Experimental report

27/04/2022

Proposal: 9-13-904 **Council:** 10/2019

Title: Refolding of the outer membrane PagP from an original surfactant-based method

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: D2O
SDS
Sodium Chloride
ethanol-d6
Methanol-d4
PagP
Sodium dodecyl-d25 sulfate
2-Methyl-2,4-pentane-d12-diol
Butanol-d10
2-Propanol-d8

Instrument	Requested days	Allocated days	From	To
D22	2	2	09/03/2021	10/03/2021
			02/06/2021	03/06/2021

Abstract:

Membrane proteins (MPs) are important therapeutic targets and of biotechnological relevance because they can improve the performance of biomaterials. However, studying MPs in vitro is challenging, because it implies their overexpression under a functional form. The development of efficient refolding techniques is urgently awaited in this field.
In that context, we have shown that specific cosolvents such as 2-Methyl-2,4-PentaneDiol (MPD) is able to protect proteins from SDS denaturation, and can refold proteins from the SDS-denatured state. Several types of MPs were already refolded by using this method. Our general objective is to understand the molecular basis of the particular assembly between SDS and MPD in order to improve its efficiency. In the present project (which is the continuation of the project 9-13-815), we would like to investigate the refolding of a model MP, PagP, from the SDS-MPD protocol. First,
several d-MPD and d-SDS concentrations will be assayed to follow the refolding of PagP. Then, a similar experiment will be performed but highlighting SDS. It will provide information on the degree of SDS-protein association as a function of d-MPD concentration

Research proposal: Refolding of the outer membrane PagP from an original surfactant-based method (experiment 9-13-904)

Context

In the framework of protein refolding, we have studied the reliability of a new method based on the synergistic association of an anionic detergent, Sodium Dodecyl Sulfate (SDS), and a diol-type organic cosolvent, the 2-Methyl-2,4-pentanediol (MPD). Remarkably, both soluble (like hen egg-white lysozyme) and membrane proteins can be refolded by this original protocol. Generally, 1 or 2M MPD is needed to refold the SDS-denatured proteins.

Objective: Understand the molecular basis underlying the particular assembly of SDS and MPD, in order to highlight its role in the refolding process.

Results

Following the experiment 9-13-815, and concerning the effect of the solvent, we have tested the effect of d-MPD versus h-MPD on the h-SDS molecules (Fig.1). The scattering signal at low angles is only observed with deuterated solvents and seems higher with higher concentrations of h-SDS.

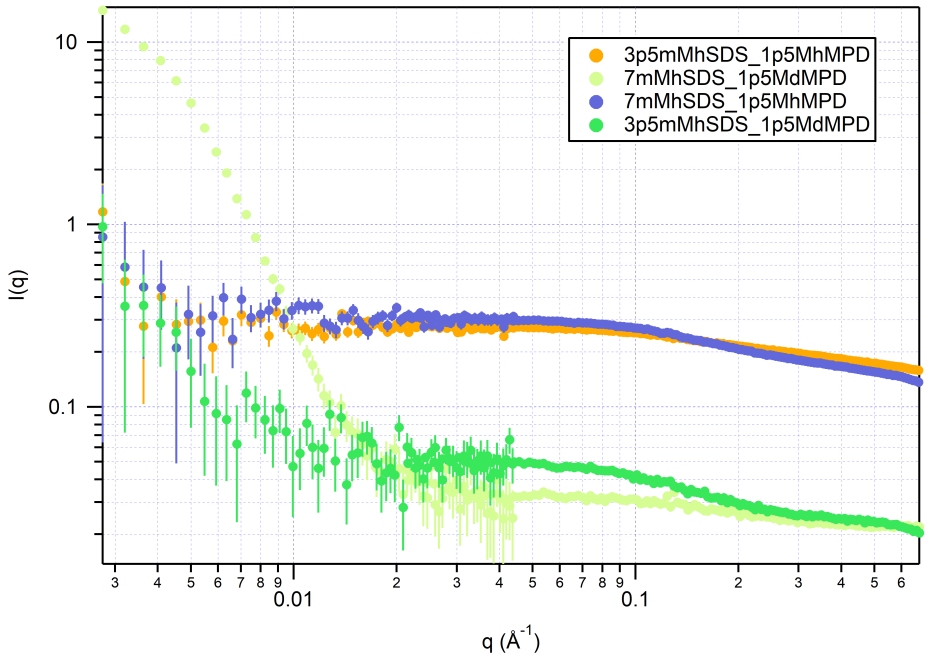
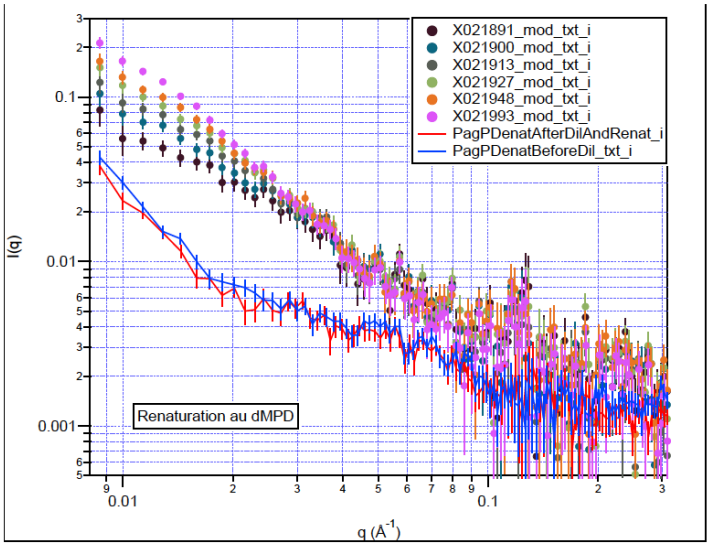


Fig.1 SANS profiles of h-SDS particles (3.5mM versus 7mM) with d-MPD or h-MPD

We then tried to follow the refolding of d-SDS-denatured PagP with 1M d-MPD, as a function of time. We did this experiment twice (Fig2A-B). We can see a change in the SANS profiles as a function of time, showing that MPD is able to change the conformation of the protein. The same was done with 0.5M d-butane-1-ol (Fig.3A-B). Smaller changes are observed between the unfolded and “refolded” state with d-butane-1-ol. Analyses are under way to try to understand these differences.

A



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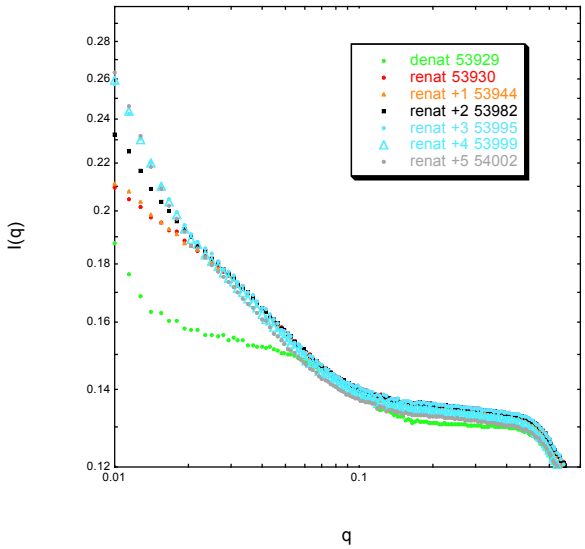
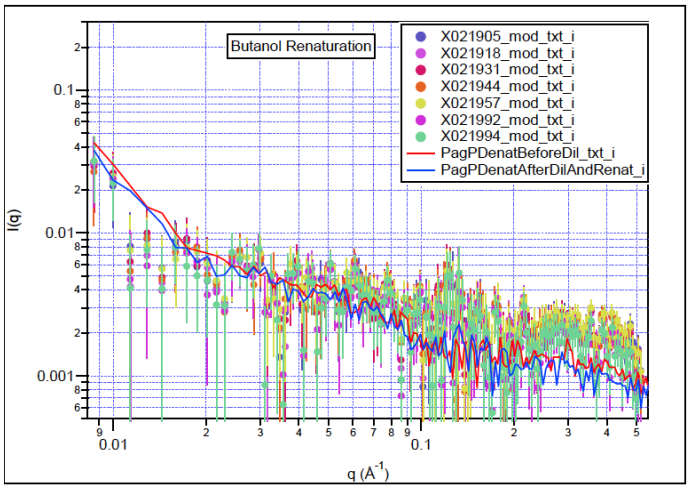


Fig.2A-B SANS profiles of d-SDS-denatured PagP refolded with 1M d-MPD, as a function of time.

A



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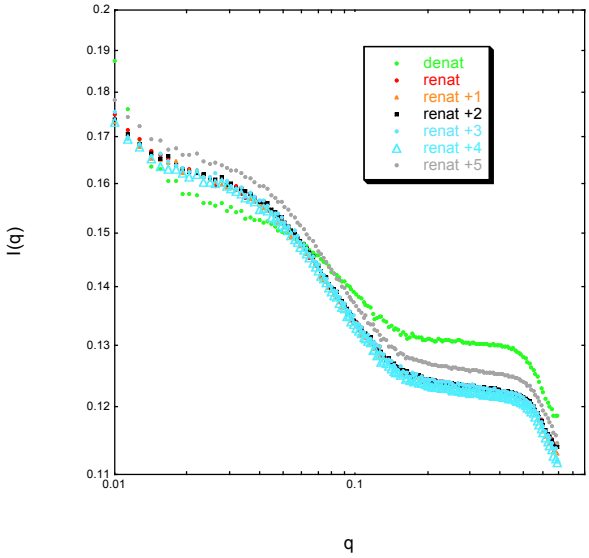

Fig.3A-B SANS profiles of d-SDS-denatured PagP refolded with 0.5M d-butane-1-ol, as a function of time.