MINISTERIO
DE CIENCIA, INNOVACIÓN
Y UNIVERSIDADES

INSTITUTO DE
BIOMEDICINA DE
VALENCIA CSIC

PROPOSAL: MX-2352
BEAMLINE: BM29
DATE: 04 / 10 / 2022
LOCAL CONTACT: Dihia Moussani

EXPERIMENT REPORT

At BM29, we collected, for the first time, SAXS data on the 1.5 MDa CAD complex. This complex is formed by oligomerization of a multidomain protein of 250 kDa. We brought two different samples: the wild-protein (WT) and an engineered version with 3 cysteine residues at strategic positions to favor the formation of disulfide bridges between the protein subunits (3Cys). The covalent linkage of the complex was intended to impede the dissociation of the sample during the preparation of grids for cryo-EM. Before cryo-EM experiments, we wanted to verify the heterogeneity and properties of the complex.

We collected several SAXS datasets of WT and 3Cys using in-line SEC separation. However, the sample after SEC was too diluted to have a good signal. Therefore, we measured the sample directly in batch at different concentrations. We are very thankful to Dihia for teaching us how to process and interpret the SAXS data.

The results indicate that the heterogeneity of the sample is larger than we predicted and, therefore, that the sample is not yet ready for cryo-EM.

Interestingly, during data collection, we discussed with Dihia the possibilities of using SAXS for screening the binding of compounds to other proteins we work with. This conversation was very helpful, and we have already started a collaboration with Dihia to do such experiments.

Santiago Ramón-Maiques
Structure of Macromolecular Targets Unit
Instituto de Biomedicina de Valencia (IBV-CSIC)
Jaime Roig, 11. Valencia-46010. Spain
Phone: +34 96 3391760 (ext. 431468); +34 697 309 193
email: sramon@ibv.csic.es
http://www3.ibv.csic.es/UED.asp