ESF Exploratory Workshop EW05-052

Life, Environmental and Earth Sciences (LESC)

Physical and Engineering Sciences (PESC)

Experimental and Computational Aspects of High-Throughput Protein NMR

Scientific Report

EUROPEAN SCIENCE FOUNDATION

Swedish NMR Centre, Göteborg, Sweden

17 - 20 June 2006

Convened by:

Martin Billeter, Vladislav Orekhov, Göran Karlsson

Göteborg University, Sweden

Co-Sponsors:

AstraZeneca, Bruker Biospin, Spectra Stable Isotopes, Varian Inc.;

Swedish NMR Centre at Göteborg University, CORDIS

Content

1. Executive Summary

Hosted by the *Swedish NMR Centre at Göteborg University*, an ESF Exploratory Workshop on "Experimental and Computational Aspects of High-Throughput Protein NMR" was held in Göteborg, Sweden, during June 17-20, 2006.

The goal of this workshop was to assemble researchers in protein NMR that work in various disciplines including spectroscopy, signal processing and algorithmic approaches, to discuss modern experimental and computational techniques that can turn NMR into an efficient and robust tool for structure genomics, and to initiate and advance the incorporation of the best tools into tested, robust and user-friendly instruments for wide-spread use in protein NMR.

Following a welcome reception on Saturday evening, the workshop was officially opened on Sunday morning. Each half-day was devoted to a specific session; however, due to the tight interconnection of experimental aspects and computational approaches on one hand, and methodology and application on the other hand, the session contents overlapped. The first four sessions covered acquisition techniques, spectra processing, resonance assignments and structure determination, always with particular weight on automation, efficiency and integrated approaches. A last session on Tuesday morning was dedicated to other high-throughput aspects, covering varied topics from synthesis of NMR-optimised amino acids to practical issues in large-scale structure genomics.

Additional sponsoring, which supplemented the ESF funds, allowed complementing the group of 22 researchers representing the relevant groups in Europe with several key persons from North America and Japan. Of the total of 29 invited participants (including the convenors), 23 presented their latest results in a lecture. The highly interdisciplinary aspects of the problem of characterising proteins by NMR were also reflected in the varying background and activities of the participants. Sophisticated (and expensive) organic chemistry can contribute in reducing the complexity of NMR spectra, which may contain many thousands of signals. Observation of NMR effects relies on very advanced electronics. Collecting the vast amount of information needed to characterise large proteins and the analysis of this data requires novel mathematical approaches and highly efficient numerical optimisation techniques. Finally, validation tools and "common sense" increase the reliability of the results.

At present, we witness significant and exciting developments of the role of NMR applied to proteins. Early results from large scale structural genomics efforts, located mostly outside Europe, demonstrate that NMR, although still applied in a rather "brute force" approach, can make critical contributions: The share of NMR is often close to 50% and, more importantly, includes many protein structures that escape crystallographic methods. A wide variety of novel methods have been presented during the last years that allow for reduction in time and data size of NMR spectra of at least one order of magnitude; this makes it possible to increase the inherent information content (by increasing the virtual dimensionality of the NMR experiments), and thus to gain in terms of efficiency, reliability and protein size. However, a lack of thoroughly tested tools implementing these novel approaches still hinders their routine application. Many NMR spectroscopists feel confused by the wealth of new ideas, and refrain from using these due to their instability and lack of user friendliness.

Central to the workshop were presentations, tests and comparisons of novel methods. A first type of approach is maybe best characterised by the title of the introductory lecture: "Hyper-dimensional NMR Spectroscopy". It comprises fast pulse sequences using specific excitation

or gradient techniques, various sampling schemes and optimally adapted processing algorithms. The goal is to obtain very high-dimensional NMR information that is not the result of trivial combination of different spectra but exhibits unambiguous couplings among all the spins involved. Depending on the experimental and computational approach, "hyperdimensional" referred to up to 13 nuclei collected into unique spin systems. Another approach for efficient NMR spectroscopy consists of optimising the sampling by deviating from regular grids and using non-uniform or sparse patterns. As a direct consequence, the standard Fourier Transform technique needs re-evaluations and alternatives were introduced.

The driving force for increasing the effective dimensionality, i.e. the number of nuclei in unambiguously defined spin systems, is of course to gain uniqueness and thus reliability of the data. This is in particular welcome for the following step, the sequence-specific resonance assignment. Although attempts of automating this step dates a number of years back, sufficient reliability is still not achieved, in particular when it comes to side chains; putting the approaches on a statistically sound basis holds strong promises.

The last step, the structure calculation, is in terms of automation most advanced. However, being the last step it obviously relies critically on the quality of the results obtained in the previous steps. It can, on the other hand, provide highly relevant feedback data to solve problematic situations arising during signal identification and resonance assignment. Thus, integration of all steps will add significantly to the reliability and the possibilities for automation of structural genomics by NMR.

Besides methodology, a number of other key issues have also been addressed. These include optimising the protein sample for NMR purposes by careful isotope labelling, validation of the resulting data (assignments, structures), and standardisation of data formats and protocols. Unbiased tests and comparisons as well as user-friendliness of the tools are a necessity when introducing new techniques to a wider NMR community. Reports from large scale structure genomics efforts show a clear interest for new ideas.

A major outcome of this workshop was formulated during a general discussion on *Follow-up activities: research, collaborations; other specific output*. With the current "inflation" on novel methods, application spectroscopists are often confused. A significant service to the community will be to (1) create a database of problems for unbiased comparisons of new tools, (2) define common data formats and construct a framework for the incorporation of new algorithms, and (3) enhance contacts and exchange among the research groups to identify "the best tool for each type of problem and for each personal taste". This issue was considered both very important and very timely, and existing attempts (e.g. the *CCPN* effort and the EU-STREP *EDLSEC*) should be coordinated within a sufficiently large community to ensure their success. The possibility for a CASP-like contest was also proposed.

NMR applied to proteins is a highly versatile method capable of providing information on structure, dynamics, interactions and function in general. Recent experimental and computational developments, such as fast spectroscopic techniques or fully automated spectra interpretation, open up new directions in its use as a highly efficient tool suitable for incorporation into high-throughput environments. Indeed, already today NMR plays a prominent role in large structural genomics projects in North America or Japan. While large-scale applications of protein NMR are often found outside of Europe, many novel developments originate in European research groups. These methodological advances are about to turn protein NMR from an approach requiring massive user intervention into an automated and streamlined processing tool.

2. Scientific Content of the Event

The following summary of the Scientific Content of the workshop is based on notes taken by the conveners (Martin Billeter, Vladislav Orekhov and Göran Karlsson).

The content of the first four workshop sessions followed the natural sequence of steps that are normally found in a NMR investigation of a protein. Session 1 entitled "Efficient spectra acquisition techniques" focused mostly on experimental approaches. The more sophisticated the experiments become, the stronger their use depends on "Novel approaches for spectra processing", which was the title for the second session. All of Sunday was devoted to these two tightly connected sessions, which comprised a total of eight speakers. The topics raised on Monday concerned the other two major steps in structural studies of proteins by NMR: "Automated resonance assignments" (session 3) and "Integrated structure determination" (session 4), with five speakers each. On Tuesday morning, the five speakers of session 5 presented various topics collected under the rather general title "Other high-throughput aspects". In addition, a general discussion on follow-up activities, with participation of all delegates, was scheduled on Monday evening; this event is presented in more detail in chapter 3 of this report.

The introduction lecture by Eriks Kupče (Varian Inc.) was actually a merging of two lectures: Prof. Ray Freeman, who was expected to present the first lecture, could not join us; he asked his close collaborator Eriks Kupče to also discuss his results. In this first presentation several criteria were used to classify fast spectroscopy techniques. A wide variety of (partly hypothetical) sampling patterns were illustrated. For the acquisition of projections, an interesting question concerns the optimal choice of these projections (i.e. of the projection angle); this question was repeatedly discussed by later speakers as well. In the context of spectra reconstruction, 10-dimensional NMR was addressed. Gerhard Wider (ETH Zürich) presented recent attempts to combine in an automated fashion projection spectroscopy, with corresponding full-dimensional spectra of up to 7 dimensions (i.e. frequencies of seven nuclei are recorded), peak identification and assignment of the protein backbone. A different approach was chosen by Vladislav Orekhov (Göteborg University): Random, although exponentially biased, sampling of the normal acquisition grid was applied to 3D and 4D spectra of proteins up to 80 kD, followed by decomposition methods to fill the gaps. Again, the optimal choice of sampling points, following preliminary analyses as the experiments proceeds was addressed and termed "target-oriented acquisition". The focus of Bernhard Brutscher's lecture (Institut de Biologie Structural Grenoble) was more shifted towards experimental aspects: the Hadamard principle and fast pulsing techniques that reduce the waiting delays after each FID.

A consequence of non-uniform sampling of various types is that standard Fast Fourier Transform cannot be readily applied. In the presentations by Dominique Marion (Institut de Biologie Structural Grenoble) and Wiktor Kozminski (Warsaw University) this shortcoming was discussed and generalisations of Fourier Transform techniques were proposed in order to overcome it. Jeffrey Hoch (University of Connecticut) provided an alternative to Fourier Transform with an up-to-date report on Maximum Entropy Reconstruction of non-uniformly sampled data. This session was completed with the presentation of yet another approach and its application, covariance NMR by Rafael Bruschweiler (Florida State University). Together, the first two sessions clearly demonstrated the applicability of fast spectroscopy techniques.

Remaining questions concern their optimal use, e.g. with target-oriented acquisition, the choice of methods in given situations, and their practical availability.

The first lecture on Monday morning considered the unambiguous characterization of very large spin system as would conventionally only be possible through up to 11-dimensional scalar coupling experiments. Martin Billeter (Göteborg University) applied decomposition methods simultaneously to several data sets from coupled evolution experiments. These large spin systems represent strongly overlapping fragments for reliable sequence-specific resonance assignments. The latter, and in particular its automation, was the topic of the lecture by Hans Robert Kalbitzer (University of Regensburg). He presented a largely complete software package that comprises many tools for computer supported interpretation of NMR data and is essentially ready for use by non-experts. While many computer-aided assignment tools more or less follow the manual approach, Hamid Eghbalnia (University of Wisconsin) implements adaptive probabilistic tools that provide a statistically more solid basis and make better use of the strengths of computers. A second comprehensive software package, combining automated procedures with interactive checks, was presented by Hunter Moseley (Rutgers University). James Masse (ETH Zürich) talked on similar efforts for automated assignment, with particular emphasis on side chains.

The second session on Monday was devoted to structure calculations. While automation in this step has already a long history, novel ideas continue to make it more robust, examples being ambiguous constraints or network anchoring. Other aspects are integration with other steps such as spectra interpretation and sequence-specific assignment, and statistically sound treatment of experimental constraints. Structure determination at high speed, usable also for non-NMR researchers and suitable for large proteins was the goal of Peter Güntert's project (Riken Genomics Sciences Centre). Of particular interest was the use of proteins built from amino acids with NMR-optimised labelling. Markus Zweckstetter (Max Planck Institut Göttingen) combined speed and protein size in structure determination specifically with the use of residual dipolar couplings. Thérèse Malliavin (Institut Pasteur Paris) discussed complementing aspects to pure structure calculation: protein dynamics, reliability and interactive communication during the calculation. Inferential structure determination, structure calculation prior to (or avoiding) the assignment step, and incorporation into the *CCPN* data model was the topic of Wolfgang Rieping (University of Cambridge). Finally, Alexandre Bonvin (Utrecht University) presented a systematic way of using unassigned resonances in NMR structure calculations: initially isolated fragments consisting of one or a few spins (unassigned resonances) are "floating around" during the calculation, to be attached and assigned towards the end of the structure determination only.

Tuesday morning was devoted to the many issues of high-throughput protein NMR that did not fit under the rather specific titles of the earlier sessions; thus this session carried the title "Other high-throughput aspects". The first lecture started where many protein NMR projects begin: with the expression of NMR-suitable proteins. Masatsune Kainosho (Tokyo Metropolitan University) presented a version of the 20 amino acids that is optimally labelled by choosing the best alternative of $^{1}H/^{2}H$, $^{14}N/^{15}N$ or $^{12}C/^{13}C$ for NMR purposes. In the resulting "SAIL" proteins, all molecular moieties are visible but double resonances from chemically equivalent groups are avoided. The results are lower spectral complexity and reduced relaxation losses. Cell-free protein expression with these amino acids is however rather costly. Wim Vranken (EBI Hinxton) gave an update of the *CCPN* project, which appears highly suitable as a general data model and software repository for testing and comparing novel experimental and computational techniques (see next chapter). Objective

validation of all new protein structures is a must; Geerten Vuister (Radboud University Nijmegen) discussed various aspects such as serious errors found in databases of structures, the risk of "confirming" wrong structures by refinement, and concluded that validation should be performed in a residue-specific manner. A very interesting insight into the situation of protein structure determination by solid state NMR and the potential of making it high-throughput was given by Bart van Rossum (FMP Berlin). While the structure calculation part is likely to follow the procedures developed in liquid NMR, the possibility for obtaining high-resolution spectra for non-soluble proteins is of course unique. The concluding lecture was presented by Cheryl Arrowsmith (University of Toronto), who summarized the entire workshop by describing the practical application of protein structure determination by NMR at a structure genomics centre. Demands on novel methods for structure genomics projects were named: flexibility, robustness and user-friendly interface.

3. Assessment of the Results

On Monday evening, all participants assembled for a round-table discussion on key issues of the workshop topic and desirable follow-up activities. The following section, "3.1 Key Issues", is essentially a summary of this general discussion, complemented with statements made on other occasions during the workshop. The conclusions regarding follow-up activities are listed in the section "3.2 Follow-up Activities".

3.1 Key Issues

Characteristic elements of the topic covered by the current workshop are (a) the multidisciplinary aspect combining spectroscopy, signal processing and algorithmics, and (b) the need for collaboration in the development of advanced tools in order to promote NMR as an efficient and robust tool of structure genomics. While many promising new methods have been presented during the past years, not all will survive while others may be combined into new tools. The ones suitable for wider use will need some sort of "marketing", which is likely to start with thorough tests and comparisons, followed by robust and user-friendly implementations, and distribution to application NMR spectroscopists.

Regarding the development of experimental and computational tools for wide use in the protein NMR community, the discussion brought about a number of issues that have to be addressed:

- reduce overlap or duplication of novel tools,
- ensure interoperability (a user should be free to combine various tools as he/she sees fit),
- provide thorough validation of all tools,
- make objective comparisons for different types of problems,
- facilitate "data harvesting",
- ensure long-term support.

On a longer perspective one may add to this list:

- implement fully integrated software,
- develop this software in wider, more international collaborations,
- organise future meetings, workshops etc. to ensure the above,
- arrange "CASP-like" competitions.

A number of initiatives exist already to cope with some of the above demands. Thus, databases like the Protein Data Bank (PDB) or the Biological Magnetic Resonance Data Bank (BioMagResBank or BMRB) fulfil some basic needs. On a higher level, the *CCPN* initiative proposes a common data model together with standard software tools. A recently initiated EU collaboration (EU-STREP named *EDLSEC*) has in part similar goals as identified in the general discussion of this workshop. Finally, one should mention that work during the last years at structure genomics initiatives such as those at RIKEN Yokohama (Japan) or Toronto (Canada) offer a wealth of data with hundreds of NMR-determined protein structures. They provide also practical experience in protein handling for NMR purposes on a large scale, covering all steps from expression to structure calculation.

3.2 Follow-up Activities

The section identifies future activities that the participants considered important for ensuring NMR a substantial role in future efforts to characterise structure, dynamics and function or proteins on a large scale. This represents, besides the extremely valuable information exchange, one of the major outputs of the workshop. The following is a list of items that should be made available or organised; detailed descriptions, e.g. the specific choice of proteins and spectra for the first point, need to be defined in future discussions.

1) A database of test problems needs to be established. This will consist of well-characterised NMR data at various levels of processing that developers of new techniques can use for real-case testing and for objective evaluation. Standardised data with reference results should be provided for:
   - raw NMR data (FIDs) in various forms: conventional spectra, projections etc.,
   - processed (Fourier-transformed) spectra,
   - resonance assignments.
   This database should include various sizes of proteins and variation of other complexity parameters such as signal to noise, resolution etc.
2) A second repository should be established containing software tools. Based on the standardised problem data of the above database, a common data model can be defined and used for objective testing of novel tools and comparison with already existing techniques. This will allow obtaining a better picture on the performance of each tool, and on the type of problems it is best suited for.
3) Future meetings (workshops, summer schools etc.) will be required for defining the exact form of the above databases, for performing and discussing comparative evaluations of the tools, and, very importantly, for teaching these to potential users.
4) Benchmarking of the tools on problems with known solutions is an important issue. Along these lines, CASP-like competitions have been proposed, where the developers of novel methodology would be confronted with new problems, the reference results of which would be made available at a meeting held only after submission of the outcome from the tools to be tested. As this would require quite a large effort and organisation, it was considered a rather long-term possibility.

Some of the above demands are already being addressed (*CCPN*, *EDLSEC* and others), but this should be coordinated with both a larger user community and with research groups outside of Europe.

4. Final Programme

Saturday 17 June 2006

Afternoon *Arrival in Göteborg; check-in at Hotel Scandic Crown*

17.00 – 19.00 *Reception and registration at the Swedish NMR Centre (welcome drink and snacks)*

Sunday 18 June 2006

Opening of the Workshop

09.30 – 09.35 Welcome by the convenors

09.35 – 09.50 **Presentation of the European Science Foundation**

09.50 – 10.00 Practical remarks

Session 1: Efficient spectra acquisition techniques

10.00 – 10.40 **Eriks Kupče**, Varian Inc., Hyperdimensional NMR Spectroscopy

10.40 – 11.10 **Gerhard Wider**, ETH Zürich, Application of automated projection spectroscopy (APSY)

11.10 – 11.30 *Coffee break*

11.30 – 12.00 **Vladislav Orekhov**, Göteborg University, Optimization of resolution and sensitivity of NMR spectra using sparse matched data acquisition and Multi-Dimensional Decomposition

12.00 – 12.30 **Bernhard Brutscher**, Institut de Biologie Structurale Grenoble, Projection, Hadamard, and fast-pulsing NMR: new methods for the study of protein structure and kinetics

12.30 – 14.00 *Lunch (Buffet at the Swedish NMR Centre)*

Session 2: Novel approaches for spectra processing

14.00 – 14.30 **Dominique Marion**, Institut de Biologie Structurale Grenoble, Non-linear acquisition and Fourier transform

14.30 – 15.00 **Wiktor Kozminski**, Warsaw University, Multidimensional Fourier Transform of arbitrarily sampled NMR

15.00 – 15.30 *Coffee break*

15.30 – 16.00 **Jeffrey Hoch**, University of Connecticut, Maximum Entropy Reconstruction of nonuniformly sampled data

16.00 – 16.30 **Rafael Bruschweiler**, Florida State University, Tallahassee, Covariance NMR: principles and applications

18.30 *Workshop Dinner: Boat trip in the southern archipelago*

Monday 19 June 2006

Session 3: Automated resonance assignments

09.00 – 09.30 **Martin Billeter**, Göteborg University, Very large spin systems from simultaneous decomposition of several projected data sets

09.30 – 10.00 **Hans Robert Kalbitzer**, University of Regensburg, Automated NMR structure determination

10.00 – 10.30 **Hamid Eghbalnia**, University of Wisconsin-Madison, Adaptive Probabilistic Tools: Applications to Rapid and Robust NMR Structure Determination

10.30 – 11.00 *Coffee break*

11.00 – 11.30 **Hunter Moseley**, Rutgers University, Automation in NMR: From Spectra to Resonance Assignments and then to Structure

11.30 – 12.00 **James Masse**, ETH Zürich, SideLink: Automated side-chain assignment of biopolymers from NMR data by relative-hypothesis-prioritization-based simulated logic

12.00 – 13.30 *Lunch (Buffet at the Swedish NMR Centre)*

Session 4: Integrated structure determination

13.30 – 14.00 **Peter Güntert**, Riken Genomics Sciences Center Yokohama, A fully automated NMR structure determination system

14.00 – 14.30 **Markus Zweckstetter**, Max Planck Institute for Biophysical Chemistry Göttingen, Fast High-resolution Structure Determination of Proteins

14.30 – 15.00 **Therese Malliavin**, Institut Pasteur Paris, Use of the protein geometry and mechanics to help the NMR structure determination

15.00 – 15.30 *Coffee break*

15.30 – 16.00 **Wolfgang Rieping**, University of Cambridge, tba

16.00 – 16.30 **Alexandre Bonvin**, Utrecht University, Direct use of unassigned resonances in NMR structure calculations with PROXY residues

Round table discussion

17.00 – 17.30 Follow-up activities: research, collaborations; other specific output

18.30 *Dinner at "Hyllan" on Chalmers Campus*

Tuesday 20 June 2006

Session 5: Other high-throughput aspects

09.00 – 09.30 **Masatsune Kainosho**, Tokyo Metropolitan University, *Optimal isotope labeling for NMR protein structure determinations*

09.30 – 10.00 **Wim Vranken**, EBI Hinxton, *Using the CCPN data model for automation and large scale analysis*

10.00 – 10.30 **Geerten Vuister**, Radboud University Nijmegen, *Structure validation of NMR-derived structures requires a per-residue approach*

10.30 – 11.00 *Coffee break*

11.00 – 11.30 **Bart van Rossum**, Forschungsinstitut für Molekulare Pharmakologie Berlin, *New developments in solid-state magic-angle spinning NMR*

11.30 – 12.00 **Cheryl Arrowsmith**, University of Toronto, *Protein structure determination from minimal data sets using ABACUS*

12.00 – 12.30 **Martin Billeter**, Göteborg University, *Closing remarks*

12.30 – 14.00 *Lunch (Buffet at the Swedish NMR Centre)*

Afternoon *Departure*

5. Statistical Information on Participants

5.1 Country of origin

The country of origin is for 22 of the participants in Europe, for 5 in North America and for 2 it is Japan.

Country	Number of participants
Britain	4
Canada	1
France	3
Germany	5
Japan	2
Netherlands	3
Poland	1
Sweden	4
Switzerland	2
USA	4
Total	*29*

5.2 Gender of participants

There were 2 female and 27 male participants (plus 1 female and 3 male local participants, who provided also administrative support). This gender distribution largely reflects the known situation in NMR research. While women are already underrepresented in the field of protein NMR, they rarely get involved in methodological research. This will hopefully change in the context of recently initiated international multi-group collaborations, which are supported for example by the EU.

5.3 Other aspects of the distribution of participants

In spite of the very limited number of manufacturers of NMR equipment, this relatively small workshop was followed by 4 industry representatives; in addition, a member of the European Bioinformatics institute contributed actively. Several outer-European structure genomics centres were represented. Besides a number of researchers directly involved in software development, the range of participants extended all the way to chemical synthesis. Finally, all members of an EU-STREP, which follows related goals, were represented.

6. Final List of Participants

This final list of participants differs from the preliminary list as follows: Prof. Ray Freeman could not participate due to health reasons (his lecture was given by Dr. Eriks Kupče), Prof. Konstantin Pervushin was replaced by Dr. James Masse, and Dr. Bruno Guigas was replaced by Dr. Peter-René Steiner.

Convenors

1. Martin Billeter
   Department of Chemistry, Göteborg University, 405 30 Göteborg, Sweden
   Tel.: +46 31 773 3925; Fax: +46 31 773 391; martin.billeter@chem.gu.se
2. Göran Karlsson
   Swedish NMR Centre, Göteborg University, 405 30 Göteborg, Sweden
   Tel.: +46 31 773 3886; Fax +46 31 773 3880; goran.karlsson@nmr.gu.se
3. Vladislav Orekhov
   Swedish NMR Centre, Göteborg University, 405 30 Göteborg, Sweden
   Tel.: +46 31 773 3886; Fax +46 31 773 3880; vladislav.orekhov@nmr.gu.se

Invited participants

4. Cheryl Arrowsmith
   Department of Medical Biophysics, University of Toronto, Ontario, Canada M5G 1L5
   Tel.: (416) 946-0881; Fax: (416) 946-0880; carrow@uhnres.utoronto.ca
5. Wolfgang Bermel
   Bruker BioSpin GmbH, Silberstreifen 4, 76287 Rheinstetten, Germany
   Tel: +49 721 5161 280; Fax: +49 721 5161 346; wolfgang.bermel@bruker-biospin.de
6. Rolf Boelens
   Bijvoet Center for Biomolecular Research, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
   Tel.: +31 30 2533859; Fax: +31 30 2637623; boelens@nmr.chem.uu.nl
7. Alexandre Bonvin
   Bijvoet Center for Biomolecular Research, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
   Tel.: +31 30 2534035; Fax: +31 30 2637623; abonvin@nmr.chem.uu.nl
8. Rafael Bruschweiler
   Dep. of Chemistry & Biochemistry, Florida State University, Tallahassee, FL 32310, USA
   Tel.: (850) 644 1768; Fax: (850) 6444 8281; rbruschw@magnet.fsu.edu
9. Bernhard Brutscher
   Institut de Biologie Structurale, Laboratoire de RMN, 38027 Grenoble Cedex 1 France
   Tel.: +33 38 78 9550; Fax: +33 38 78 5494; bernhard.brutscher@ibs.fr
10. Hamid Eghbalnia
    Department of Biochemistry, University of Wisconsin-Madison, WI 53706 USA
    Tel.: (608) 262-8528; Fax: (608) 262-3759; eghbalni@nmrfam.wisc.edu

11. Rutger Folmer
    AstraZeneca Structural Chemistry Laboratory, 43183 Mölndal, Sweden
    Tel.: +46 31 776 1000; Fax: +46 31 776 37 00; rutger.folmer@astrazeneca.com
12. Peter Güntert
    RIKEN Genomics Center, C-519, 1-7-22, Suehiro-cho, Yokohama 230-0045, Japan
    Tel.: +81 45 503 9345; Fax: +81 45 503 9343; guentert@gsc.riken.jp
13. Jeffrey Hoch
    Department of Molecular, Microbial, and Structural Biology, University of Connecticut Health Center, Farmington, CT 06030-3305 USA
    Tel.: (860) 679 3347; Fax: (860) 670 1239; hoch@uchc.edu
14. Masatsune Kainosho
    Tokyo Metropolitan University, 1-1 Minami-ohsawa, Hachioji, Tokyo 192-0397 Japan
    Tel.: +81 426 77 2544; Fax: +81 426 77 2544; kainosho@nmr.chem.metro-u.ac.jp
15. Hans Robert Kalbitzer
    Institute for Biophysics and Physical Biochemistry, University of Regensburg, 93053 Regensburg, Germany
    Tel.: +49 941 943-2594; Fax: +49 941 943-2479; hans-robert.kalbitzer@biologie.uni-regensburg.de
16. Wiktor Kozminski
    Department of Chemistry, Warsaw University, Pasteura 1, 02-093 Warszawa, Poland
    Tel.: +48 22-8-220-211; Fax: +48 22 8225996; kozmin@chem.uw.edu.pl
17. Eriks Kupče
    Varian Limited, Oxford Industrial Park, Oxford OX5 1QU, UK
    Tel.: +44 1865 435402; Fax: +44 1865 841945; eriks.kupce@varianinc.com
18. Ernest Laue
    Department of Biochemistry, University of Cambridge, Cambridge CB2 1RX, UK
    Tel.: +44 1223 766894; Fax: +44 1223 332355; edlaue@bioc.cam.ac.uk
19. Therese Malliavin
    Institut Pasteur, Unité de Bioinformatique Structurale, 75724 Paris CEDEX 15, France
    Tel: +33 1 45 698 8230; Fax: +33 1 45 68 8719; terez@pasteur.fr
20. Dominique Marion
    Institut de Biologie Structurale Jean-Pierre Ebel, C.N.R.S - C.E.A., 41, Avenue des Martyrs, 38027 Grenoble Cedex 1, France
    Tel.: +33 38 78 9550; Fax: +33 38 78 5494; Dominique.Marion@ibs.fr
21. James Masse
    Laboratorium für Physikalische Chemie, ETH Zürich, 8093 Zürich, Switzerland
    Tel.: +41 044 633 4464; Fax: +41 44 632 1021; jmasse@bionmr.phys.chem.ethz.ch
22. Hunter Moseley
    Rutgers University, CABM Structural Bioinformatics Lab., 679 Hoes Lane, Piscataway, NJ 08854, USA
    Tel.: (732) 235-5321; Fax: (732) 235-5633; hunter@cabm.rutgers.edu

23. Wolfgang Rieping
    Department of Biochemistry, University of Cambridge, Cambridge CB2 1RX, UK
    Tel.: +44 1223 766022; Fax: +44 1223 332355; wolfgang.rieping@mole.bio.cam.ac.uk
24. Peter-René Steiner
    Bruker BioSpin GmbH, Silberstreifen 4, 76287 Rheinstetten, Germany
    Tel: +49 721 5161 451; Fax: +49 721 5161 480; peter-rene.steiner@bruker-biospin.de
25. Bart van Rossum
    Forschungsinstitut für Molekulare Pharmakologie (FMP), Robert-Rössle-Str. 10, 13125 Berlin, Germany
    Tel.: +49 30 94793 224; Fax: +49 30 94793 169; brossum@fmp-berlin.de
26. Wim Vranken
    EBI, EMBL Outstation Hinxton, Cambridge, CB10 1SD, UK
    Tel.: +44 1223 494 682; Fax: +44 1223 494 487; wim@ebi.ac.uk
27. Geerten Vuister
    Department of Physical Chemistry, Radbout University, 6525 ED Nijmegen, The Netherlands
    Tel.: +31 24 365 2321; Fax: +31 24 365 2112; g.vuister@science.ru.nl
28. Gerhard Wider
    Institute of Molecular Biology and Biophysics, ETH Zürich, 8093 Zürich, Switzerland
    Tel.: +41 44 633 3455; Fax: +41 44 633 1073; gsw@mol.biol.ethz.ch
29. Markus Zweckstetter
    Department of NMR–based Structural Biology, Max Planck Institute for Biophysical Chemistry, Am Fassberg, 37077 Göttingen, Germany
    Tel.: +49 0551 201-2220; Fax: +49 0551 201-2202; mzwecks@gwdg.de

Local participants providing also administrative support

1. Jonas Fredriksson
2. Victor Jaravin
3. Patrik Jarvoll
4. Anastasia Zhuravleva