

Advanced Protein Folding Technology

Many recombinant proteins expressed in bacteria form inactive precipitates called inclusion bodies. Inclusion bodies are not soluble in non-denaturing buffers and they do not refold automatically into the native protein conformation. However, expression of proteins as inclusion bodies has a number of advantages because inclusion bodies are: (1) not toxic to the host cells (2) resistant to proteolysis; (3) produced in a high yield; and (4) easily isolated from the soluble impurities and cell membranes. Therefore, expression of proteins as inclusion bodies often yields large quantities of almost pure materials. The challenging part is to find an effective protein folding method to convert the inclusion body materials into biologically active proteins.

Gene expression yields insoluble and inactive proteins are not limited to bacterial expression systems. It can also happen to yeast or higher eukaryotic expression systems or *in vitro* protein synthesis. Inactive and insoluble proteins can also result from denaturation of active and soluble proteins during purification, storage, transportation and special biophysical or biochemical processing such chemical modification. *In vitro* protein folding provides possibilities to make biologically active proteins using the inactive materials from different sources.

In prior to the folding process, the proteins must be solubilized under denaturing conditions. The denaturing conditions (or factors) can be high concentrations of a denaturant such as urea and guanidine hydrochloride, extreme pH, or denaturing detergent [Clark. *Current Opinion in Biotechnology*. 12:203-207 (2001)]. After solubilization, the proteins are brought into a native (non-denaturing) environment by removal of the denaturing factors. The most popular methods for denaturant removal are dialysis and dilution [Middelberg. *TRENDS in Biotechnology*. 20:437-443 (2002)]. In the dialysis process, dialysis membranes are used to allow the small molecules (denaturant and buffer components but not proteins) to pass through the membrane so that the denaturant concentration is reduced in the protein sample. In the dilution process, both the protein and denaturant are diluted with a folding buffer. Other methods for denaturant removal include diafiltration [West et al. *Biotechnology and Bioengineering*. 57:590-599 (1998)], preparative size exclusion chromatography [Batas et al. *Journal of Biotechnology*. 68:149-158 (1999)] and reversible absorption on a solid support such as immobilized metal affinity resin or and ion exchange resin [Li et al. *Protein Expression and Purification*. 33: 1-10 (2004)].

Since different proteins fold under different conditions, there is no universal folding condition that is suitable for folding different proteins. Some additives such as sugars, polyethylene glycol (PEG), glycerol [Meng et al. *The International Journal of Biochemistry and Cell Biology*. 33: 710-709 (2001)], detergent [Donate et al. *Protein Science*. 7:1811-1820 (1998)], organic solvent, arginine, proline [Samuel et al. *Protein Science*. 9:344-352 (2000)] and some organic molecules [Rozenma et al. *Biochemistry*. 35:15760-15771 (1996)] facilitated protein folding. In addition to including additives in the folding solution, macromolecules such as chaperon protein GroEL [Dong et al. *Journal of Chromatography A*, 878: 197-204 (2000)] or macro-assembly of small molecules like liposomes [Yoshimoto et al. *Journal of Chromatography B*, 743:93-99 (2000)] were employed to help protein folding. In some cases, the folding solution was treated with high pressure to facilitate protein folding [St. John et al. *Journal of Biological Chemistry*. 276:46856-46863 (2001)]. However, one condition good for folding one particular protein often does not apply to another protein. The first fractional factorial protein folding screen was introduced by Chen et al in 1997 in folding the ligand binding domain of the glutamate receptor in a dialysis mode [Chen et al. *Proc. Natl. Acad. Sci., USA*, 94:13431-13436 (1997)]. The folding screen was further optimized and tested for different proteins [Armstrong et al. *Protein Science*.8:1475-1483 (1999)].

The protein folding screen method using spin columns developed by ProFoldin has the following advantages:

- **It is easy to use:** The guanidine hydrochloride- or urea-solubilized proteins are loaded on the columns and the folding protein products are collected after a spin and incubation procedure.
- **It is effective:** The denaturant is completely removed and the protein is transferred into various folding environments in the spin-column process. The chromatographic and folding conditions were optimized to minimize protein aggregation and disulfide bond formation where necessary.
- **It is easy to scale-up for preparative protein folding:** Once the optimal folding condition is identified from the spin-column screen, large protein folding columns with the specified conditions are available for large-scale preparative folding.
- **Comparing with dilution and dialysis methods:** The advantage of the dilution method is that any additives in the folding buffer simultaneously interact with the protein when the denaturant is diluted. The disadvantage of the dilution method is that the dilution process results in a large volume of solution with a very

dilute protein concentration since the molar concentration of the denaturant is high (8 M urea or 6 M Gdn-HCl), the dilution ratio must be high enough to get a low denaturant concentration. In a dialysis mode, the protein concentration is more controllable than the dilution mode. However, the disadvantage of dialysis is that different molecules on the two sides of dialysis membranes have different kinetics of passing through the membrane. Some additives are not able to get into the protein solution as quickly as needed. Dialysis of protein solutions against many different solutions in a large volume for protein folding screen can be tedious. The column-based protein folding screen method allows the additives simultaneously interact with the protein while the denaturant is removed completely by the column. Since no large volumes of buffers are needed to remove the denaturant, high protein concentrations in the folding product are achievable. In addition, the column-based protein folding screen process is simple and saves time.