Experimental report

13/09/2023

Proposal: 8-03-1069 **Council:** 10/2022

Title: Solution structure of mTSPO membrane protein in biomimetic systems

Research area: Biology

This proposal is a continuation of 8-03-993

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Samples: DMPC
mTSPO protein
lipid nanodiscs

Instrument	Requested days	Allocated days	From	To
D22	2	2	19/05/2023	20/05/2023
			01/06/2023	02/06/2023

Abstract:

We focus on mouse TSPO (mTSPO), a ubiquitous and functionally important translocator membrane protein, used as a marker in many brain and cancerous diseases in human. Recently, we determined, using SANS and ab initio modeling, the low resolution structure of mTSPO without ligand in SDS (the detergent used to extract and purify the protein) and in DPC (used to solve mTSPO structure by NMR, but in the presence of a stabilizing ligand). We intend now to characterize the solution structure of mTSPO, with and without ligand, in different biomimetic membrane environments: lipid/detergent mixtures and lipid nanodiscs.

Experiment 8-03-1069 report on D22 (May 2023)

mTSPO/lipid/detergent complexes characterized by SEC-SANS

TSPO is a ubiquitous and functionally important membrane protein of about 18 kDa expressed throughout the body and used as a marker in many brain diseases. The first high resolution 3D structure of a mammalian TSPO was resolved by liquid NMR in DPC but only in presence of a stabilizing ligand[1]. No crystals could yet be obtained for mammalian TSPO.

We study the structure/function of mouse TSPO (mTSPO) in different amphiphilic environments. We found recently that mTSPO is partially unfolded and very flexible in sodium dodecyl sulfate (SDS) (its solubilisation and purification detergent from *E. coli* inclusion bodies), where the protein is stable and monodisperse but in a nonfunctional form[2]. In contrast, in dodecylphosphocholine (DPC), mTSPO is more compact but has still a significantly larger conformation compared to its ligand-binding NMR structure[2].

We present below the data from the SEC-SANS measurements we performed on D22 in May 2023 on mTSPO with lipid/detergens DMPC/DPC mixed micelles.

SEC-SANS experiments:

We used the size-exclusion chromatography (SEC, Superdex 75i (24 mL) column) sample environment on D22 to eliminate possible protein aggregates and be able to correctly subtract the elution buffer containing micelles that can scatter.

We aimed to measure the mTSPO/lipid/detergent complexe with hydrogenous or deuterated detergent DMPC/DPC in buffer (50 mM HEPES, 150 mM NaCl, pD 7.8) with different H_2O/D_2O ratios in the three following contrast conditions:

- *(i)* **mTSPO/h-DMPC/h-DPC in 100% D_2O** buffer to measure the whole protein/lipid/detergent **complex**.
- *(ii)* **mTSPO/d-DMPC/d-DPC in 42% D_2O** buffer to contrast-match the hydrogenous protein and measure the **lipid/detergent belt** only.
- *(iii)* (*iii*) **mTSPO/ d-DMPC/d-DPC in 100% D_2O** buffer to contrast-match the deuterated detergent and measure the **protein** only.

We previously checked that, in 100% D_2O buffer, both d-DMPC micelles and a mixture of 86% d-DPC/14% h-DPC micelles are well contrast-matched.

Results:

Due to the ILL reactor shutdown last March, the experiment has to be postponed from March to May. Meanwhile, the samples of mTSPO in DMPC/DPC, prepared just before the scheduled date of March, have unfortunately been denaturated after freezing (Fig. 1 A-B). We observed this protein denaturation during the experiment itself and could not repeat these measurements due to both absence of beamtime and the very expensive cost of deuterated DMPC.

A **B**

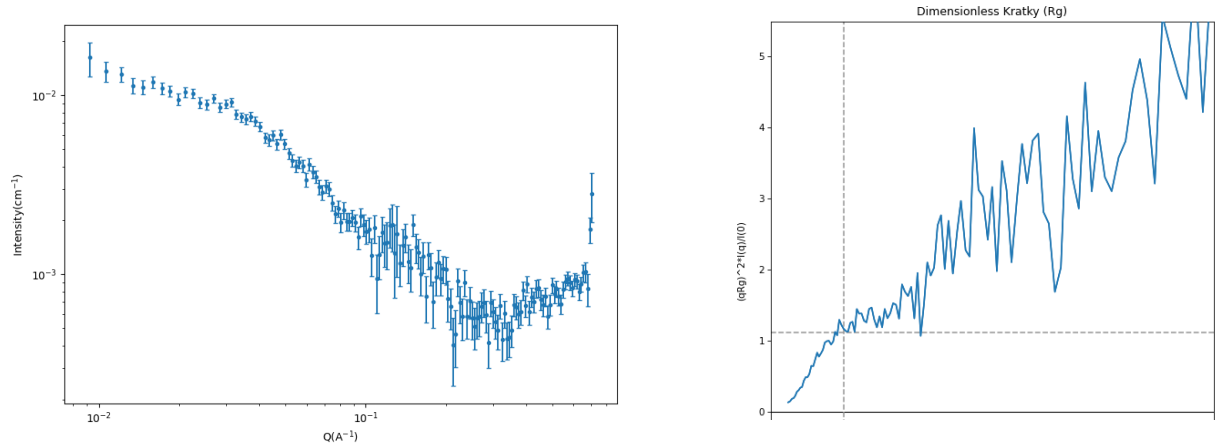

Figure 1. (*A*) SEC-SANS data from mTSPO/d-DMPC/d-DPC samples in 100% D_2O buffer, corresponding to mTSPO protein SANS signal. **(*B*)** The Kratky plot of the corresponding data shows a completely unfolded protein.

References:

1. Jaremko Ł, Jaremko M, Giller K, Becker S, Zweckstetter M. Structure of the Mitochondrial Translocator Protein in Complex with a Diagnostic Ligand. *Science*. 2014;343(6177):1363-1366. doi:10.1126/science.1248725.

2. Combet S, Bonneté F, Finet S, et al. Effect of amphiphilic environment on the solution structure of mouse TSPO translocator protein. *Biochimie*. 2023;205:61-72. doi:10.1016/j.biochi.2022.11.014