Internship project Master M2

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Title: Development of an image analysis approach to analyze conformational variability of biomolecular complexes by X-ray free electron laser (XFEL) technology

Goals: Adaptation of a cryo-electron microscopy (cryo-EM) data analysis methodology to XFEL data

Recent progress in instrumental and software developments for single-particle cryo-electron microscopy (cryo-EM) has allowed near-atomic structural resolution of various biomolecular complexes [1, 2]. This progress has made cryo-EM competitive with X-ray crystallography that used to be the primary high-resolution structural biology technique. While X-ray crystallography allows obtaining structures of complexes in their crystallized form, cryo-EM allows obtaining their structures in solution under cryogenic conditions (vitrified samples), which facilitates studying large and flexible complexes that are difficult to crystallize. Such large and flexible complexes could ideally be studied under physiological conditions using X-ray free-electron laser (XFEL) technology [3]. Single-particle XFEL experiments in solution are currently being developed to allow studying structure and dynamics (conformational changes) of complexes. This M2 internship project aims at developing a new data analysis approach for characterizing conformational changes of complexes using single-particle XFEL data collection. The conformational changes are linked to biological functions of complexes (e.g., protein synthesis, cellular transport, etc.). To achieve these functions, the complexes undergo large conformational transitions. Different conformations can coexist and their characterization is crucial to understand the functional mechanisms and to develop new drugs. The new single-particle XFEL data analysis approach to conformational heterogeneity will be inspired by a single-particle cryo-EM data analysis approach developed in our group.

The principle of single-particle XFEL is similar to the principle of single-particle EM, but there are also some important differences between the two techniques. The main difference is that data collected by EM are real-space images while data collected by XFEL are diffraction patterns, meaning that only amplitudes are measured by XFEL and the corresponding phases must be determined computationally. Also, diffraction-pattern pixels have low intensities and many zero values further from the detector center. As in single-particle EM, the single-particle XFEL acquisition of the entire 3D information requires combining 2D data from many copies of the complex in different orientations and determining the orientation of each 2D data computationally [4]. Averaging data from many copies of the complex increases the signal beyond that available in the data of a single copy of the complex. In the case of conformational heterogeneity of the sample imaged by EM, 2D and 3D classifications can be performed to sort data into different conformations under the hypothesis that the conformational heterogeneity can be described in terms of discrete conformational changes (conformational changes with a countable number of intermediate states). However, particularly challenging is the problem of interpreting data in terms of continuous conformational changes of complexes (conformational changes with an uncountable number of intermediate conformational states).

Our group has pioneered the development of cryo-EM image analysis approaches to continuous conformational variability. Our Hybrid Electron Microscopy Normal Mode Analysis (HEMNMA)

methodology interprets the conformation in each cryo-EM single-particle image by comparing it with 2D projections of a 3D reference model deformed using Normal Mode Analysis (NMA, which is a method for molecular mechanics simulation) and it has been used with complexes of various sizes and architectures [5, 6]. Such cryo-EM approaches could be used to analyze XFEL data but should be modified to take into account the different nature of the two types of data.

This M2 internship project will be focused on establishing the basis of the new single-particle XFEL data analysis approach that will be inspired by HEMNMA. The methods developed during this project will be validated using synthetic and experimental data. This research can be continued in the framework of a PhD thesis on new approaches to analyzing continuous conformational changes of complexes by single-particle XFEL.

REFERENCES

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