EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:
https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Structural analysis of early Eumelanin intermediates and their growth mechanisms	**Experiment number:** SC-5172
Beamline: ID02	**Date of experiment:** from: 01/19/2022 to: 01/21/2022	**Date of report:** 26.08.2022
Shifts: 3	**Local contact(s):** Lauren Matthews	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Fabian Kollmann*
Anne Büngeler*
Prof. Dr. Klaus Huber

Report:

The build-up of eumelanin particles and their final structure was investigated in pure water and in aqueous MES buffer under various conditions. A biomimetic synthesis starting with L-Dopa was used in all cases. The enzyme tyrosinase served as catalyst for the oxidation of L-Dopa, which is accompanied by the consumption of dissolved oxygen.

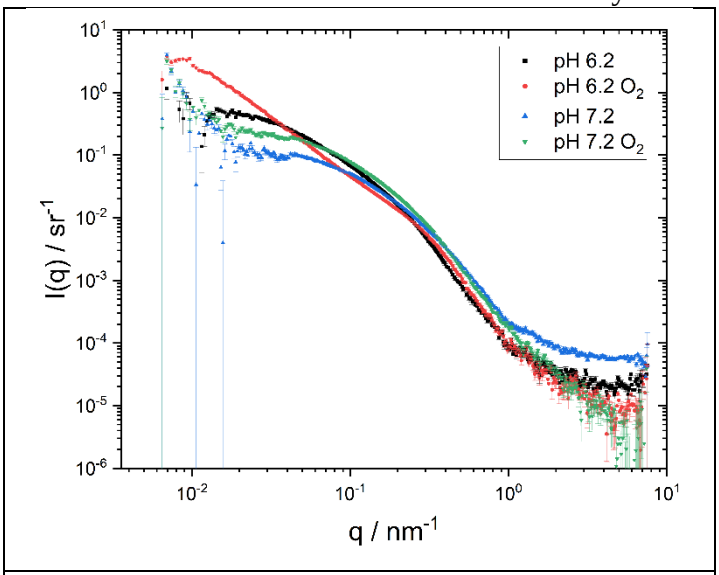

Figure 1: SAXS measurements of eumelanin particles prepared in MES buffer at pH 6.2 and 7.2. Samples indicated with "O_2" were saturated with oxygen.

Table 1: Radii of gyration determined with Guinier-plot

Sample	pH 6.2	pH 7.2	pH 7.2 O_2
R_g / nm	36	17	20

Characterization of particles at final state

Eumelanin particles synthesized in MES buffered aqueous solution at pH values of 6.2 and 7.2 were characterized after completion of the process. Since during the formation reaction of eumelanin dissolved oxygen is consumed, an experiment was performed at ambient O_2 content and another one at a saturated O_2 level at both pH values respectively. All four samples were measured at detector distances of 1 and 10 m. Evaluation yielded radii of gyration (R_g) given in Table 1. In case of the sample prepared at a pH of 6.2 under oxygen saturation aggregation of the particles occurred, which prohibited an evaluation of R_g. Kratky plots indicate a compact structure of the particles.

Time-resolved experiments on particle formation

The particle formation was observed over time in water at pH = 4 and in MES buffer at pH = 6.2. Figures

2-4 show a selection of intermediate states observed during the respective time-resolved SAXS experiment. Firstly, particle formation was investigated in pure water. Previous light scattering experiments indicated a fast particle growth ending up with spherical particles with a diameter close to 400 nm. The goal of the time-resolved SAXS experiments was to observe intermediate stages of the growing eumelanin particles at two different O_2 contents, one corresponding to the state equilibrated with ambient air and one corresponding to the state saturated with O_2. Measurements carried out with ambient O_2 content are shown in Figure 2 and measurements of samples saturated with O_2 are presented in Figure 3. Initially, a sample detector distance of 3 m was used (upper plot of Figure 2 and 3). Directly after the beginning of the reaction particles were detected. However, the scattering signal did not significantly increase with time and the q-regime was not suitable to determine the size of the particles. Therefore, additional experiments were carried out at a sample-detector distance of 10 m (bottom plot of Figure 2 and 3). For both oxygen concentrations the scattering signal was rather weak, even after several hours of the reaction. Based on previous light scattering experiments this was unexpected. Comparison with a supplementary measurement of the same reaction solution which was kept ex-situ (Figure 2 bottom) indicates that a radiation damage of the enzyme or eumelanin might have occurred during the time-resolved measurement. Secondly, the eumelanin particle formation was observed in MES buffered solution at a pH of 6.2 (Figure 4). Under these conditions the formation of the particles was additionally characterised after completion of the growth reaction (Figure 1) as has been done in the case of ambient oxygen content at. The sample-detector distance of 3 m turned out not to be suitable to determine the size of the formed particles (Figure 4, top). A second experiment at a sample-detector distance of 10 m was followed for 5 h. However, the scattering signal remained on a low level, which again might be caused by radiation damage. A sample of the same reaction solution was kept ex-situ and was measured after 24 hours (Figure 4, bottom). This experiment corresponds to the one shown in Figure 1 denoted as pH 6.2 O_2 and yields very similar results.

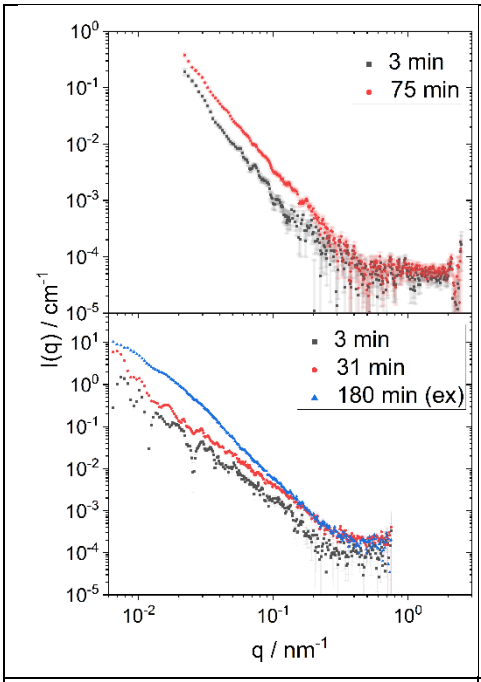

Figure 2: SAXS measurements of eumelanin particle growth in water (pH=4) at detector distances of 3 m (top) and 10 m (bottom). "180min (ex)" indicates a sample withdrawn from stock after 180 minutes.

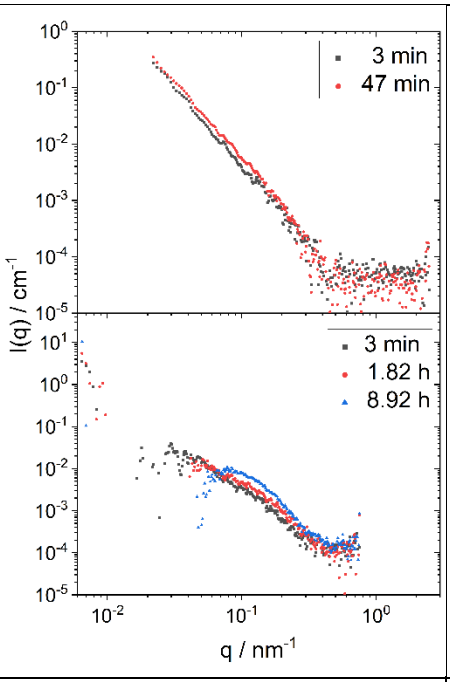

Figure 3: SAXS measurements of eumelanin particle in water (pH=4) at detector distances of 3 m (top) and 10 m (bottom). All samples were saturated with oxygen.

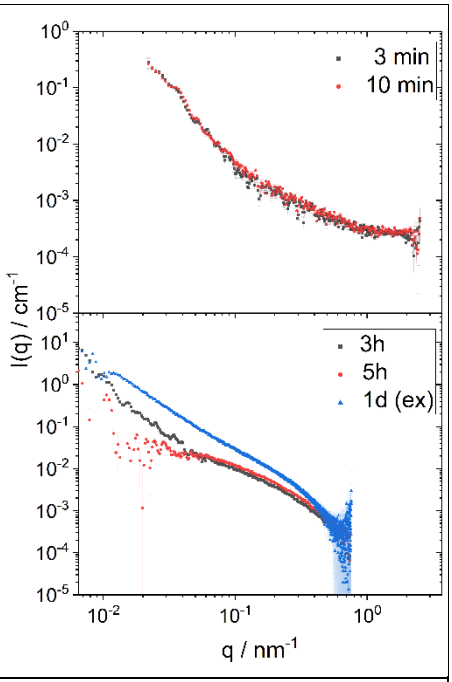

*Figure 4: SAXS measurements of eumelanin particle in MES buffer at pH 6.2 at detector distances of 3 m (top) and 10 m (bottom). All samples were saturated with oxygen. "*1d (ex)*" indicates a sample withdrawn from stock after 1 day.*