Universität Basel

Research Project

Fast protein-complex isolation, sample preparation and data processing for high-resolution structural analysis and visual proteomics

Third-party funded project

Project title Fast protein-complex isolation, sample preparation and data processing for high-resolution structural analysis and visual proteomics
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Organisation / Research unit
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Department
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Major bottlenecks in structure determination of macromolecules by cryo-EM are: (i) the protein complexes must be produced in significant amounts for subsequent structural analysis. Unfortunately, many protein complexes of (biomedical) interest are sparsely produced in eukaryotic cells. (ii) The destabilization of complexes during isolation must be countered. Protein assemblies are significantly diluted on isolation and their inter-molecular interactions are destabilized. This leads to the dissociation of many complexes formed transiently during biological processes. (iii) The data analysis of heterogeneous samples, as they arise due to the stochastic interaction networks, is still cumbersome. Nevertheless, precisely these “interactomes” are of great interest in biological research. The above difficulties can be avoided by, first, minimizing the overall sample consumption, and, second, by reducing the time required to isolate the target complexes and prepare samples for cryo-EM. Protein isolation should be fast (one step, approx. 1h) and produce samples clean enough for imaging by cryo-EM. Ideally, the protocol should be independent of protein modifications, such as tags, as these can interfere with the biological function and assembly of the target complex. Additionally, stabilization of the complex by new cross-linking methods might be desirable directly after cell lysis. Last but not least, sample preparation for cryo-EM should be accomplished in a loss-less manner: to date, more than 99% of the protein is lost during blotting steps when classical cryo-EM grid preparation methods are used. This project aims (i) to establish a method to rapidly extract target proteins and their complexes from minimal amounts of cell lysate, and, (ii) to develop a loss-less cryo-EM grid preparation system that only consumes minute amounts of sample (5 nl) and does not involve any blotting steps. In this framework we will also explore the use of microfluidic cross-linking strategies to stabilize protein-complexes. In addition, methods for structural analysis by the single particle cryo-EM approach and de novo identification of complex subunits or interaction partners will be developed and tested on a single particle level (“visual proteomics”). Major bottlenecks in structure determination of macromolecules by cryo-EM are: (i) the protein complexes must be produced in significant amounts for subsequent structural analysis. Unfortunately, many protein complexes of (biomedical) interest are sparsely produced in eukaryotic cells. (ii) The destabilization of complexes during isolation must be countered. Protein assemblies are significantly diluted on isolation and their inter-molecular interactions are destabilized. This leads to the dissociation of many complexes formed transiently during biological processes. (iii) The data analysis of heterogeneous samples, as they arise due to the stochastic interaction networks, is still cumbersome. Nevertheless, precisely these “interactomes” are of great interest in biological research.ă

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