

## Protein Aggregation Diseases (PAD) B/D Working Group

Protein aggregation diseases (PAD), exemplified by Parkinson's or Alzheimer's disease and systemic amyloidoses, are characterized by an abnormal deposition of protein aggregates of regular three-dimensional structure (amyloid). The B/D PAD working group aims at developing proteomics assays for proteins that are relevant to the study, diagnosis and therapy of protein aggregation diseases. These assays will be tested and refined on a set of relevant patient samples (for clinical applications) and on samples from model organisms and cell culture (for basic research). The assays will be made publicly available through web-based databases such as the SRMAtlas or PRIDE. Initially the assays will consist of standardized (scheduled) SRM coordinates from triple-quadrupole instruments and spectral libraries from shotgun proteomics measurements. We will also consider generating SWATH/data-independent fragment ion maps for relevant samples. Besides developing assays for measuring protein abundances, a peculiarity of our initiative is that it will attempt also the development of proteomics assays for "aberrant protein conformations", those typically generated in PADs. The idea relies on a recently published method involving SRM and proteolytic markers (LiP-SRM, Feng et al., Nat. Biotechnology, 2014). The conformational assays for "functional" and "pathological" conformational states of each amyloidogenic protein will also consist of SRM coordinates.

Current members of the group are:

1. Paola Picotti (ETH Zurich, CH, chair) (Proteomics, MS)
2. Bouke Hazenberg (Univ. of Groningen, NL) (Clinician)
3. Susan Lindquist (Whitehead Inst., MA, USA) (Biologist)
4. Gyorgy Marko-Varga (Univ. of Lund, Sweden) (Proteomics, MS)
5. Melinda Rezeli (Univ. of Lund, Sweden) (Proteomics, MS)
6. Roman Zubarev (Karolinska Inst., Sweden) (Proteomics, MS)
7. Giampaolo Merlini (University of Pavia, Italy) (Clinician)
8. Catherine Costello (Boston Univ., MA, USA) (Proteomics, MS)
9. Roger Nitsch (Univ of Zurich, CH) (Clinician)

We launched the PAD group in Madrid. The kick-off meeting was relatively well attended (25-30 people). The chair (Paola Picotti, ETH Zurich) introduced the purpose of B/D working groups in general, and the specific aims of the PAD initiative. She presented the current composition of the working group, based on an exchange of emails occurred before the meeting with clinicians and proteomics researchers working on PAD. She also presented her proposal on the organization of the activities of the group (workpackages, priorities, interactions between the different subgroups) and raised a number of discussion points. The introduction was followed by four presentations by members of the PAD group. **Dr. Bouke Hazenberg** (clinician, University of Groningen, Netherlands) presented the clinical background of protein aggregation diseases, with a focus on systemic amyloidoses, clinical needs and areas of application of proteomics in a clinical context. **Dr. Giampaolo Merlini** (clinician, Director of the Amyloidosis Research and Treatment Center of the University of Pavia, Italy) presented the need for proteomics assays in the diagnosis of amyloidosis to compensate for flaws of immunoassays. He also described the progress of his unit in using (shotgun) proteomics for the diagnosis of amyloidosis and the identification of a proteomics signature that enables discrimination of amyloidosis patients and healthy individuals. **Dr. Paul Boersema** (proteomics researcher, ETH Zurich, Switzerland) presented the results of a recently initiated collaboration with Bouke Hazenberg within the PAD group, aimed at the development of SRM assays for the complete set of amyloidogenic proteins and their application to clinical samples. **Dr. Melinda Rezeli** (University of Lund) presented the progress of the Marko-Varga group in the development of SRM assays for the amyloidogenic protein alpha-synuclein involved in Parkinson's disease and the importance of developing assays for its truncated forms. **Paola Picotti** presented a novel proteomics workflow enabling quantitation of conformationally altered proteins in complex samples (Feng Y et al. Nat Biotech. 2014) and how this can be applied to the detection of aberrant protein conformations involved in PADs, specifically PD.

The session was concluded with a discussion involving members of the group and people from the audience. The chair wrapped-up the results of the discussion and the action points. The audience was encouraged to join the working group if interested.

Action points:

1. Two target protein lists were identified. The first list includes all known amyloidogenic proteins, based on a reference nomenclature paper by *Sipe et al, Amyloid 2014*. This protein set has already been clearly defined and agreed on. The second list includes proteins that are not amyloidogenic per se, but are involved in the development of the different PADs. Proteins to be included in this second list will be identified by different PAD subgroups.
2. The activities of the group were divided into workpackages focusing on different PAD diseases (currently: systemic amyloidoses, Parkinson's disease and Alzheimer's disease). Groups involved in each workpackage will identify proteins to be included in the second list for the specific disease and develop the corresponding assays.
3. The group will start collecting available shotgun proteomics data to guide the design of targeted proteomics assays and then proceed with the development and testing of SRM assays for the target protein lists.
4. Collaborations between clinicians and proteomics researchers were initiated (Picotti-Hazenberg, Picotti-Merlini and Picotti-Nitsch) to support testing of the generated assays on relevant samples and for identifying protein targets.
5. The possibility of raising funds to support the work of the group was discussed. The subgroup focusing on systemic amyloidosis (Picotti, Merlini, Hazenberg) will probably apply for funding from "e-Rare".
6. The group agreed on trying to interface with the Luxembourg initiative on Parkinson's disease. Researchers working on the project will be contacted by the PD subgroup of PAD.
7. Dr. Melinda Rezeli (Lund) volunteered to co-chair the PAD group.

**Note from the chair:** The presence of the two clinicians was crucial to the success of this initiative. They clearly presented clinical needs and areas where proteomics measurements would make a big difference for a clinician, providing inspiration and novel ideas. They also strongly committed to the support of the working group by providing clinical samples to test the developed assays. Such measurements and interactions are currently ongoing. Their contributions will also be important for the next meetings of the group. Both clinicians came to Madrid at their expenses: this is remarkable, since the only session of the HUPO they attended was our meeting. I think it would be important for the future to make sure that instruments are in place to provide at least partial financial support to clinicians (or biologists without proteomics background) interested in joining HPP initiatives. I know that few fellowships were available in Madrid for this purpose, but the general understanding was that they were meant for young(er) scientists.