Self-reflection II

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Topic: Atomic resolution cryo-EM
By: Sriram Subramaniam on 8 – May – 2017 / Seminar: Exploring the Complexity of Life by Cryo-EM

Introduction

Cryo-EM (Cryo Electron Microscopy) presents an alternative to more established X-Ray Crystallography for determining 3 dimensional structures of bio-molecules (specially for proteins). It allows the examined molecule to stay in near native conditions while scanning, unlike X-Ray crystallography, where the molecule is required to be isolated and formed into a crystal. Sriram Subramaniam talked about advancements in Cryo-EM[1], specifically about increased resolution and its application. I selected this presentation for my self reflection because 3 dimensional structures are important for in-silico analysis, specially for homology modeling. Knowing the advantages and limitations of the method is therefor essential for selecting best possible input for analysis pipeline.

Background

Cryo-EM is a form of transmission electron microscopy (TEM), which involves imaging of sample using electron beams at cryogenic temperatures where the sample is embedded in a carbon based grid[2]. The protocol is less “invasive" than X-Ray crystallography, mainly because the sample is studied in conditions similar, if not identical to the original biological conditions. On the contrary, X-Ray crystallography requires the sample to go through drastically different conditions, which does not necessarily correlate well with biology and may alter the structure itself, which is detrimental to the whole structure analysis pipeline. Sriram Subramaniam gave examples[3] from his lab where his group was able resolve structures with resolution $<$3Å, and illustrated the degree of "fineness" in the structures(noticing how they were able to se the rings and empty spaces in between). Coupled with this, he also talked about another technique, Atomic-force microscopy[4] which employs a radically different method and gives even finer resolution, but at cost of control and is not yet suitable for complex biomolecules. Atomic-force microscopy can resolve structure in a sequential manner, by determining atoms, and then assembling the data to form pictures with the primary difference being that it can detect the 3 dimensional arrangement natively, unlike Cryo-EM, which infers the 3 dimensional structure from multiple 2 dimensional images.

Reflection

Cryo-EM has many benefits over X-Ray crystallography, with relatively less drawbacks[5]. In Bioinformatics and computational biology, where the source data determines how accurate predictions are, it is imperative that the source itself is relatively error free. Cryo-EM presents structures which are more closer to their native states than the states used in X-Ray crystallography. This has many benefits, including but not limited to, computer aided drug discovery[6]. In drug discovery, the quality of protein structure is of paramount importance because most of the algorithms keep the structure stable, hoping that it reflects biological state and scans the surface for potential drug binding site. In case of high resolution Cryo-EM, structures are resolved with enough confidence to determine subtle peculiarities in binding sites, thus aiding in drug discovery and better understanding of disease causing agents (Sjors Scheres talked about application of Cryo-EM in Alzheimer’s disease[7] in same symposium).
It also presents an interesting opportunity in field of software development and optimization. Since increase in Cryo-EM resolution is relatively new, available applications for analyzing image data don’t necessary form consensus and may give different output. This presents an opportunity for further analysis of method itself, and creation of better tools to analyze the data. Cryo-EM can generate data for multiple "states" in each run, giving a rough overview of the multiple states available for a particular molecule by isolating different images corresponding to different states. This is computationally intensive, and is another area of active software development in Bioinformatics.

In conclusion, I would say that Cryo-EM is a promising method, one which can not only identify the 3 dimensional structure, but can also help in identification of transition between structures at high resolution. More interestingly, Atomic-force microscopy was a new method for me, and is even more promising for future development, if not for the software for the method itself.

References

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