Experimental report

13/09/2018

Proposal: 8-03-927 **Council:** 4/2017

Title: Catching protein unfolding by AAA+ATPases in the act: a SANS study of an intermediate unfolding state of GFP

Research area: Biology

This proposal is a new proposal

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Samples: proteins in solution

Instrument	Requested days	Allocated days	From	To
D22	1	1	30/03/2018	31/03/2018

Abstract:

The specific recognition, unfolding and degradation of faulty proteins is an essential process in any living cell in order to guarantee protein homeostasis and keep the proteome in a healthy and functional state. Dysfunction of this process can lead to accumulation of misfolded proteins that are potentially toxic and lead to multiple diseases. Despite the crucial importance of understanding this process, detailed structural insights, at a molecular level, are limited, mainly due to specific flexibility and conformational changes involved.
Here, we propose to study the intermediate unfolded state of a specifically tagged model protein, GFPssrA-biotin/avidin, by SANS in solution. The presence of a biotin/avidin-tag will allow to block GFP within the unfoldase complex PAN. Using alternate deuteration of either partner will allow us to both describe the specific conformation of GFP and PAN during the process. In addition, an online fluorescence device on D22 will monitor the folded state of GFP. By arresting the unfoldase/substrate complex, the structural interpretation/analysis can be much more advanced and detailed than in a previous pioneering study carried out at ILL.

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AAA+ ATPases are a large family of ubiquitous enzymes with multiple tasks, including the remodeling of the cellular proteome. A subfamily, so-called unfoldases, recognize, unfold, and address misfolded or dysfunctional proteins towards proteolytic complexes, which eliminate these potentially toxic proteins for a healthy, functional state of the cellular proteome. Energy-dependent proteases are found across the three domains of life: bacteria, archaea and eukaryotes. In these systems the AAA+ proteins form a hexameric ring complex that associates with the catalytic 20S core particles which hydrolyze the unfolded proteins into small oligo-peptide chains of about 5-10 residues.

In a collaboration between ILL and IBS a novel approach was developed combining time-resolved small angle neutron scattering (TR-SANS) with online-fluorescence spectroscopy on D22 (developed by Anne Martel), in order to monitor the PAN unfoldase and 20S core particle from the deep-sea *Methanocaldococcus jannaschii* organism and a Green Fluorescent Protein (GFP) model substrate. By mixing perdeuterated (d) d-GFP (produced at the ILL DLAB) with hydrogenated (h) h-PAN and h-20S in 42% D_2O buffers, it was possible to follow the population and state of d-GFP during the active unfolding and proteolysis process at a time resolution of 1min and to mask the signal from PAN and 20S (see Fig. 1). To this end, the enzymatic activity of the hyperthermophilic PAN system was controlled and tuned by adjusting the temperature to 55°C.

The results show the progressive disappearance of native d-GFP, *via* the diminution of its forward scattered intensity, I(0) (see inset in lower left-hand corner). The final loss of native population was about 15%. Unfortunately, the 20S complex was not in an optimal state (see next page) and the effect was small.

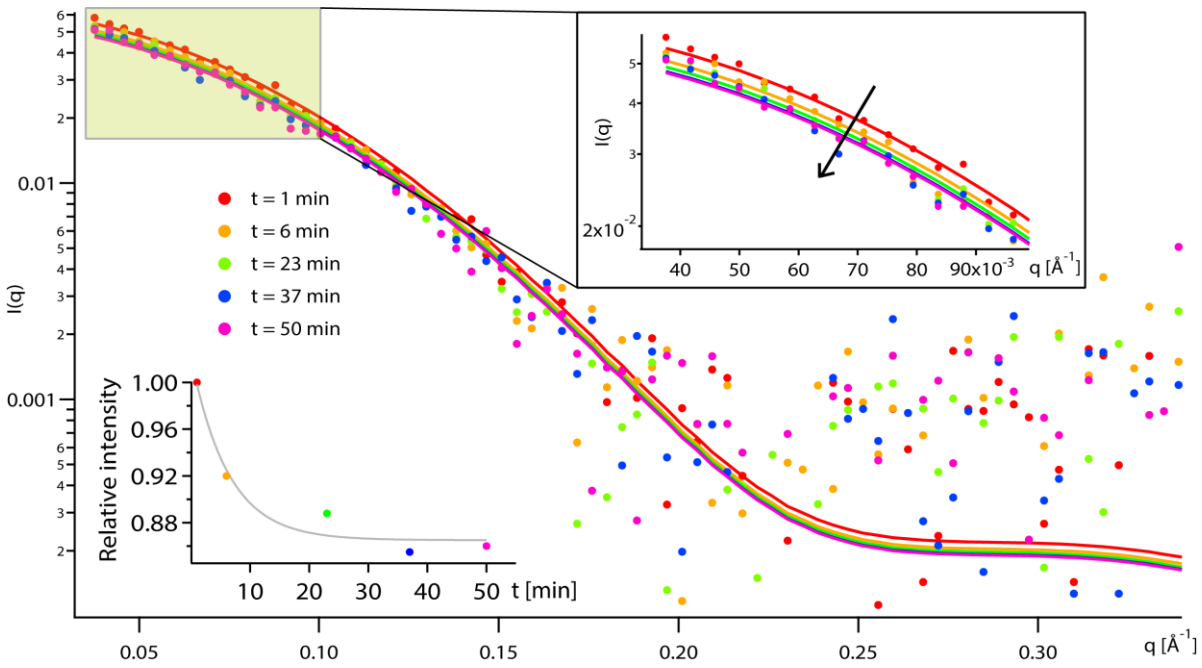

Fig. 1: Progressive diminution of the intensity of the native d-GFP SANS signal in a 42% D2O solution in the presence of h-PAN and h-20S. Please note that the form factor of the native GFP population (represented by the continuous lines, calculated from the native PDB model) remains unchanged. The intensity seems to diminish at an exponential rate up to a level of about 85-86%.

The catalytic 20S core particles previously produced in our lab were not entirely pure and active. The two subunits constituting the core particle, α and β, used to be expressed separately in *E.coli* before assembling them into complete and active 20S proteasome. Unfortunately, this protocol was leading to an irreversible assembly of the α subunits, preventing the assembly with the β subunits into complete 20S particles. The 20S particles produced during the last experiment were therefore contaminated by these inactive, self-assembled α subunits. Therefore, we developed a new protocol to co-express the α and β subunits together in *E.coli*, improving significantly the quality of the final 20S core particles solution for the next experiment. The improved quality was assessed by SEC-MALLS (Fig. 2A) and SAXS (Fig. 2B & C).

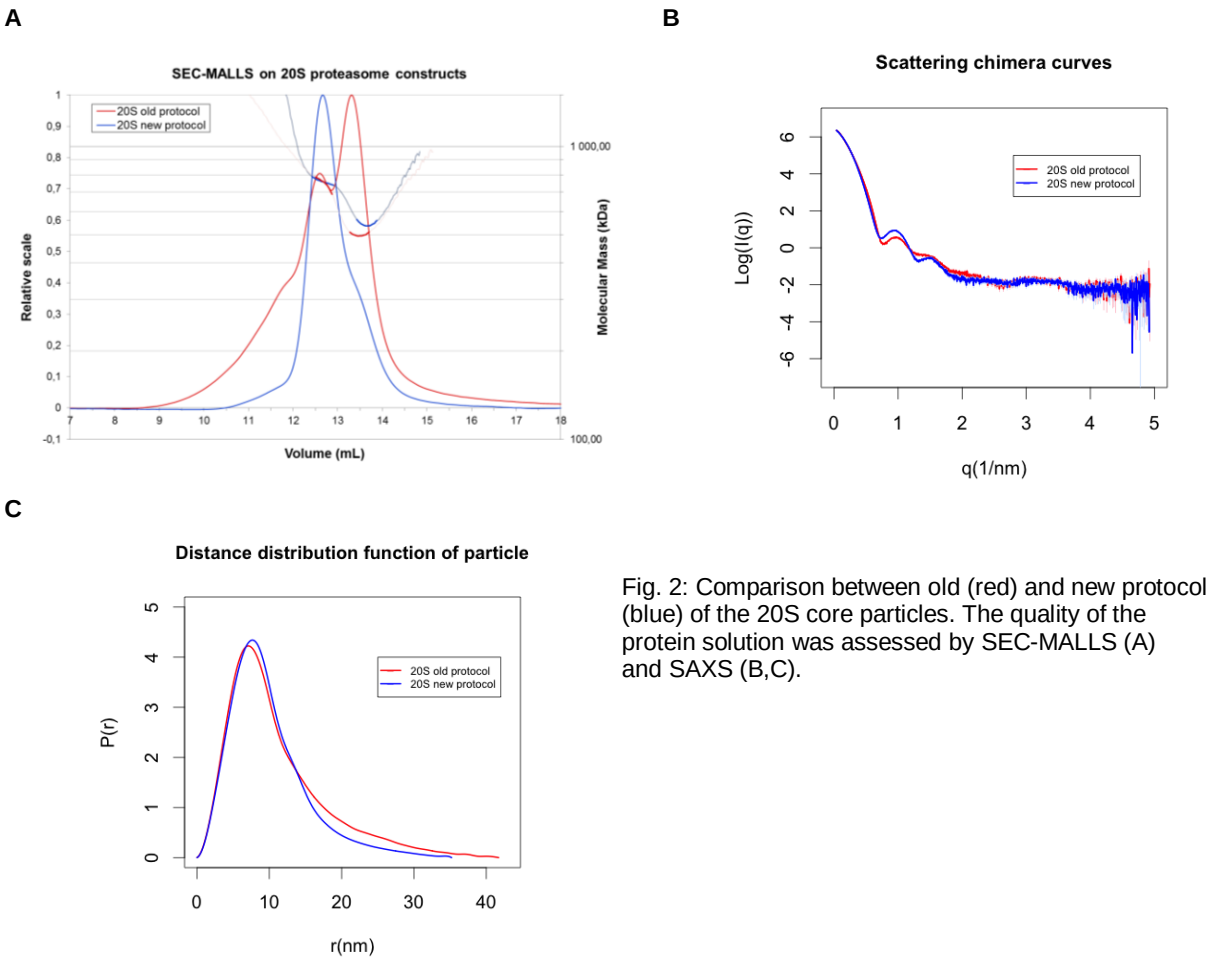

Fig. 2: Comparison between old (red) and new protocol (blue) of the 20S core particles. The quality of the protein solution was assessed by SEC-MALLS (A) and SAXS (B,C).