

The EU-NMR project, which covers Networking, Transnational Access, and Joint Research Activities for the European Bio-NMR community, is entering its final year of activity. The current edition of the EU-NMR newsletter highlights some of the project's accomplishments, which may set the stage for the next steps forward.

SCIENTIFIC HIGHLIGHTS

GAINING INSIGHTS INTO THE MECHANISM OF CYTOCHROME C OXIDASE ASSEMBLY

Copper is essential for the proper functioning of cytochrome c oxidases, and therefore for cellular respiration in eukaryotes and many bacteria. Their mechanism of assembly and the insertion of copper ions into the proper protein domains is the subject of intense investigation. The periplasmic protein that selectively inserts Cu(I) ions into subunit II (CuA) of *Thermus thermophilus* ba3 oxidase to generate a native CuA site of CcO has now been identified and completely characterized. It has also been established that the purported metallochaperone Sco1 is unable to deliver copper ions; instead, it works as a thiol-disulfide reductase to maintain the correct oxidation state of the CuA cysteine ligands. This work provides the molecular mechanism of CuA site assembly in bacteria, defining the specific functional role of each protein involved in CuA site maturation.

NEW APPROACH TO IMPROVE SENSITIVITY FOR MEMBRANE PROTEIN STUDIES

Improved sensitivity is of central importance for the solid-state NMR spectroscopy of membrane proteins. A new solid-state NMR concept in which the measurement time can be shortened substantially compared to conventional experiments has been devised. This approach relies on the selective excitation of polypeptide backbone resonances in uniformly labeled samples. Their magnetization REgenerates quickly due to the LOwer spin temperature of ADjacent spins (RELOAD). This "RELOADing" process allows more scans to be acquired in a much shorter period of time, and has been incorporated into 2D homo- and heteronuclear CP-MAS experiments, proving very successful in structural and kinetic solid-state NMR studies on different membrane proteins.

METHODS FOR ACCURATE STRUCTURE DETERMINATION OF PROTEINS IN SOLUTION STARTING FROM X-RAY DATA

A novel approach to accurately refine solution structures has been applied to two complexes of calmodulin (CaM) with target peptides. Starting from the crystal structures, new structural models in solution have been calculated that are in agreement with the paramagnetic restraints and differ significantly from the starting crystal structures. The paramagnetic restraints, pseudocontact shifts and residual dipolar couplings have been made available after lanthanide substitution to the calcium ion in one of the four calcium binding sites of CaM. In this way, the global orientation tensor could be independently and precisely determined from pseudocontacts, which are less sensitive than residual dipolar coupling to the presence of local structural inaccuracies. The relative rearrangement of a domain or a secondary structure element with respect to the metal-bearing domain could therefore be detected.

CHARACTERIZATION OF WEAK PROTEIN-PROTEIN INTERACTIONS BY TITRATION OF RESIDUAL DIPOLAR COUPLINGS

NMR spectroscopy is the technique of choice for studying low affinity protein-protein interactions with dissociation constants above 100 μM . Residual dipolar couplings (RDCs) provide powerful constraints on intermolecular orientation in molecular complexes, but the combination of free and bound contributions to the measured RDCs seriously complicates their exploitation for weakly interacting partners. In collaboration with the RALF NMR, Prof. van Nuland and his group have for the first time used an approach developed in Grenoble for the exploitation of the unique orientational information available from RDCs in the case of weak interaction and rapid exchange between free and bound forms of the proteins. The approach relies on the measurement of RDCs in different equilibrium mixtures of partially aligned free and complex forms of the proteins, and on novel analytical approaches to determine and normalize the effective level of alignment in all equilibrium mixtures. The authors show that this results in the simple extraction of precise RDCs for bound forms of both partner proteins, even when the bound form can not be isolated. The approach is applied to determine the three dimensional structure of the weakly interacting CD2AP SH3-C:Ubiquitin complex ($K_d=132\pm 13 \mu\text{M}$).

TRANSNATIONAL ACCESS

The following table shows the statistics reflecting the performance of EU-NMR in the provision of transnational access; we are proud to note that the goals have been exceeded for the third consecutive year!

	Access provided (days)	Number of users	Number of user groups
Stipulated/year	790	80	60
1 st year	1028	100	66
2 nd year	1068	150	90
3 rd year	921	142	94

According to the Users' Group Document prepared by Profs. Michael Assfalg and Henriette Molinari, the general consensus is that access has greatly improved in response to the growing strength of the user community. The new issues emerging among users are related to participation in the construction of larger European schemes and in the enhancement of the impact of the NMR community in the Life Sciences.

SOFTWARE STANDARDIZATION AND PROVISION OF AN E-INFRASTRUCTURE

Biomolecular NMR traditionally suffers from extensive fragmentation of the computational methods adopted by individual research teams, presently preventing full interoperability among different laboratories and detailed comparison of results derived from bio-NMR data. This may limit the scientific impact of European bio-NMR research. Another consequence of this fragmentation is that it can slow down the spread of newly developed methodologies and strategies within the community, as it is unclear how to then incorporate these with more standard parameters e.g. for structure determination purposes. The latter point is specifically addressed by one of the JRAs of EU-NMR, in which orientation dependent structural parameters are incorporated into a common package for 3D structure determination of biomolecules. With regard to the general problem of data and software standardization, EU-NMR has stimulated the e-NMR initiative, which aims at deploying and unifying the NMR computational infrastructure in systems biology, using a Grid infrastructure. Access to the programs is provided via the web, upon obtaining a personal certificate for authentication (more details at www.enmr.eu).

EU-NMR WORKSHOPS

Workshops constitute an important tool to stimulate technological improvements and transfer of knowledge and to promote scientific awareness of the potential of NMR to solve challenging scientific questions emerging in the Life Sciences.

To achieve these goals, different kinds of workshops have been organized throughout the years in the frame of EU-NMR. Since the core of the project consists of providing access to NMR, a series of workshops involving the staff members (local operators) at the research infrastructures (RIs) have been devoted to discussing the key aspects of monitoring instrumentation performance, promoting the development of new experiments, and ensuring that the most advanced experiments are made readily available to the RI users. As a result, a common operating procedure, which also includes the use of standard samples, has now been developed and is used by the RIs to ensure a high and common standard of NMR access. More general workshops, either on aspects of interest to the bio-NMR community ("New pulse sequences for proteins, nucleic acids and protonless NMR", Tarragona, Spain, 2007) or on specific emerging areas such as metabolomics ("NMR-based metabolomics", Lyon, France, 2008) have been organized with the primary goals of training and transfer of knowledge. Finally, in order to get in closer contact with a larger share of the scientific community and expand the outreach as well as the user base of EU-NMR, a new series of workshops was started to present the possibilities offered by NMR for addressing challenging scientific problems to an audience of non-NMR experts through several examples of high quality scientific presentations. These workshops constitute a satellite symposium of one of the most important events for European biologists ("Biological magnetic moments", Athens, Greece, 2008, Prague, Czech Republic, 2009).

UPDATE OF INSTRUMENTATION

The wide variety of instrumentation available at each of the EU-NMR Research Infrastructures comprises all of the highest-end bio-NMR instrumentation that is commercially available or in advanced stages of development world-wide. The high field instruments include one 950 MHz, five 900 MHz, two 850 MHz, five 800 MHz, one 750 MHz, and six 700 MHz magnets, and are completed by 24 NMR spectrometers at lower fields (600-360 MHz). The equipment also includes cutting-edge technologies in areas such as EPR and relaxometry.

Most of the high-field instruments are equipped with state-of-the-art hardware, such as cryogenically-cooled multiple-channel probeheads, continuously upgraded consoles and all of the ancillary equipment necessary for the most disparate applications in biological NMR. The complete list is reported at <http://www.eu-nmr.eu/NMRspec/>.

CONCLUSION OF THE CA NMR-LIFE

The Coordination Action NMR-Life was recently concluded, following a number of successful activities that gained impact from the evolution of the international scientific scenario. The final meeting, entitled "NMR to Lay the Bricks for Molecular Systems Biology" was held in Montecatini, Italy on December 14-18, 2008. During this meeting, a number of documents were discussed within the context of prestigious lectures and released on the CA web site. The final act of the project was the presentation of this collection of documents at the EU-NMR user meeting on January 26-29, 2009.

This volume will soon be available on the CA website at www.postgenomicnmr.net, and print copies can also be provided upon request by writing to Ivano Bertini at bertini@cerm.unifi.it.

LABORATORY STANDARDIZATION

All of the laboratories participating in EU-NMR have been involved in the optimization of their NMR instrumentation. Indeed, this is one of the most relevant points for the improvement of services to external users. To monitor the performance of spectrometers, a protocol has been proposed and tested during the first years of activity by all of the laboratories. A final proposed protocol was defined and consists of the acquisition of a set of experiments on standard samples. Each laboratory participating in EU-NMR has compared the acquired spectra and defined the best conditions to obtain optimal results from their spectrometers. Moreover, the exchange of information between the laboratories has permitted the set-up of forefront NMR experiments which can be proposed to external users.

