

Potential of protein x-ray crystallography in drug design



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THE IMMENSE POTENTIAL OF PROTEIN X-RAY CRYSTALLOGRAPHY IN DRUG DESIGN

Crystallography is an indispensable technique in many areas of science, enabling us to determine molecular structures in a deeper fashion than other available methods, such as mass spectroscopy. Although at first it was mainly a field of study for mineralogy, dealing with simple shapes and crystal lattices, in 1895, Wilhelm Röntgen discovered X-rays, marking the transition to a new era in the domain. Less than two decades later, the X-ray irradiation of salt crystals was shown to produce diffraction patterns, revealing the atomic periodicity of the compound. About 20 years later, protein crystallography started, uncovering a new generation of discoveries in the macromolecular structures of the organisms.(1)

Many years the standard laboratory X-ray sources were sealed tubes. A lamp filament shaped cathode emitted electrons that were accelerated to energies of hundreds of kilovolts, and the rays were produced by their impact on metal anodes. Later, the classic anodes were changed with rotating-anodes as they could resist more and give more intense rays. Nowadays, where synchrotron radiation is not accessible, the rotating-anode sealed tubes are still used. . Synchrotron is one of the newer technologies, as it was proven that it can be used as a much stronger X-ray source, thus generating a higher resolution.(1)

Early on, the data was collected by photographic films and precession cameras. Given the weak diffraction of protein crystals and their faster decay, it is unquestionably necessary to have a simultaneous collection of

the weak diffraction spots spread along a large area together with a high spatial resolution. This was accomplished using X-ray-sensitive film.

Nowadays, as a result of the cutting-edge developments in the domain, the most widespread methods for PX (protein crystallography) data-collection are CCDs (charge-coupled devices) and CMOS (complementary metal oxide semiconductor).(1)

Even if the new technology is very beneficial, the actual preparations for the experiments are often difficult. Biomolecules' crystallization is made in 2 indivisible steps. Firstly, the molecules have to achieve an ordered state from their disordered one, in a difficult process called nucleation. The next phase is the crystal's growth, an easier step because of its better-known mechanisms.(7)

A crucial step while crystallizing a protein is that the species remain the same within the crystallization procedure. This implies that the molecule needs to have a good compositional stability, or, in other words, to be homogenous. The homogeneity of a protein specimen is typically determined using electrophoresis or mass spectroscopy. For example, if the protein leads to a single band on a SDS-PAGE electrophoresis gel, this is a proof of its stability. Still, there is no guarantee that if the protein is shown to be stable on the experiments' conditions, it will be stable as well over the period of a crystallization procedure.(3)

Compositional homogeneity is an essential requirement, but not a sufficient one. The protein must also possess conformational stability or conformational order to be able to crystallize. A vast variety of proteins,

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called intrinsically disordered proteins or IDPs show a considerable amount of conformational disorder. Consequently their crystallization is very hard, thus they are generally avoided by crystallographers. For determining those proteins' structures, the most convenient method is using NMR spectroscopy.(3)

After a crystal is formed, it has to be harvested, which means to move it from its growth place to the X-ray apparatus. The first step is looping, which implies the placement of the crystal in a microloop found on a mounting pin. Then, usually the crystal undergoes treatments like cryoprotection, reducing the destructive effects of the X-rays on the molecular structure. After all these procedures, the X-ray machine can be used, the diffraction patterns of the protein crystal can finally be collected and analyzed.(4)

Because biological macromolecules are dynamic systems, knowing the static three-dimensional structures is not always enough. Time-resolved crystallography produces some sort of films of the proteins' behavior. One of the methods is the Pump-Probe Method, where a short laser pulse initiates a reaction. After a previously known time, an X-ray beam is sent, enabling us to see the reaction status at that given moment. Then, the sequence is repeated. In other situations, the laser pulse is followed by subsequent X-ray beams, enabling us to see the protein's state at more than one given time. (6)

The 3D structures of biological macromolecules are considered the uppermost standard of characterizing their architecture. The obtained

structures can give a richness of information for drug discovery attempts utilizing CADD (computer-aided drug design). (2)

Although there are many methods for determining molecular structures, X-ray crystallography is especially good one for drug discovery, because this method is capable of producing very high resolutions, sometimes even at atomic-scale, and this method enables the mapping of large heteromeric complexes, such as ribosomes. Furthermore, and one of the most important advantages is that X-ray crystallography can grant evidence of the binding way of small ligands in the crystal.(2)

The amount of macromolecular structures available is growing exponentially. The wholly use of the flourishing structure data, along with the abundance of other types of biological data available (such as amino acids, metabolic pathways, expression patterns) is a substantial challenge for data mining in a variety of medical applications, such as drug design.(2)

Although the structures deposited to the Protein Data Bank (PDB) are generally high quality ones, not all structural details are optimum. The precise spatial coordinates of all the atoms in a structure can be derived exclusively from the experimental maps for high-resolution structures. If the structure has a lower resolution, previous information, like chemical identity and stereochemistry, must be used to figure out the atoms positions in the molecule.(2)

Selenium was shown to have a use as an anomalous scatterer. This has been a huge progress as it made multi-wavelength anomalous dispersion (MAD)

the main technique for determining new protein structures. The advantage is <https://assignbuster.com/potential-of-protein-x-ray-crystallography-in-drug-design/>

that the sulfur in methionine can be replaced with selenium generating selenomethionine, thus the element will be incorporated in the protein. For nucleic acids, bromine can be used to modify the nitrogenous bases, having a similar effect as selenium for proteins.(5)

One of the advances in drug design is in the understanding of body receptors. For example, the structurally-based design of ligands for GPCRs (G protein-coupled receptors) had a considerable increase, mainly because of the clarification of the X-ray produced structures.(8) These receptors can be found in the cell plasma membrane, mediating the amount of received stimuli by secreted signal molecules. Improvements in GPCR crystallography headed the scientists to structures for more receptors, offering a better understanding of body mechanisms.(9)

X-ray crystallography was shown to be a good approach for finding cures for deadly diseases, such as HIV. The creation of viral enzymes and other factors necessary to produce mature virions is due to HIV-1 protease. The investigations using X-ray imaging contributed deeply to the understanding of the enzyme's function, helping the experts find drugs that can disrupt the protease's activity.(10)

Neurological diseases are also a domain where structure-based drug design (SBDD) using X-ray crystallography and spectroscopic methods (such as NMR) has appliances. For instance, GABA-AT protein, a neurotransmitter responsible for muscular activity control, has been studied using SBDD.(11)

X-ray crystallography is a very important technique enabling us to see macromolecular structures, and with the new innovative technologies, at an <https://assignbuster.com/potential-of-protein-x-ray-crystallography-in-drug-design/>

unprecedented amount of detail. This helps in the search for new drugs by knowing the target they should affect, thus having an idea about the chemical characteristics of the drug that is to be synthesized. The developments in the past decades in crystallography and spectroscopy helped tremendously in finding cures for many diseases, therefore revolutionizing medicine.

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