A denatured protein may be brought back to its native form by in vitro folding. The folding process is often slow, and yields can be poor. As each protein is unique, the in vitro folding conditions must be determined case by case, often using specific cosolvents as additives. An example is the group of proteins in which disulfide bonds must be reestablished as part of the renaturation process.

Hydrogen bonds and intramolecular interactions (electrostatic and van der Waals) stabilize the native structure of the protein in a cooperative manner. On denaturation, the cooperative effect is lost, resulting in unfolding of the molecule and exposure of the inner hydrophobic core to the hydrophilic aqueous environment. For small globular proteins, denaturation is an almost all-or-none process approximated rather well by the two-state transition. Thermodynamically, the denaturation process can be observed by an increase of molar heat capacity and a rapid enthalpy increase with increasing temperature. The primary structure (amino acid sequence) is not affected by denaturation. The indicators of denaturation include loss of structure and loss of biological activity and aggregation. Preventive actions against denaturation are described in Table 9.12.

TABLE 9.12

Preventive Actions Against Denaturation

Factor	Comment
pH	Loss of tertiary structure is expected at pH above 9.5. Proteins tend to be most stable near the isoelectric point.
Temperature	The unfolding process is a function of temperature. Many proteins have optimal stability in the temperature range 10°C–30°C. Loss of structure is expected both at low temperatures (cold denaturation) and at elevated temperatures. The reason that many protein biopharmaceuticals are stored at low temperatures is to minimize chemical degradation (e.g., deamidation).
Time	The denaturation reaction can be very fast.
Conductivity	No data available.
Redox potential	Cleavage of disulfide bonds is expected under reducing conditions. A redox potential below 100 mV is considered unstable for some proteins (e.g., insulin). Not all proteins undergo conformational changes upon reduction of the disulfide bond(s).
Co-solvents	Sucrose, mannose, glucose, glycine, alanine, glutamine, and ammonium sulfate are examples of compounds acting as protein stabilizers (weak or not binding to the protein surface).
	Magnesium sulfate, guanidinium sulfate, sodium chloride, and other weakly interacting salts exhibit an effect depending on protein charge and concentration.
	PEG and MPD act as stabilizers due to steric exclusion and repulsion from charged groups. Both PEG and MPD may destabilize the protein under certain circumstances, where binding is favored over exclusion.
	Co-solvents, such as urea or guanidinium chloride, which bind strongly to the protein surface, are strong denaturants.

Source: *Handbook of Biogeneric Therapeutic Proteins: Regulatory, Manufacturing, Testing and Intellectual Property Issues*, Taylor & Francis Group, Boca Raton, FL, 2005.

Abbreviations: MPD, 2-methyl-2,4 pentanediol; PEG, polyethylene glycol.