Protein Renaturation and Folding

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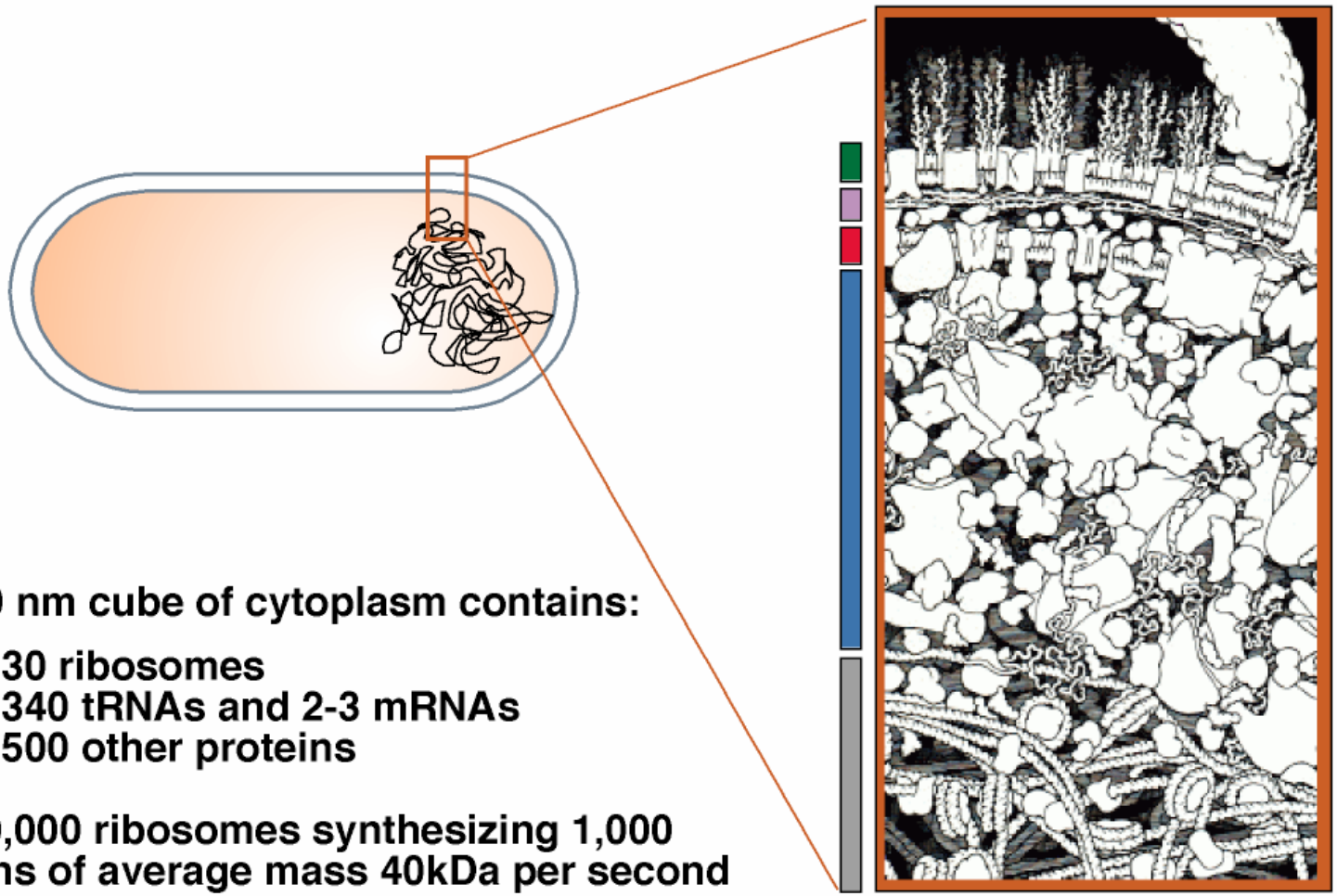
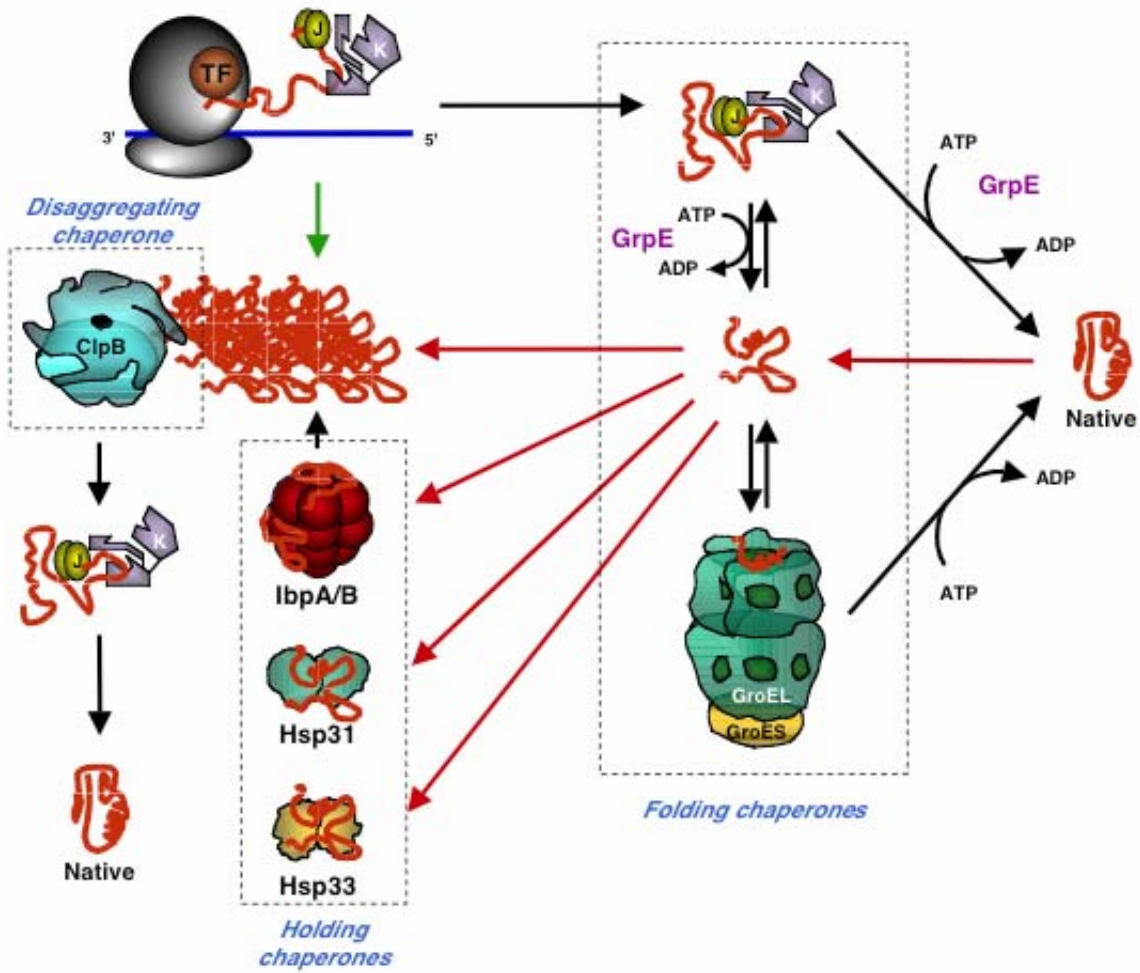
E. coli intracellular environment and protein synthesis



- A 100 nm cube of cytoplasm contains:
  - 30 ribosomes
  - 340 tRNAs and 2-3 mRNAs
  - 500 other proteins
- 15-30,000 ribosomes synthesizing 1,000 chains of average mass 40kDa per second

http//www.scripps.edu/pub/goodsell/gallery/cell.html

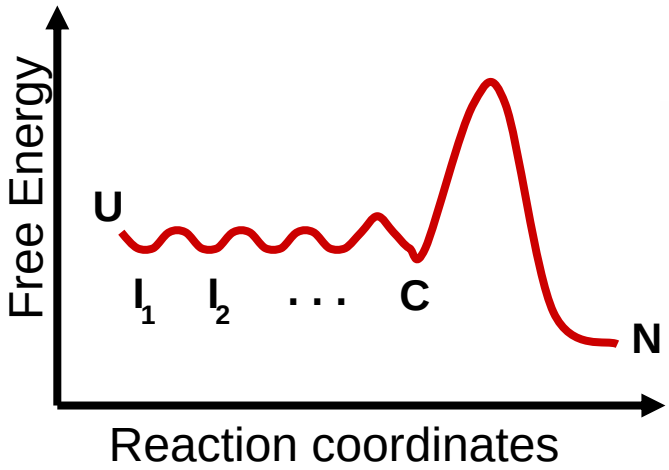
Chaperone-assisted protein folding in the *E. coli* cytoplasm

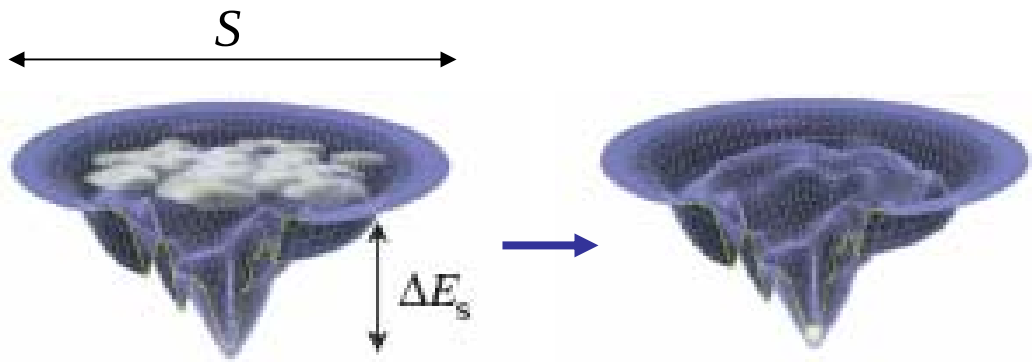


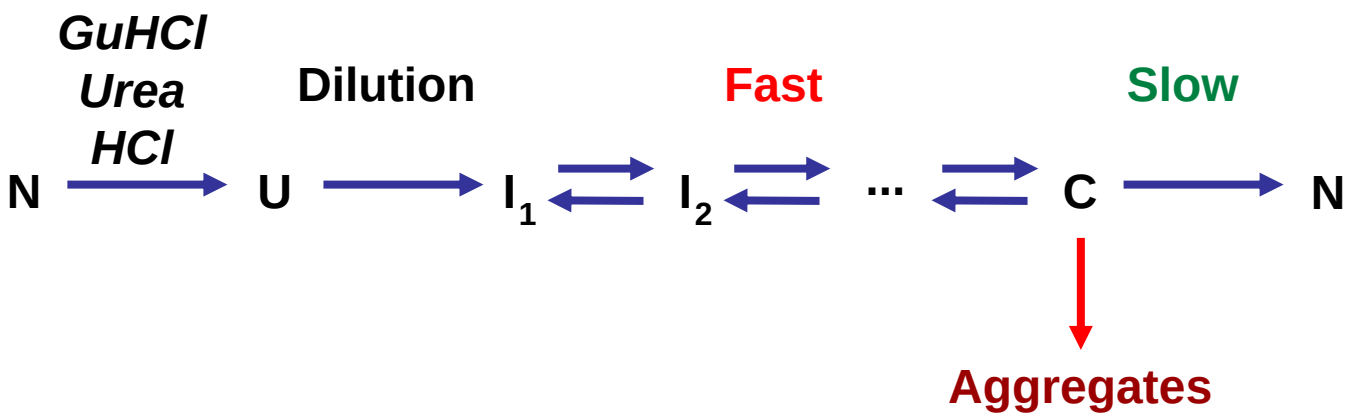
Baneyx and Mujacic (2004) *Nat. Biotechnol.* **22**:1399

In vitro protein folding

- Energy-independent
- All information necessary for folding is specified in amino acid sequence

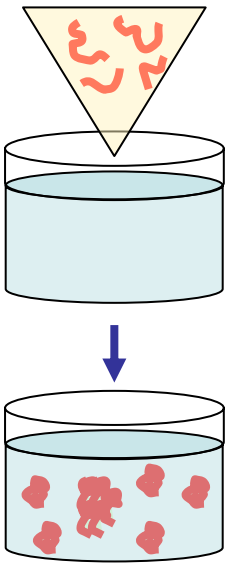






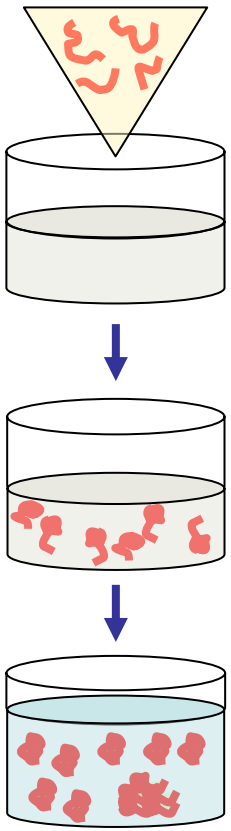
Batch refolding

Rapid dilution



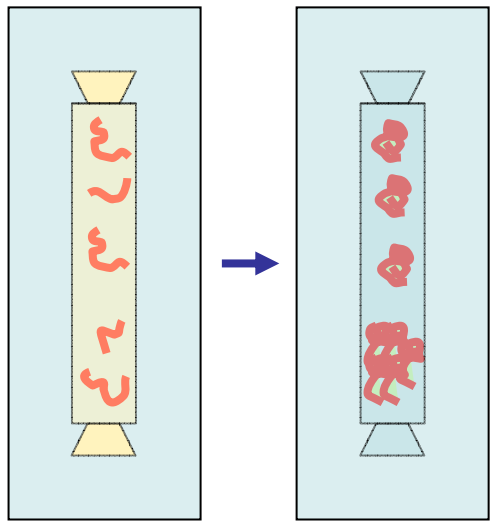
Aggregation
Product is dilute

Stepwise dilution



Aggregation may be reduced
Product is dilute

Dialysis (one-step or stepwise)



Aggregation
Time-consuming
Product is dilute

Variations: pulsed dilution; reverse dilution; mixing

Improving refolding efficiencies with chemical additives

- **Aggregation suppressors**

  Reduce folding intermediate interactions without affecting refolding rates

  - Arginine-HCl (generic); Proline
  - Cyclodextrin; polyethylene glycol
  - Mild detergents

- **Folding enhancers**

  Increase protein stability and folding rates but also protein-protein interactions

  - Sugars/polyols (sucrose; sorbitol; glycerol; ethylene glycol…)
  - Salts (ammonium sulfate; magnesium chloride)
  - Glycine and Alanine

- **Chemical chaperones**

  Detergent prevents aggregation and are removed with cyclodextrin/cycloamylose (which may be conjugated to a solid phase)

  - Detergent (e.g. Triton X-100, Tween 80, CTAB…) + cyclodextrin
  - Detergent + cycloamylose

Daugherty et al. (1998) *J. Biol. Chem.* **273**:33961
Mashida et al. (2000) *FEBS Lett.* **486**:131
Tsumoto et al. (2003) *Protein Express. Purif.* **28**:1-8
Mishra et al. (2005) *J. Biol. Chem.* **280**:15553

Improving refolding efficiencies with protein additives

- **Molecular chaperones**

  Interact with hydrophobic domains exposed by folding intermediates

  - ***Folding chaperones***: promote active folding intermediate remodeling via ATP-fueled conformational changes (e.g., DnaKJ-GrpE, GroELS)
  - ***Holding chaperones***: interact with and stabilize folding intermediates (e.g., small Hsps)

- **Foldases**

  Accelerate rate limiting steps along the folding pathway and often exhibit chaperone activity

  - ***Cysteine-thiol oxidoreductases***: promote formation and isomerization of disulfide bonds
  - ***Peptidyl-prolyl cis/trans isomerases***: promote the isomerization of X-Pro bonds

Thomas et al. (1997) *Appl. Biochem. Biotech.* **66**:197
Baneyx and Mujacic (2004) *Nat. Biotechnol.* **22**:1399

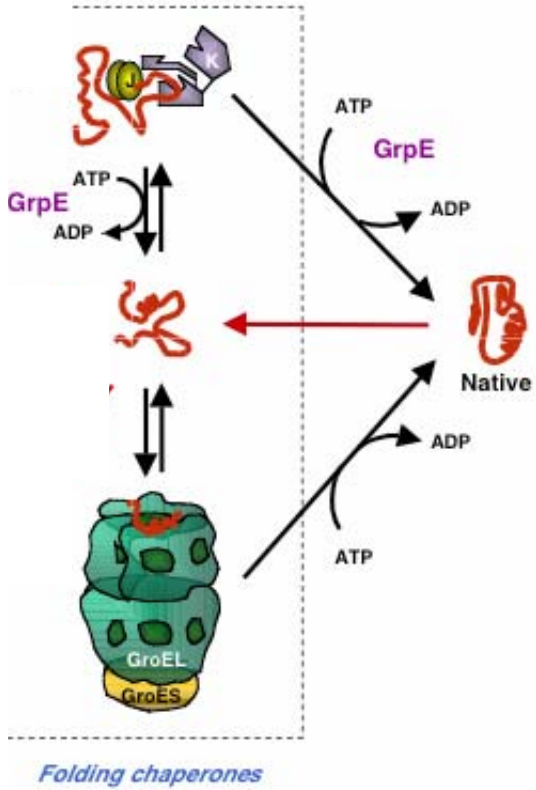
Improving refolding efficiencies with protein additives

Examples

- DnaK/DnaJ/GrpE in solution
- GroEL/GroES in solution
- DnaK/DnaJ/GrpE/GroEL/GroES in solution
- Trigger factor in solution

- Immobilized DnaK
- Immobilized GroEL
- Immobilized GroEL (apical fragment)

- GroEL + polyols
- GroEL in reverse micelles

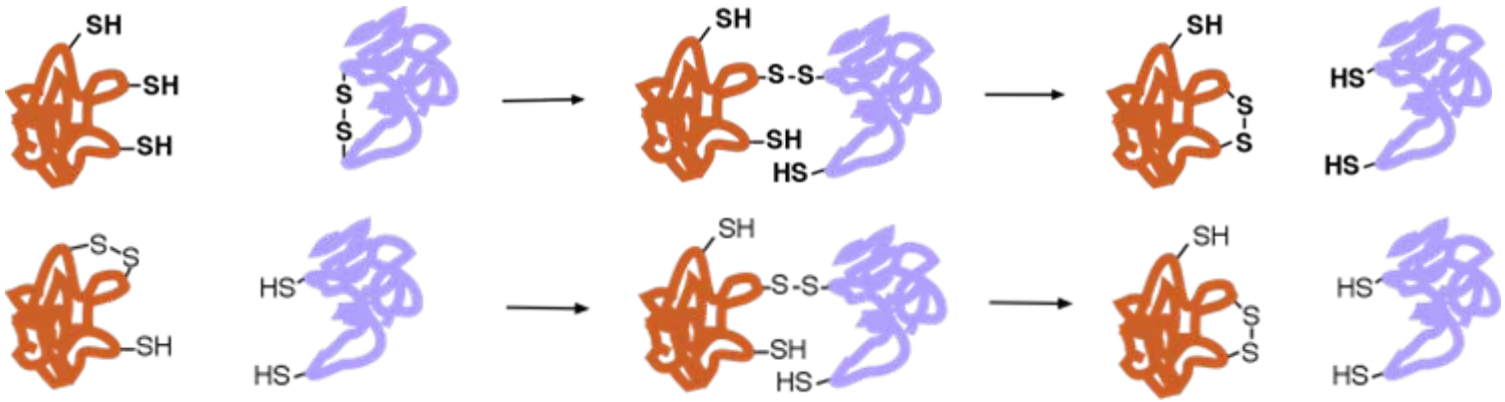


Liu and Zhou (2005) *BBRC* **313**:509
Martens et al. (2000) *Eur. J. Biochem.* **267**:6679
Buchner et al. (1992) *Biotechnology* **10**:682
Altamirano et al. (1999) *PNAS* **94**:3576

Voziyan and Fisher (2002) *Arch. Biochem. Biophys.* **397**:293
Sakono et al. (2003) *J. Biosci. Bioeng.* **96**:6679275
Dong et al. (1999) *J. Chromatogr A* **878**:197

Improving disulfide bond formation

- **Chemical additives**
  - Oxidized glutathione or GSSG/GSH couple (e.g., 2:1)
  - Copper sulfate (oxidant)
- **Protein additives**

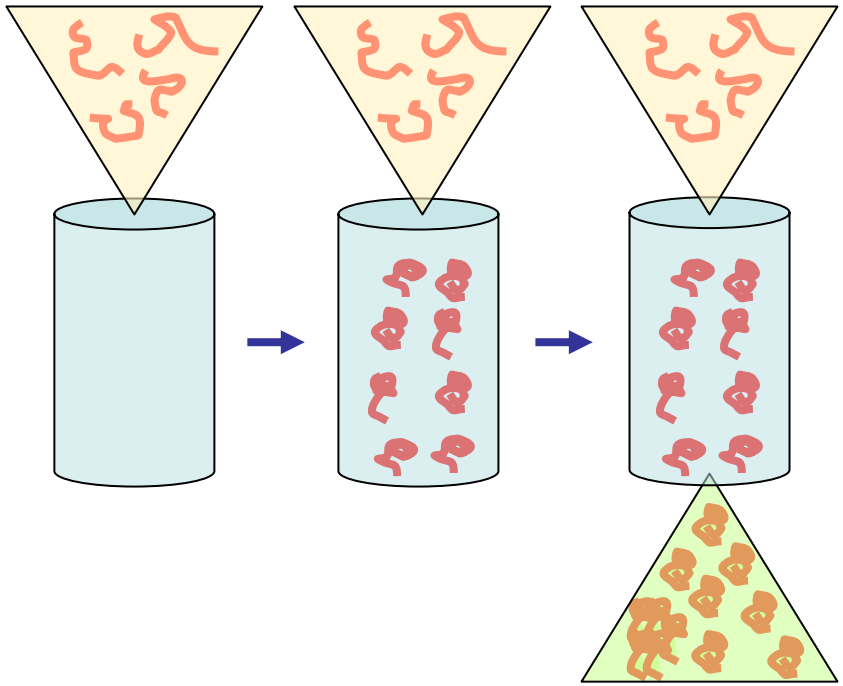


  - Oxidase (e.g. DsbA)
  - Protein disulfide isomerases (e.g. eukaryotic PDI or bacterial DsbC)
  - Combination of PDI with BiP or DnaK & DsbAC with apical GroEL
  - May be immobilized

Zhao et al. (2005) *J. Biol. Chem.* **280**:13470
Mashida et al. (2000) *FEBS Lett.* **486**:131
Tsumoto et al. (2003) *Protein Express. Purif.* **28**:1-8
Mishra et al. (2005) *J. Biol. Chem.* **280**:15553
Rosenfeld et al. (1997) *Arch. Biochem. Biophys.* **342**:298
Mayer et al. (2000) *J. Biol. Chem.* **275**:29421
Martens et al. (2000) *Eur. J. Biochem.* **267**:6679
Altamirano et al. (1999) *PNAS* **94**:3576
Buchner et al. (1992) *Biotechnology* **10**:682
Tsumoto et al. (2003) *Protein Eng.* **16**:535

Matrix-assisted refolding

- Unfolded proteins are loaded at high concentration on column packed with ion exchange (IEX) , size exclusion (SEC), or hydrophobic interaction (HIC) resin
- Partial refolding may occur upon adsorption or folding may occur upon release into the eluate



- Continuous/high productivity
- Control of mobile phase composition (additives and chaotrope)

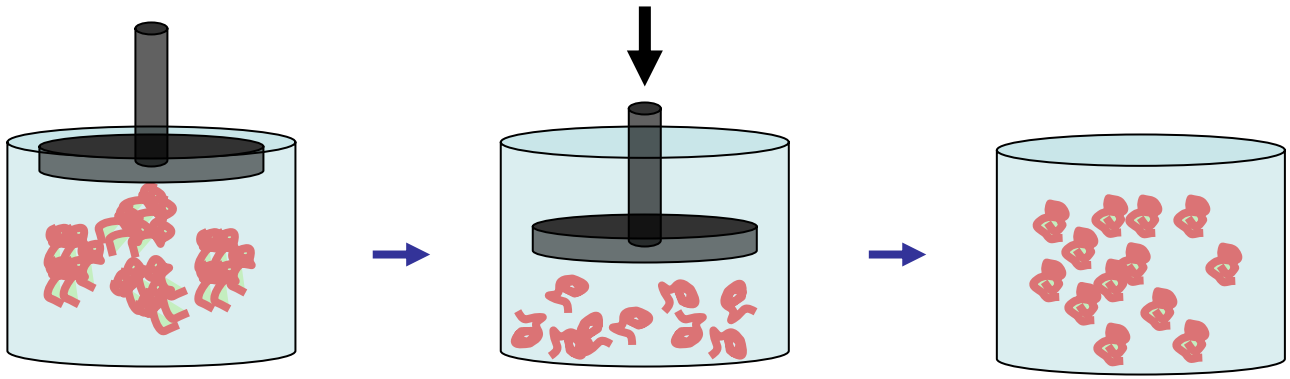
HIC: load in salt; GuHCl/urea may affect solubility

SEC: progressive refolding & one-step aggregate separation but low velocity & small sample load

IEX: preferred for high load & buffer control but not suitable for GuHCl-denatured samples

Pressure-assisted refolding

- Inclusion bodies are solubilized by pressurization to 100-200 MPa and refolding takes place over hours or days following depressurization
- Likely first disrupts salt bridges but also nonnative H-bonds and van der Waals contacts



- Works at high protein concentrations (mg/mL)
- Refolding buffer can be supplemented with osmolytes and/or intermediate concentrations of denaturant
- Discontinuous process

St. John et al. (1999) *PNAS* **96**:13029
Randolph et al. (2002) *BBA* **1595**:224
Lefebvre et al. (2004) *Biotechnol. Prog*. **20**:623

Summary

- Many strategies are available but none is universal
- Refolding parameters must be optimized on a case by case basis (>50% yields are usually required for a viable industrial process)
- In developing refolding processes a number of variables must be considered

  - Cost, stability and reusability of additives (chaperones, foldases, ATP)
  - How much (if any) contamination is acceptable
  - Numbers of steps in the refolding train
  - Conformational quality of the product
  - Desired productivity (mg/mL.h)