116a Improving Success Rate in Protein Crystallization

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Predicting successful protein crystallization conditions is not a trivial matter. Protein crystals are needed in structural biology to elucidate the molecular 3D structure by means of X-ray diffraction. In practice screening kits are used with large arrays of different solvent conditions to induce protein crystallization. This method is basically a trial and error method and could be improved if more theoretical knowledge of protein crystallization can be developed. One handle to couple experiments to theory is the second osmotic virial coefficient B_{22}. This is a measure of the degree of interaction between individual protein molecules, and can consequently be used to analyze, predict and optimize crystallization processes where the protein interactions play an important role. Solution conditions under which proteins tend to crystallize correspond theoretically to slightly negative osmotic second virial coefficients, resulting from weak attractive protein-protein interactions. An experimental range of B_{22} values, between -1×10^{-4} to -8×10^{-4} mol-mL/g^2, was named the 'crystallization slot' [1], but it is still undocumented how the width of the 'crystallization slot' is affected by protein and solution properties. In this work we will present a thermodynamic analysis of the relation between the 'crystallization slot' and the protein and solution properties.

A crucial element in the development of this technique is the availability of systematic B_{22} measurements. B_{22} is traditionally measured using colloidal characterization techniques, such as static light scattering, small-angle X-ray or neutron scattering, membrane osmometry, and sedimentation equilibrium. These approaches are inefficient in terms of both required time and required amount of proteins. Self-interaction chromatography (SIC) was recently adapted to measurement B_{22} values. In this paper the application of SIC aided predictive crystallization to crystallize proteins is demonstrated for model proteins as well as pharmaceutically active proteins. Furthermore, miniaturization approaches are described that make this technique suitable for High Throughput Screening.

1. George A. and Wilson W.W. 1994. Acta Crystallographica D, 50 (4): 361-365.