# Experimental report

14/09/2019

**Proposal:** 9-13-782 **Council:** 4/2018

**Title:** An original surfactant-based protein refolding method

**Research area:** Soft condensed matter

**This proposal is a new proposal**


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**Samples:** CaCl2
D2O
SDS
NaCl
hen egg-white lysozyme
2-Methyl-2,4-pentanediol

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| D22 | 2 | 2 | 05/09/2018 | 06/09/2018 |
| | | | 03/07/2019 | 04/07/2019 |


## Abstract:

Major issues in biochemistry include mastering the denatured proteins refolding and understanding the mechanisms. The refolding step is often an inefficient empirical process, time and resources-consuming. The development of new refolding techniques is therefore urgently awaited. In that context, we have successfully shown the reliability of a new procedure based on the association of Sodium Dodecyl Sulfate (SDS) and 2-Methyl-2,4-pentanediol (MPD). To our knowledge, this is the only protocol described proved to efficiently refold several types of proteins. The goal of the project is to understand the molecular basis of the particular assembly and highlight its relevance in the refolding process. Deeper analyses are requested to determine the influence on the specific interactions and complex stability of parameters such as SDS/MPD concentrations, pH, ionic strength and temperature. SANS is the perfect technique to investigate the micelle structure, micelle-solvent interactions and structural changes upon self-aggregation. We shall also investigate the refolding of a model protein, i.e. lysozyme, and give some insights in the interplay of protein-SDS and protein-MPD interactions.

**Research proposal**: An original surfactant-based protein refolding method (experiment 9-13-782)

**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**

To analyse the SDS-MPD interactions, a SANS run was first focussed on the structure of SDS/MPD aggregate for a SDS concentration (7mM) above the critical micelle concentration (which is ca. 2.6mM in a phosphate buffer), and MPD concentrations ranging from 0 to 3M (Fig.1A). The use of deuterated MPD in a deuterated solvent of the same SLD allowed a precise determination of the SDS micelle aggregates. It appears that from 0.1 to 1M MPD, the size of the SDS micelle is gradually decreasing while a transition is observed at 1.25M MPD, showing the formation of large size aggregates or some sort of local phase separation (Fig.1A). Interestingly, such a MPD amount precisely corresponds to the requested concentration for protein refolding. Fig.1B shows the radius of gyration of SDS micelles as a function of MPD concentration. These preliminary results agree with the dynamic light scattering (DLS) and molecular dynamics simulations previously performed, clearly evidencing the SDS micelle dissociation in the presence of molar concentrations of MPD.

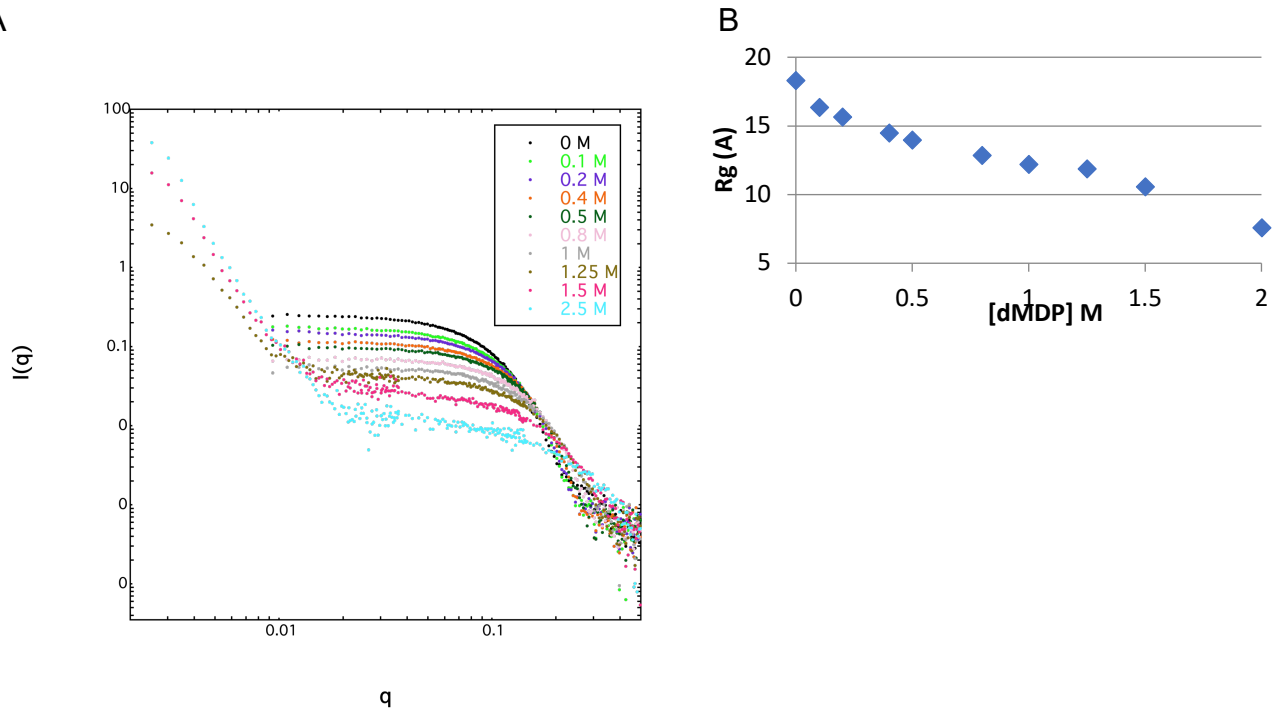


Fig.1 A) SANS profiles of SDS particles as a function of d-MPD concentration; B) Radius of gyration (Rg) as a function of d-MPD concentration

To further clarify the origin of the scattering at low angles, we hypothesized that MPD, at high concentration, could give rise to a nanophase separation. From DLS experiments, we observed, at 0.5M MPD, particles with a 92.7nm diameter while with 2M MPD, the particle size reaches 164.4 nm, meaning that MPD molecules would form significantly larger particles. This phenomenon was also tested by SANS (Fig.2). The non-deuterated with respect to the deuterated MPD shows a higher signal at low angle, particularly at 1M MPD, confirming our hypothesis.

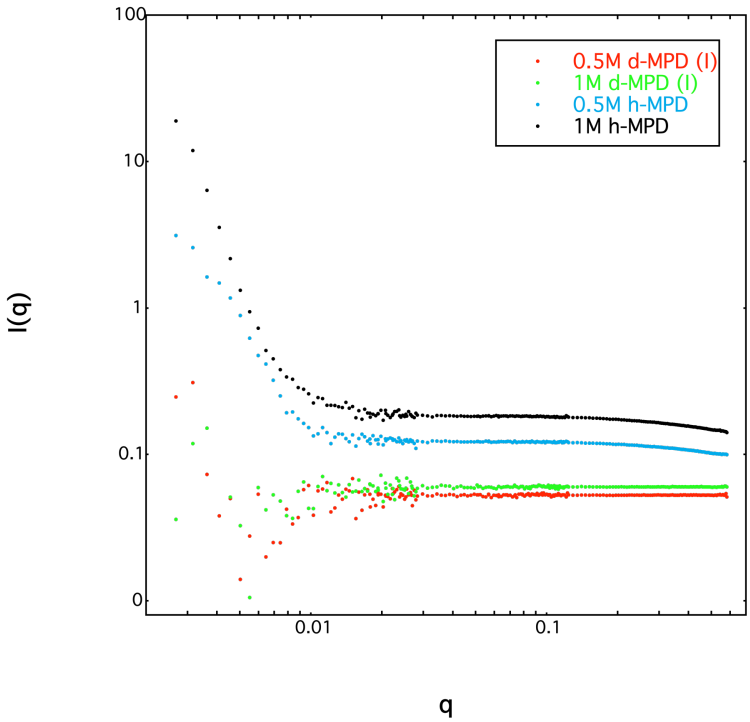


Fig.2 SANS profiles of h-MPD and d-MPD

In a second part of the SANS experiments, we have investigated the refolding of a model soluble protein, the hen egg-white lysozyme, which was previously refolded in 7mM SDS and 2M MPD. Solvent, SDS and MPD had the exact same scattering length densities in order to only highlight the protein signal. Despite the small protein concentration (0.5mg/ml), we were able to observe a significant difference between the SDS-unfolded state and its native state (Fig.2A). Various MPD concentrations (from 0 to 2 M) were added *in situ* to the initial denatured-SDS protein in order to unveil the refolding process. In order to enhance the resolution and avoid free SDS micelles in solution, we performed a second in situ experiment with a higher protein concentration (1mg/ml) and less detergent (3.5mM SDS) (Fig.2B). From the second experiment, we could observe a lowering of Rg from the denatured (24.5Å) to the refolded lysozyme (13.95Å). This is in agreement with the values observed in literature.

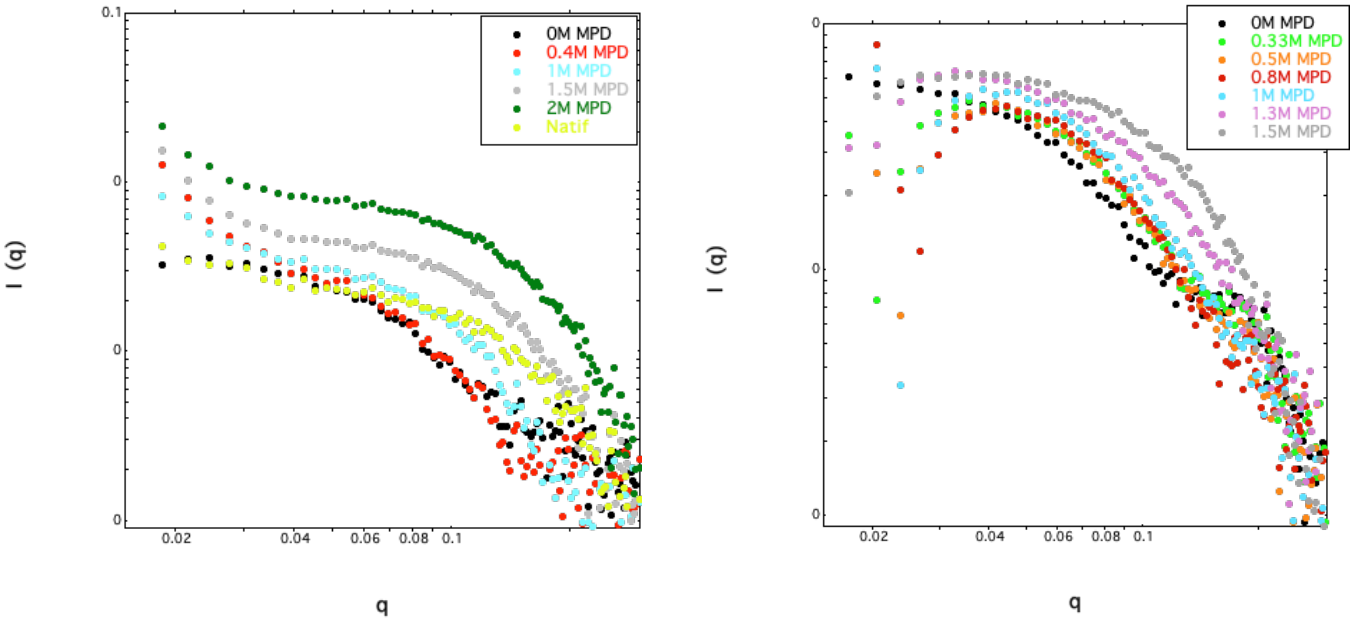


Fig.2 A) SANS profiles of 7mM SDS-unfolded Lysozyme (0.5mg/ml) as a function of d-MPD concentration; B) SANS profiles of 3.5mM SDS-unfolded Lysozyme (1mg/ml) as a function of d-MPD concentration

**Prospects**

In the next experiments, we will consider the structure of SDS/MPD aggregates for a 3.5mM SDS concentration (used for the second in situ refolding experiment) and MPD concentrations varying from 0 to 2M. Then we plan to evaluate the effects of other cosolvents such as ethanol. Finally, the refolding of other SDS-denatured proteins, such as the membrane protein PagP, will be followed upon MPD addition.