1-11-8. Protein Crystallization

Overview

Protein crystallization is a fundamental technology underpinning structural biology and drug discovery. However, obtaining high-quality crystals has been a long-standing challenge, traditionally relying on trial-and-error methods. This process is crucial for a deeper understanding of life and for elucidating protein structures that have previously been inaccessible. Various innovative protein crystallization methods utilizing light have recently been developed, enabling precise spatio-temporal control over nucleation and crystal growth. This has significantly improved efficiency and controllability, transforming the conventional empirical process into a more scientific approach.

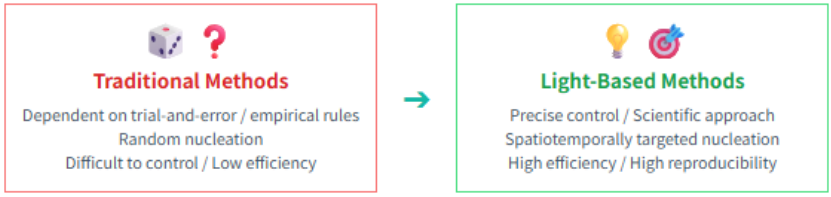

Figure 1. Conceptual evolution of protein crystallization approaches.

Current Status and Frontiers

In recent years, innovative approaches to overcome the long-standing challenges in protein crystallization have been successively developed, focusing on the excellent spatiotemporal controllability of light. Light possesses the characteristic of transmitting energy and information non-contact, allowing for precise external manipulation of nucleation and crystal growth dynamics by acting directly or indirectly on protein molecules in solution. The following describes four primary methods in detail.

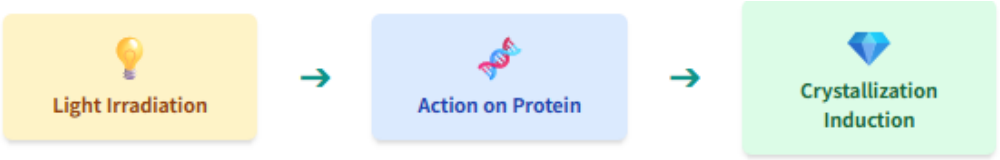

Figure 2. Light-utilizing protein crystallization technology.

1. Photochemical induced crystallization (PIC)

This method involves irradiating a protein solution with weak UV or visible light to promote protein nucleation through photochemical reactions. It applies to various proteins involving radical intermediates and sensitizers. This method precisely controls the timing of nucleation initiation by light irradiation, contributing to improved crystallization efficiency.

2. Non-photochemical laser-induced nucleation (NPLIN)

This technique promotes protein nucleation by irradiating the solution with non-

focused nanosecond or picosecond laser pulses without absorption of laser light. Proposed mechanisms include enhanced molecular alignment due to the optical Kerr effect and the formation of microcavitations. A significant advantage of this method is the extremely low risk of thermal or photochemical damage to proteins, as it does not involve light absorption.

3. High-intensity laser-induced nucleation (HILIN)

This method utilizes focused high-intensity femtosecond laser pulses. The formation and collapse of cavitation bubbles due to optical breakdown create localized high-concentration regions, promoting protein nucleation. It is effective in promoting protein crystal growth. This method is particularly effective in promoting nucleation, even for proteins with poor crystallizability.

4. Optical trapping-induced crystallization (OTIC)

This method utilizes the gradient force of optical tweezers (laser trapping) to capture and concentrate proteins to high concentrations, thereby promoting protein nucleation. Key aspects include the formation of localized high-concentration domains and anisotropic control of the solution through laser polarization. It enables precise in-situ observation of crystal growth. The crystal growth process can be monitored in real-time, allowing for the adjustment of optimal conditions.

These diverse light-based protein crystallization technologies each promote crystallization through different mechanisms, leading to a more scientific and precise control of the process.

References

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Future Perspectives and Directions

Challenges expected to be solved or realized within the next 10 years include: optimization of optical parameters for various proteins and a deeper understanding of the underlying mechanisms. Further advancements involve highly controlled and efficient processes through integration with holographic optical trapping, AI, and microfluidic devices. Additionally, scaling up from laboratory scale to industrial applications and developing new application fields are anticipated.

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