

HIGH-THROUGHPUT METHOD FOR OPTIMUM SOLUBILITY SCREENING FOR HOMOGENEITY AND CRYSTALLIZATION OF PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application No. 60/666,738, filed on Mar. 29, 2005, which is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] The present invention relates to high throughput methods for screening for optimal solution conditions for crystallizing proteins.

[0004] 2. Related Art

[0005] Advances in X-ray crystallography have provided three-dimensional structures of thousands of proteins. In spite of these advances, protein aggregation continues to be a common problem that can lead to unsuccessful crystallization of proteins. This problem is becoming more prominent in attempts at crystallizing many different proteins and protein complexes in a structural genomics scale.

[0006] At the Berkeley Structural Genomics Center (BSGC), a purified protein sample is obtained after one or more chromatography steps (immobilized metal affinity chromatography (IMAC), ion-exchange chromatography, and size exclusion chromatography), and the sample is analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1971) to determine the chemical purity of the protein. In the process of developing techniques to automate protein purification, one tries as much as possible to use a set of generic buffers. Very little is known about the properties of the proteins a priori, except for theoretical pI, molecular weight and amino acid composition. The general practice is to use one or two favorite buffers where pH and salt concentration are some of the variables. However, a protein has complex properties and its condition and behavior depend very much on the environment surrounding it. In the past when faced with difficulties of aggregation and precipitation, one would try to change purification parameters, add or remove fusion tags, test some additives (e.g., DTT, glycerol, etc.). After purification, the protein is concentrated and the presence of aggregates is assessed using for example, the dynamic light scattering method. This method as described by Zulauf & D'Arcy (1992) has shown that the presence of aggregates in the protein solution may inhibit crystal nucleation or growth (Habel et al., 2001, Ferre-D'Amare & Burley, 1997).

[0007] In order to grow crystals, one must identify the conditions under which proteins will precipitate out of solution. The technique of vapor diffusion is commonly used to analyze this controlled precipitation and by using a sparse matrix approach (Jancarik and Kim, 1991 and further expanded by Hampton Research (Aliso Viejo, Calif.)), one can test a large number of crystallization conditions. This assumes that the starting protein solution is not aggregated or precipitated. In both the preparation for NMR or X-ray crystallography samples, one must start with a protein solution that is homogeneous and monodisperse. Lepre and Moore (1998) developed a modified vapor diffusion method

to efficiently screen solvent conditions for NMR samples in order to optimize solubility. Collins et al. have developed preliminary solubility screens using different buffer components and focus on the combination of best anion and cation buffers for solubility improvement (*Acta Cryst.* (2004) D60, 1674-1678).

STATEMENT OF GOVERNMENTAL SUPPORT

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BRIEF SUMMARY OF THE INVENTION

[0009] The present invention provides a method of screening for optimal solubilization (OS) conditions to crystallize proteins comprised of the following steps: (1) providing a reservoir buffer in a container, wherein the container has a cover slip; (2) depositing an aliquot of said buffer either on the cover slip or on a pedestal in the container above the buffer reservoir in each container; (3) placing a protein in said aliquot of said buffer to form a protein solution drop; (4) inverting the cover slip onto the container; (5) incubating the container at a given temperature for a sufficient period of time; and (6) evaluating said protein solution drop for solubility and monodispersity. The method for screening is repeated or performed in parallel with a set of at least 4 buffers for each protein provided in order to screen the optimal solubilization conditions. The set of buffers can be comprised of 24 buffers, and in preferred embodiments, the set of 24 buffers is the set in Table 1.

[0010] Thus, the present invention further provides for a high-throughput method to screen for optimum solubility condition to crystallize proteins comprising the following steps: (1) providing a set of 24 buffers as shown in Table 1; (2) providing a multi-well container comprised of at least 24 wells, wherein each well has a cover slip; (3) adding each of the 24 buffers to each respective well of said container; (4) depositing on each cover slip or reservoir pedestal, a protein solution drop comprised of an aliquot of the protein and an aliquot of the same buffer in the well; (5) inverting the cover slips onto each respective well; (6) incubating the container at a given temperature for a sufficient period of time; and (7) evaluating any clear protein solution drops to select the optimum solubility condition.

[0011] If the protein solution drop is not clear, an aliquot of an additive is added to each protein solution drop to find the best condition. The clear protein solution drops are evaluated to determine the monodispersity of the sample. Therefore, the method can further comprise the step of depositing an aliquot of an additive in the protein solution drop to help screen for the optimum solubility condition.

[0012] Herein is described methods to test a panel of buffers and many additives in order to obtain the most homogeneous and monodisperse protein conditions for proteins that aggregate and cannot be concentrated prior to setting up crystallization screens. In a preferred embodiment, a panel of twenty-four buffers is tested using the hanging-drop method and vapor diffusion equilibrium. After monitoring precipitation, the conditions leading to clear drops are selected for dynamic light scattering (DLS) char-