	Experiment title: Foldase or chaperone role of ciclophyllin A in the interaction with TDP-43	**Experiment number**: MD 1318
Beamline: ID02	**Date of experiment**: from:02/12/21-03/02/22 to: 03/12/21-04/02/22	**Date of report**:
Shifts: 6	**Local contact(s)**: Lauren Matthews	*Received at ESRF:*

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Report:

The RNA-binding protein TDP-43 is a 43 kDa protein that helps regulate many aspects of RNA processing, such as splicing, stabilization and mRNA production. It has been identified in an abnormal phosphorylated state in cellular inclusions and has been linked to the pathogenesis of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). In biomedical studies targeted to the development of effective therapeutic agents, we observed that PPIA is an interacting partner of TDP-43 and governs its functions, probably by influencing its folding and localization. From a structural point of view, PPIA is a very well characterized protein, while less is known about TDP-43.
We measured both proteins in aqueous environment, following their interaction in time. Moreover, we synthetized in our laboratories (Istituto di Ricerche Farmacologiche Mario Negri IRCCS) a paradigmatic peptide within the LC domain, the 341-371 that encompasses the entire hnRNP binding region that is fundamental for TDP-43 function in the assembly of the RNA-protein complexes and an analogue peptide carrying the G348V mutation in the LC domain. We investigated the structure of the different peptides and the effect of this punctual mutation on their interaction and on the extent and kinetics of aggregation of the peptides.
We could characterize the different peptides (WT, mutated and scrambled for reference) in physiological environement (Fig.1 left). The WT peptide seems to be more prone to aggregate, and not in a monomeric form at steady state.

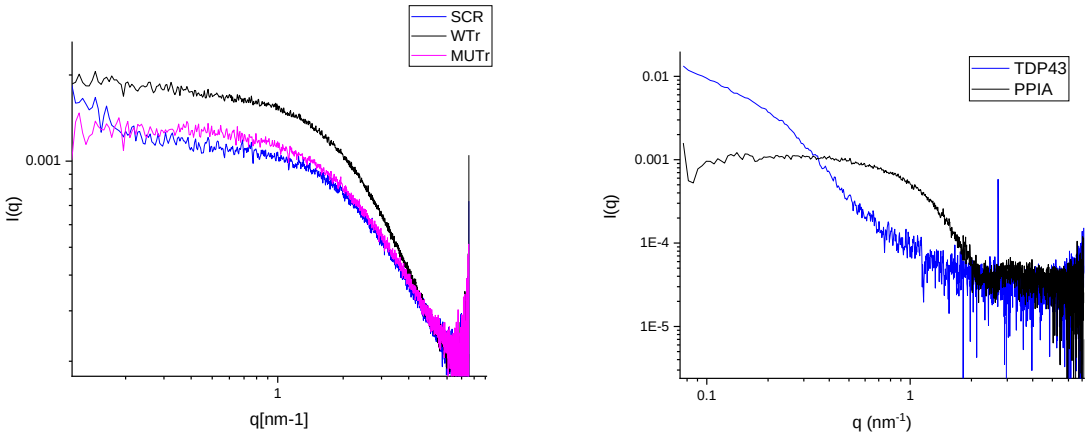

Figure 1: (left) In solution peptides: WT in black, the point mutated one in pink and the scrambled synthetized sequence in blue.(right) PPIA (black line) and TDP43 (blue line) in solution

We characterized PPIA and the whole TDP43 (Fig.1, right). The PPIA curve depicts a monomeric protein with gyration radius comparable to the one found in literature (about 15Å).
TDP43 spectrum show instead the shape of an elongated object, more similar to a long cylinder than a sphere.
The interaction between TDP43 and PPIA has been observed, with an evolution in time.
In Figure 3 the complexation of the proteins is evident in the violet curve, representing the time zero interaction. Comparing this curve with the red one, that represent the simulation of a spectrum in which PPIA and TDP43 are not interacting, an effective binding can be observed. After 30 minutes however the curve resembles more the theoretical one, demonstrating that the interaction that occurs is fast and reversible.

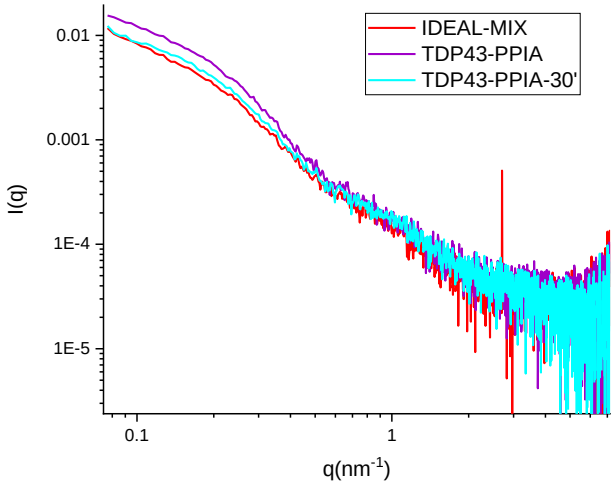

Figure 2: Interaction between TDP43 and PPIA at t=0 (violet curve) and t=30' (cyan curve) compared to non-interacting theorical one (red curve).

This interesting result needs to be confirmed by further analysis, but suggests a foldase effect of PPIA towards TDP43.