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FOCUS: 31st ASILOMAR CONFERENCE, NATIVE MS-BASED STRUCTURAL BIOLOGY: EDITORIAL AND REVIEW

Editorial and Review: 31st ASMS Asilomar Conference on Native Mass Spectrometry-Based Structural Biology

The 31st Asilomar Conference on Mass Spectrometry, organized by Albert Heck (Utrecht University) and Joseph Loo (University of California, Los Angeles), was held on October 16–20, 2015 at the Asilomar Conference Center in Pacific Grove, CA. The focus of the conference was on "Native Mass Spectrometry-Based Structural Biology." Approximately 160 attendees representing over 15 countries listened to presentations on the latest advancements in sample handling, ionization techniques, analyzers, ion activation methods, and new biological applications to study the structures and interactions of large biomolecules and assemblies. A majority of the attendees contributed to the conference directly, with 27 invited lectures, eight shorter "hot topic" talks, 42 rapid 150-s poster highlight summaries, and 56 poster presentations.

Native mass spectrometry provides a means to interrogate the structure of large biomolecules and complexes from a nondenaturing solution environment. The method has now matured and found its way into integrated structural biology projects, primarily to establish the unknown architecture of large protein complexes and molecular machineries. "Biomolecular Interactions: Identification and Characterization of Protein Complexes" was the topic of the 19th Asilomar Conference held in 2003 (organized by Carol Robinson and Joseph Loo), but there have been tremendous advancements since then, including the introduction of new mass analyzers, improvements in sample handling and ionization techniques, the rapid adoption of ion mobility spectrometry, and the application of different ion activation methods to elucidate structure of and interactions within protein assemblies. New areas of applications have emerged, focusing on the structures of, for example, viruses, membrane proteins, biopharmaceuticals, and signaling nodes. The 2015 Conference highlighted the current state-of-the-art in native mass spectrometry and especially how it can impact structural biology studies, and provided a view of possible future advances in this field. The growth of the field and its interest level are reflected in the larger number of attendees, given that the 2003 Conference attracted slightly over 100 delegates.

The opening Friday evening session started with Joe Loo giving a historical perspective of the Asilomar conference and describing some of the traditions established by past year's conferences, including the "cutting of the necktie," and after a rejuvenating hibernation, the return of Po-Po, the timekeeping monkey (Figure 1) (courtesy of Laszlo Tokes and Sharon Pitteri). Albert Heck summarized the field of native MS and reiterated the goals of the conference to encourage discussion and an open exchange of ideas. The first plenary discussion was delivered by Carol Robinson (University of Oxford) on "Structural Biology in the Gas Phase—the First 25 Years" on her pioneering efforts to bring native MS to the structural biology community and her recent efforts to extend native MS to address membrane protein assemblies (Figure 2). Her talk was followed by a historical perspective on the very early, but already exciting, days of measuring intact viral particles using mass spectrometry, "From Native Virus Analysis to Metabolomics," by (a tie-less) Gary Siuzdak (The Scripps Research Institute).

The Saturday morning session started with a spirited discussion of ionization methods used for native MS, including the most commonly used electrospray ionization (ESI) ("Fundamentals of Protein Electrospray Ionization;" Lars Konermann, University of Western Ontario), and those less commonly used for native MS, such as matrix-assisted laser desorption/ionization (MALDI) ("MALDI Mass Spectrometry for Studying Biomolecular Complexes;" Renato Zenobi, ETH-Zurich), and laser induced liquid bead ion desorption (LILBID) ("New Developments for Native LILBID-MS;" Nina Morgner, Goethe-Universität Frankfurt). Although ESI is the most mature desorption/ionization technique used for native MS, the capabilities of techniques that generate mostly singly charged molecules like MALDI and LILBID were shown to be promising for native MS, especially the combination of chemical cross-linking (to stabilize protein complexes) with MALDI-MS.

Throughout the history of mass spectrometry, the general concept of "breaking" molecules, from small to large, to derive structure information has been a consistent theme. More recently, this strategy has been extended to native protein complexes. It is hoped that fragmenting gas-phase protein complexes will yield structural insight into the topology of large assemblies. Talks in the afternoon featured discussions on more novel alternative activation/dissociation methods, such as surface induced dissociation (SID) ("SID of Model Systems with Known Interfaces: What Have We Learned?" Vicki

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Figure 1. The return of Po-Po, the timekeeping monkey (and Mike Gross, Washington University-St. Louis) to Asilomar

Wysocki, The Ohio State University), 193-nm ultraviolet photodissociation (UVPD) ("Ultraviolet Photodissociation for Characterization of Proteins and Protein Complexes;" Jenny Brodbelt, University of Texas – Austin), and electron capture dissociation (ECD) ("ECD of Native Proteins and Assemblies;" Joe Loo, University of California, Los Angeles) that are used for probing the structure of native proteins and complexes. Compared with more conventional collision-induced dissociation (CID), these fragmentation methods appear to yield more structural information that is consistent with elements from a given protein complex's known native structure.

Figure 2. The first speaker of the conference, Carol Robinson (University of Oxford; right), with Brian Chait (Rockefeller University; left), the last speaker of the conference

The afternoon ended with a short session on "Macromolecular Assemblies," in which Albert Heck (Utrecht University, "Probing the Limits in High Mass Range and Resolution in Native Mass Spectrometry with Applications into Structural Virology") presented his group's work on extending the mass range of native MS to the megaDalton regime for accessing large viral assemblies, using mostly Orbitrap instruments. Justin Benesch (University of Oxford, "Quantifying Protein Self-Assembly") discussed his efforts to quantify protein subs-toichiometries of complexes and the dynamics of quaternary organization of protein assemblies.

Native mass spectrometry can be used to contribute to an integrative approach to gain insights into the structure of macromolecular assemblies. Distance constraints can be generated by chemical cross-linking and the MS-based identification of cross-linked residues can further aid the determination of the structures of large complexes. After dinner, talks on "Merging Native and Cross-Linking Mass Spectrometry" were given by two leaders in this growing field, Andrea Sinz (Martin-Luther University Halle-Wittenberg, "What You Always Wanted to Know about Chemical Cross-Linking/MS"), and Alexander Leitner (ETH-Zurich, "Applications of Chemical Cross-Linking/Mass Spectrometry in Integrative Structural Biology").

Native MS has by now gained an important prominence in the pharmaceutical industry, particularly for the characterization of intact therapeutic monoclonal antibodies. This was featured in the Sunday morning session on "Native MS in Pharma Research." Sarah Cianferani (CNRS Strasbourg, "Native MS Methods for In-Depth mAbs and ADC Characterization") presented her group's work on the analysis of bi-specific antibodies (that are composed of parts of two different monoclonal antibodies and as a result binds to two different types of antigens) and antibody-drug conjugates (ADCs). The application of native MS in small molecular drug discovery, e.g., protein–drug interactions, was highlighted by Rachel Garlish (of the company UCB London, "Using Mass Spectrometry to Screen for Protein-Drug Interactions").

The relatively new application of native MS towards difficult-to-analyze membrane proteins and their complexes, as discussed by Carol Robinson's opening plenary talk, was further emphasized by talks by Michael Gross (Washington University-St. Louis, "Membrane Proteins: Native MS, Lipid Nanodiscs, and Footprinting"), and Frank Sobott (University of Antwerp, "Dynamic Protein Structure: from Protein Disorder to Membrane Pores"). Gross described the characterization of a protein solubilized in nano-discs, self-assembled discoidal fragments of lipid bilayers stabilized in solution by two amphipathic helical scaffold proteins, using fast photochemical oxidation of proteins (FPOP).

The Sunday evening session focused on "Developments in Mass Analyzers for Native MS." The improvements in sensitivity and resolving power, especially for large molecule detection at high mass-to-charge ratio (m/z), have played a significant role in the growing popularity of native MS (with ESI). Alexander Makarov from Thermo Scientific updated the

conference attendees on the enhancements made to Orbitrap analyzers for measuring ions beyond *m/z* 40,000. Jan Commandeur from MS Vision discussed improvements made to quadrupole time-of-flight (QTOF) analyzers for better desolvating large protein ions to *m/z* 70,000. A new type of hybrid instrument that involves the coupling of a Thermo Velos source with a Waters QTOF instrument (referred to as an "unholy marriage") was described by Commandeur. Ralf Hartmer from Bruker Daltonics showed impressive native MS results with high resolving power for monoclonal antibodies using their latest QTOF technology.

Ion mobility spectrometry (IMS) has already had a significant impact on native MS for over 10 y. Much work by many labs has shown that the determined collision cross-sections by IMS have the potential for defining the sizes and shapes of gas-phase heterogeneous macromolecular assemblies. The conference's last day began with a presentation by Alison Ashcroft (University of Leeds) on "Exploring Protein Function Using Ion Mobility-Mass Spectrometry;" among many systems described, amphipols were shown to be useful solubilizing agents for native MS/IMS of membrane proteins. Brandon Ruotolo (University of Michigan, "Collisional Unfolding of Protein Complexes: from Domain-Specific Stability Measurements to High-Throughput Drug Discovery") showed how fingerprints from his collision induced unfolding (CIU) method, derived from collisional heating in the gas phase with subsequent IMS separation, is sensitive to the analyte's structure and ligand binding. The virtues of differential mobility analysis (DMA) of very large protein complexes were presented by Guenter Allmaier (Vienna University of Technology, "Nano ES Differential Mobility Analysis of V's (Viruses, Virus-like Particles and Vaults)").

Other detection methods that could impact future native MS research include coupling nano-electromechanical systems with MS to achieve single-molecule detection of large proteins (Michael Roukes, California Institute of Technology, "Nanomechanical MS: Toward Native Single-Molecule Analysis"). Another single-particle detection technique is charge detection mass spectrometry (CDMS). Martin Jarrold (Indiana University) presented his latest work on CDMS to analyze the assembly products of coat protein variants of bacteriophage P22 with masses extending beyond 20 MDa. Jeff Brown from Waters Corporation discussed improvements to native MS with a QTOF analyzer, including electron transfer dissociation and UVPD.

The after-dinner plenary talk on Monday evening was delivered by Brian Chait of Rockefeller University, who gave an engaging talk entitled "Fundamentals of Native ESI for Macromolecules Then and Now." The Chait lab was among the few groups in the early 1990s to investigate the application of ESI-MS for measuring noncovalently bound protein complexes. His talk featured the work in those early days to his current research to elucidate the higher order structure of proteins and protein assemblies such as the huge nuclear pore complex.

There was a lot of science to absorb in 3+ days of the conference. In addition to the invited presentations, the sponsoring vendors gave short talks of their products. Eight poster presenters were selected to give short 12-min "hot topic" talks, in addition to the other poster presenters giving a 2.5-min *rapid* summary of their work (before the music abruptly plays them off of the stage). For many of the attendees, this was their first time attending an Asilomar meeting at "the refuge by the sea." However, the pleasant weather, ocean breezes, and relaxing environment helped to stimulate discussions and hopefully encouraged new "interactions" amongst the conferees. Some new fun events were also introduced to the conference that hopefully will become new traditions for future meetings. (Can you make "s'mores" when it's raining outside?) Other highlights of the meeting, including many "JASMS selfies" that were contributed by the attendees, can be found in the slides (see Supplemental Materials) that Joe Loo presented at the end of the conference.

It has been 12 y (2003–2015) between the initial Asilomar meeting on this topic and this latest one. The general feeling of the community present was that the field has now been expanded and matured to an extent that the next meeting on this topic should already be in a few years. The interest from the pharmaceutical industry and the more conventional structural biology community make the topic very lively and exciting.

This Special Focus Issue features developments related to the topic of "Native Mass Spectrometry-Based Structural Biology," and the articles are contributed by those who presented their work at the 31st Asilomar Conference on Mass Spectrometry. In addition, a special article by Leney and Heck describes the origins and a (re)definition of the term, "native MS."

(1) Account & Perspective – "Native Mass Spectrometry: What is in the Name?" by Aneika C. Leney and Albert J. R. Heck
(2) Account & Perspective – "Staying Alive: Measuring Intact Viable Microbes with Electrospray Ionization Mass Spectrometry" by Erica Forsberg, Mingliang Fang, and Gary Siuzdak
(3) Account & Perspective – "Are Charge-State Distributions a Reliable Tool Describing Molecular Ensembles of Intrinsically Disordered Proteins by Native MS?" by Antonino Natalello, Carlo Santambrogio, and Rita Grandori
(4) "Quantitation of the Noncovalent Cellular Retinol-Binding Protein, Type 1 Complex Through Native Mass Spectrometry" by Wenjing Li, Jianshi Yu, and Maureen A. Kane
(5) "Action-FRET of a Gaseous Protein" by Steven Daly, Geoffrey Knight, Mohamed Abdul Halim, Alexander Kulesza, Chang Min Choi, Fabien Chirot, Luke MacAleese, Rodolphe Antoine, and Philippe Dugourd
(6) "FPOP-LC-MS/MS Suggests Differences in Interaction Sites of Amphipols and Detergents with Outer Membrane Proteins" by Thomas G. Watkinson, Antonio N. Calabrese, James R. Ault, Sheena E. Radford, and Alison E. Ashcroft
(7) "Dissociation Behavior of a TEMPO-Active Ester Cross-linker for Peptide Structure Analysis by Free Radical Initiated Peptide Sequencing (FRIPS) in Negative ESI-MS" by Christoph Hage, Christian H. Ihling, Michael Götze, Mathias Schäfer, and Andrea Sinz

Figure 3. Attendees of the 31st Asilomar Conference on Mass Spectrometry

(8) "Conformational Space and Stability of ETD Charge Reduction Products of Ubiquitin" by Frederik Lermyte, Mateusz Krzysztof Łącki, Dirk Valkenborg, Anna Gambin, and Frank Sobott
(9) "nES GEMMA Analysis of Lectins and their Interactions with Glycoproteins – Separation, Detection, and Sampling of Noncovalent Biospecific Complexes" by Nicole Y. Engel, Victor U. Weiss, Martina Marchetti-Deschmann, and Günter Allmaier
(10) "Native Mass Spectrometry Characterizes the Photosynthetic Reaction Center Complex from the Purple Bacterium *Rhodobacter sphaeroides*" by Hao Zhang, Lucas B. Harrington, Yue Lu, Mindy Prado, Rafael Saer, Don Rempel, Robert E. Blankenship, and Michael L. Gross
(11) "Gas-Phase Analysis of the Complex of Fibroblast Growth Factor 1 with Heparan Sulfate: a Traveling Wave Ion Mobility Spectrometry (TWIMS) and Molecular Modeling Study" by Yuejie Zhaoa, Arunima Singh, Yongmei Xu, Chengli Zong, Fuming Zhang, Geert-Jan Boons, Jian Liu, Robert J. Linhardt, Robert J. Woods, and I. Jonathan Amster
(12) "Structural Characterization of Monomers and Oligomers of D-Amino Acid-Containing Peptides Using T-wave Ion Mobility Mass Spectrometry" by Xueqin Pang, Chenxi Jia, Zhengwei Chen, and Lingjun Li

We thank Jennifer Watson for helping to make the conference proceed smoothly and to encourage a fun atmosphere. Although the meeting occurred last year, we hope that those who attended still have fond memories (Figure 3). And to those who didn't attend…….too bad, *you missed a good one*. But reading the papers in this issue should help fill in some of the gaps.

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