



Improved Method and Apparatus for Determining Crystallization Parameters

Protein crystallization, whether for aqueous or membrane proteins, is a major bottleneck limiting determination of protein structure. A recent international survey of protein crystallization rates at 22 structural genomics centers around the world indicated that for 10,204 soluble proteins obtained, less than 1700 were of diffraction quality. The low success rate for producing diffraction-quality crystals can be largely attributed to two factors: limited amounts of purified protein coupled with an incredible number of possible crystallization conditions.

The number of possible crystallization conditions is particularly problematic, as a huge number of variables impact the crystallization process, including: protein purity, concentration, homogeneity, stability, flexibility, the selection of precipitating agent, buffer, pH, temperature, light, magnetism, gravity, atmosphere identity, atmospheric pressure, counterion, organic moment and additional additives to aid in crystallization. For membrane proteins, additional factors must be considered regarding any lipids or detergents to be used. Each of the above factors must be considered both alone and in combination in order to determine a set of crystallization conditions that yield high quality crystals. Although new high throughput testing systems have allowed scientists to test thousands of crystallization conditions for a given protein, success rates remain low, suggesting that brute force combinatorial methods are not the answer.

Researchers at Colorado State University, University of Alabama at Birmingham, and Mississippi State University have developed a technique for predicting optimal crystallization conditions. Using either self-interacting chromatography (SIC) or light scattering techniques, a parameter known as the second virial coefficient (B) may be determined. After performing a limited number of these experiments under varying conditions, the algorithm is able to predict the likelihood of crystallization for the entire matrix of possible combinations of each variable, thereby avoiding the long and costly process of performing experiments under all possible conditions.

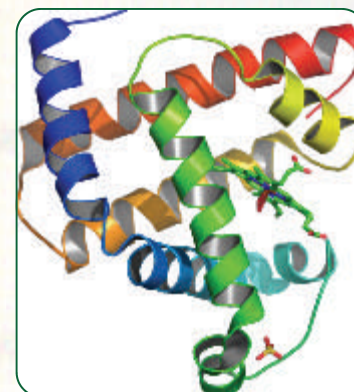
Furthermore, a microfluidic device has been developed which can automate the initial SIC experiments. After feeding the data to a computer, the algorithm is performed and B may be determined for all of the untested conditions.

Conversely, this technology may be employed in order to determine the optimal conditions for maximum solubility of a protein.

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Patent Information
Patent pending.

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Features and Benefits

- Rational method for determining conditions that will yield diffraction-quality crystals of protein.
- Low amounts of protein required as only minimal experiments are performed.
- This technology solves the lignin problem by employing enzymes from white-rot and other fungi.
- Novel algorithm predicts a crystallization parameter for the entire matrix of possible combinations.
- Automated device technology allows for rapid results with minimal supervision.

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