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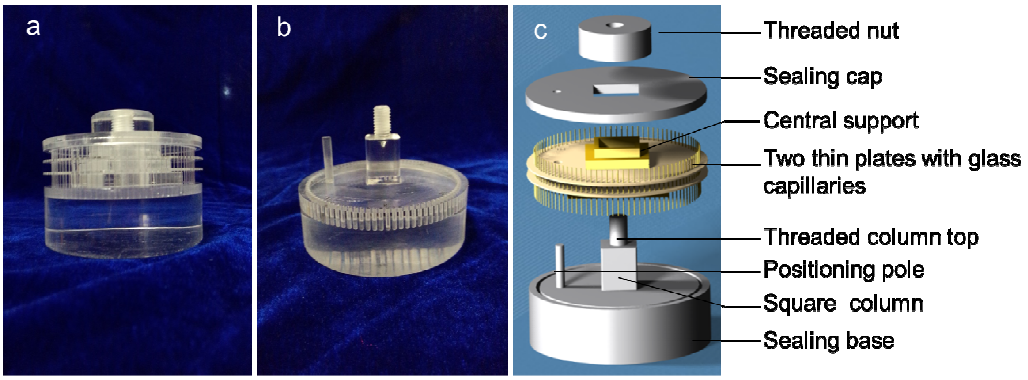

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A new method to realize high-throughput protein crystallization in a superconducting magnet

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We present a new method for the realization of high-throughput protein crystallization screening using an array of 96 capillaries aligned in a circle. In this method, each capillary represents a single crystallization condition, and all capillaries experience an identical magnetic field condition. After crystallization, the crystals in the capillary can be directly diffracted without harvesting. This method proved easy to perform and was applicable for use in the magnetic field and may be further extended for use in other circumstances, for example, in space microgravity conditions.

Introduction

Protein crystallization is important because it provides diffraction-quality protein crystals for X-ray crystallography, one of the main methods of obtaining structural information regarding protein molecules.[1, 2] However, due to their structural complexity and large size, proteins are more difficult to crystallize than small molecules and hence protein crystallization is still a bottleneck in proteomics.[3-5] The main obstacles usually occur during two steps: screening and optimization of the crystallization conditions. Many attempts have been made to overcome these problems, and new methods have been proposed. The use of special environments has attracted a great deal of attention. Among such special environments, high magnetic fields have been extensively studied in recent decades;[6-22] both magnetic fields and the simulated microgravity environments generated by gradient magnetic fields have improved the quality of protein crystals. Thus, magnetic fields are potentially useful for protein crystallization. Furthermore, magnetic fields represent additional physical environments for protein crystallization in that their use creates crystallization conditions that differ from conventional conditions. Although some studies have reported that the use of magnetic fields can reduce the probability of crystallization,[23] the availability of these additional crystallization conditions might provide new opportunities to obtain crystals that are not possible under normal conditions; this alternative is exceptionally useful in cases when crystallization is very difficult under normal conditions. Therefore, screening for crystallization under the influence of magnetic fields should be explored.

Superconducting magnet technology is developing rapidly, and magnet facilities are readily available in many laboratories. Many national magnet laboratories provide free access to machine time for researchers. Hence, it becomes easier to use strong magnetic fields than before.

Unfortunately, despite the merits that this technique might have regarding protein crystallization, magnetic fields are not widely utilized by protein crystallographers. One reason for this is that the experimental space provided by high-field superconducting magnets is usually limited and is thus not applicable for high-throughput protein crystallization, which is currently the most common method of obtaining protein crystals. To fully exploit the beneficial effects of magnetic fields, high-throughput crystallization in a magnetic field must be realized.

In this communication, we present a novel crystallization procedure that aims to reduce the size of crystallization setup so that it can be accommodated into the magnet bore and high-throughput protein crystallization screening and optimization can be easily carried out in such special environment. Each of the crystallization units shall be at identical magnetic field condition to avoid any uncertainties in crystallization process. To realize the purpose, we designed a crystallization setup that uses capillaries as the containers for holding crystallization solutions. The capillaries were arranged into an array, and the number of the capillaries in the array was 96 to be compatible with most commercially available crystallization screening kits. To ensure that each capillary experiences identical magnetic field conditions, the capillaries were aligned in a circle. The protein was first dissolved in pure water or buffer and then introduced into each capillary via capillary action. The

capillaries were then placed into a temperature-controlled environment, and the solution was allowed to dry. After drying, the capillaries were assembled into a circle to form the array; then, chemical reagent solutions (from a crystallization screening kit) were drawn up through capillary action from a pre-filled 96-well (round) plate. The filled capillaries were then sealed and placed into the magnetic field for crystallization. In the following sections, we will describe the design of the setups used, preliminary crystallization screening results, and the applicability of this method for in-situ diffraction.

Design of the high-throughput crystallization setup

Due to the limitations of superconducting magnet technology, most high-field superconducting magnets provide room-temperature bores of limited size. Hence, it is necessary to design a suitably sized setup. Commercially available crystallization plates (e.g., 96-well and 24-well Intelli-Plates) are widely used. These plates can be placed into the room-temperature bore of some superconducting magnets. However, the rectangular shape of commercial plates makes it impossible to achieve identical magnetic field environments for each crystallization unit in the magnet bore. If the crystallization units are aligned to form a circular array, then each unit will experience identical magnetic field conditions if the array is placed coaxially with the magnetic field. We therefore designed a crystallization setup with a circular array of crystallization units. To reduce the size, we based the setup on capillary tubes. The entire system for high-throughput crystallization in a high magnetic field comprises the following parts: a superconducting magnet, a temperature control subsystem, and a crystallization setup.

A. Overall configuration

Fig. 1 shows the overall configuration of the experimental system. The magnet provides a magnetic field for the crystallization experiments. A water jacket, which is used for temperature control, is inserted into the magnet bore, and the high-throughput crystallization setup is placed into the water jacket. The crystallization setup can be moved along the magnet bore but is usually placed where the magnetic field is maximal. Both ends of the water jacket are sealed using insulating foam. The water bath is connected to the water jacket to allow temperature-controlled water to circulate in the jacket, and the temperature of the space enclosed by the water jacket can be controlled.

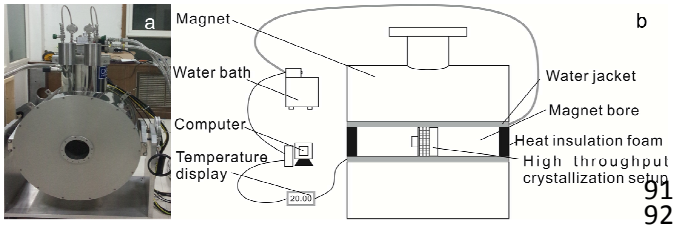

Fig. 1. Experimental configuration. (a) The superconducting magnet used in the experiment; (b) a schematic illustration of the system.

B. The magnet

The facility uses a cryogen-free superconducting magnet [OXFORD12T150MM, OXFORD Instruments, UK]. Fig. 1a shows a picture of the magnet; the magnet is rather strong, and the large room-temperature bore has a diameter of 150 mm; the maximum magnetic field is 12T. The main specifications of the magnet are summarized in Table 1. The magnet bore can rotate from a horizontal to a vertical position. The magnet is horizontally set in Fig. 1.

Table 1. The main specifications of the magnet.

Items	Specifications
Guaranteed maximum central magnetic field	12.0 T
Current required for 12.0T	179.658 A
Field / Current ratio	0.06679 T/A
Homogeneity	Better than 0.1%
Field variation over ± 55 mm on the bore axis (Z-axis)	< 1 T
Room-temperature bore diameter	150 mm

C. High-throughput crystallization setup

Fig. 2 shows the high-throughput crystallization setup and its accessory, which are designed based on the ideas mentioned at the beginning of this section. In detail, the high-throughput crystallization setup and its accessory include the following parts:

(1) The high-throughput crystallization setup:

Fig. 2a is a photograph of the setup that is placed into the magnet bore. The outer diameter of the setup is designed to fit into the water jacket. Details of the setup assembly are shown in Fig. 2c. The core of the setup is a circular array of 96 capillaries that are fixed using two thin plates (Fig. 2c). The thin plates are attached to a central support. At the center of the central support, a square hole is designed to fit with the square column that is raised from the sealing base. The sealing base contains a circular groove filled with grease, and the grease seals the capillaries when the capillaries are inserted into the groove. A positioning pole on the sealing base is used to guide the assembly of the thin plates and to guide the sealing cap to the sealing base to ensure that assembly is easy and accurate. At the upper part of the setup is a sealing cap, which is similar to the sealing base in that it contains a grease-filled groove to seal the top of the capillaries. On top of the setup is a threaded nut, which is compatible with the threaded column top. This nut is used to fix the assembly when complete.

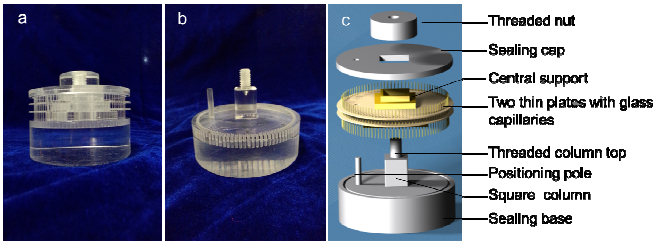

Fig. 2. The accessory used for the high-throughput crystallization setup. (a) The high-throughput crystallization setup, which is placed into the magnet; (b) the reservoir base is pre-filled with chemical reagents from a crystallization

screening kit; (c) details of the structure of the high-throughput crystallization assembly.

(2) The reservoir base

The reservoir base is similar to the sealing base of the crystallization assembly. There are only two differences between the two parts: the reservoir base is thicker than the sealing base, and the grease-filled groove in the sealing base is replaced by an array of 96 wells that aligned in the circle. The positions of the wells match those of the capillaries such that the capillaries can be inserted into the wells. To use the reservoir base, the chemical reagents from the crystallization screening kit are pre-filled into the wells such that the chemical reagents are introduced into the capillaries through capillary action.

Except for the glass capillaries, the material used for the setup assembly and the reservoir base is polymethyl methacrylate (PMMA).

The procedures to use the setup assembly and its accessory are very easy and simple. First, the capillaries are filled with dried protein. In this step, capillary action is used to fill the capillaries with a protein solution; then, the capillaries are allowed to dry in a drying container. Second, the capillaries are assembled into the two thin plates as shown in Fig. 2c, and the capillaries are filled with solutions of the crystallization screening reagents, again using capillary action. Third, the setup is assembled and placed into the magnetic field.

D. Temperature control subsystem

Temperature is an important parameter for protein crystallization.[24, 25] A temperature control subsystem is used to control the crystallization temperature. The design of this subsystem is the same as that used in our laboratory to control the temperature in another superconducting magnet.[11] Temperature-controlled water is circulated to control the temperature of the sample, which is placed inside the space enclosed by the water jacket. To ensure the accuracy of the temperature control, a closed-loop control strategy is used, which has been described previously.[11] The temperature of the sample can be controlled within ±0.1K.

Testing of the high-throughput protein crystallization setup in a high-field magnet

To test the applicability of the experimental system, we conducted a verification experiment using three model proteins: lysozyme, proteinase K and glucose isomerase.

Hen egg-white lysozyme (HEWL) (Seikagaku Corporation, six times recrystallized), proteinase K (pK) (Sigma-Aldrich Corporation), and glucose isomerase (GI) (Hampton Research) were dissolved in pure water to obtain protein solutions at concentrations of 20 mg/ml, 15 mg/ml and 10mg/ml, respectively. Each of the protein solutions was then introduced into 96 capillaries using capillary action. The filled capillaries were then dried in a sealed chamber in which desiccant (silica gel) was distributed at 293K. The dried capillaries were then assembled into the thin plates, and crystallization reagents were added from the reservoir base, which was filled with crystallization reagents from the screening kit Index™ (Hampton Research, USA). The crystallization setup was then assembled and placed in the magnet bore; the temperature was controlled at 293K. Two days later, the assembly was removed from the magnet, and the capillaries were inspected using a stereomicroscope (Olympus SZX 16, Japan). Some crystals were then diffracted using an X-ray diffractometer (MarμX, Mar Research, Germany) to test the diffraction capability.

The results of this experiment were as follows:

(1) Crystallization hits (conditions that yielded visible protein crystals under the microscope) were observed in the capillaries. Fig. 3 schematically shows the screening results. Crystals of lysozyme, proteinase K and glucose isomerase were found in 35, 63, and 23 capillaries, respectively, indicating that the setup was practical and efficient for protein crystallization screening.

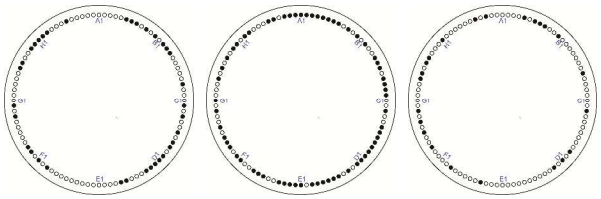

Fig. 3. Crystallization screening results in a preliminary test of the experimental system using lysozyme (left), proteinase K (middle) and glucose isomerase (right) as the model proteins. "○" represents the condition in which no crystals were found; "●" represents the condition that yielded crystals. "A1, B1, …, H1" are markers to indicate the serial number of the chemical reagent in the screening kit Index™.

(2) Fig. 4 shows crystal images of the three proteins obtained using the crystallization setup in the magnetic field. The morphology of the crystals varied in size and shape, depending on the crystallization conditions. No obvious orientation phenomenon was observed, verifying that the theory that the orientation is achieved during the sedimentation of the crystals to the bottom of the crystallization container is correct.[26]

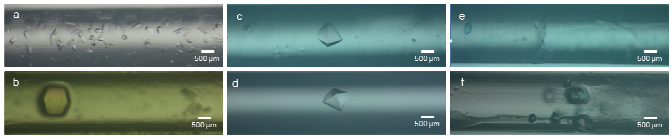

Fig. 4. Typical morphology of protein crystals obtained in the capillaries in the magnetic field. (a) and (b): HEWL crystals obtained with A2 and B2 conditions; (c) and (d) pK crystals obtained with A6 and B5; (e) and (f) GI crystals obtained with B2 and D1, respectively. "A1, B1, …, H12" are markers to indicate the serial number of the chemical reagent in the screening kit Index™.

(3) We arbitrarily chose several capillaries containing crystals and tested the diffraction capability of the crystals using an X-ray diffractometer. Diffraction patterns were obtained using simple in-situ diffraction (i.e., without the need to harvest the crystals, use cryoprotectant, and flash-cool the crystals). Fig. 5 illustrates a typical diffraction pattern of a lysozyme crystal; this crystal easily diffracted to 2.8Å using the home facility.

Obviously, the in-situ diffraction feature of this method is significantly advantageous.

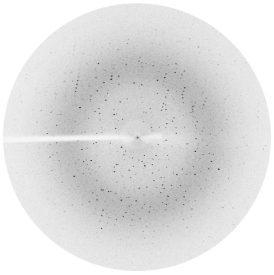

Fig. 5. A typical diffraction pattern of HEWL crystals obtained using the high-throughput crystallization setup in a magnetic field. The diffraction pattern was obtained using in-situ diffraction.

Concluding remarks

In this communication, we present a high-throughput protein crystallization setup based on a new idea to fully exploit the magnetic fields generated by a superconducting magnet. The experimental system was tested using lysozyme crystallization experiments, and the results showed that the method and the design were successful. The method exhibits obvious use for application in protein crystallization.

(1) High-throughput protein crystallization (both crystallization screening and optimization) can be rapidly and easily achieved. Currently, most laboratories use automated liquid-handling systems to rapidly set up crystallization screening trials. In the method presented in this paper, no automated machine is required; however, the speed with which crystallization trials can be set up can be increased even further (within several seconds using the current method versus more than 1 minute for most automated systems).

(2) The method presented here is easy to perform. Because no automation system is needed, the cost can be greatly decreased. Furthermore, the consumables used in the current method are cheaper than those used in prevailing protein crystallization screening methods, i.e., the glass capillaries used are cheaper than commercial crystallization plates.

(3) The size of the crystallization screening array can be made smaller, and a different shape can be used; in this way, the method can be extended for use under many conditions. Because the size of the capillaries can be reduced, the total size of an array of capillaries can be made much smaller than conventional crystallization plates. Therefore, this method can be easily applied in situations (such as the small-bore magnetic field shown in this paper and other conditions, such as in space microgravity experiments) when limited space is available.

(4) In-situ diffraction is easy to realize in this method. This represents a great advantage because handling protein crystals can be difficult. In-situ diffraction obviates the need for crystal handling; hence, the deleterious effects of crystal handling on diffraction quality can be avoided.

The design of the high-throughput protein crystallization setup is clearly suitable for use in strong magnetic fields. More extensive studies will continue in our laboratory to compare the crystallization success rates and crystal quality obtained inside and outside of magnetic fields. Apart from applications in strong magnetic fields, we also suggest that this method be tested in other physical environments, especially in a space microgravity environment.

Acknowledgements

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Notes and references

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