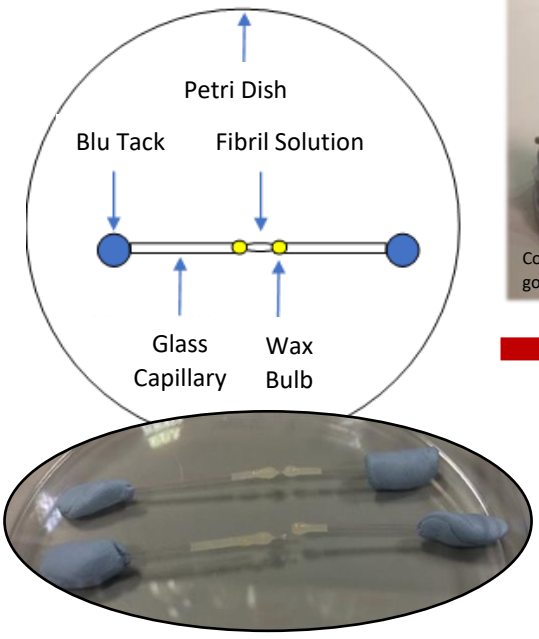
X-ray Diffraction Studies for Protein Fibrils by WAXS

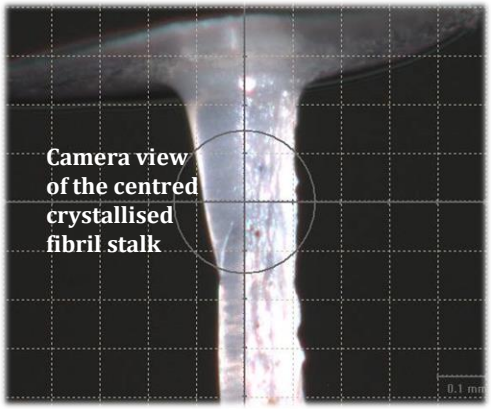
Introduction

Wide angle X-ray scattering (WAXS) is the method of choice to provide internal structural information and sample characteristics of protein fibrils. It can also show the degree of crystallinity. The typical angular range is between 5°- 60° in 2Θ.

Experimental details

An alpha-synuclein fibril sample was dried to form stalks via the stretch frame technique[1] which can be applied to all protein fibril samples. Two glass capillaries were cut to approximately 3 cm in length and dipped in melted beeswax to create a wax bulb. The capillaries were then mounted parallel to the base of a petri dish using Blu Tack®. The bulbs are placed ca. 3 mm apart, and 10 µL of 500µM/mL alpha-synuclein fibrils are suspended between the bulbs. The solvent was left to evaporate overnight to allow the formation of a dried, aligned fibril stalk for analysis.



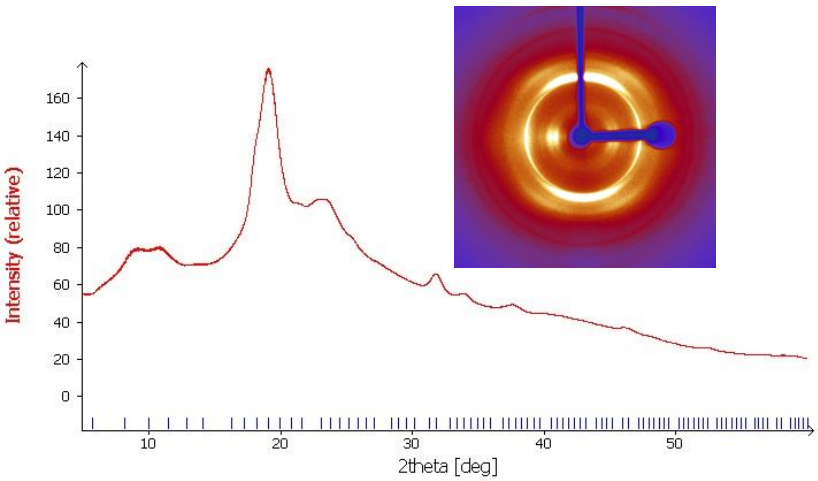


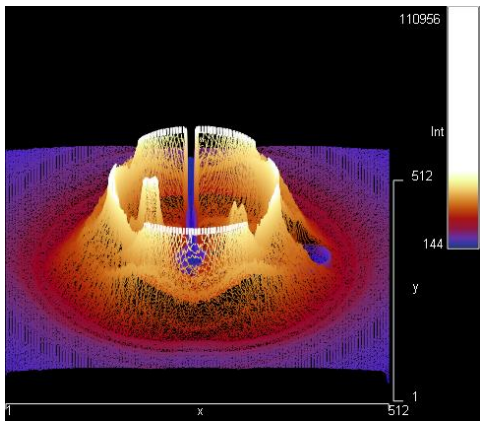
Data Collection

- RIGAKU SuperNova Dual single crystal X-ray Diffractometer
- Source: Microfocus monochromated Cu-Kα_1 radiation (λ = 1.54184 Å)
- Generator: 50kV, 0.8mA
- 2D-Detector : EosS2
- Powder Diffraction mode

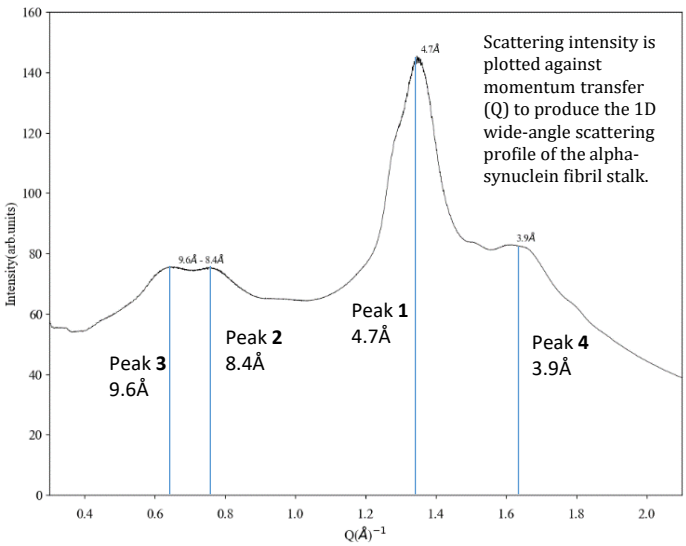
- Scan range: 3°- 100° in 2 Θ / 3 frames @ 60 sec exposure each
- Data collection software: CrysAlisPro version 1.171.40.67a (software is updated frequently)

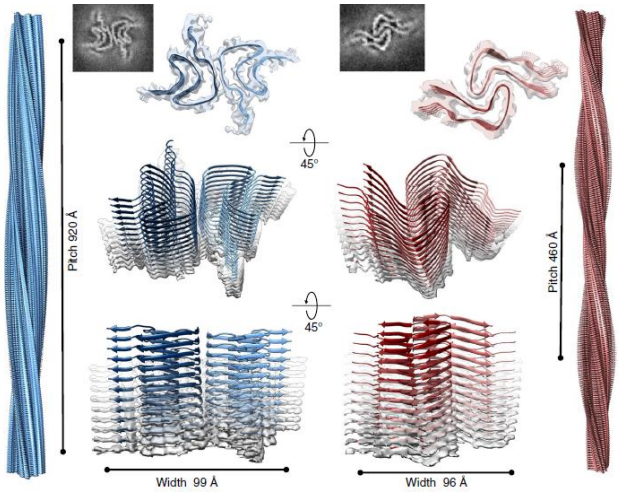
Results





Data were processed using CrysAlisPro 1.171.40.67a and were visualised as 1D, 2D and 3D scattering pattern of the α-synuclein fibril. Out of the three scans the centre frame was chosen for visualization. 1D scattering data were converted to real space using the equation **Q = 2π/d** where **Q** is the momentum transfer and **d** is the real space as described in Bragg's law. The real space of the major peaks was calculated and compared with established literature values.





The recent Cryo-EM study on alpha-synuclein fibrils confirms the findings from WAXS. The figure is a 3D reconstruction of the fibril to illustrate the packing of misfolded alpha-synuclein, the spacing between beta strands and beta sheets, and fibril polymorphism[5].

Conclusion

The 1D pattern displays **4 peaks** of interest. **1**: The outer ring (at **~4.7Å**) has the most intense meridional reflections, these indicate the spacing of beta-strands which are parallel to the fibril axis[3]. **2+3**: The intensity of the inner ring shifts towards the equatorial direction (**~8.4 Å** and **~9.6 Å**). These inner equatorial reflections are created due to the spacing between stacked beta-sheets that are perpendicular to the fibril axis. **4**: One additional peak that is based around ~ **3.9Å** is less well known, but is thought to be caused by the twisting of beta-sheets within the fibril structure[5]. The pattern is expected for alpha-synuclein fibrils[4,5]. While some peak positions may vary slightly to fibril polydispersity, the key reflection at ~ **4.7 Å** is universal across all fibril species and a major indicator of fibril formation[1,3,5].

[1] Gras SL, Squires AM. Dried and hydrated x-ray scattering analysis of amyloid fibrils. Protein Folding, Misfolding, and Disease. 2011(752), pp. 147-163.
[2] CrysAlisPro 1.171.40.67a (Rigaku Oxford Diffraction, 2019).
[3] Morris, K.L. & Serpell, L.C., 2012. X-ray fibre diffraction studies of amyloid fibrils. Methods in Molecular Biology, 849, pp. 121-135. Morton, K. (unpublished work). Using small angle x-ray scattering to observe hen egg-white lysozyme amyloidogenesis.
[4] Søren Bang N, Francesca M, Samuele R, Annette Eva L, Lise G, Anders K, et al. Wildtype and A30P mutant alpha-synuclein form different fibril structures. PLoS ONE. 2013;8(7): e67713.
[5] Binsen L, Peng G, Kevin AM, Phorum S, Meng Z, Gayatri N, et al. Cryo-EM of full-length α-synuclein reveals fibril polymorphs with a common structural kernel. Nature Communications. 2018;9(1):1-10.