MOLECULAR TOOL

A high-throughput system for the determination of ideal buffer conditions for maintaining proteins in solution.

- Tool to determine optimal buffer to keep protein in solution
- Saves time and effort in finding buffer
- Avoids delay in HTS
- <96 test conditions
- Simple to use
- Inexpensive to produce

A method to enable the rapid identification of optimal or desirable conditions for maintaining a selected protein in solution for further investigation has been developed by the University of Dundee. This overcomes current problems in maintaining sufficient quantities of protein in solution for subsequent evaluation.

Background

The current drive toward high-throughput structural analysis of proteins is constrained by several bottlenecks including the maintenance of sufficient levels of protein in solution.

Initial cloning and soluble expression of the protein of interest is increasingly being tackled by robotic systems to create large numbers of potential constructs and expression systems. However, even when these lead to sufficient levels of target protein expression, maintaining the protein in a stable soluble form can become problematical with proteins often falling out of solution.

Selection of the initial buffer system is largely based around the predicted ph of the protein, and is often biased by the previous experience of the scientists. High concentrations of salt (up to 1M NaCl), with perhaps the addition of a reducing agent such as DTT are employed as typical extensions from the simple buffer. While for many proteins this may be sufficient, in a significant number of cases proteins are observed to aggregate and fall out of solution. This occurs most commonly when

- High-salt is removed prior to ion-exchange chromatography
- At post purification dialysis to remove high concentrations of imidazole used to elute the protein in His-tag affinity chromatography

There are many additives which are known to aid in maintaining proteins in a soluble form. However to test for optimal permutation of protein characteristics

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against an array of factors such as pH would require significant time and effort whilst consuming large amounts of sample.

To help combat this the University of Dundee has developed a rapid high-throughput method which tests for up to 96 different conditions simultaneously with minimal consumption of sample.

The technology

A method of screening for conditions suitable for maintaining a protein in a soluble form has been developed which involves mixing a protein with a test solubilising solution and then measuring the amount of soluble protein using a commercially available protein quantification kit.

The current method is conducted in a multi-well plate in order that a significant number of different test solubilising solutions and controls may be carried out simultaneously allowing a variety of pHs, buffers and additives to be tested.

This method enables identification of the optimal or desirable conditions for maintaining a selected protein in solution to allow such proteins to be kept in solution for further investigation.

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Patent Pending

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