ISIS@MACH ITALIA

Science and Technology Facilities Council
ISIS Neutron and Muon Source

Experiment Proposal

Experiment number GP2023016

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Experiment title	Structural characterization of protein fibrils and microcrystals
MRF Instrument	**SAXS Xenocs Xeuss**
Access Route	Direct Access
Science Areas	Biology and Bio-materials, Chemistry, Materials, Medicine, Physics
Sponsored Grant	None
Grant Title	-
Start Date	-
Similar Submission?	High resolution TEM
Industrial Links	-

Days requested: 2
Previous GP Number: GP2023015
DOI: -
Sponsor: -
Grant Number: -
Finish Date: -

Non-Technical Abstract

The goal of the project is to observe and classify the stability of various types of fibrils and crystals, formed by proteins in diderent conditions. Fibrils and crystals are indeed widely studied at synchrotrons, FELs and neutron sources. We wish to establish reliable protocols to prepare, ship, store, and finally deliver them under photon or particle beams to perform experiments in properly characterized, reproducible conditions. The proposed experiments aim at understanding the edect of freezing/unfreezing cycles on α-synuclein protein (α-syn) fibrils and lysozyme microcrystals. The former is involved in the pathogenesis of Parkinson's disease (PD). Fibrils and crystals will be pre-characterized by electron microscopy, and frozen. Samples will be unfrozen and re-characterized by electron microscopy in order to observe possible morphological changes due to aging and freezing.

Publications -

ISIS neutron and muon source

IM@IT E-platform: No

Instruments	
Access Route	
Science Areas	
Sponsored Grant	
Grant Title	
Start Date	
Similar Submission?	
Industrial Links	

Days Requested:
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Sample record sheet

Principal contact Dr Francesco Stellato, Università degli Studi di Roma Tor Vergata, ITALY
MRF Instrument **SAXS Xenocs Xeuss** **Days Requested:** 2
Special requirements:

SAMPLE

Material	(1) α-syn fibrils in PBS buffer (2) Lysozyme microcrystals grown in batch in NaCl + acetic acid	-	-
Formula	CHNOS	-	-
Forms	Liquid		
Volume	1 ml		
Weight	mg		
Container or substrate	SAXS liquid sample containers	-	-
Storage Requirements	-	-	-

SAMPLE ENVIROMENT

Temperature Range	- K	-	-
Pressure Range	- mbar	-	-
Magnetic field range	- T	-	-
Standard equipment	-	-	-
Special equipment	-	-	-

SAFETY

Prep lab needed	Yes	-	-
Sample Prep Hazards	-	-	-
Special equip. reqs	-	-	-
Sensitivity to air	No	-	-
Sensitivity to vapour	No	-	-
Experiment Hazards	-	-	-
Equipment Hazards	-	-	-
Biological hazards	No, all samples are biosafety 1 level	-	-
Radioactive Hazards	-	-	-
Additional Hazards	-	-	-
Additional Details	-	-	-
Sample will be	Removed By User	-	-

Structural characterization of protein fibrils and microcrystals

Abstract:

The goal of the project is to observe and classify the stability of various types of aggregates, namely fibrils and crystals, formed by proteins in different conditions. Fibrils and crystals are indeed widely studied at large-scale facilities such as synchrotrons, free electron lasers (FELs) and neutron sources. It is therefore essential to establish reliable protocols to prepare, ship, store, and finally deliver them under photon or particle beams in order to perform experiments in properly characterized, reproducible conditions.

The proposed experiments aim at understanding the effect of freezing/unfreezing cycles on protein fibrils and crystals. We will concentrate on α-synuclein protein (α-syn) fibrils and lysozyme microcrystals in particular. Because it is involved in the pathogenesis of Parkinson's disease (PD), α-syn is a protein of great biological interest. Lysozyme is a soluble protein that is abundant and easy to purify, and it is therefore widely used as a model system in structural biology experiments. In order to simulate realistic conditions for experiments performed at large-scale facilities, fibrils and crystals will be prepared weeks before the planned experiments, pre-characterized by electron microscopy, and frozen. Within the proposed experimental campaign, the samples will be unfrozen and re-characterized by electron microscopy in order to observe possible morphological changes due to aging and freezing.

1. Background and Context

Protein (mis-)folding and aggregation is a major biological and medical concern because it is at the root of a group of severe pathologies known as protein conformational disorders. Many of these pathologies are neuro-degenerative, devastating diseases that have a major impact on human health [Selkoe 2003], including Alzheimer's disease and PD. PD is the second most common neurodegenerative disease after Alzheimer's disease and the most common movement disorder, affecting nearly 3% of the population over the age of 70 [Pringsheim 2014]. In current PD pathogenesis hypotheses, the synuclein protein (α-syn) in its oligomeric, aggregated, and fibrillar forms has taken center stage as potentially toxic. The aggregates are known to be involved in various cellular processes leading to neurodegeneration. Although cell-to-cell transmission of aggregated α-syn has been demonstrated in cell culture and animal models, the precise molecular mechanisms and evolution of Parkinson's disease neuropathology remain unknown [Walsh 2016]. Unravelling the structure of α-syn fibers is therefore critical from a biomedical standpoint. In this context, α-syn fiber diffraction measurements at synchrotrons, FELs, and neutron sources can aid in understanding the structure of α-syn under different conditions.

On the other side, lysozyme microcrystals can be considered a standard for serial crystallography measurements performed at synchrotrons and FELs. Therefore, providing a characterization of their stability under realistic aging and transport conditions would be beneficial for experiments at large-scale facilities.

2. Proposed experiment

We aim at characterizing the samples by SAXS (Small Angle X-ray Scattering) technique, which provide information on interfaces of the sample through 3D chemical mapping *in solution. We therefore request access to the following instruments for a total of two working days, in order to allow a tuning of the ideal experimental conditions.*

- *SAXS (Small Angle X-ray Scattering)*

We will measure two different samples, both prepared weeks in advance and kept frozen.

- *α-syn fibrils in PBS buffer*
- *Lysozyme microcrystals grown in batch in NaCl + acetic acid*

3. Summary of previous experimental proposals or characterisation

- *α-syn fibrils in PBS buffer samples have been pre-characterized by electron microscopy (TEM) and SAXS.*

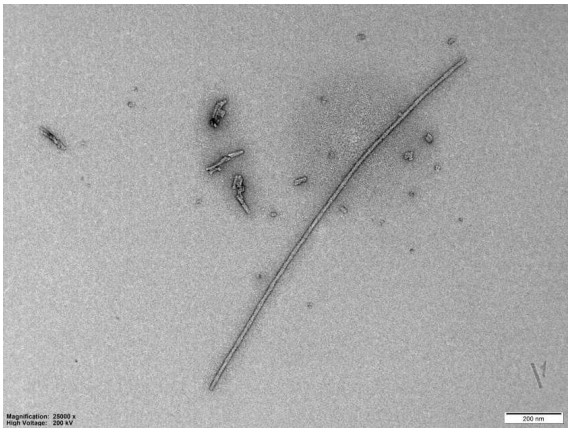

Figure 1 – *A single α-syn fiber imaged by transmission electron microscopy*

- *Lysozyme microcrystals samples have been pre-characterized by SEM and X-ray diffraction.*

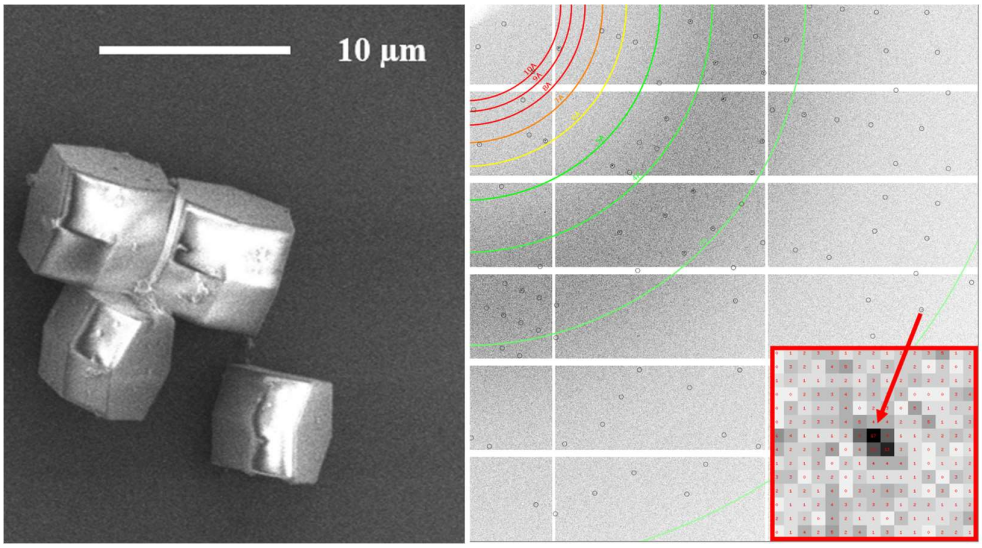

Figure 2 – *Lysozyme microcrystal grown in batch and imaged by scanning electron microscopy (left panes); single crystal diffraction pattern (from Stellato 2014).*

4. References

[1] Pringsheim T et al (2014) Mov Disord 29: 1583-1590.

[2] Schulz J et al. (2019) Journal of synchrotron radiation 26(2).

[3] Selkoe DJ (2003) Nature 900-904.

[4] Stellato F et al. (2014) IUCrJ 1: 204-212.

[5] Walsh DM et al., (2016) Nat Rev Neurosci. 17(4):251–260.