GUP1 proposal- LmDHPS preliminary data

We performed the protein expression in *Escherichia coli* BL21(DE_3)-R_3-pRARE2 and obtained a highly pure LmDHPS heterocomplex formed by the isoforms A and B (LmDHPSA and LmDHPSB), suitable for being used in Cryo-EM experiments. Procedure: After growing and harvesting the cultures, the cells harboring LmDHPSA and LmDHPSB were lysed by sonication. For purification, the following sequence of methods was applied: (1) Immobilized Metal Affinity Chromatography (IMAC) run on an Akta purification machine; (2) Protein digestion with TEV (Tobacco Etch Virus) and SUMO proteases to remove the tags from the purified proteins. (3) Reverse IMAC, in which the target proteins now without the tag. (4) The target proteins also underwent a Size Exclusion Chromatography (Gel filtration) for further purity improvement. The final purified proteins were analyzed by SDS-PAGE and electrospray-ionization mass spectrometry (Waters ESI-TOF instrument) to verify the correct mass. Refeyn analysis also indicates a oligomeric state organization. The small data set provides 2D classes of the non-polymerized particles, indicating a good variety of orientation. The assembled filamentous are also notable, but ware not included on the 2D classification.

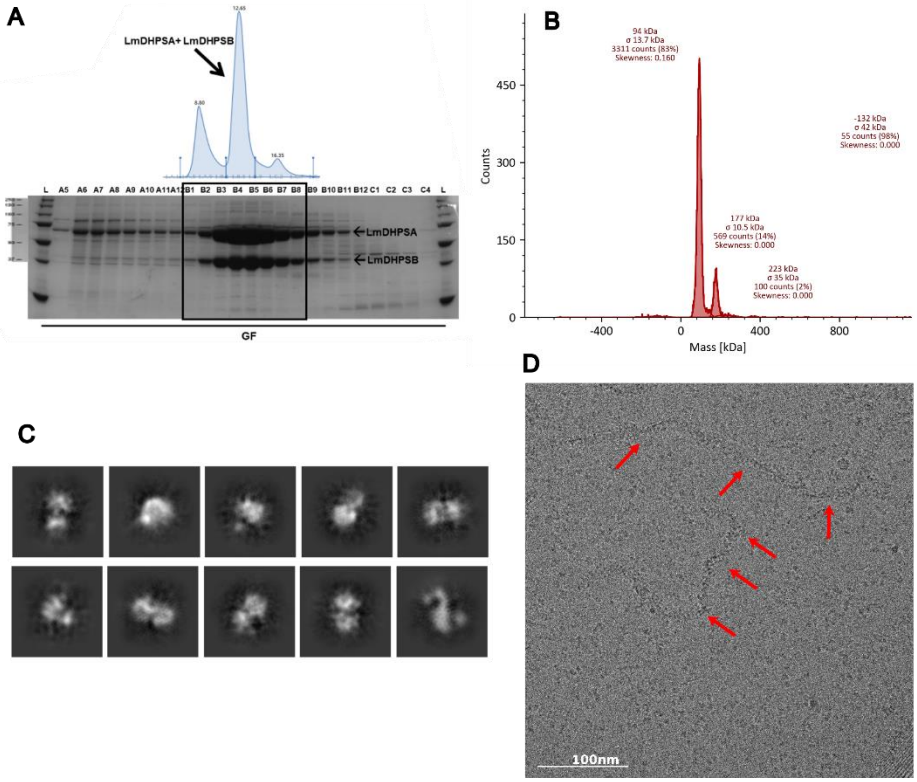

Figure 1 (A) and (B) show that the LmDHPS heterocomplex was obtained with high purity. The correct mass for the isoforms was also determined: LmDHPSA - Expected: 61510.55 Da, Found: 61511 Da; LmDHPSB- Expected: 40796.24 Da, Found: 40796 Da. (C) 2D classes generated from a small data set collected at Glacios microscope, not considering the polymerization. (D) Micrograph of LmDHPS cryo-em grids, assembled filamentous polymer are visible (red arrows).