Experimental report

08/09/2016

Proposal: 8-03-868 **Council:** 4/2016

Title: Seeing protein unfolding and degradation by an archaeal proteolytic machinery by fluorescence-coupled real-time SANS

Research area: Biology

This proposal is a new proposal

Main proposer: Frank GABEL

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Local contacts: Anne MARTEL

Samples: Hyrdogenated and deuterated proteins in solution

Instrument	Requested days	Allocated days	From	To
D22	2	1	07/07/2016	08/07/2016

Abstract:

A tightly regulated proteome (i.e. the ensemble of proteins in a cell) is of paramount importance throughout the life cycle of any biological cell. An important step, apart from transcriptional and translational control mechanism, is proteolysis, i.e. the controlled degradation of proteins. A major key player in this mechanism is the proteasome and a dysfunction of proteolysis can have severe impact on cells and lead to health problems or death in humans. In the present proposal we build on a previous real-time SANS project that has studied the dynamics of an isolated proteasome partner, the archaeal PAN unfoldase complex, and its activity on a tagged GFP substrate. Now, we propose to widen this study by including the crucial 20S proteasome partner in solution. By combining SANS with online fluorescence spectroscopy (to follow the unfolding of GFP) and temperature activation, we expect to gain unprecedented insights into the kinetics of protein unfolding in solution and into the concomitant conformational changes of PAN at a time resolution of about 30 seconds.

Report on the SANS proposal 8-03-868
"Seeing protein unfolding and degradation by an archaeal proteolytic machinery by fluorescence-coupled real-time SANS"

by Emilie Mahieu, Bruno Franzetti and Frank Gabel

Introduction and general context

This is a report on the results obtained from protein unfolding by an ATP-dependent proteasome regulator (PAN), a long-term project that uses time-resolved small-angle neutron scattering (SANS), coupled with online fluorescence spectroscopy (Fig. 1, top right). In this project we aim to study the protein unfolding and degradation process by an archaeal unfoldase machinery, and at the same time promoting instrumental developments on the D22 ILL SANS beamline (collaboration with beamline responsible Anne Martel).
After having successfully finished an ILL PhD project observing the unfolding of Green fluorescent protein (GFP) by PAN in the presence of ATP, we now focus on the fully functional and more complex system, including the proteolytic partner proteasome 20S (see Fig. 1, middle). Its presence in solution, in addition to GFP and PAN promotes degradation (proteolysis) of the unfolded GFP polypeptide chains into small polypeptides. The anticipated results will complement and complete previous results (Fig. 1, right).

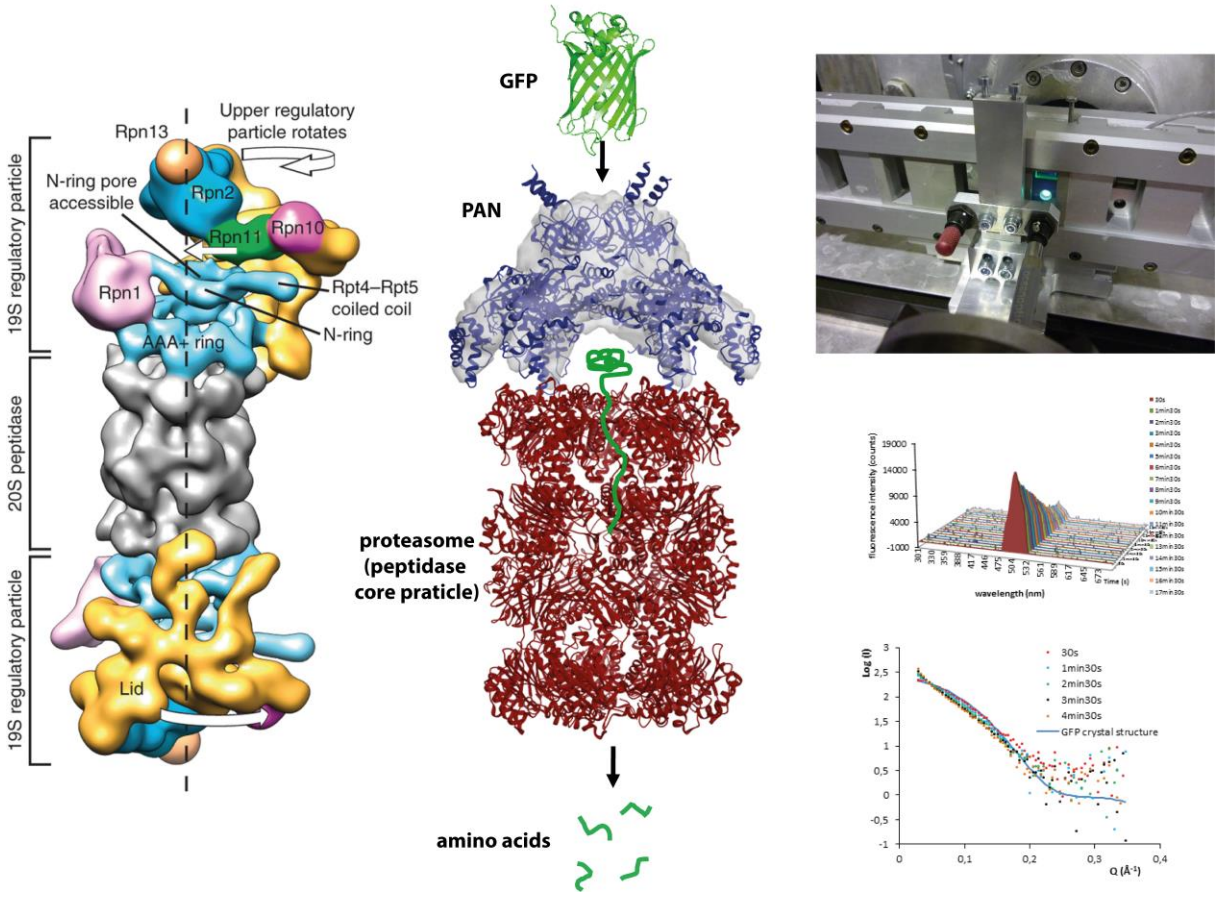

Figure 1: Left: EM structure of the eukaryotic proteasome complex. Middle: model of its archaeal homolog based on data obtained by our group. Right, from top: Online fluorescence setup, GFP fluorescence and SANS curves measured on the instrument D22 at ILL (Ibrahim et al. under review).

In a first preliminary D22 experiment in July during Emilie Mahieu's master internship, we have measured isolated, hydrogenated proteasome 20S ("h20S") in 42% D2O solution (the molecule's theoretical match point). Our data show that it is indeed well-matched with respect to the 42% D2O buffer signal, i.e. it is "invisible" at 42% D2O (Fig. 2, two bottom flat lines). This is important since its signal measured in a SAXS experiment at BM29 (ESRF) is very strong (Fig. 2, middle pink curve with several subsidiary minima and maxima). We have also checked that deuterated GFP ("dGFP") is not degraded in the presence of h20S (but in the absence of PAN) and keeps its compact, folded structure: when measured at 42% D2O (match point of h20S), the SANS curves of isolated dGFP and dGFP+h20S superpose very nicely (Fig. 2, two top curves), indicating that GFP is not degraded by 20S alone, as expected in the absence of the unfoldase PAN. Indeed, unfolding of protein substrates by PAN is required for them to access the 20S proteolytic core (Fig. 1, middle) in order to avoid unspecific degradation of globular, well-folded proteins.

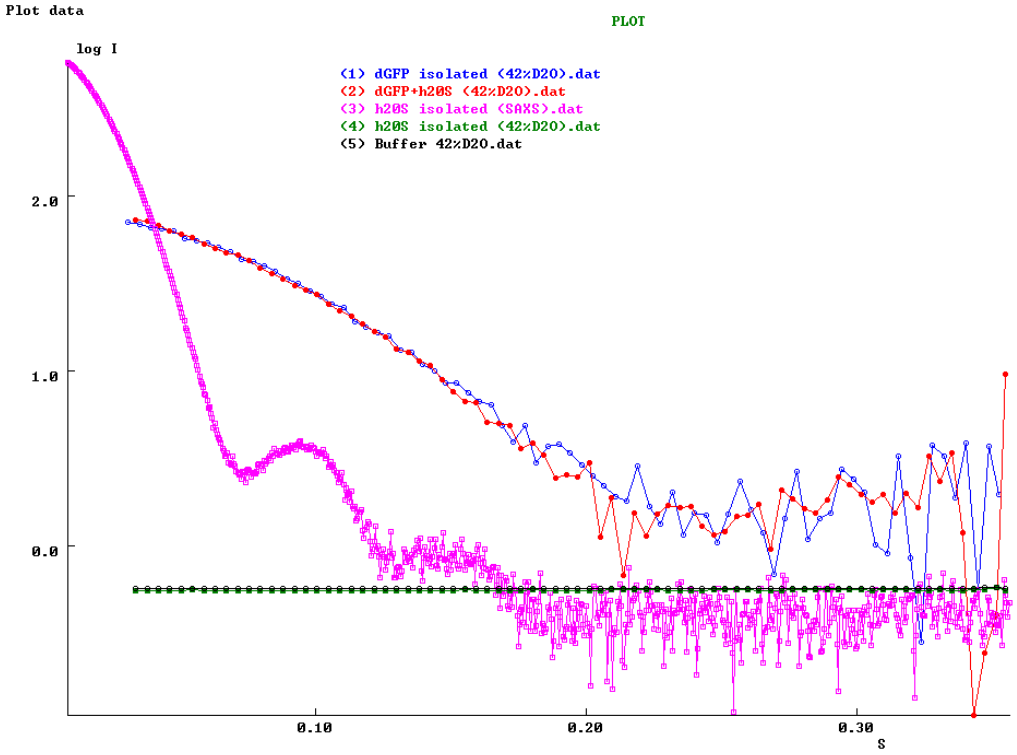

Figure 2: Several SANS control curves measured in the preliminary D22 experiments carried out in July 2016 by Emilie Mahieu during her master internship. The two top curves (blue and red) compare isolated GFP and GFP in the presence of 20S. The middle (pink) curve represents 20S as seen by SAXS. The two bottom flat lines (green and black) show the isolated 20S SANS data at its contrast match point (42% D2O).

After the successful proof-of-principle preliminary experiments, we would like now to start the more elaborated SANS experiments during Emilie Mahieu's PhD thesis (starting in October 2016) by mixing and reconstituting the fully functional ternary complex (GFP:PAN:20S), and by applying several deuteration schemes (dGFP:hPAN:h20S, hGFP:dPAN:h20S) to focus on either the conformational changes of PAN or GFP during the unfolding/degradation process (see our submitted proposal in this round).