Check for updates

FEBS Letters

FEBS PRESS
science publishing by scientists

THE SCIENTISTS' FORUM

iNEXT: a European facility network to stimulate translational structural biology

iNEXT Consortium[†]

Magnetic Resonance Centre (CERM), University of Florence, Sesto Fiorentino, Italy

iNEXT, 'Infrastructure for NMR, EM and X-rays for Translational research', is a EC-funded Horizon 2020 network of national infrastructures aiming to facilitate EC-supported, trans-national user access to several high-end structural biology infrastructures and to expand the use of structural biology technologies towards new research communities. iNEXT has developed different access routes offering cutting edge technologies and expertise, thus targeting the diverse needs of research groups that desire to perform X-ray crystallography, small-angle X-ray scattering, solution or solid-state NMR, single particle or cell tomography electron microscopy, biophysics, and imaging experiments. Specific Joint Research Activities aim at improving the user experience in ever advancing methods, and are focused on structure-based drug discovery, membrane protein characterization and structural cell biology initiatives.

Keywords: cellular structural biology; membrane proteins; structural biology; structure guided drug discovery

Structural biology has proven indispensable for research and development in the life sciences, biotechnology and biomedical fields. High-end equipment is essential for structural biology: synchrotrons to produce intense, tunable and focused X-ray beams for macromolecular X-ray crystallography (MX) and small-angle X-ray scattering (SAXS) studies; high-field superconducting magnets for NMR; new-generation electron microscopes with powerful optics and fast direct readout electron detectors for cryo-electron microscopy (cryo-EM) applications in single particle studies and cell imaging. The use of such structural biology instrumentation has been imperative, but also very expensive. In the past decades, the European Commission research policy has put forward the need to support and widen opportunities for the European research community to access such extremely expensive state-of-the-art instrumentation, with the evident goal of strengthening the European Research Area by enhancing mobility and by stimulating collaborations among European researchers, thus promoting the rational use of infrastructural resources and avoiding duplication of investments.

The 4-year Research Infrastructure Integrating Activities Project iNEXT ('Infrastructure for NMR, EM and X-rays for Translational research') has been granted in 2015 as part of the current EC Framework Programme 8 (widely known as Horizon 2020). The iNEXT Consortium embeds 23 well-known structural biology teams from different European countries[1] and aims to facilitate EC-supported, trans-national user access to several facilities established for high quality research and state-of-the-art equipment. Access proposals can come from both academia and industry users, and are evaluated by external reviewers. Excellent proposals that aim to contribute to the development of innovative therapeutics and diagnostics, to the engineering of biotechnology tools and materials, and to expand the use of structural biology technology towards biochemical and biomedical research are the target for iNEXT support.

Besides NMR and synchrotron equipment, that both have been accessible to European users since long time through previous EC-funded projects, iNEXT allows for the first time access to modern Electron Microscopy equipment for single particle studies, electron tomography and Correlative Light-Electron Microscopy (CLEM). It is noteworthy that the 2017 Nobel Prize in chemistry was given in recognition of the spectacular developments in cryo-EM in recent times, showing the foresight of the Consortium in opening access also to this technology to all European scientists. iNEXT is also the first EC Infrastructure project

Abbreviations

CLEM, Correlative Light-Electron Microscopy; EM, Electron Microscopy; ESFRI, European Strategic Forum for Research Infrastructures; FBDD, fragment-based drug discovery; HT, high-throughput; IMP, integral membrane protein; JRA, joint research activity; LBDD, ligand-based drug discovery; MX, micromolecular X-ray crystallography; SapNP, Saposin-Derived NanoParticle; SAXS, small-angle X-ray scattering; SEC, size exclusion chromatography; ssNMR, solid-state NMR; SXRM, soft X-ray microscopy.

to facilitate the study of macromolecular interactions using a variety of biophysical techniques, and to offer access to advanced light imaging. iNEXT makes focused efforts to attract also researchers that have limited experience in structural biology but are leaders in other life science fields, and wants to ensure that the open access, but also ongoing research developments, will impact the industry sector.

Users can choose from different access modalities to exploit the techniques available through iNEXT, which take into account both their research needs and the available expertise. iNEXT access is provided at three different levels:

1 The Structural Audit level allows nonstructural biologists to mail-in samples to characterize macromolecular systems in terms of their biophysical stability and propensity to crystallize, of their shape and low resolution structure by SAXS, of their suitability for NMR experiments by e.g. examining the [^{1}H;^{15}N]-HSQC spectrum, and of their potential for successful cryo-EM studies by negative stain EM.
2 The Enhanced Support level allows users to receive full hands-on support with their projects by experts working at the research facilities of the iNEXT Consortium. Enhanced support can be requested for: extensive crystallization trials that may lead to high quality crystals; beamlines use for acquisition of structural data for MX; structural characterization of complex samples by SAXS; data acquisition, assignments, structure calculations and/or ligand binding studies with solution NMR and solid-state NMR (ssNMR); optimization of samples for single particle EM; steady-state and transient kinetics underlying macromolecular interactions by fluorescence methods (biophysical characterization); advanced light imaging in structural studies with in-cell measurements of protein interaction kinetics; ligand and fragment screening.
3 The High-End Data Collection level allows experts that need access to state-of-the-art equipment but require limited assistance to: access synchrotron beamlines for MX or SAXS studies for single or multiple visits; acquire solution NMR or ssNMR spectra on high-field instruments at host institutes; gain access to state-of-the-art Electron Microscopes for single particle studies, tomography and CLEM.

Interested users can obtain access by registering at the iNEXT website (http://www.inext-eu.org/access/) and subsequently submitting a short proposal via the Access portal. Applicants can also indicate their possible preference for a particular research infrastructure. The proposal will be reviewed by external expert reviewers, evaluating on scientific quality, feasibility and translational aspects (as one of iNEXT's objectives). After a positive evaluation, the indicated facility will contact the user and plan a suitable timeslot for the requested measurements. A typical application procedure will take 2–3 weeks, also depending on timeslot availabilities at the involved facilities. Depending on the requested activity, users may wish to contact the staff of the involved Research Infrastructures before proposal submission, for example to discuss feasibility of planned activities or to plan measurements in collaboration with researchers of the Research Infrastructure. Since access in iNEXT is to highly advanced state-of-the-art instrumentation in Structural Biology for translational research, successful applications generally require demonstration of project feasibility at less advanced instruments, for example at the home institutions. However, preliminary basic screening capacity for project feasibility is also available via iNEXT (Structural Audit modality). Whereas iNEXT has been primarily setup to address the needs of researchers working in European states or in EU associated countries, it can also provide (limited) access to researchers with excellent projects from other parts of the world. Within the first 2.5 years of activity, iNEXT processed more than 500 different external peer-reviewed user projects in the different access modalities.

In addition to the provision of access to cutting edge technologies, iNEXT promotes the organization of meetings and beginner- and expert-training activities, in order to secure and expand Europe's leading international role in structural biology. Moreover, productive interactions between the academic iNEXT partners and researchers from industry are cherished, exploring and contributing to the societal impact of structural biology in the vital fields of biomedicine and biotechnology. Furthermore, the iNEXT Consortium members also designed three joint research activities (JRAs) to advance the development and integration of structural biology techniques in different relevant research field and to improve the quality as well as the quantity of access provision to the iNEXT user community.

iNEXT's JRAs are devoted at 'Developing structure guided drug discovery workflows', 'Enabling technologies for integral membrane protein systems', and 'Enabling integrative methodologies for cellular structural biology'. These three highly interactive JRAs have already proven to be extremely successful. The selected research areas produced highly relevant output, with several interesting developments becoming available for the benefit of external users. Some highlights that have emerged from the iNEXT JRAs are described below.

All activities of iNEXT are carried out synergistically with several infrastructure initiatives of the

18733468, 2018, 12, Downloaded from https://febs.onlinelibrary.wiley.com/doi/10.1002/1873-3468.13062 by Weizmann Institute Of Science, Wiley Online Library on [16/02/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

European Strategic Forum for Research Infrastructures (ESFRI) Roadmap. In particular, iNEXT services are complementary to those offered by the European Research Infrastructure INSTRUCT-ERIC [1] that provides access to a large portfolio of structural biology technologies and specifically supports research that uses integrated, multidisciplinary approaches and technologies. Besides INSTRUCT-ERIC and the European Spallation Source ESS, which are Partners of the iNEXT Consortium, iNEXT also cultivates productive interactions with the ESFRIs EU-OPENSCREEN (for efforts towards screening of small chemical compounds for biological activity, for instance in drug development) and EuroBioImaging (combining biomedical imaging approaches).

Developing structure guided drug discovery workflows

The venture for drug discovery and for the proof of concept for the therapeutic potential of specific targets are often linked to structure-based approaches. While ligand-based drug discovery (LBDD) practices are well-established in the industry and the academia, developments in fragment-based drug discovery (FBDD) are rapidly changing the field, but are often beyond the reach of most academic laboratories, as they require skilled experts, specialized instrumentation and expertise in different areas of research. iNEXT sets out to contribute to FBDD by assembling and validating a fragment library and to impact both FBDD and LBDD practices by enabling technologies that allow the high-throughput (HT) processing of crystals in synchrotrons and fragments in NMR facilities and by providing innovative and highly automated pipelines for screening to scientists both from the academia and from the industry.

Within a FBDD approach, typically a few hundred fragments (building blocks of low chemical complexity) can provide effective sampling of a large swathe of chemical space and have been proven valuable tools in drug development [2]. A key factor for FBDD success is the composition of the fragment library itself; we analysed computationally a large collection of potential fragments (11 677 in total) from various sources. From this collection, 782 fragments were selected based on the concept of 'poised fragments' [3] with the aim to streamline the downstream synthesis of more complex, higher affinity inhibitors. iNEXT facilities performed a series of validation and quality control experiments on this library including liquid chromatography-mass spectrometry and ^{1}H-NMR characterization, and solubility measurements. In particular, the quality of the library and the buffer solubility was assessed by NMR for the entire library, also exploiting the relevant software for HT data acquisition and data analysis software and for synchronizing the activities across the different NMR partner sites. This high quality Diamond-Structural Genomic Consortium-iNEXT Poised Library is currently available at different iNEXT facilities to support FBDD campaigns for user projects.

To perform FBDD campaigns by X-ray crystallography, hundreds of crystals are needed. Crystal mounting, which could constitute a bottleneck for HT-FBDD, has been fully automated with the CrystalDirect™ technology (Fig. 1) [4]. Efficient and automated X-ray data collection is also key to support large scale FBDD. Some synchrotrons available to iNEXT users provide fully automated synchrotron-based macromolecular crystallography [5]. A fully automated HT system for screening is also indispensable for an efficient FBDD by NMR. Some of the iNEXT NMR facilities have implemented robotic systems for sample loading and for data acquisition. State-of-the-art NMR-based software is also available to facilitate automatic NMR data analysis. Collectively, iNEXT facilities are supporting already 14 fragment screening projects through the iNEXT user access program.

The services developed for the FBDD pipelines can be attractive not only to academic researchers, but also to pharmaceutical companies and small-medium (start-up) enterprises. They are also crucially strengthening the Europe-wide biomedical research sector, to contribute to an ever faster and more efficient progress in structure guided drug design projects.

Enabling technologies for integral membrane protein systems

Integral membrane proteins (IMPs) are perhaps the most recalcitrant targets for structural biologists, requiring a lipid environment for both protein stability and function. This requirement makes structure determination of IMPs a complicated task. However, as these proteins are the focus of close to 50% of currently used therapeutics [6–8] the societal benefit of overcoming obstacles in their structural characterization is obvious. One of the objectives of iNEXT is to enable technologies for IMP research, delivering protocols for modular membrane protein reconstitution, strategies for optimizing expression and isotopic labelling for ssNMR and solution NMR, and providing infrastructure for structural studies across a range of access modalities.

Detergent solubilization is commonly used for both functional and structural studies of IMPs [9–11].

18733468, 2018, 12, Downloaded from https://febs.onlinelibrary.wiley.com/doi/10.1002/1873-3468.13062 by Weizmann Institute Of Science, Wiley Online Library on [16/02/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

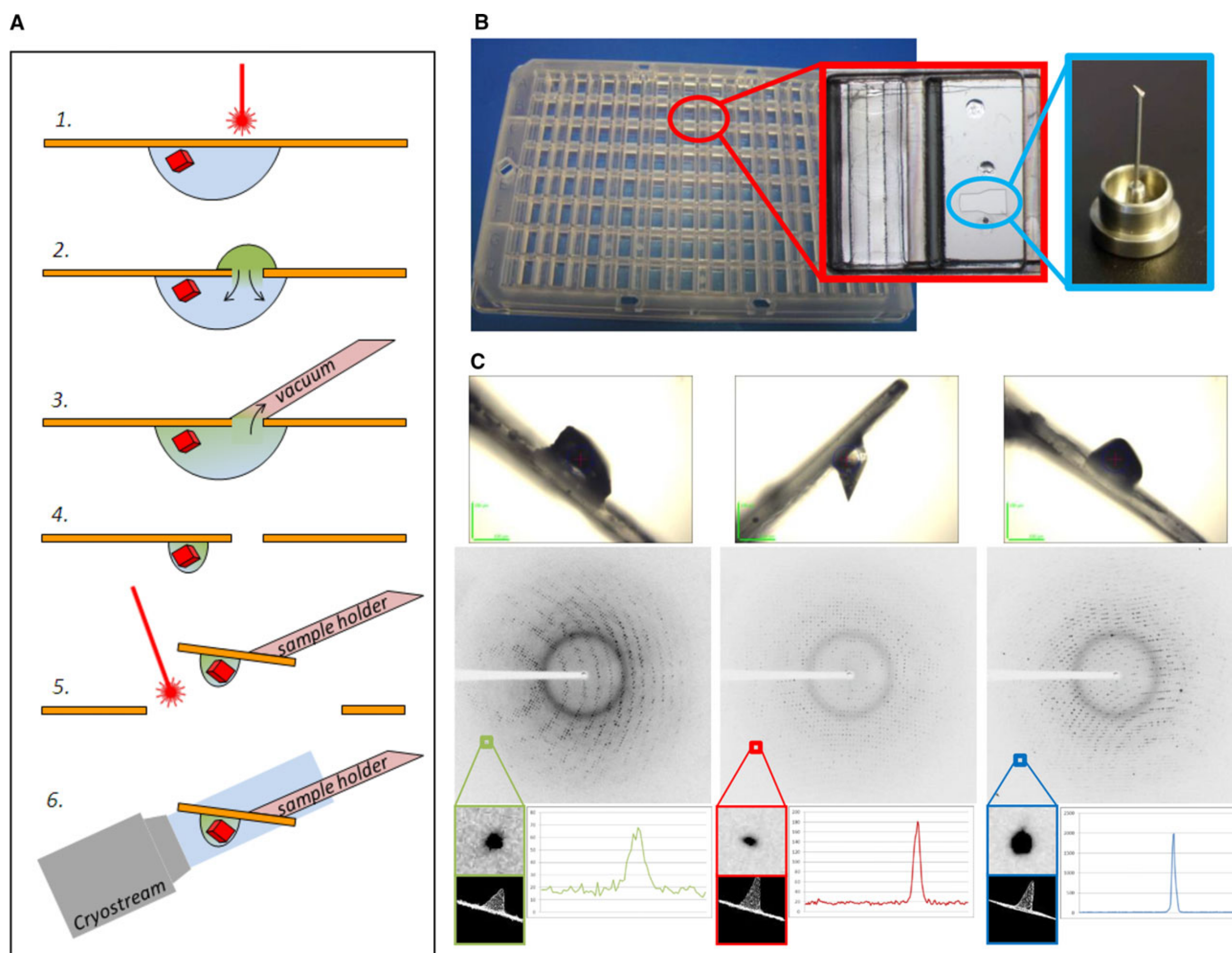

Fig. 1. The CrystalDirect™ approach. (A) Schematic representation of the methods for automated crystal harvesting, chemical delivery and cryo-cooling. From top to bottom, crystals are grown on the surface of a low X-ray-background film which is directly compatible with diffraction data collection. A laser beam operating in the photoablation regime is used to produce an aperture in the film (1). Chemicals can be delivered to crystals through diffusion by applying a small amount of solution on the outside of the opening that enters into contact with the crystallization drop (2). After incubation, or immediately after producing the aperture, if no chemicals are delivered both the externally applied and the crystallization solution are gently aspirated through the aperture by applying a vacuum (3–4). The sample can then be mounted by excising the film around the crystal with the laser and gluing it to the tip of a data-collection pin (5). The crystal is then moved to a cryo-jet for flash-cooling (6). (B) The 96-well CrystalDirect microplate. One of the cells of the microplate is shown in detail (outlined in red). Crystals from one of the drops have been harvested and mounted on a pin (blue outline). (C) X-ray diffraction analysis of crystals prepared using the automated CrystalDirect harvesting and cryo-cooling method. Reproduced with permission of the International Union of Crystallography from [4].

Classical approaches involving surfactant molecules based on maltosides (e.g. Dodecylmaltoside) and glycosides (e.g. Octylglucopyranoside) have been successful, but often impact or even abrogate function [12]. An alternative near-native like membrane scaffold system was explored, namely the Saposin-derived NanoParticle (SapNP) or SALIPRO™ system (Fig. 2 [13]). Target IMPs can be assembled in a saposin nanoparticle in the presence of lipids, stabilized using the modular nature of the small (~8 kDa) helical saposin-A protein. SAXS and Size Exclusion Chromatography-Multi-Angle Light Scattering (SEC-MALS) on the discoidal SapNPs reveal a low polydispersity and a native-like phospholipid bilayer structure encircled by lipophilic saposin monomers [14] allowing to directly reconstruct low resolution shapes from SAXS data in a detergent free environment. We showed that SapNP reconstituted IMPs are more stable against heat denaturation and bind their respective ligands. We can now offer a highly modular system for implementing the SapNP approach and a procedure for the rapid structural characterization of IMPs by SAXS and EM.

The technologies necessary for HT structural characterization of stabilized IMPs in solution are deployed

18733468, 2018, 12, Downloaded from https://febs.onlinelibrary.wiley.com/doi/10.1002/1873-3468.13062 by Weizmann Institute Of Science, Wiley Online Library on [16/02/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

18733468, 2018, 12, Downloaded from https://febs.onlinelibrary.wiley.com/doi/10.1002/1873-3468.13062 by Weizmann Institute Of Science, Wiley Online Library on [16/02/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

at different iNEXT facilities. Specifically, HPLC HT-SEC-SAXS systems equipped with autoloaders and additional detectors for complementary data analysis (dynamic and static light scattering, refractive index, and UV) were installed at the PETRA-III (Germany) and SWING (SOLEIL, France) synchrotron SAXS beamlines, offering fully automated data collection, data reduction and analysis. Using the SEC-SAXS infrastructures, detergent solubilized IMPs can also be studied, with robust buffer subtraction and separation of protein-detergent complexes from free micelles and additional components [15].

Enabling integrative methodologies for cellular structural biology

To fully harvest the power of structural analysis for cellular systems, the use of approaches enabling the characterization in a cellular context is essential.

Solution NMR is the technique of choice to obtain structural information at atomic resolution in physiological conditions. In-cell NMR can uniquely provide such data directly in living cells. We developed a protocol for performing protein in-cell NMR in cultured human cells [16,17]. This approach leverages existing mammalian protein expression technologies to allow the proteins of interest to be directly expressed and isotope-labelled within the cells (Fig. 3), and is especially useful to characterize protein folding and maturation [18,19], cofactor binding, redox state changes [20,21] and other physiological processes occurring after protein synthesis. An in-organello NMR approach was also developed, in which protein expression was targeted to mitochondria via a specific targeting sequence [22]. Furthermore, a protocol in which the timing of expression of two different proteins is controlled by coupling the silencing of a stably expressing gene with the transient expression of the second gene was set up, allowing the selective labelling of only one protein, which is critical to study protein-protein interactions [23]. These approaches start to become available to iNEXT users.

Solid-state NMR can probe membrane protein assemblies at an atomic level, studying proteins both in artificial bilayer settings and in cellular preparations. So-called 'cellular ssNMR' protocols were developed few years ago using the model bacterium *Escherichia coli*. They involved general protein expression and cell fractionation procedures that lead to uniformly ^{13}C,^{15}N-labelled preparations of whole cells and cell envelopes [24–26]. These approaches can allow the characterization of rigid membrane-associated molecular components, the conformation of the protein target, and also components that surround the target protein. Increasing levels of molecular complexity were tackled by enhancing spectroscopic sensitivity using Dynamic Nuclear Polarization and proton detection. These together with the development of dedicated isotope-labelling schemes enable the study of proteins in bacterial or eukaryotic membranes, while (U-^{13}C,^{15}N)-whole cell preparations could also reveal signals from nucleic acids, notably from RNA, that are particularly abundant during bacterial exponential growth phase. We are now working on developing approaches for integrating cellular ssNMR methodologies with single-particle cryo-EM [27], high-resolution fluorescence [28], and possibly with cryo-electron tomography to study complex cellular systems across different length and temporal scales [29].

The combination of different signals in cellular imaging, e.g. from electrons (EM), photons (light) and X-rays [soft X-ray microscopy (SXRM)], can allow

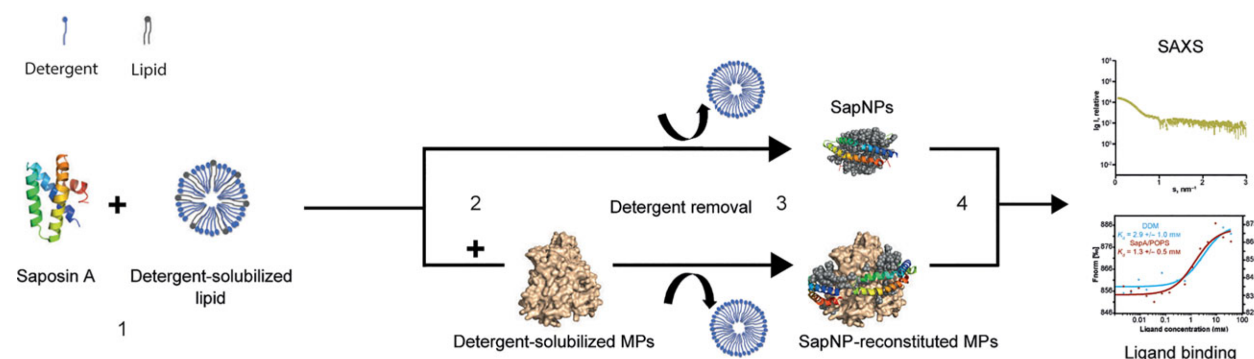

Fig. 2. Workflow of the assembly of SapNPs with target IMPs. (1) Saposin A and detergent solubilized lipids are mixed together, (2) detergent solubilized IMP is added to the solution, (3) detergent is removed via SEC purification and/or incubation with biobeads and (4) SapNP-IMP is formed and separated from empty SapNPs as required. Figure adapted from Ref. [14].

18733468, 2018, 12, Downloaded from https://febs.onlinelibrary.wiley.com/doi/10.1002/1873-3468.13062 by Weizmann Institute Of Science, Wiley Online Library on [16/02/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

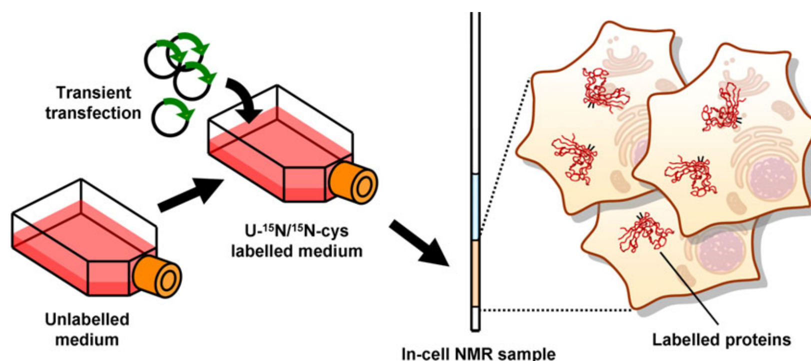

Fig. 3. Sample preparation workflow for in-cell NMR in mammalian cells [17]. From left to right: cells are first grown in unlabelled growth medium; transient transfection with the gene(s) of interest is performed, and the unlabelled medium is replaced with isotope-labelled medium; cells containing the labelled protein(s) of interest are collected for in-cell NMR analysis.

significant insight into many cellular processes. Typically, one needs to first obtain images using light microscopy to highlight the areas of interest by using fluorescent proteins or marker dyes. A platform to integrate fluorescence and X-ray images rapidly at the X-ray microscope facility, to enable fast and accurate data acquisition was created for SXRM using the USFC CHIMERA software [30,31]. Volumes and 2D images, as well as fluorescence images are loaded on the same coordinate interface, permitting fast and easy pixel size integration for the user. This workflow can also be used in most of the microscopies that are potentially relevant for cellular structural analysis (Light; soft X-ray; hard X-ray; transmission electron microscopy; scanning electron microscopy). As the workflow is independent on the software that controls the system, users are only required to extract the images acquired in the microscope and load them in their own laptops, allowing them to work across many facilities.

Finally, iNEXT has catalysed the development of protocols and reagents that use the CRISPR/Cas technology to knock in fluorophores in the native locus of proteins of interest. Together with vector collections that allow integration of the same fluorophores in purified proteins, users can get access to a toolset that can allow the study of the exact same molecule both in the cell and by biophysical methods, exploiting the same fluorophore tags.

Accelerating science output

While the iNEXT partners are engaged in developing new technologies for the users, a number of European scientists have already exploited the iNEXT Consortium facilities. They accessed well established techniques available at iNEXT platforms for performing experiments at high-end instrumentation, and benefited of the unique expertise of the facility researchers for advancing their research and/or for getting trained in specific technologies.

Several scientific publications, reporting data obtained through iNEXT access opportunities, show the impact of this project.

Understanding how to reduce side effects during immunotherapy is a highly relevant topic which was addressed by a research group from the Medical University of Vienna [32]. In particular, the work focused on the characterization by NMR, at the CERM facility, of the lipid binding properties of the major peach allergen named Pru p 3, providing evidence that upon ligand binding, the allergen undergoes local structural changes which modulate its allergenic activity.

Deciphering microbial virulence mechanisms is of fundamental importance for the treatment of infectious diseases. In this frame, the studies conducted by Romano-Moreno *et al*. [33], uncovered the molecular mechanism by which the *Legionella pneumophila* targets the host proteins. The X-ray structural characterization, performed at Diamond synchrotron, contributed to explain how *L. pneumophila*, the causative agent of Legionnaires' pneumonia, utilizes the endosomal sorting machinery to facilitate the targeting of effector proteins.

Florin *et al*. [34], through the access to CEITEC high-resolution cryo-EM facility, solved the structure of the ribosome complexed with release factor 1 and with the derivative of the insect-produced antimicrobial peptide apidaecin named Api137. The latter is an 18-amino-acid derivative of the natural apidaecin 1b, which was optimized to have improved antibacterial properties and serum stability. This study revealed the molecular interactions that lead to release factor 1 trapping, providing a starting point for the rational

design of specific inhibitors of eukaryotic translation termination.

As a further example, we mention the NMR structure determination of the high-affinity complex of a G-quadruplex binding a potential new anticancer agent [35]. This structure characterization provided a starting point for rational drug design for a class of targets, i.e. telomeric G-Quadruplexes, that has received increasing attention in the last years.

Conclusions

iNEXT is enabling research activities that lead to innovative therapeutics and diagnostics, better engineering of biotechnology tools and materials and at the same time promoting the use of structural biology in biochemical and biomedical research studies. In addition to service provision initiatives, specific research efforts have been focused to successfully develop protocols and methods to benefit the research community. Many technological achievements have already been implemented at different research facilities that are involved in the project and are already offered to iNEXT users.

We welcome researchers who are interested in submitting their projects to make use of the available structural biology facilities as well as of our recent technological developments. Our access procedures are equally straightforward for both experts and nonstructural biologists. More details about the iNEXT project and procedures to obtain access to our activities can be found on the iNEXT website [36].

We think that the services and technologies offered by iNEXT have and will have an increasing impact on a broad range of biological studies, and we are keen to be the promoters of effective scientific integration among different domains of science.

Acknowledgement

This manuscript is meant to be just a glance at the opportunities made available, and being implemented, by the iNEXT Consortium to the European scientific community. Thus, it is obviously far from being a complete description of all the activities that have and are being carried out in the frame of the iNEXT project. I acknowledge all Partners and Users for making the iNEXT project successful, and Marc Baldus, Rolf Boelens, Francisco Javier Chichón, Irina Cornaciu, Enrico Luchinat, Frank Löhr, Christian Loew, José Antonio Márquez, Haydyn Mertens, Francesca Morelli, Anastassis Perrakis, Marie Renault, Christian Richter, Harald Schwalbe, Sridhar Sreeramulu, Dmitri Svergun and Hans Wienk for supporting me in writing this review. This work has been supported by iNEXT, grant number 653706, funded by the Horizon 2020 programme of the European Commission.

References

1 INSTRUCT-ERIC website: www.structuralbiology.eu

2 Arrowsmith CH, Audia JE, Austin C, Baell J, Bennett J, Blagg J, Bountra C, Brennan PE, Brown PJ, Bunnage ME *et al.* (2015) The promise and peril of chemical probes. *Nat Chem Biol* **11**, 536–541.

3 Cox OB, Krojer T, Collins P, Monteiro O, Talon R, Bradley A, Fedorov O, Amin J, Marsden BD, Spencer J *et al.* (2016) A poised fragment library enables rapid synthetic expansion yielding the first reported inhibitors of PHIP(2), an atypical bromodomain. *Chem Sci* **7**, 2322–2330.

4 Zander U, Hoffmann G, Cornaciu I, Marquette JP, Papp G, Landret C, Seroul G, Sinoir J, Röwer M, Felisaz F *et al.* (2016) Automated harvesting and processing of protein crystals through laser photoablation. *Acta Crystallogr D* **72**, 454–466.

5 Nurizzo D, Bowler MW, Caserotto H, Dobias F, Giraud T, Surr J, Guichard N, Papp G, Guijarro M, Mueller-Dieckmann C *et al.* (2016) RoboDiff: combining a sample changer and goniometer for highly automated macromolecular crystallography experiments. *Acta Cryst* **D72**, 966–975.

6 Yin H and Flynn AD (2016) Drugging membrane protein interactions. *Annu Rev Biomed Eng* **18**, 51–76.

7 Rask-Andersen M, Almen MS and Schioth HB (2011) Trends in the exploitation of novel drug targets. *Nat Rev Drug Discov* **10**, 579–590.

8 Overington J, Al-Lazikani B and Hopkins AL (2006) How many drug targets are there? *Nat Rev Drug Discov* **5**, 993–996.

9 Hiruma-Shimizu K, Shimizu H, Thompson GS, Kalverda AP and Patching SG (2015) Deuterated detergents for structural and functional studies of membrane proteins: properties, chemical synthesis and applications. *Mol Membr Biol* **32**, 139–155.

10 Moraes I, Evans G, Sanchez-Weatherby J, Newstead S and Stewart PDS (2014) Membrane protein structure determination – the next generation. *Biochim Biophys Acta* **1838**, 78–87.

11 Breyton C, Gabel F, Lethier M, Flayhan A, Durand G, Jault JM, Juillan-Binard C, Imbert L, Moulin M, Ravaud S *et al.* (2013) Small angle neutron scattering for the study of solubilised membrane proteins. *Eur Phys J E Soft Matter* **36**, 71.

18733468, 2018, 12, Downloaded from https://febs.onlinelibrary.wiley.com/doi/10.1002/1873-3468.13062 by Weizmann Institute Of Science, Wiley Online Library on [16/02/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

12 Seddon AM, Curnow P and Booth PJ (2004) Membrane proteins, lipids and detergents: not just a soap opera. *Biochim Biophys Acta* **1666**, 105–117.
13 Frauenfeld J, Löving R, Armache JP, Sonnen AF, Guettou F, Moberg P, Zhu L, Jegerschöld C, Flayhan A, Briggs JA *et al*. (2016) A saposin-lipoprotein nanoparticle system for membrane proteins. *Nat Methods* **13**, 345.
14 Flayhan A, Mertens HDT, Ural-Blimke Y, Martinez-Molledo M, Svergun DI and Loew C (2018) Saposin lipid nanoparticles: a highly versatile and modular tool for membrane protein research. *Structure* **26**, 345–355.
15 Pérez J and Vachette P (2017) A successful combination: coupling SE-HPLC with SAXS. *Adv Exp Med Biol* **1009**, 183–199.
16 Banci L, Barbieri L, Bertini I, Luchinat E, Secci E, Zhao Y and Aricescu AR (2013) Atomic-resolution monitoring of protein maturation in live human cells by NMR. *Nat Chem Biol* **9**, 297–299.
17 Barbieri L, Luchinat E and Banci L (2016) Characterization of proteins by in-cell NMR spectroscopy in cultured mammalian cells. *Nat Protoc* **11**, 1101–1111.
18 Luchinat E, Barbieri L, Rubino JT, Kozyreva T, Cantini F and Banci L (2014) In-cell NMR reveals potential precursor of toxic species from SOD1 fALS mutants. *Nat Commun* **5**, 5502.
19 Luchinat E, Barbieri L and Banci L (2017) A molecular chaperone activity of CCS restores the maturation of SOD1 fALS mutants. *Sci Rep* **7**, 17433.
20 Mercatelli E, Barbieri L, Luchinat E and Banci L (2016) Direct structural evidence of protein redox regulation obtained by in-cell NMR. *Biochim Biophys Acta* **1863**, 198–204.
21 Barbieri L, Luchinat E and Banci L (2018) Intracellular metal binding and redox behavior of human DJ-1. *J Biol Inorg Chem* **23**, 61–69.
22 Barbieri L, Luchinat E and Banci L (2014) Structural insights of proteins in sub-cellular compartments: in-mitochondria NMR. *Biochim Biophys Acta* **1843**, 2492–2496.
23 Luchinat E, Secci E, Cencetti F and Bruni P (2016) Sequential protein expression and selective labeling for in-cell NMR in human cells. *Biochim Biophys Acta* **1860**, 527–533.
24 Renault M, Tommassen-van Boxtel R, Bos MP, Post JA, Tommassen J and Baldus M (2012) Cellular solid-state nuclear magnetic resonance spectroscopy. *Proc Natl Acad Sci U S A* **109**, 4863–4868.
25 Renault M, Pawsey S, Bos MP, Koers EJ, Nand D, Tommassen-van Boxtel R, Rosay M, Tommassen J, Maas WE and Baldus M (2012) Solid-state NMR spectroscopy on cellular preparations enhanced by dynamic nuclear polarization. *Angew Chem Int Ed* **51**, 2998–3001.
26 Baker LA, Daniëls M, van der Cruijsen EAW, Folkers GE and Baldus M (2015) Efficient cellular solid-state NMR of membrane proteins by targeted protein labeling. *J Biomol NMR* **62**, 199–208.
27 Kaplan M, Cukkemane A, van Zundert GCP, Narasimhan S, Daniëls M, Mance D, Waksman G, Bonvin AMJJ, Fronzes R, Folkers GE *et al*. (2015) Probing a cell-embedded megadalton protein complex by DNP-supported solid-state NMR. *Nat Meth* **12**, 649–652.
28 Kaplan M, Narasimhan S, de Heus C, Mance D, Van Doorn S, Houben K, Popov-Celeketic D, Damman R, Katrukha EA, Jain P *et al*. (2016) EGFR dynamics change during activation in native membranes as revealed by NMR. *Cell* **167**, 1241–1251.e11.
29 Baker LA, Sinnige T, Schellenberger P, de Keyzer J, Siebert CA, Driessen AJM, Baldus M and Grünewald K (2018) Combined 1H-detected solid-state NMR spectroscopy and electron cryotomography to study membrane proteins across resolutions in native environments. *Structure* **26**, 161–170.e3.
30 Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC and Ferrin TE (2004) UCSF Chimera – a visualization system for exploratory research and analysis. *J Comput Chem* **25**, 1605–1612.
31 Yang Z, Lasker K, Schneidman-Duhovny D, Webb B, Huang CC, Pettersen EF, Goddard TD, Meng EC, Sali A and Ferrin TE (2012) UCSF Chimera, MODELLER, and IMP: an integrated modeling system. *J Struct Biol* **179**, 269–278.
32 Dubiela P, Aina R, Polak D, Geiselhart S, Humeniuk P, Bohle B, Alessandri S, Del Conte R, Cantini F, Borowski T *et al*. (2017) Enhanced Pru p 3 IgE-binding activity by selective free fatty acid-interaction. *J Allergy Clin Immunol* **140**, 1728–1731.e10.
33 Romano-Moreno M, Rojas AL, Williamson CD, Gershlick DC, Lucas M, Isupov MN, Bonifacino JS, Machner MP and Hierro A (2017) Molecular mechanism for the subversion of the retromer coat by the Legionella effector RidL. *Proc Natl Acad Sci USA* **114**, E11151–E11160.
34 Florin T, Maracci C, Graf M, Karki P, Klepacki D, Berninghausen O, Beckmann R, Vázquez-Laslop N, Wilson DN, Rodnina MV *et al*. (2017) An antimicrobial peptide that inhibits translation by trapping release factors on the ribosome. *Nat Struct Mol Biol* **24**, 752–757.
35 Wirmer-Bartoschek J, Bendel LE, Jonker HRA, Grün JT, Papi F, Bazzicalupi C, Messori L, Gratteri P and Schwalbe H (2017) Solution NMR structure of a ligand/hybrid-2-G-quadruplex complex reveals rearrangements that affect ligand binding. *Angew Chem Int Ed Engl* **56**, 7102–7106.

18733468, 2018, 12, Downloaded from https://febs.onlinelibrary.wiley.com/doi/10.1002/1873-3468.13062 by Weizmann Institute Of Science, Wiley Online Library on [16/02/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

36 iNEXT website. www.inext-eu.org. The iNEXT Consortium is part of the Horizon 2020 programme of the European Commission, grant 653706 titled "iNEXT: Infrastructure for NMR, EM and X-rays for Translational Research". The Consortium involves 23 partners from 14 different European countries: (1) Utrecht University, Utrecht, Netherlands; (2) EMBL-Heidelberg and EMBL-Hamburg, Germany, and EMBL-Grenoble, France; (3) Diamond Light Source, Didcot, UK; (4) Consorzio Interuniversitario Risonanze Magnetiche di Metallo Proteine, Florence, Italy; (5) Goethe University Frankfurt, Frankfurt, Germany; (6) The Netherlands Cancer Institute, Amsterdam, Netherlands (7) CEITEC – Masaryk University, Brno, Czech Republic; (8) CSIC – Spanish National Research Council, Madrid, Spain; (9) FVB-FMP – Leibniz-Institut für Molekulare Pharmakologie, Berlin, Germany; (10) Synchrotron SOLEIL, Gif-sur-Yvette, France; (11) MAXIV, Lund University, Lund, Sweden; (12) NeCEN, Leiden University, Leiden, Netherlands; (13) Leiden University Medical Center, Leiden, Netherlands; (14) European Synchrotron Radiation Facility, Grenoble, France; (15) RALF-NMR, Centre National de la Recherche Scientifique, Lyon/Grenoble, France; (16) ESFRI Instruct, Oxford University, Oxford, UK; (17) Aarhus University, Aarhus, Denmark; (18) European Spallation Source, Lund, Sweden; (19 Eötvös Loránd University, Budapest, Hungary; (20) Instituto de Tecnologia Química e Biológica, Lisbon, Portugal; (21) University of Oulu, Oulu, Finland; (22) University of Patras, Patras, Greece; (23) Weizmann Institute of Science, Rehovot, Israel.

Correspondence
L. Banci, Magnetic Resonance Centre (CERM), University of Florence, Via Luigi Sacconi 6, 50019 Sesto Fiorentino, Italy
Fax: +39 055 4574923
Tel: +39 055 4574270
E-mail: banci@cerm.unifi.it

[†]iNEXT Consortium members are in Appendix.

Appendix

iNEXT Consortium members

Rolf Boelens (Coordinator): Universiteit Utrecht, Utrecht, The Netherlands; Dmitri Svergun, Jan Ellenberg, Stephen Cusack: European Molecular Biology Laboratory (Hamburg, Heidelberg and Grenoble sites, respectively); Martin A. Walsh, Diamond Light Source Limited, Didcot, United Kingdom; Lucia Banci: Consorzio Interuniversitario Risonanze Magnetiche di Metallo Proteine, Firenze, Italy; Harald Schwalbe, Johann Wolfgang Goethe Universitaet, Frankfurt am Main, Germany; Anastassis Perrakis: Stichting het Nederlands Kanker Instituut - Antoni van Leeuwenhoek Ziekenhuis, Amsterdam, The Netherlands; Vladimír Sklenář, Masarykova Univerzita, Brno, Czech Republic; Jose Maria Carazo Garcia: Agencia Estatal Consejo Superior de Investigaciones Cientificas, Madrid, Spain; Hartmut Oschkinat: Forschungsverbund Berlin E.V., Berlin, Germany; Andrew Thompson: Société Civile Synchrotron SOLEIL, Gif sur Yvette, France; Marjolein Thunnissen: Lunds Universitet, Lund, Sweden; Bram Koster: Universiteit Leiden and Leids Universitair Medisch Centrum, Leiden, The Netherlands; Gordon Leonard: Installation Europeenne de Rayonnement Synchrotron, Grenoble, France; Bernhard Brutscher: Centre National de la Recherche Scientifique, Grenoble/Lyon, France; David I. Stuart: INSTRUCT Academic Services Limited, Oxford, United Kingdom; Poul Nissen: Aarhus Universitet, Aarhus, Denmark; Hanna Wacklin: European Spallation Source ESS AB, Lund, Sweden; András Perczel: Eotvos Lorand Tudomanyegyetem, Budapest, Hungary; Margarida Archer: Instituto de Tecnologia Quimica e Biologica, Oeiras, Portugal; Rik Wierenga: Oulun Yliopisto, Oulu, Finland; Georgios A. Spyroulias: University of Patras, Rio Patras, Greece; Joel Sussman: Weizmann Institute of Science, Rehovot, Israel.

18733468, 2018, 12, Downloaded from https://febs.onlinelibrary.wiley.com/doi/10.1002/1873-3468.13062 by Weizmann Institute Of Science, Wiley Online Library on [16/02/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License