Experimental report

18/02/2021

Proposal: 8-03-993 **Council:** 10/2019

Title: Structural determination of mTSPO/detergent complexes

Research area: Biology

This proposal is a resubmission of 8-03-991

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Samples: TSPO protein
SDS detergent
DPC detergent

Instrument	Requested days	Allocated days	From	To
D22	2	2	15/02/2020	16/02/2020
			04/02/2021	05/02/2021

Abstract:

Our project is to determine the solution structures of TSPO (translocator protein outer membrane), an ubiquitous and functionally important membrane protein involved in neurodegeneration and cancer in human. No XR crystallography structure is available for the mammal form (mouse mTSPO) of this protein. The objective is to characterize the interactions between mTSPO and detergents, by coupling molecular dynamics simulations and scattering experiments, in order to better understand the influence of the surfactant on the crystallization of the membrane complexes. We would like to measure mTSPO with SDS (its solubilisation detergent) or DPC (used to solve its NMR structure). We will use different contrast matching conditions to probe selectively the whole complex, the detergent belt or the protein alone. We will need to combine SANS with the size-exclusion chromatography (SEC-SANS), a unique setup available at the ILL. With membrane proteins, the chromatographic setup is absolutely necessary to separate the protein/detergent complexes from the detergent free micelles.

Experiment 8-03-993 report on D22 (2020 Feb. 15th and 2021 Feb. 5th)

mTSPO/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 (mouse mTSPO) was resolved by liquid NMR in DPC but only in the presence of a stabilizing ligand [1]. No crystals could yet be obtained for mammalian TSPO. Our aim is to compare the structure of mTSPO in different lipid/detergent solutions to get the best crystallization condition: in SDS (the anionic detergent used in mTSPO extraction and purification [2]), in DPC (the zwitterionic detergent used in mTSPO NMR study [1]), and finally in a mixture of lipid/detergent bio-mimetic environment in which mTSPO activity is the highest one [9].

We present below the data we obtained from SEC-SANS measurements on D22 performed in Feb. 2020 and Feb. 2021 with **SDS** or **DPC** detergents, respectively.

SEC-SANS experiments:

We used the size-exclusion chromatography (SEC, Superdex 200 Hiload 300/100 (24 mL) column) sample environment on D22 to eliminate putative protein aggregates and be able to correctly subtract the elution buffer containing detergent micelles. We used in Feb. 2020 two instrument configurations on D22: 6 Å-2 m (off-centered by 300 mm) and 5.6 m detector-sample distances. In Feb. 2021, we could benefit from the installation on D22 of a second detector allowing to perform only one 6 Å-5.6 m experiment to get in one injection the same Q-range. Such experiments are very time-consuming due to the SEC environment (setup installation and instrument calibration, column equilibration, very slow flow rate to get statistics).

We measured the mTSPO/detergent complexes with hydrogenous or deuterated detergent (SDS or DPC) in buffer (50 mM HEPES, 150 mM NaCl, pD 6.6) with different H_2O/D_2O ratios in the three following contrast conditions: (*i*) **mTSPO/h-detergent in 100% D_2O** buffer to measure the whole protein/detergent **complex**; (*ii*) **mTSPO/d-detergent in 42% D_2O** buffer to contrast-match the hydrogenous protein and measure the **detergent belt** only; **mTSPO/d-detergent in 100% D_2O** buffer to contrast-match the deuterated detergent and measure the **protein** only. We checked that, in 100% D_2O, d-SDS and a mixture of 86% d-DPC/14% h-DPC are contrast matched.

mTSPO/SDS experiment:

The radii of gyration (R_g) we obtained from the SANS curves (**Fig. 1**) are the following ones: 25.41 ± 0.08 Å for mTSPO/SDS complex, 25.3 ± 0.3 Å for the SDS belt, and 32 ± 2 Å for mTSPO protein. The high value of mTSPO R_g (the theoretical R_g of the compacted liganded mTSPO should be ~18 Å) in the SDS condition strongly suggests mTSPO does not have a completely globular structure after its extraction, as already expected from other studied made by circular dichroism [3] and fluorescence [4].

We fitted the SANS curves together with the DANVILLE software [5], in collaboration with A. Koutsioumpas (MLZ, Germany) (**Fig. 2**). The obtained SDS aggregation number in the detergent belt associated to the protein was 134 molecules, confirming our own previous SEC-MALLS results. The obtained protein volume is 20,043 Å^3, close to what is expected from its molecular weight (~22,000 Å^3), giving confidence the final fit is reliable. We used DAMAVER software (ATSAS, [6]) for finding the most probable model and the filtered envelope from a pool of 10 reconstructions (**Fig. 2E**).

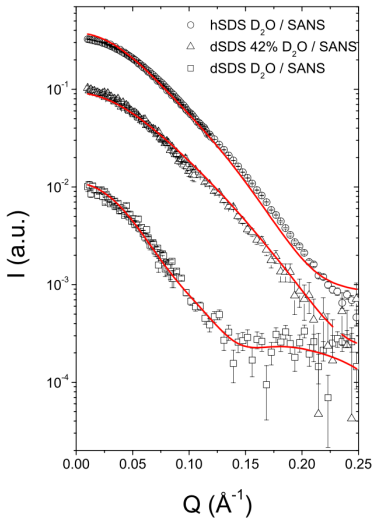

Figure 1. SEC-SANS data from **mTSPO/SDS** samples corresponding to the different contrasts used: mTSPO/hSDS in 100% D_2O buffer (**whole complex, circles**), mTSPO/dSDS in 42% D_2O (**dSDS belt, triangles**), and mTSPO/dSDS in 100% D_2O buffer (**protein, squares**). All curves are adequately fitted (red curves) with the *ab initio* algorithm (best model, from 10 reconstruction procedures).

The results show that mTSPO seems to be partially unfolded (**Fig. 2E**) and not fully embedded in the detergent belt (**Fig. 2B**). Work is in progress to study by coarse-grained simulation how the protein "unfolds" inside this envelope.

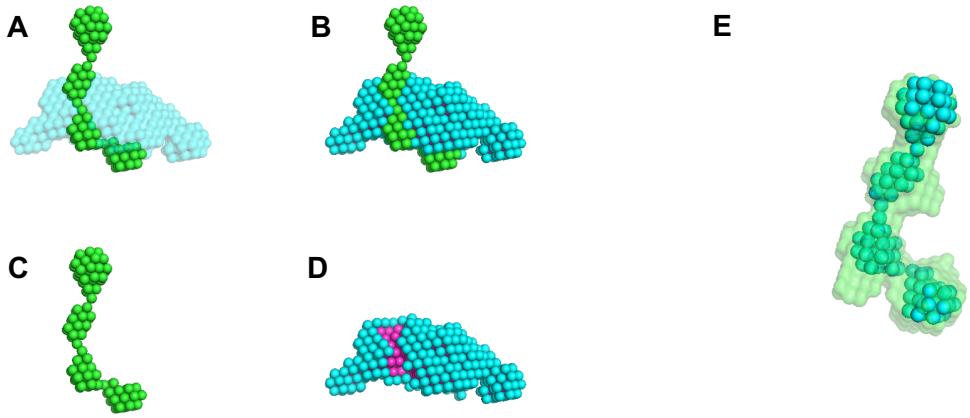

Figure 2. *Ab initio* reconstructed shapes of mTSPO/SDS complex with SDS tails (magenta beads), SDS heads (cyan beads), and mTSPO protein (green beads). (***A***) mTSPO with its semi-transparent SDS detergent belt; (***B***) the full model; (***C***) the protein only; (***D***) the SDS belt only. **(*E*)** The best model for mTSPO protein and the filtered model from 10 reconstructions according to DAMAVER [6].

mTSPO/DPC experiment:

At first analysis (the experiment was performed one week ago), the radii of gyration (R_g) are significantly different compared to the SDS condition (**Fig. 3**): 30.34 ± 0.06 Å for the protein/DPC complex, 28.0 ± 0.8 Å for the DPC belt (higher than for the SDS one, in agreement with a higher number of DPC molecules in the belt observed by SEC-MALLS), and 24.7 ± 0.5 Å for mTSPO protein (smaller than in the SDS condition). This R_g value for mTSPO is therefore more compatible with a more compact form of mTSPO.

First calculations using CRYSON software (ATSAS [7]) give a fit to the protein theoretical SANS curve (calculated from the liganded 2MGY.PDB structure [1]) of the SANS data closer in DPC than in SDS conditions (**Fig. 4**). The fit is not "good" even with DPC detergent. However, we have to keep in mind that the unliganded native protein should be less compact compared to the 2MGY.PDB NMR structure stabilized by PK-11195 ligand. Moreover, multiconformers of

mTSPO have been reported in DPC condition, that can lead to SANS polydispersity [8]. Work is in progress to make *ab initio* simulations using DANVILLE to compare with the SDS results.

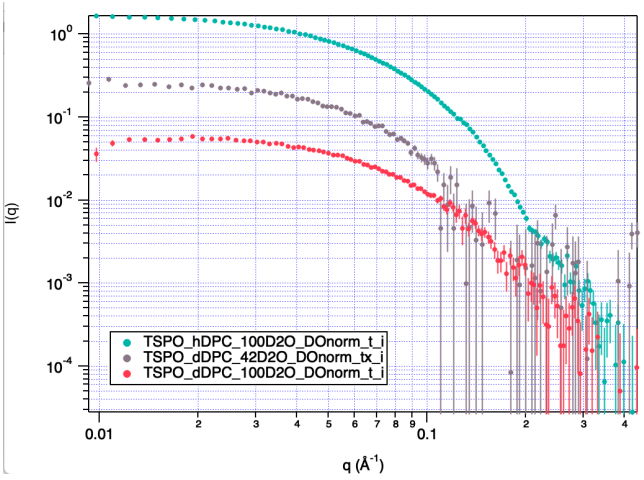

Figure 3. SEC-SANS data from **mTSPO/DPC** samples corresponding to the different contrasts used: mTSPO/hDPC in 100% D_2O buffer (**whole complex, cyan**), mTSPO/(84% dDPC/6% hDPC) in 42% D_2O (**DPC belt, grey**), and mTSPO/(84% dDPC/6% hDPC) in 100% D_2O buffer (**protein, red**).

Conclusions

We conclude from these two SEC-SANS experiments that mTSPO membrane protein, when extracted from bacterium inclusion bodies by SDS detergent, does not have a globular structure but rather a partially unfolded one. The exchange of SDS by DPC detergent allows mTSPO to recover a more globular form, which made it possible to determine its NMR structure in the presence of the specific PK-11195 ligand [1].

However, the affinity of mTSPO for this ligand in DPC detergent remains low (several micromolar affinity), whereas this affinity is nanomolar in the presence of lipids [9]. We wish now to continue mTSPO characterization by SEC-SANS in the presence of a mixture of lipids (DMPC) and DPC detergent (see the proposal).

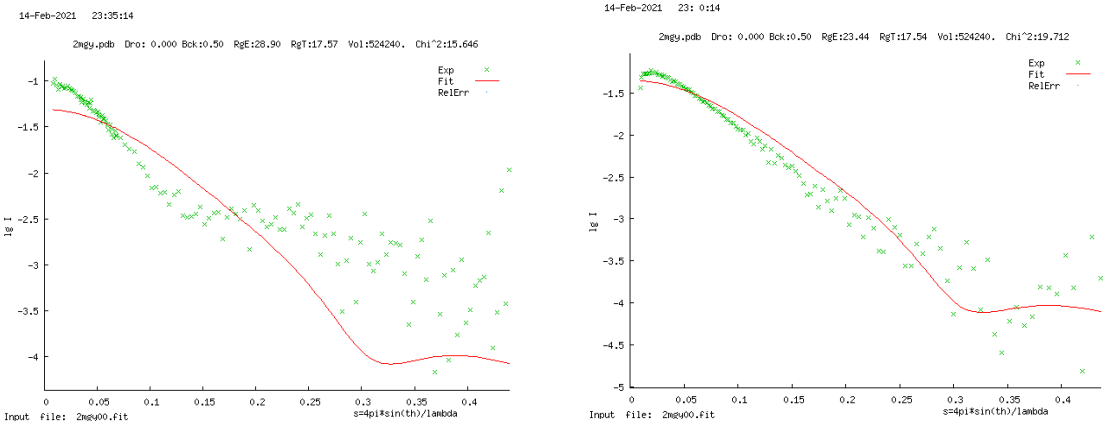

Figure 4. CRYSON (ATSAS) calculations using 2MGY.PDB liquid NMR structure of the liganded compact form of mTSPO in DPC environment and the SANS data obtained for mTSPO solubilized in SDS (left) or DPC (right) detergents.

References

[1] Jaremko Ł. *et al.,* Science. 343: 1363-1366, **2014**. **[2]** Lacapere J.J. *et al.*, Biochem Biophys Res Commun 284: 536-541, **2001**. **[3]** Murail S. *et al.*, Biochimica et Biophysica Acta 1778: 1375-1381, **2008**. **[4]** Lacapere *et al.*, Springer pp. 393, **2014**. **[5]** Koutsioubas A., Biophys Journal. 113: 2373-2382, **2017**. **[6]** Volkov V.V. and Svergun D. I., J. Appl. Cryst. 36: 860-864, **2003**. **[7]** Svergun D.I. *et al.* Proc. Natl. Acad. Sci. USA 95: 768-773, **1998**. **[8]** Jaremko Ł. *et al.,* Chemistry 21: 16555-16563, **2015**. **[9]** Iatmanen-Harbi S *et al.* Int J Mol Sci 20: 1444, **2019**.