Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXV

SAXS APPROACHES IN BIOLOGY AND ENVIRONMENTAL SCIENCES

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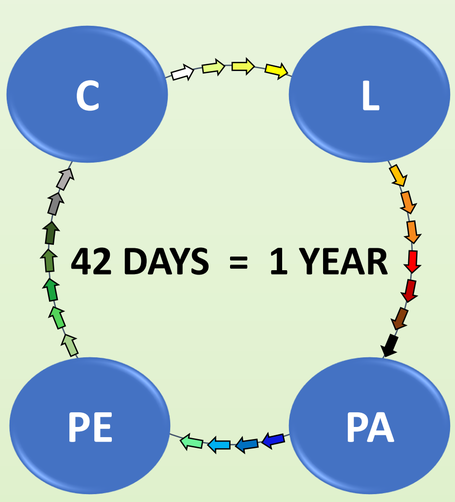
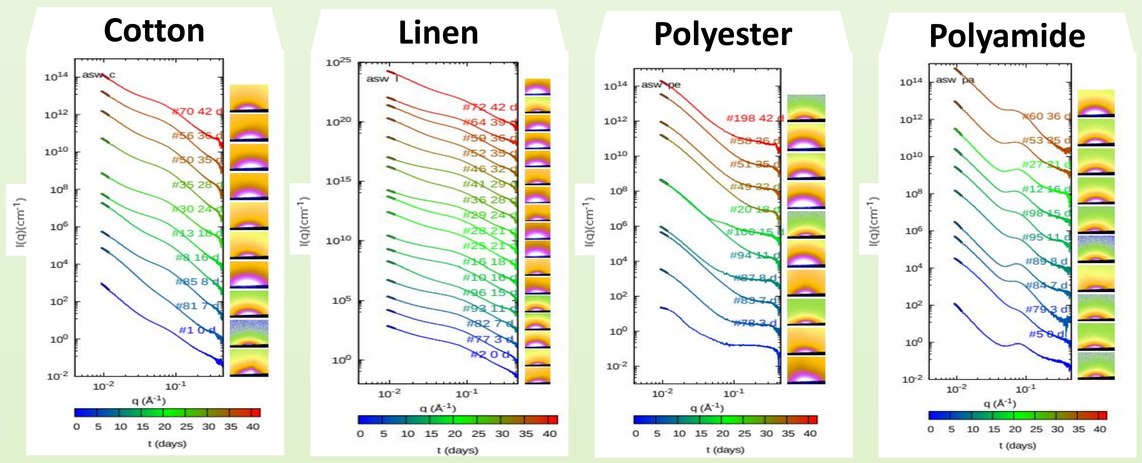
Small Angle X-ray Scattering (SAXS) is a non-conventional technique that allows getting information about nano-objects, concerning their structure, shape, and interaction in physiological environment. It can be used for both biological and environmental samples, such as: proteins, sugars and textile fibers.

SIMULATED LONG-TERM SOLAR IRRADIATION

Textile microfibers derived from the laundering of clothes are one of the most threatening emerging pollutants nowadays, because they are not trapped from wastewater plants. Once in the sea, they undergo different degradation processes that break them into smaller fibers able to enter the food chain and eventually reach the bloodstream. A long-term solar irradiation has been simulated in order to better understand the mechanism of degradation exerted by the sun.



Fresh water and artificial sea water bottles filled with 1g/L of textile microfibers under the solar simulator lamp.



Experimental scattering curves of fibers in artificial sea water as a function of solar exposing time. The bidimensional images on the right have been used to calculate the radial average required to obtain the plotted curves.

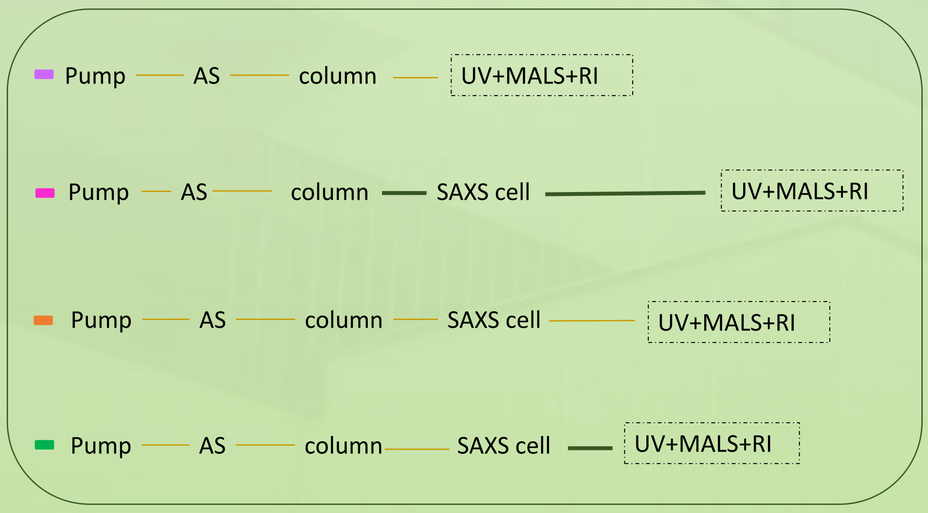
SAXS measurements were carried out in December 2021 at the Elettra synchrotron in Trieste and they are still under analysis.

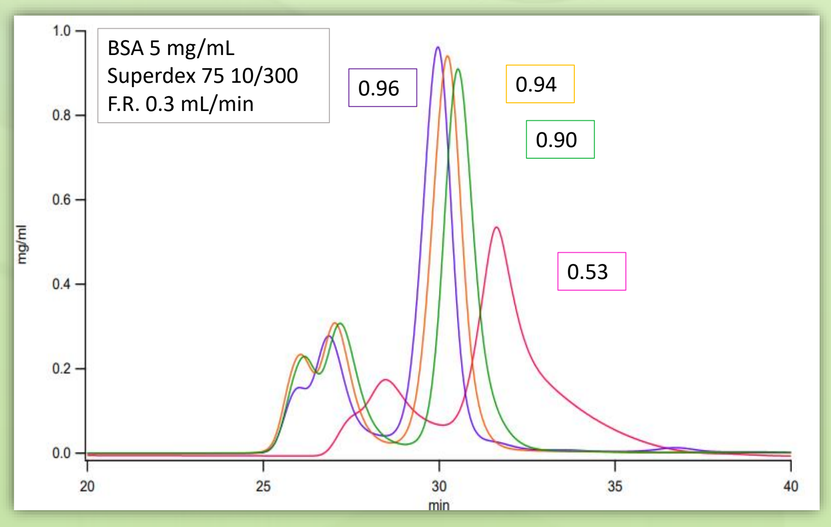
SEC-SAXS

Most proteins are present in solution as aggregates of monomers or oligomers and this condition does not allow to have a clear SAXS signal, and interferes also with the following data analysis. Thanks to Size Exclusion Chromatography coupled with Small Angle X-ray Scattering this problem can be easily overcome, allowing experiments to be performed in monodispersed systems.

SAXS CELL TEST

In order to obtain the best SAXS data, which are highly dependent on sample concentration, different tests have been performed to underline the degree of dilution that occurs during the SEC experiment.



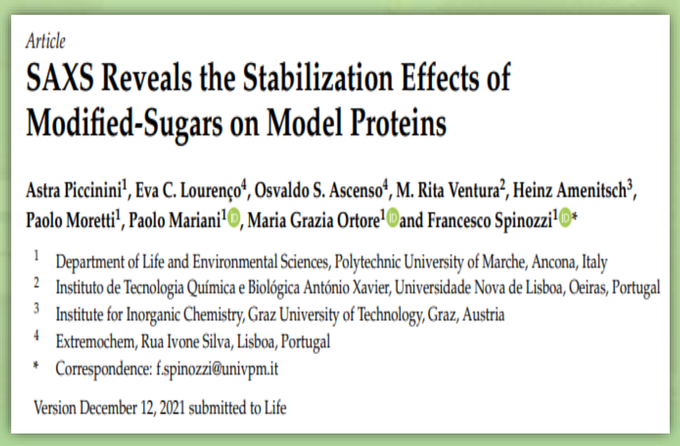


The curves shown in the graph represent the chromatograms derived by multi-angle-light-scattering. It is clear how the set up configuration influences the dilution of the sample, starting with a basic dilution that is not referred to the SAXS cell, but to the system itself (purple curve). The insertion of the capillary increases the dilution factor up to a maximum of ten times. The best result that has been achieved without any leaks or breaks of the cell is represented in green.

HPLC Agilent 1260II Infinity with Wyatt Mals detectors 18 angles, Quasi-Elastic Light Scattering – DLS and DRI Optilab Detector

SAXS AND SUGARS

Several proteins are not stable at room temperature and need to be stored in a cold regime, which is costly and sometimes tricky. For this reason stabilizers are one among the best solutions to preserve proteins in different conditions.



Article

SAXS Reveals the Stabilization Effects of Modified-Sugars on Model Proteins

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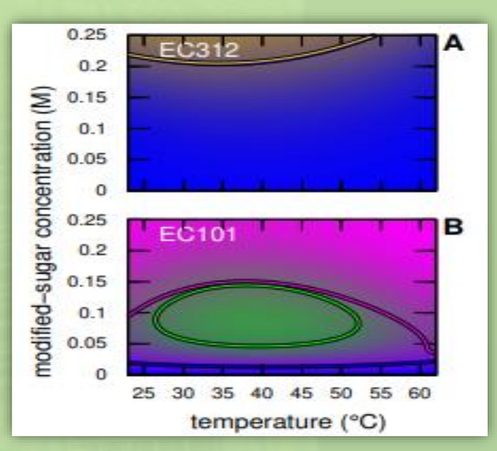
Several sugar-derived compounds were developed by the Lisbon University spin-off Extremochem, and they were used together with two commercial proteins: myoglobin and insulin. Different range of temperatures were investigated in order to understand the sugar stabilization effect on proteins structure.



Phase-diagrams obtained by the global fit analysis of 2 g/L insulin, with EC-312 and EC-101. Each colour represents the oligomeric form of insulin at increasing temperature and sugar concentration. The solid lines indicate the thermodynamic condition in which the oligomer is present in 50%.

EC-312 stabilizes the monomeric state of insulin up to a concentration of 0.2M, whereas EC-101 behaves differently because for higher concentrations it promotes the tetrameric form of the protein, and between 0.05M and 0.15M it gradually brings to an increase in hexamers.

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