

Scientific Life

N-term 2017: Proteostasis via the N-terminus

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N-term 2017 was the first international meeting to bring together researchers from diverse disciplines with a shared interest in protein N-terminal modifications and the N-end rule pathway of ubiquitin-mediated proteolysis, providing a platform for interdisciplinary cross-kingdom discussions and collaborations, as well as strengthening the visibility of this growing scientific community.

N-term 2017 – Protein Specialists Meet in Halle

In September 2017, scientists from around the world met at the Leibniz Institute of Plant Biochemistry in Halle/Saale to participate in ‘N-term 2017: Proteostasis via the N-terminus’, a scientific conference on the emerging topic of protein turnover and homeostasis via co- and post-translational modifications that take place at or involve the amino (N) terminus of proteins. N-terminal amino acids can undergo a wide range of modifications, which influence protein function, targeting, and stability in diverse ways. A thematic emphasis for N-term 2017 was placed on the diversity of modifications that occur at the N terminus, and their consequences on protein fate, most notably via proteolysis through the N-end rule pathway.

The N-end rule pathway is represented in eukaryotes as part of the ubiquitin proteasome system and in prokaryotes as

part of ubiquitin-independent protein degradation systems [1–4]. The ‘pathway’ in eukaryotes in fact comprises several different branches [5–7], which collectively contribute to cellular protein quality control and signaling, through targeting both regulatory and misfolded proteins for destruction [1,8,9]. In all organisms studied, the N-end rule pathway has been shown to regulate a diverse range of growth, developmental, and stress-associated processes, and has also been linked to human disease [10–12]. N-terminal modifications are not solely linked to proteolysis, however; they can also influence protein behavior through other modes of action. For example, N-terminal acetylation is extremely prevalent and has been linked to a variety of functions in addition to its influence on protein half-life [6], such as facilitating protein folding and interactions [13], whilst N-terminal lipidation (e.g., myristoylation) can direct subcellular protein targeting [14].

In the last few years, the number of research groups investigating protein N-termini and the N-end rule pathway has dramatically increased, and a vibrant global N-end rule research community has developed. In 2015 a small, plant-specific meeting focused on N-terminal protein modifications was held as part of the Society of Experimental Biology’s general meeting in Prague. The objective of the N-term 2017 organization team, consisting of the authors of this report, was to significantly expand upon this, and hold the first international, interdisciplinary, and cross-kingdom conference focused exclusively on protein N-termini. A central aim of the meeting was to initiate and establish an open network, to foster discussion, interactions, and collaborations, as well as potentially promote the exchange of researchers, knowledge, and techniques. The conference attracted over 100 scientists with 40 talks

in the fields of animal, plant, and bacterial sciences, as well as medicine and biotechnology (Figure 1).

Plenary Lectures: Historical Perspectives and Emerging Themes

N-term 2017 featured two plenary talks from key scientists in the field of N-end rule pathway research. The first of these was delivered by Andreas Bachmair of the Max F. Perutz Laboratories at the University of Vienna, who alongside Daniel Finley discovered the N-end rule pathway whilst in the lab of Alexander Varshavsky [5], and who has since made many significant contributions to the identification and dissection of enzymatic components of the pathway in plants. The final talk of the meeting was given by Yong Tae Kwon of the Protein Metabolism Medical Research Center at Seoul National University in Korea, also a former postdoc of Alexander Varshavsky’s, who has since gone on to actively research the pathway in mammals and demonstrate its relevance for autophagy [15] in addition to proteasome-mediated proteolysis. These two plenary lectures provided comprehensive and clear views of historical aspects, conceptual novelties, and future directions for the field, and in doing so perfectly opened and closed the conference.

Molecular Mechanisms and Processes Governed by Protein N-Termini

Research presented over the 3 days was centered on molecular enzymology, biochemistry, genetics, and structural biology, as well as the significance of N-terminus-mediated proteostasis in a physiological context across organisms from diverse kingdoms of life. The meeting was organized such that talks were not divided into specific themes, to encourage full participation and maintain



Trends in Biochemical Sciences

Figure 1. N-term 2017: Proteostasis via the N-terminus. Clockwise from left: (1) N-term 2017 poster. (2) The participants of N-term 2017 shortly after the prompt: '... and now, show your favorite N-terminal amino acid in single-letter code!' (3) (left to right) Daniel Gibbs, with the poster prize winners Sjon Hartman, Leah Taylor-Kearney, and Su Hyun Lee. (4) Nico Dissmeyer, opening the conference. Photo credits: Antje Hellmuth and Pavel Reichman, IPB, Halle.

scientific pace. Some of the topics discussed, amongst others, included:

- Advances in proteomic and bioinformatic techniques for large-scale analysis, assessment, and quantification of N-terminal identities and modifications.
- Medically relevant functions for the N-end rule, including during neurodegenerative disease, organ development, the regulation of programmed cell death, as well cancer and genetic disorders such as the Johanson–Blizzard syndrome.
- New substrates and functional insight into the oxygen-sensing division of the N-end rule pathway in plants, and its importance for development, environmental interactions, and stress responses.

- The development of N-end rule-based biotechnological tools, including temperature-dependent N-degrons for conditional protein (de)stabilization.
- N-terminal protein acetylation and myristoylation, the plasticity of these modifications, and their effects on protein behavior and stability.
- The N-end rule pathway and N-terminal processing events in prokaryotes and plastids.
- Novel functions and branches of the N-end rule, including structural and functional insight into the emerging cross-talk between N-terminal arginylation and regulation of autophagy.

One key outcome over the course of the talks and intensive discussions between the participants was that it is becoming increasingly clear that the N-end rule is far

more plastic and complex than previously thought. For example, N-terminal post-translational modifications classed as 'destabilizing' do not always lead to recognition by the molecular components of the N-end rule pathway, nor trigger proteolysis. By contrast, residues previously considered stabilizing are now emerging as destabilizing in certain sequence and structural contexts. It seems that the N-end rule, rather than being a set of 'hard and fast' rules, should instead be considered as a 'passport' checkpoint, through which N-terminal residues may pass, dependent on many intrinsic and extrinsic factors.

Young Scientists Take the Stage

The organizers specifically invited participants at various career stages, thereby

creating an atmosphere that enabled dynamic discussions among groups with different levels of expertise and areas of interest. For example, from over 30 submitted posters, 10 doctoral candidates were selected to give short 'snapshot' presentations to highlight the key take-home messages of the work presented in their posters. Three poster prizes for the contributions of the students were sponsored by EMBO and Springer Nature, and were selected by conference delegates based on their presentation methods, clarity, and experimental approach. The EMBO poster prize went to Sjon Hartman (Utrecht University) for his poster 'NO problem: ethylene-induced regulation of nitric oxide confers flooding tolerance in plants'. The two book prizes from Springer Nature went to Leah Taylor-Kearney (University of Oxford) for the topic of 'Conserved Function of a Plant Cysteine Oxidase from *Marchantia polymorpha*' and to Su Hyun Lee (Seoul National University) for her work on 'p62 is an N-recognin of the N-end rule pathway which modulates autophagosome biogenesis'. The methods, problems, and approaches of the winners underscore current trends in the community, where plant research in particular has seen an unprecedented increase in popularity and attention over the last 10 years.

Looking to the Future

N-term 2017 was a great success; it provided a fantastic opportunity for people to put names to faces and highlighted how much this field has grown. One participant was overheard saying 'this is the first conference I've attended where I feel like my research is mainstream'. However, it was clear from the breadth of topics presented that protein N-terminal research is mainstream. It is hoped that the N-term network will continue to strengthen, and that research into the N-end rule and N-terminal modifications will be promoted more widely throughout the scientific community. A special and lasting achievement of

N-term 2017 is that the foundations were laid for a recurring gathering, with the 'N-term baton' being passed to Yong Tae Kwon, who will serve as the lead organizer for the next proposed meeting. 'N-term 2019' has been tentatively scheduled to take place at Seoul National University in South Korea, where it is hoped that the number of participants and range of topics will expand even further.

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Forum

Overview of Biochemical Assays in Lipidic Cubic Phase

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The development of novel biochemical methods to efficiently characterize membrane protein (MP) properties in lipidic cubic phase (LCP) is important for studying complicated MPs and their multimeric complexes. Here, we summarize recent LCP-related assays and provide an outlook on their applications in structure and function studies of MPs.