

Supervisor(s):

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Host laboratory:

Lab: Institut de Biologie Structurale

Host group/team:

MICA (Microscopic Imaging of Complex Assemblies)

Title of the M2 research internship:

Investigating Endogenous Cellular Filaments Using Cryo-EM of Cell Extracts

Project summary:

This project will utilize an innovative, biochemistry-minimal approach to structurally characterize cellular filaments directly from cell extracts. Leveraging the cutting-edge method of integrative cryo-electron microscopy (cryo-EM), the student will enrich and identify endogenous filamentous structures, such as actin filaments previously observed in *Physarum polycephalum* (see Figure 1, structures 6 and 14). This approach avoids classical biochemical purification and overexpression, preserving native interactions and complexes. The student will explore strategies to selectively concentrate filamentous assemblies in cell extracts, optimize cryo-EM imaging conditions, and analyze resulting data to obtain high-resolution structures. This pioneering work promises insights into native filament organization and dynamics, significantly advancing our understanding of cellular architecture.

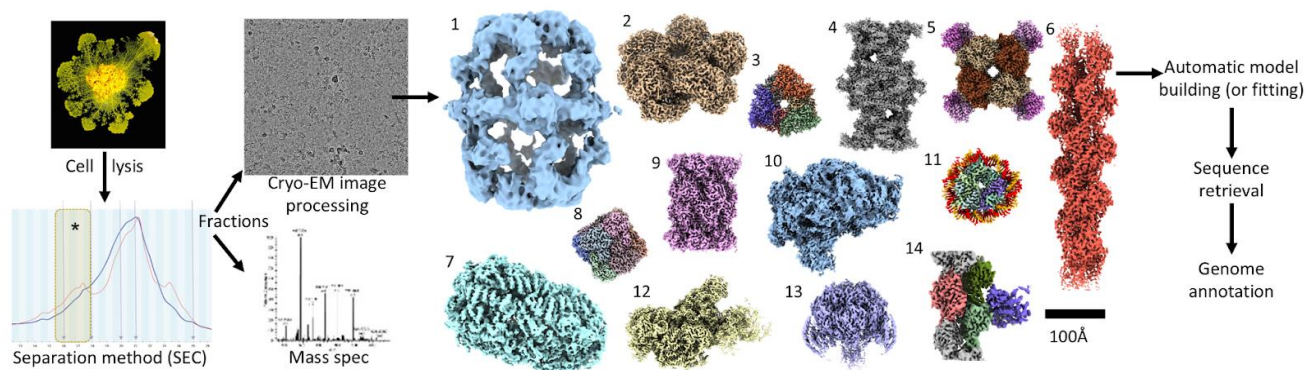


Figure 1: Simplified cell extract analysis workflow and application to *P. polycephalum*. The soluble part of the cell lysate was separated by size-exclusion chromatography and one MW range (zone marked with an asterisk) was used for cryo-EM grid preparation and mass spectrometry analysis. From a 45k micrographs dataset, the obtained structures, their corresponding number of particles (ptcls) and resolution are as follows: **1.** Fatty acid synthase - 52 ptcls - 12 Å **2.** Amino acid decarboxylase - 28 481 ptcls - 2.3 Å ; **3.** Glutamate decarboxylase - 131 989 particles - 2.6 Å ; **4.** 3-methylcrotonyl-CoA carboxylase - 2 179 ptcls - 3.3 Å ; **5.** Thiamine synthase - 125 602 ptcls - 2.0 Å ; **6.** Actin filament - 74 660 ptcls - 2.9 Å ; **7.** Unknown "cage" protein - 5 865 ptcls - 4 Å ; **8.** PDX1.2/1.3 - 32 021 ptcls - 2.6 Å ; **9.** 20S proteasome - 50 407 ptcls - 2.6 Å ; **10.** Ribosomal large subunit - 8 338 ptcls - 3.9 Å ; **11.** Nucleosome - 76 583 ptcls - 2.8 Å ; **12.** Ribosomal small subunit - 47 092 ptcls - 2.6 Å ; **13.** V1-ATPase - 36 021 ptcls - 3.1 Å ; **14.** Actin-myosin - 13 189 ptcls - 3.4 Å

Keywords:

Cryo-EM, Integrative Structural Biology, Cell extracts, Filaments

Relevant publications of the team:

This new research project has not yet given publications (several in preparation).