Development Status of Protein Crystal Automounting Robot System

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Abstract

This paper introduces the working environment and functional requirements of the current protein crystal automounting robot system. According to the sample positioning method, the system is divided into two categories for review. The hardware composition and functional characteristics of the current automounting robot system in the world are summarized and analyzed, and the future development trend is prospected.

Keywords

Automounting robot; Crystal diffraction; Synchrotron radiation source; High-throughput protein crystallography.

1. Introduction

Proteins play a key role in various important basic biological processes, such as being able to play important functions in the cell cycle by regulating processes such as signal transduction, metabolism, and molecular recognition [1-3]. Studies have found that proteins are directly linked to many important diseases, such as Alzheimer disease (AD), cardiovascular disease, and cancer. Therefore, it is necessary to understand their functions by studying their structure [4]. More than 90% of the known protein structures in the protein data bank (PDB) are analyzed by X-ray crystal diffraction. Therefore, improving the efficiency of protein crystal diffraction has become one of the key points in protein structure research [5-7] . Designing a high-throughput crystallographic automation system, from protein purification, crystal growth, crystal diffraction, to data modeling, has become a hot topic in the current cross-research of structural biology, automation control, and robotics [8-11].

There are currently two effective ways to conduct crystal diffraction experiments, indoor X-ray machines and synchrotron X-ray diffraction line stations. The world's well-known synchrotron light sources include American ALS and APS, European ESRF, Japanese Spring-8, Swiss SLS, and Shanghai Synchrotron Radiation Facility (SSRF). These light sources can provide support for multidisciplinary innovation research. With the rapid development of structural biology, the number and size of research groups are increasing rapidly. At present, there is a huge gap in demand for X-ray diffraction stations each year. In addition to building more crystallographic beamlines, improving the efficiency of current beamlines is also an effective solution [12].

The traditional mounting mode for crystallographic diffraction experiments is that the experimenter uses a metal clip and other tools to quickly remove the needle holder with a protein crystal sample from liquid nitrogen and install it on the angle measuring head. The loading speed of the sample is limited by the user's experience and the operation time of the rebuilding safety interlock protection of the diffraction experiment line station, and the utilization efficiency of the machine is low. Compared with the traditional manual loading mode, the use of the automounting system can effectively avoid the waste of machine time caused by interrupting the experiment and rebuilding the safety interlock. The user no longer needs to frequently enter and exit the experimental shed with

radiation danger and the operation is stored in the Crystal samples in low-temperature liquid nitrogen achieve accurate and fast protein crystal mounting, which significantly improves the experimental efficiency.

2. Robot system working environment and functional requirements

2.1 Characteristics of the working environment

2.1.1 Sub-section Headings

The biological macromolecule experimental beamline station uses high-intensity X-rays, and its photon energy range can reach 5-18keV. The protein crystal sample is exposed to high-intensity X-rays, so the arrangement order inside the crystal will be quickly destroyed, and sufficient diffraction data cannot be collected. Therefore, the crystal sample needs to be stored in an ultra-low temperature environment for freezing and storage A liquid nitrogen dewar was set up to store experimental crystal samples.Consequently, the main working environment characteristics of the biological macromolecular experimental beamline are strong ionizing radiation and ultra-low temperature environment.

2.2 Functional requirements of automounting robot

According to the above experimental requirements and characteristics of the experimental environment, the functional requirements of the crystal automatic sample loading robot areunder the remote instruction of the experimenter, and it can be accurately and quickly grasped from liquid nitrogen in a beam line station with a small space and strong ionizing radiation. The sample is placed at the sample loading point, and the crystal sample replacement process is completed safely and efficiently. Therefore, the damage to the surrounding precision instruments due to improper human operation is avoided, and the experimenters are kept away from the strong ionizing radiation and low temperature liquid nitrogen environment, ensuring the safety of equipment and experimenters.

It takes an average of 3 minutes for a skilled laboratory technician to manually load a sample. After the automounting robot is used, the process of experimenters entering and exiting the experimental beamline station, interrupting the experiment and re-establishing the safety interlock is eliminated, greatly reducing the mounting time, and using the robot is 3 to 4 times more efficient than manual mounting.

3. Basic laboratory equipment for beamline stations

3.1 Beamline station basic equipment

The main basic equipment of the biological macromolecule experimental line station is a diffractometer and a detector. The diffractometer is equipped with a sample goniometer system to rotate the sample. The angle of the goniometer is the sample point of the crystal sample. The detector is a cube located at the end of the beamline and used to receive the diffraction signal [13].

3.2 Sample container

The sample pin base is put into a sample container for storage and experiment. The sample hole on the sample container is numbered. The coordinate position of the sample on the container can be determined according to the number, which can be used for positioning and grasping by the robot. Currently, it is based on the type of sample needle holder There are 6 common sample containers: SSRL Cassette, ACTOR Magazine, Cryo-vial Basket, ALS Puck, Uni-Puck, MiniSPINE Puck.

3.3 Crystal sample pin base

The current mainstream crystal sample pin base is shown in Figure 1. It adopts a uniform size. The base has a large outer diameter of 12 mm, a small outer diameter of 9.7 mm, and a height of 21.5 mm. The ring at the front is used to hold the crystal and can be placed in liquid nitrogen Cryopreserved crystals. In the X-ray diffraction experiment, the purpose of changing the crystal sample is achieved by transferring the sample needle holder. There is a two-dimensional code mark on the bottom of the

needle base for easy identification and magnetism. So far, there are 7 types of sample needle holders: Hampton magnetic, Hampton copper magnetic, ALS-style, Mitegen B3S, SPINE, MiniSPINE, New Pin.

Figure 1. Sample Pin Bases

(left: Hampton copper magnetic; medium: Hampton Magnetic; right: SPINE)

Mitegen B3S features a unique design compatible with MicroRT ™ capillaries for room temperature screening and crystal dehydration studies.

MiniSPINE and NewPin, which are in the promotion and testing stage, were developed by EMBL and tested and used in ESRF. Its unique compact design greatly increases sample capacity. Under the storage space of Uni-puck size, it can accommodate up to 16 mainstream specifications sample holders, 36 MiniSPINE needle holders, and 64 NewPin. NewPin uniquely uses radio frequency identification (RFID) tags for identification to track and identify crystal information [14].

4. Research status of protein crystal automatic mounting robot system

The protein crystal automatic mounting robot system is mainly composed of 3 parts: an auto-filled liquid nitrogen sample storage part, a sample acquisition part, and a sample transfer part. According to the hardware configuration of the sample transfer part, the currently used automatic mounting system is mainly divided into two categories according to the sample positioning and grasping methods: Dewar mobile positioning and robotic arm positioning.

4.1 Dewar mobile positioning type automounting robot system

4.1.1 SPACE

The RIKEN structural gene beamline of Spring-8 in Japan developed the automatic loading robot SPACE in 2002. SPACE consists of a three-axis robotic arm and an XY translation stage[15].

SPACE sample pin has a 'turnbuckle-like' structure, which consists of a cylindrical body and two screw rods along the revolving axis[16]. One of the two screws is left-handed and the other is right-handed. The mount arm is a stainless steel rod with a left-handed screw hole at the end. The sample goniometer also has a right-handed screw hole on the spindle axis. Therefore the pin can be mounted or dismounted on the goniometer by a simple rotation of the rod. The method of rotating the thread ensures that the direction and position of the sample pin mounted on the goniometer are uniquely determined. This feature is used in conjunction with the XYZ translation stage of the goniometer to perform unmanned automated data collection at night through the crystal sample position determined during the day sample evaluation process.

4.1.2 ALS automounting robot

The first generation of automatic loading robot developed by ALS uses a three-axis Dewar translation stage solution. A MySQL database system is configured to record crystal information, such as Puck ID, location tag, and affiliated unit. The liquid nitrogen control device is in a dynamic Dewar, so it is difficult to ensure the reliability of its control system, and the condensed water in the sample Dewar can often accumulate on the translation stage, which may cause the malfunction of the translation stage and the motor [17].

In order to solve the shortcomings of ALS I, the second-generation automounting robot of ALS has improved the sample Dewar tank. The Puck support plate is suspended in the Dewar tank by a 3-axis translation bracket. It is removed from the area where condensed water is easy to form, and an auxiliary liquid nitrogen chamber connected with the liquid nitrogen chamber of the sample is added to play a certain buffer function, better controlling the liquid nitrogen level.

4.2 Dewar mobile positioning type automounting robot system

4.2.1 SAM from Stanford Synchrotron Radiation Laboratory

In 2002, SSRL released the SAM automounting robot. SAM uses EPSON's ES553 4-axis robot with a pneumatic gripper[18]. The end of the gripper is similar to the traditional cold forceps, used to keep the crystal sample cold during the transfer process. The pliers also include two clamping rods for gripping the dumbbell magnet tool. The tool has been working in a liquid nitrogen environment. Two strong magnets, one strong and one weak, are used at both ends of the tool to remove and return the sample pin from the sample container.

The sample container has a cylindrical design and can hold up to 96 sample pins. The Dewar can hold 3 sample containers and is equipped with a dumbbell magnet holder for placing dumbbell magnets. The Dewar tank is also equipped with a thermal insulation cover, which can be automatically opened and closed when the gripper enters and exits, to prevent long-term exposure of liquid nitrogen in Dewar to the ambient temperature environment to generate ice crystals that affect the sample.

To further improve system efficiency, an air-knife (EXAIR Super-Air-Wipe # 2402) was added during the heating/drying of the gripper. It is used to quickly remove ice particles or moisture attached to the surface of the grasping arm and prevent frost from damaging the grasping hand and the crystal sample [19].

4.2.2 ACTOR

Rigaku's ACTOR is the first commercial automatic crystal mounting robot. As shown in Figure 2, using Mitsubishi's RV-6SD industrial robot can screen up to 30 crystal samples per hour. Dewar support plate supports ACTOR, ALS, Uni-Puck sample container[20]. The heating cover plate is used to prevent liquid nitrogen frost in Dewar, and the center hole position of Dewar cover is calibrated by the conical cover to ensure that the gripper can enter and exit Dewar smoothly.

Figure 2 ACTOR developed by Rigaku

4.2.3 ISARA

ISARA is the latest generation of automatic loading robot of IRELEC[21], which uses Staubli's TX60L industrial robot arm. The system is compatible with Uni-Puck, MiniSPINE, Cryo-vial Basket, and is equipped with SBS crystal plate gripper, which can perform crystal in-situ diffraction experiments.

The ISARA dewar can isolate the cooling area of the gripper from the sample area to prevent the ice crystals on the gripper from falling to the sample storage area and affecting the crystal sample. This design also limits the boiling area of liquid nitrogen when the gripper cools, helping to maintain the liquid nitrogen level evenly. The system is equipped with a precise laser sensor to accurately and automatically learn the position of each reference point from the experimental environment.

4.2.4 BART

In order to prevent the Dewar cover from freezing, Diamond Light Source's BART is equipped with 12 heating elements on the edge of the Dewar tank, which are divided into 2 groups of 6 each, and are monitored by thermocouples[22].

BART also adds a vision system to the robot. It enables it to identify the opening in the Dewar cap and track its position. This position can be fed back to the robot, so that the robot can autonomously find the position to enter the dewar. In addition, a second camera is configured to read the QR code information of the sample pin base.

4.2.5 RoboDiff

ESRF's RoboDiff includes a high-capacity dewar (HCD) that is able to accommodate 240 SPINE standard sample holders, a Stäubli TX60L six-axis robot for sample transfer and a high-accuracy air-bearing rotation axis acting as the goniometer.

RoboDiff controls all I/O devices associated with the system through the Ethernet controller of the robot CS8C controller [23], which facilitates the control system to achieve cross-checking and synchronization, and improves system reliability. Integrate error diagnostic information at all stages of equipment operation to track errors and enable equipment to recover from these failure conditions.

RoboDiff innovatively combines the sample gripper with a goniometer and directly uses the magnetic head on the goniometer head to adsorb and transfer the sample [24]. This method reduces the complexity of the system and integrates all functions into a single device, greatly reducing difficulty in system troubleshooting and recovery.

For the high accuracy requirements of diffraction experiments of small crystals, the movement of the RoboDiff robot arm is only used during loading and unloading. Once the robotic arm is in a pre-set position, the sample can be optically aligned with the X-ray beam position using the Y/Z translation stage at the bottom of the robotic arm. This translation table has a range of ±1.4 mm for both vertical and horizontal movements, which are encoded to 120 and 20 counts mm^{-1}, respectively.

4.2.6 FlexED8

The FlexED8 developed by the European Molecular Biology Laboratory is based on a six-axis industrial robot, equipped with a tool changer, can be configured with multiple tool grippers, supporting the new MiniSPINE and NewPin.

The system's sample storage Dewar uses a new open structure with a built-in ice crystal filtration system [25]. Ice crystals floating in the sample Dewar can flow from the top edge, and the sunken ice crystals are discharged from the bottom of the Dewar. The liquid nitrogen contaminated with ice is returned to the main Dewar tank, filtered and de-iced, and reinjected into the sample dewar. This design not only avoids ice damage to the sample, but also greatly simplifies the operation process of the manipulator, eliminating the need to open and close the dewar cover of the traditional sample changing process, and improving system efficiency.

5. Conclusions and prospects

The protein crystal automounting robot system can be superior to manual operation forrealizing rapid and accurate transfer of protein crystal samples under strong radiation and ultra-low temperature environment. Not only improves the experimental efficiency, but also prevents the artificial equipment from being damaged due to misoperation.

This article introduces the characteristics and application of the mainstream automatic sample loading robot systems of major light sources in the world, and summarizes the related technology and development issues:

(1) The existing automounting system can be divided into two types according to the positioning method of the sample, that is, the direct positioning of the robotic arm to grab the specified sample and the movement of the dewar to move the specified sample to the grasping position.

(2) Except for the latest NewPin, which uses radio frequency identification (RFID) tags, other sample needle holders use two-dimensional code identification to record sample information.

(3) Sample storage Dewar is divided into two types: open and closed. The open type reduces the effect of ice crystals on the sample through a complex chassis structure and filters. The closed type reduces the generation of ice crystals by reducing the contact time of low-temperature liquid nitrogen with the external environment through the Dewar cover.

(4) In order to ensure the stability and safety of the manipulator in and out of Dewar, a vision module has been added to the manipulator to determine the coordinates of Dewar's grip hole in real time through the camera for the manipulator to safely enter and leave Dewar.

(5) Fault diagnosis is also added to the automatic sample loading system to track system faults and make it possible for the equipment to recover from the faults automatically.

(6) Due to the high-throughput requirements of protein crystal diffraction experiments, it is necessary to increase the volume of the dewar to increase the sample reserve. The operation of mounting/dismounting Puck with large-capacity dewar requires the laboratory personnel to manually mounting/dismounting those via the mounting rod. The compact Pucks arranged in the liquid nitrogen environment greatly increase the difficulty and failure rate. Once the position deviation occurs, the gripper will collide during the gripping process of the robot, causing damage to the gripper, equipment and samples. The limited and narrow experimental environment also hinders the configuration of large-capacity dewar tanks. Therefore, experimenters need to frequently enter and exit the experimental line station to replace the Puck. This process will also reduce the experimental efficiency.As a result, a manipulator for Puck can be designed. The experimenter can push the required puck into the beamline station through the small window of the experiment beamline station, and the robot can replace the puck by identifying the puck information. This operation can be carried out in the experiment, which greatly improves the automation degree of the beamline station and improves the experimental efficiency.

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