Reconstitution of human EXOG into Salipro nanoparticles as a platform to investigate the effect of lipid environment on its functionality

Adela Bledea, Laboratory of Structural Biology

Intercollegiate Faculty of Biotechnology UG & MUG, Gdansk

Principal Investigator: Dr. hab. Michał R. Szymański

Collaborator: Dr. Bartosz Szczesny

EXOG is a mitochondrial inner membrane nuclease that plays an essential role in human mitochondrial DNA repair. Its 5′exonuclease activity, as well as substrate specificity conferred by the C-terminal Wing domain allow for precise processing of DNA damage. Its N-terminal transmembrane domain promotes its exclusive mitochondrial localization and confines the enzyme to the inner membrane of mitochondria.

It is not well understood how the lipid environment of the mitochondrial inner membrane affects EXOGs activity. Activity tests performed on deletion mutant that lacks the transmembrane domain showed that its activity is modulated in the presence of detergent solubilized lipids. Detergents are usually used to purify membrane proteins, but they have a denaturing effect, therefore affecting the stability and activity of membrane proteins. Thus, my aim is to create a detergent free platform using Saposin A-lipoprotein nanoparticle system to reconstitute full-length EXOG in a native-like environment and examine the effects of the lipids on its stability and activity. To this end, I successfully reconstituted Salipro nanoparticles using a lipid composition similar to the one from mitochondria. AFM analysis shows homogeneous nanoparticles with a size range between 30-50 kDa, which corresponds to the estimated molecular weight from size exclusion chromatography. Preliminary results of EXOG-

nanoparticles reconstitution trials show modulation of EXOGs activity in the presence of different lipids. We are currently investigating how the density of the lipid bilayer influences EXOG incorporation into the nanoparticles and further, how it modulates its functional properties.

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