Cyclodextrins Self-assembling for cryo-EM structure of flexible proteins

CORSET

Abstract

The project aims to surpass the current state-of-the-art methods in cryo-EM by enabling the determination of complete 3D structures of proteins, including their flexible domains. We propose a novel strategy: encapsulating proteins within cyclodextrin-based supramolecular scaffolds of precisely defined dimensions. This approach will impose constraints on their flexibility, thus limiting the range of conformations they can adopt and facilitating the resolution of their 3D organization.

Our methodology leverages the advanced supramolecular chemistry of cyclodextrin-based assemblies to design functional, dimensionally precise scaffolds. These scaffolds will be modular, adaptable, and capable of selectively binding specific protein domains modified with tags commonly used in biochemistry. In the field of supramolecular chemistry, the proposed self-assembly of functional building blocks is completely original, and the application involving attachment to a protein to constrain its flexibility is unprecedented.

We will develop this strategy to determine the structure of proteins involved in specific region of contact between organelles in eukaryotes. These regions, named membrane contact sites (MCSs), are constituted by protein that bridge apposed organelles and perform major cellular functions, such as lipid transfer, calcium signaling, and organelle dynamics. MCS dysfunction is involved in human diseases, including cancer, obesity, and neurological disorders. The determination of the structure of these proteins remains limited to rare proteins by the presence of numerous flexible domains. We will study VAP-A-OSBP complex that translocate cholesterol from ER to Golgi apparatus.

This highly interdisciplinary approach is organized around a new collaboration between P1, expert in supramolecular and synthetic chemistry (M. Sollogoub, UMR 8232 SU CNRS) and P2 with expertise in structural biology and biophysics (D. Lévy UMR 168 Institut Curie, CNRS, SU). Both teams have the human resources, expertise, instruments, including a state-of-the-art cryo-electron microscope, and computing power to provide a favorable framework for the realization of a PhD thesis.