Internship offered in M2
2018-2019

Responsible for internship

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Internship topic: **Development of a new X-ray free electron laser data analysis approach to conformational variability of biomolecular complexes**

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 should ideally be studied under physiological conditions. Single-particle X-ray free-electron laser (XFEL) experiments in solution are currently being developed to allow such studies [3]. This M2 internship project aims at developing new data analysis approaches for characterizing structure and dynamics (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 approaches to conformational heterogeneity will be inspired by the existing single-particle cryo-EM approaches. 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 (a countable number of conformational states). However, particularly challenging is the problem of interpreting data in terms of continuous conformational changes (an uncountable number of conformational states).

The M2 internship project will be done in the group that pioneered the development of single-particle cryo-EM image analysis approaches to continuous conformational variability. Their Hybrid Electron Microscopy Normal Mode Analysis (HEMNMA) methodology interprets the conformation in each cryo-EM

single-particle image by comparing this image with 2D projections of a 3D reference model deformed using Normal Mode Analysis (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 internship project will be focused on establishing the basis of a 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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2. Khatter, H., Myasnikov, A.G., Natchiar, S.K., and Klaholz, B.P. (2015). Structure of the human 80S ribosome. Nature *520*, 640-645.
3. Miyashita, O., and Joti, Y. (2017). X-ray free electron laser single-particle analysis for biological systems. Curr Opin Struct Biol *43*, 163-169.
4. Yefanov, O.M., and Vartanyants, I.A. (2013). Orientation determination in single-particle x-ray coherent diffraction imaging experiments. J. Phys. B: At. Mol. Opt. Phys *46*, 164013.
5. Jin, Q., Sorzano, C.O., de la Rosa-Trevin, J.M., Bilbao-Castro, J.R., Nunez-Ramirez, R., Llorca, O., Tama, F., and Jonic, S. (2014). Iterative elastic 3D-to-2D alignment method using normal modes for studying structural dynamics of large macromolecular complexes. Structure *22*, 496-506.
6. Jonic, S. (2017). Computational methods for analyzing conformational variability of macromolecular complexes from cryo-electron microscopy images. Curr Opin Struct Biol *43*, 114-121.

Techniques involved: Image analysis methods development, molecular mechanics simulation, statistical analysis, X-ray free-electron lasers

Paid internship: Yes
Can this internship be continued for a PhD? Yes
If yes, type of PhD funding envisaged is: TBD