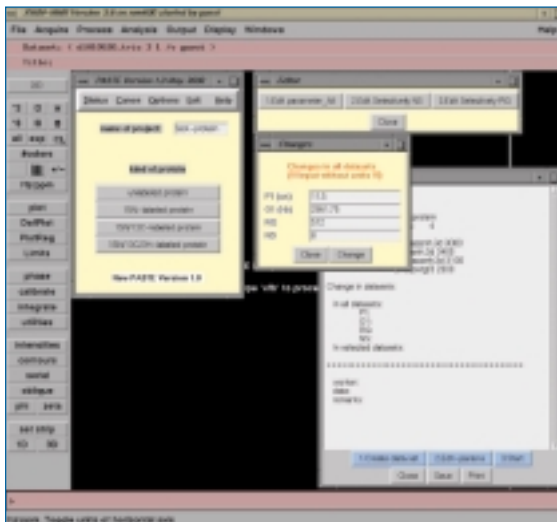


Figure 3 Individual acquisition parameters are selected and can be altered in all or some NMR experiments. Acquisition parameters that differ from each spectrometer session are entered in the Changes window.

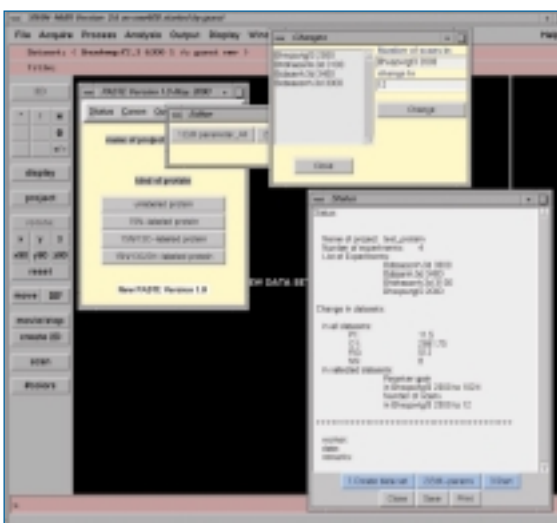


Figure 4 Acquisition parameters that alter their values in selected NMR experiments only are corrected. All changes are reported in the Status window.

As an option, notes by the operator can be written in the Status window, which can be saved and/or printed for documentation. After finishing the setup procedure, the set of NMR experiments can be executed by pressing the “Start” button. The total measurement time is calculated and shown on the display (*Figure 5*). During data acquisition, the user is informed of the permanent status of his/her experiments.

PAPST

Many experimental acquisition (eg., nitrogen and carbon pulses) and processing (eg., offsets) parameters need to be changed within a time frame of about 3–6 months, depending on the quality and age of the spectrometer components. Therefore, the operator

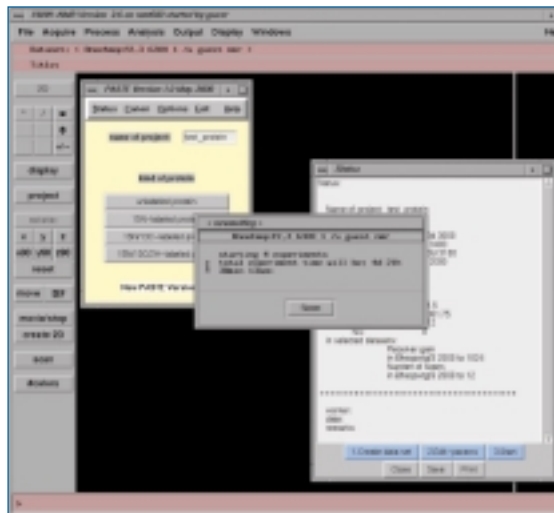


Figure 5 The set of selected experiments is started from the Status window with the “Start” button. The number of executed NMR experiments is reported, and the total acquisition time of all experiments is calculated.

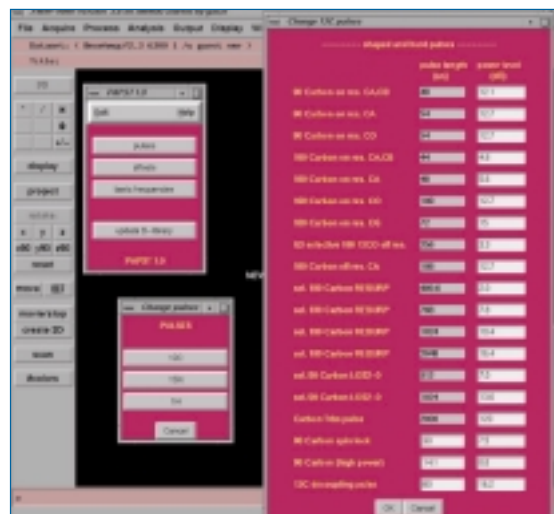


Figure 6 GUI of PAPST started from XWINNMR software (upper left). After selecting the type of parameter to be updated (eg., ^{13}C pulses on the lower left), a listing of all used carbon pulses is given, which can be altered (right).

does not confront these parameters each time when setting up an NMR experiment. PAPST was developed for this kind of NMR experiment maintenance. It can change all relevant parameters in the B-library globally.

Graphical interface and handling

As with PASTE, PAPST is initiated from XWINNMR. In the open GUI of PAPST, three different groups of parameters (pulses, offsets and basic frequencies) appear (*Figure 6*). The button pulses control different pulses that occur in the pulse programs used in the experiment library. Once the operator chooses the nucleus for which the pulses should be updated, a listing of pulse lengths and power levels is attained.

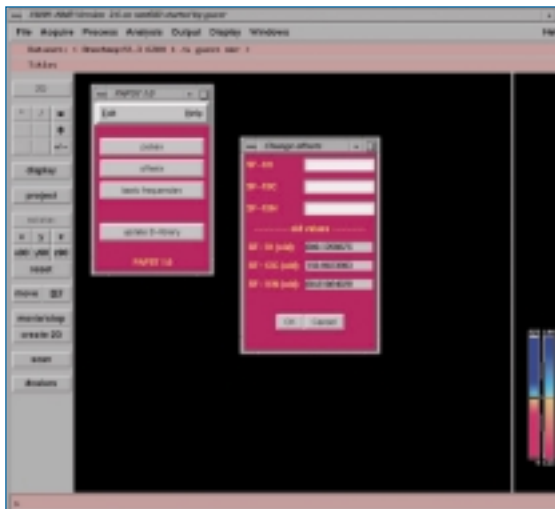


Figure 7 Editing frequency offsets for the three nuclei used in protein NMR is performed after choosing "offsets." The current offset values are copied in the processing files of all NMR experiments in the B-library. The previous offset values are reported below.

For correct referencing of the acquired spectra, it is necessary to have the offset parameter (SF) for each nucleus (*Figure 7*). PAST and PAPST allows the operator to adjust these parameters, which must be recalibrated within about six months. It is also necessary from time to time to correct the basic frequencies. These must be adjusted less frequently than the offset parameters, depending on the drift rate of the magnet. Considering the fact that the B-library contains 265 NMR experiments, editing the basic spectrometer frequency (*Figure 8*) automatically saves a great deal of tedious work. All changes are executed by the command "update B-library," which replaces the old values for the respective acquisition and processing parameters by the new ones in the B-library.

Conclusion

Reducing manual interference in the setup of NMR experiments is crucial for high-throughput protein-structure determination within structural genomics projects. The software programs described offer two major benefits: 1) time savings in the setup and adjustment of NMR experiments, and 2) minimisation of operator error.

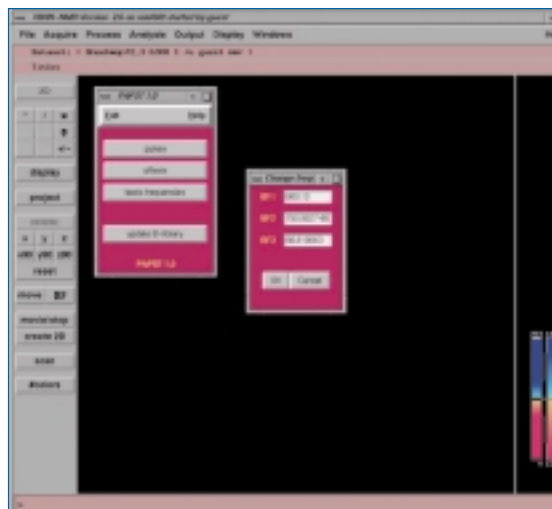


Figure 8 The basic spectrometer frequency can be adjusted in the acquisition files of all NMR experiments in the B-library by activating "basic frequencies."

PASTE and PAPST aid the user in setting up numerous NMR experiments chosen from an experiment library. In addition, special acquisition parameters can be adjusted in one instance in all selected data sets.

The experimental parameters of the experiment library are maintained by PAPST. PAPST is used to adjust crucial acquisition and processing parameters that need to be changed within a medium time range (months). PAPST are implemented in XWINNMR software and can be executed easily. In addition, the software may help novice technicians set up and execute complete data acquisition and processing within an NMR protein project.

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