Experimental report

12/02/2018

Proposal: 8-03-904 **Council:** 10/2016

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

Research area: Biology

This proposal is a continuation of 8-03-868

Main proposer: Frank GABEL

Experimental team: Frank GABEL
Emilie MAHIEU

Local contacts: Anne MARTEL

Samples: Proteins in aqueous solution

Instrument	Requested days	Allocated days	From	To
D22	1	1	21/02/2017	22/02/2017

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.

Experimental Report 8-03-904:

Neutron Scattering Provides New Insight into Protein Substrate Processing

AAA+ ATPases are a large family of ubiquitous enzymes with multiple tasks, including the remodelling 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 core particles. The common feature believed to underlie the diverse functions of the AAA+ family of ATPases is their ability to undergo structural alterations during the ATP power stroke that cause unfolding of proteins or disassembly of protein complexes. Given the intrinsic flexibility of ATPases and the transient character of the interaction with their protein substrates, it is challenging in structural biology to follow the conformational changes of these enzyme-substrate complexes during the active unfolding process.

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 (contact: Anne Martel), in order to monitor the PAN unfoldase from the deep-sea *Methanocaldococcus jannaschii* organism and a Green Fluorescent Protein (GFP) model substrate. By using alternating perdeuteration of both partners (ILL DLAB) and by controlling the enzymatic activity of the hyperthermophilic PAN system by temperature activation at 55-60°C, it was possible to follow conformational changes of both PAN and GFP separately and individually during the active unfolding process at a time resolution of 30 seconds.

The results show the progressive unfolding and aggregation of GFP as well as a reversible contraction of the PAN unfoldase during the active reaction. Concomitant with the unfolding of its substrate, the PAN complex underwent an energy dependent transition from a relaxed to a contracted conformation, followed by a slower expansion to its initial state at the end of the reaction. The results support a model in which AAA ATPases unfold their substrates in a reversible power stroke mechanism involving several subunits and demonstrate the general utility of this time-resolved approach for studying the structural molecular kinetics of multiple protein remodelling complexes and their substrates on the sub-minute time scale.

While the methodological approach developed has been designed for this specific project, it is expected that it can be applied to a wide range of biological macromolecular complexes, and provide structural information from individual partners at a time resolution of some seconds.

Reference: Ibrahim, *et al.* (2017), Scientific Reports **7**: 40948.

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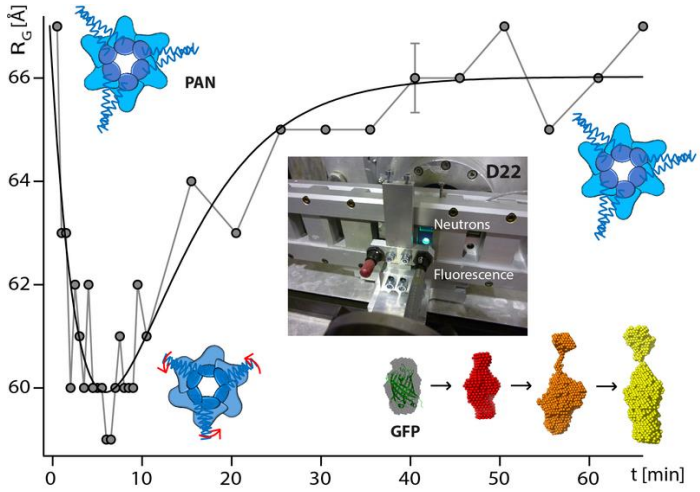

Figure legend : Reversible contraction of the PAN unfoldase during the active unfolding process of GFP. The picture inset shows the instrumental setup at D22 which allows the study of biomacromolecules in solution simultaneously by neutrons and by optical spectroscopy.