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Editorial

Dear AMPERE colleagues,

this issue contains the report on EUROMAR 2015 in Prague (p.2) as well as an article by Jean-Philippe Demers on the work that earned him the Raymond Andrew Prize 2015 (p.5). If you want to nominate a young scientist for the 2016 Award, please note that the deadline is February 15th 2016.

Have you already seen our newly designed homepage at www.ampere-society.org? On this page you can find the electronic version of this Bulletin, information on past award winners, calls for award nominations, and up-to-date information on AMPERE conferences, conferences of subdivisions, and other conferences in the field. If you want to have your favourite conference listed there, please send information on the date, location, and homepage to contact@ampere-society.org. AMPERE also offers the possibility to announce open positions. For this, please send an e-mail with the information to job@ampere-society.org.

For those of you who are interested in good food and, in particular, the magnetic resonance aspects of characterizing it, the MR Food Conference will take place June 7-10 2016 in Karlsruhe, Germany under the auspices of Groupement AMPERE.
You find more information on; http://mrfood2016.gvt.org/

Secretary General of Groupement AMPERE

Report on Euromar 2015
Prague, Czech Republic

EUROMAR, a joint conference on magnetic resonance phenomena, took place on July 5-10, 2015 at the Prague Congress Centre, a venue with stunning panorama view of Prague Castle, in the Czech Republic. The meeting was chaired by Prof. Vladimír Sklenář from CEITEC Masaryk University, Brno, who is one of the pioneers of nuclear magnetic resonance (NMR) spectroscopy of nucleic acids.

Auditorium of a parallel session

EUROMAR 2015, gathering leading scientists from all around the world, offered a unique opportunity to report and witness the latest scientific breakthroughs in magnetic resonance in a broad range of scientific fields, stretching from physics and chemistry to biology and medicine.

The program of congress consisted of 1 tutorial session, 8 plenary sessions dedicated to the renowned experts in the field, and 24 parallel sessions on various topics, where new methods and instrumentation developments in established areas and novel technologies and applications in emerging fields were presented. Altogether, 136 lectures were delivered during the congress.

Prof. Vladimír Sklenář, chair of the EUROMAR 2015

Young scientist and students had the opportunity to present and discuss their research at 3 poster sessions with more than 360 posters and during numerous social events. The congress fully accomplished its main goal to serve as a stimulating forum for sharing experience, exchanging ideas, and establishing fruitful collaborations.

Auditorium of the Raymond Andrew Prize lecture held by Jean-Philippe Demers

Raymond Andrew Prize 2015

Jean-Philippe Demers
Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

Structural determination of large assemblies at atomic resolution using solid-state NMR and electron microscopy: the Type-Three Secretion Needle of *Shigella* bacteria

The Type-Three Secretion System (T3SS) is a large supra-molecular assembly found in pathogenic Gram-negative bacteria. Bacteria use this system to deliver toxic proteins into a target host cell, such as human intestinal epithelial cells. The T3SS contains a needle which extends into the extracellular space. Upon contact of the needle tip with the host cell, translocator proteins form a pore through which effector proteins enter and subsequently alter host cell processes during infection[1-2]. The first molecular model of the T3SS needle was proposed in 2006 by docking the crystal structure[3] of a truncation mutant the *Shigella flexneri* needle subunit protein MxiH into a 16-Å negative stain EM density map[4]. Following improvements in the sample production protocols[5-6], the atomic structure of the *Salmonella typhimurium* T3SS needle has been determined by solid-state NMR (ssNMR)[7]. Shortly after, a cryo-electron microscopy (cryoEM) density map of the *Shigella flexneri* T3SS needle was determined with a resolution of 7.7 Å[8]. The two models of the *Shigella* needle, determined on the basis of cryoEM data, have the N-terminus of the needle subunit protein (MxiH) located on the inside of the assembly, pointing to the lumen of the needle. The model of the *Salmonella* needle, determined on the basis of solid-state NMR (ssNMR) data and scanning transmission electron microscopy (STEM) data, has the N-terminus of the needle subunit protein (PrgI) located on the outside surface of the assembly, pointing towards the extra-cellular space. In addition, the secondary structure identified in the cryoEM model of MxiH contains an additional β-hairpin to account for a "protrusion" in the EM density map while the model of PrgI contains only α-helix—loop—α-helix as secondary structure elements. This created a controversy in the study field of the Type-III Secretion System: since the primary sequence of the homologous proteins PrgI and MxiH share 60% identity, it was expected that the two needles would have similar structure and organization.

We thus aimed to answer the following questions:

- What is the secondary structure of MxiH in the assembled needle?
- What is the needle subunit orientation within the assembly?
- Is there a conserved architecture among bacterial needles?

We recorded ssNMR spectra on uniform and sparse labeled MxiH needles, enabling us to extract the secondary chemical shifts of backbone nuclei. This established that the MxiH protein contains a rigid segment (M1-T11), an N-terminal α-helix (L12-A38), a loop followed by the C-terminal α-helix (Q45-R83). The presence of a β-hairpin was ruled out by this analysis. Immuno-gold labeling experiments on *in vitro*-polymerized needles as well as needles present *in vivo* on *Shigella* bacteria revealed that the N-terminus of the MxiH subunit is present on the outside surface of the needle assembly, similar to the needle organization proposed for PrgI needles[7]. For the amino acids L12 to R83 of MxiH, we observed that the pattern of secondary chemical shift is highly similar between MxiH and PrgI. A homology model of the MxiH needle, produced for residues L12 to R83, could fit well (correlation of 0.66) in the 7.7-Å cryoEM density of MxiH needles[8], suggesting a single common architecture for all bacterial T3SS needles[9].

We then tackled the following questions:

- Do the two contradicting models of the *Shigella* T3SS needle reflect true differences in needle structure, for example due to the use of different sample production protocols?
- Is it possible to produce an atomic structure which satisfies all experimental data: ssNMR, cryoEM, and immuno-gold labeling?
- What is the conformation of the non-conserved N-terminal region from M1-T11? How can the "protrusion" in the cryoEM density map be explained?

To address these questions, we recorded an extensive set of ssNMR spectra for the purpose of collecting long-range distance restraints. Using unambiguous long-range correlation, we were able to produce *de novo* a preliminary description of the MxiH protein fold and of the inter-molecular interfaces present in the MxiH needle. Using this description, we could complete the assignment of distance cross-peaks. In total, 12,350 cross-peaks were analyzed in long-range spectra and over 17,850 cross-peaks were analyzed for the full project. We classified the distance cross-peaks either as unambiguous correlations or as ambiguous correlation using a strategy based on the symmetric geometry of helical assemblies.

We developed with our collaborators a hybrid structure calculation approach based on Rosetta modeling to integrate both NMR and EM structural constraints[10]. In order to avoid the introduction of any bias by the initial NMR assignment, we introduced an automatic procedure for cross-validation of PDB models using NMR constraints prepared for the purpose of validation.

This procedure is similar to the use of R_{free} used in crystallography. The structure calculations include the 7.7-Å cryoEM density map as restraint. The final structural models are determined to a precision of 0.4 Å backbone RMSD. The final models produced by this hybrid approach (Figure 1) are compatible with all experimental data available: both with the ssNMR constraints, with few NMR constraint violations (4.5%) for the validation set, and with the cryoEM density map, with correlation in the range 0.62–0.67. The MxiH structural model produced by the fit to the cryoEM density map alone[8] however does not satisfy the constraints of the independent NMR validation data set. Using STEM images independently recorded on the *in vitro* polymerized MxiH needles, we confirmed that the polymerized needles and the hybrid models have the same helical symmetry and compatible axial subunit displacement along the helical axis.

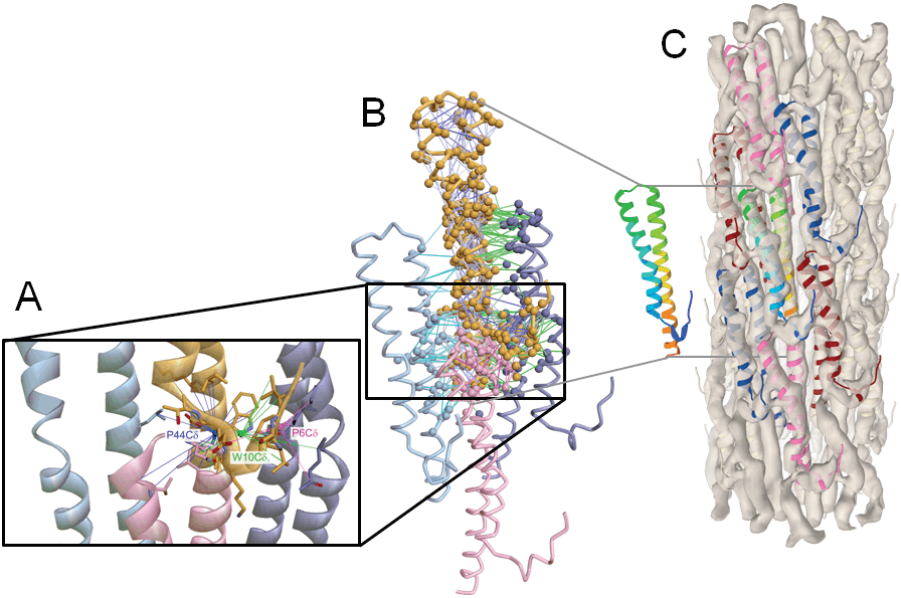

Figure 1: Atomic structure of the T3SS needle of *Shigella flexneri*. A) Detailed view of the N-terminal region (M1-T11) occupying the "protrusion" in the EM density. B) Four adjacent MxiH subunits with intra-molecular and inter-molecular distance restraints represented as colored lines. C) Six adjacent MxiH subunits fitted in the cryoEM density map.

This work resolves the controversy in the field regarding the architecture of bacterial T3SS needles: it is now accepted by the community that T3SS needles adopt an α-helix–loop–α-helix motif with the N-terminus located at the outside surface of the assembly. The final models clearly define the position and conformation of the rigid N-terminus of the MxiH subunit, occupying the protrusion present in the cryoEM density map. This collaborative work has led to the development of a new hybrid structural determination approach which integrates ssNMR and EM data. The final models of the T3SS needle reveal an electrostatically balanced surface, suggesting a mechanism for the transport of toxic substrates through the T3SS needle.

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Raymond Andrew Prize 2016 – Call for nominations

Call for Nominations for the Raymond Andrew Prize for an outstanding PhD thesis in the field of magnetic resonance:

For the Raymond Andrew Prize 2016 the AMPERE Prize Committee is seeking your help in searching for qualified candidates who completed their dissertation during the period of 2014/2015. The prize will be presented during the EUROMAR in Aarhus (Denmark) from July 3rd to July 7th 2016.

You are kindly invited to submit nominations by e-mail to
andrewprize@nmr.phys.chem.ethz.ch

Suggestions must be received by 15th February 2016 and should include the following documents:
- Nomination letter
- Curriculum vitae
- List of publications and presentations at conferences
- PhD thesis in PDF

The thesis should be written in English. In exceptional cases, the thesis may also be submitted in triplicate as a hardcopy to the AMPERE Secretariat. Please note that the nomination letter cannot be written by the candidate herself or himself.
Submissions that arrive too late will automatically be transferred to the next year. The prize committee will reconsider excellent contributions for two years in a row.
For a list of past Andrew Prize winners see:
http://www.ampere-society.org

Sincerely yours,
Gunnar Jeschke

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Future conferences

Ampere events

2016

Food MR	Karlsruhe (Germany)	June 7-10 2016
Euromar 2016	Aarhus (Denmark)	July 3-7 2016

2017

Euromar 2017	Warwaw (Poland)	July 2-6 2017

Other events

2016

49th Annual International Meeting of the ESR Spectroscopy Group	Colchester (England)	April 3-7 2016
57th ENC	Pittsburgh (USA)	April 10-15 2016
Interpore Conference	Cincinnati (USA)	Mai 9-11 2016
ICMRBS 2016	Kyoto (Japan)	August 21-26 2016
APES 2016	Irkutsk (Russia)	August 28 - Sept. 2, 2016

2017

20th ISMAR conference	Québec City (Canada)	July 23-28 2017