LINXS Amyloid Workshop: **Mind the gaps in amyloid fibre structure analyses**

Date: Nov 21 and 22, 2019
Place: LINXS, 5th floor, Scheelevägen 19, Lund, Sweden
Number of Participants: up to 30 participants

Area and Aims of the workshop: Amyloid diseases are caused by aggregation of proteins and their accumulation as amyloid fibrils in cells and tissues. Much research is focused on understanding the basic biology of amyloidosis, and how it relates to disease aetiology. Integrative structural biology is an integral part in order to achieve this but also very challenging. The workshop will therefore address several aspects of amyloid structure analysis ranging from sample preparation, over amyloid fibre diffraction and complementary methods such as single particle cryoEM, to data collection and modelling. The workshop will discuss how different methods and length scales in the structure analyses can be bridged, and also how in vitro and in vivo specimens are linked. As is described below, the workshop will be composed of five sessions that will address the different topics

Who should participate: The workshop wishes to bring to together researchers from different scientific areas to discuss existing and novel experimental approaches to determine amyloid structures. The participants may have specific knowledge in methodology that relates to the different topics, or may have a specific biological or medical research question that can be addressed by structure analysis methods.

Format: Meeting is divided into five major and connected topics. Each topic is introduced by a keynote speaker and followed by 2-3 contributed talks/scientific question/solution descriptions. The topic is then open for discussions and summary.

Deadline for registration is October 30.

- ➔ Registration should include short motivation letter (max half a page) on why attending the meeting. Please specify your science case/methodology and which session(s) that are of highest interest for you, and if you are willing to contribute with a short talk. This information will be used to make the final program.

➔ We also ask the participant to send us questions prior to the meeting that you would wish to have addressed, and that can be discussed during the meeting.

Agenda

Thursday November 21

9.30 – 10.00	Arriving and coffee/tea
10.00 – 10.10	Welcome and LINXS overview – Jens Lagerstedt
10.10 – 10.30	**Solving the structure of natural amyloid fibrils: 60 years of progress** – Speaker: Vittorio Bellotti
10.30 – 12.30	**Session one:** Sample preparation for Xray, CryoEM, and other structure analysis methods **Focus area to be discussed**: Recent advances of structural characterization of amyloid fibrils will be discussed focusing on natural fibrils and highlighting crucial critical issues regarding the heterogeneity of the material and the procedures of fibrils preparation
10.30 – 11.20	**Keynote 1:** Francesca Lavatelli, University Hospital Policlinico San Matteo Pavia, *Preparation of natural amyloid fibrils: strategies and pitfalls* **Biographical note:** Francesca Lavatelli, MD, PhD, is a Researcher at the Amyloidosis Research and Treatment Center, University Hospital Policlinico San Matteo Pavia. Dr. Lavatelli has been involved in basic and translational research on amyloid diseases since 2004, in the group of Giampaolo Merlini. The current research interests of Dr. Lavatelli are mainly centered on the proteomic and mass spectrometry analysis of purified amyloid fibrils and affected tissues, and on the assessment of the molecular mechanisms of

proteotoxicity in systemic amyloidoses. This research, in collaboration with major centers worldwide, has led to the development of novel diagnostic proteomics approaches for typing amyloid proteins and to the clarification of important mechanisms behind cell dysfunction in these diseases, including mitochondrial damage due to interaction with pathogenic immunoglobulin light chains. Dr. Lavatelli is a member of the Board of the International Society for Amyloidosis and is coordinator of research projects funded by national and international organizations.

Short abstract: Amyloid fibrils isolated from affected tissues are the ideal material for biophysical and biochemical studies aimed at dissecting their molecular features in vivo. Amyloid deposition in the biological environment, however, is heterogeneous and occurs in tight connection with the extracellular matrix and other tissue structures, making fibril purification a complex and delicate step. Approaches and methodologies for amyloid fibril isolation from different sources will be discussed, along with an overview of strategies to optimize ex vivo and in vitro fibril preparation for distinct downstream applications.

11.20 – 11.40 Per Zetterström, Umeå University, *SOD1 prions in ALS*

11.40 – 12.00 Veronica Lattanzi, Lund University, *Structural determinants of amyloid-β fibrils by small-angle neutron and x-rays scattering techniques.*

12.00 – 12.30 Discussion and Summary

12.30 – 13.15 **Lunch**

13.15 – 15.15 **Session two:** Amyloid as a partially ordered system – experimental and analytical approaches using synchrotrons, neutrons, FELs.

Focus area to be discussed: Amyloid structures as observed in vitro and ex vivo are often characterised by X-ray fibre diffraction. Despite the direct relevance of these data, there are major challenges for the way in which the diffraction experiments are carried out, the complexity of the data, and the analytical techniques available. These issues will be discussed in the context of future needs and priorities.

13.15 – 13.50 **Keynote 2:** Theyencheri Narayanan, European Synchrotron Radiation Facility: *New opportunities for X-ray fiber diffraction with extremely brilliant synchrotrons.*

Biographical note: Dr. Narayanan obtained his Ph.D.in Physics from the Indian Institute of Science, Bangalore, in 1991. After several postdoc positions including at CEA Saclay and the University of California, Berkeley, he joined the European Synchrotron Radiation Facility (ESRF) in 1997. He is presently a Senior Scientist at ESRF and he was also head of the Soft Matter group from 2008–2015. His main research interests are soft matter self-assembly, dynamics of active colloidal systems and structure-function relationship in large biomolecular assemblies. Dr. Narayanan is leading the development of time-resolved small-angle and ultra small-angle X-ray scattering methods for studies of soft matter and biophysical systems.

Lecture abstract: This presentation will give an overview of new possibilities for biological X-ray fiber diffraction studies offered by the combination of extremely brilliant synchrotron sources and advanced X-ray detectors. In situ fiber diffraction experiments can be performed with unprecedented resolution and precision covering a broad size scale from several Ångströms to micron range. A key advantage of synchrotron method is that it allows combining X-ray fiber diffraction with physiologically relevant biochemical and mechanical

protocols, and probe the structure-function relationship. This will be illustrated using different biological specimen with emphasis on studies of cardiac muscle. An important property of the new synchrotron sources is the high degree of transverse coherence that has not been exploited very much in fiber diffraction work. This will be a way forward for enhancing the information content in fiber diffraction diagrams. While the instrumentation has well advanced, a major bottleneck is the data analysis. Concerted effort is required to overcome the current stalemate with respect to fiber diffraction data analysis and modeling

13.50 – 14.10 Annette Langkilde, Copenhagen University, *Can we use fiber diffraction to distinguish different forms of α-synuclein fibers?*

14.10 – 14.30 Yoshi Nishiyama, Cermav / CNRS, *Analyzing textured diffraction patterns at different legth scales: case of fibrous polysaccharides and implications for other partially ordered systems including amyloid*

14.30 – 14.50 Sergei Grundinin, Inria / CNRS, *Extending Pepsi-SAXS for scattering on oriented objects*

14.50 – 15.15 Discussion and Summary

15.15 – 15.30 **Short break**

15.30 – 17.30 **Session three:** Cryo EM structures of amyloid fibrils
Focus area to be discussed: Purification and electron cryo-microscopy analysis of amyloid fibrils

15.30 – 16.20 **Keynote 3:** Marcus Fändrich, Ulm University, *Cryo EM structures of amyloid fibrils.*
Biographical note: Prof. Marcus Fändrich studied Biology and Biochemistry at the Universities of Heidelberg and Cambridge, before he went to Oxford to obtain his Doctorate

degree. In 2012 M. Fändrich become full professor at Ulm University where he is now a head of the Institute of Protein Biochemistry. His main research interest is the structure and formation of amyloid fibrils in neurodegenerative diseases and systemic amyloidosis.

Lecture abstract: Systemic amyloidosis is a group of protein misfolding disease in which the production site of the fibril precursor protein does not necessarily correspond to the deposition site in the body. In systemic AA amyloidosis, the fibril precursor serum amyloid A protein is produced mainly in liver but deposits in spleen, kidney and other organs. Systemic AA amyloidosis, which affects humans and many animal species, is one of the best cases of a prion-like disorder in mammals. We have used cryo-EM to determine the molecular structures of AL and AA fibrils purified from human and AA amyloidotic mice.

16.20– 16.40 Mattias Törnquist, Lund University, *Ultrastructural evidence for self-replication of Alzheimer-associated Aβ42 amyloid along the sides of fibrils.*

16.40– 17.00 Kaituo Wang, University of Copenhagen, *My first cryoEM attemp on amyloid fibrils.*

17.00– 17.30 **Discussion and Summary:** Purification and electron cryo-microscopy analysis of amyloid fibrils, recommendations.

18.00 – **Pizza and beverages at LINXS**

<u>Friday November 22</u>

09.30 – 10.00 First day wrap-up

10.00 – 13.00 **Session four**: Interpretation + Synergies

Focus area to be discussed: X-rays, neutrons, crystallography, fibre diffraction, NMR, cryo-EM, molecular modelling. How do these techniques link for the study of amyloid *in vitro* and *in vivo*?

10.00 – 10.40 **Keynote 4:** Trevor Forsyth, Institut Laue Langevin/Keele University, *Bridging the gaps in amyloid characterisation – interdisciplinarity and multi-technique approaches.*

Abstract. The history of amyloid structural studies is populated with difficulties of interpretation that arise from the limitations of individual methods - often combined with the fact that individual researchers and groups have high levels of expertise in a small number of techniques. In addition, just as there is a huge gap between the molecular and cellular levels of organization in structural biology generally, there has been a dearth of information connecting *in vitro* and *in (or ex) vivo* studies. The integrating approaches necessary to address this situation will be discussed.

10.40 – 11.00 Helena Rasmussen, Aarhus University, *Multi-Method Approach to investigate the aggregation mechanism of FapC*

11.00 – 11.20 Christofer Lendel, KTH Royal Institute of Technology, *Multiscale structural analysis of protein nanofibril materials*

11.20 – 11.40 Sofie Nyström, Linköping University, *Accessing amyloid polymorphism with conformation sensitive fluorescent probes.*

11.40 – 12.20 **Lunch**

12.20– 14.20 Discussion and Summary

14.20 – 14.30 **Summary and Next Steps** - Oxana Klementieva

14.30 -	Coffee/tea and Farewell
15.00 – 16.30	Work group meeting – Only WG members