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STABILITY AND STORAGE CONDITIONS OF PROTEIN

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Proteins comprise an extremely heterogeneous class of biological macromolecules. Proteins are multifunctional in the sense that their specific amino acid sequence simultaneously determines folding, function and degradation. Regarding stability, proteins, due to their delicate balance of attractive and repulsive weak interactions, are only marginally stable if physiological conditions are considered as standard state. Therefore, it seems appropriate to briefly summarize some of the relevant methods that are available to study the stability and folding of proteins (Table 1). In the vast majority of examples it is established that proteins have the intrinsic capacity to acquire their unique three-dimensional structure in a spontaneous and autonomous way, depending solely on their amino acid sequence and the (native-like) environment. They are often unstable when not in their native environments, which can vary considerably among cell compartments and extracellular fluids (Damaschun *et al.,* 1999). The spectrum of experimental approaches suitable for the characterization of the structure-function relationship of proteins is described in Table 1.

If certain buffer conditions are not maintained, extracted proteins may not function properly or remain soluble. Proteins can lose activity as a result of proteolysis, aggregation and suboptimal buffer conditions. Purified proteins often need to be stored for an extended period of time while retaining their original structural integrity and/or activity. The extent of storage 'shelf life' can vary from a few days to more than a year and is dependent on the nature of the protein and the storage conditions used. Optimal conditions for storage are distinctive to each protein; nevertheless, it is possible to suggest some general guidelines for protein storage and stability.

Common conditions for protein storage are summarized and compared in Table 2. Generally, there are tradeoffs associated with each method. For example, proteins stored in solution at 4°C can be dispensed conveniently as needed but require more diligence to prevent microbial or proteolytic degradation; such proteins may not be stable for more than a few days or weeks. By contrast, lyophilization allows for long-term storage of protein with very little threat of degradation, but the protein must be reconstituted before use and may be damaged by the lyophilization process (Taschner *et al.,* 2001).

General Considerations for Protein Storage

Generally, proteins are best stored at ≤ 4°C in clean, autoclaved glassware or polypropylene tubes. Storage at room temperature often leads to protein degradation and/or inactivity, commonly as a result of microbial growth. For short term storage (1 day to a few weeks), many proteins may be stored in simple buffers at 4°C. Some osmolyte helps to extend the shelf-life of most proteins for storage at 4° C or -20°C compared to storage in simple phosphate or Tris buffers (Taravati *et al.,* 2007; Rösgen *et al.,* 2007).

Frozen at -20°C or -80°C is the more common form of cold protein storage. Because freeze-thaw cycles decrease protein stability, samples for frozen storage are best dispensed and prepared in single-use aliquots so that, once thawed, the protein solution will not have to be refrozen. Alternatively, addition of 50% glycerol or ethylene glycol will prevent solutions from freezing at -20°C, enabling repeated use from a single stock without warming (i.e., thawing).

Dilute protein solutions (< 1 mg/ml) are more prone to inactivation and loss as a result of low-level binding to the storage vessel. Therefore, it is universal to add "carrier" or "filler" protein, such as purified bovine serum albumin (BSA) to 1-5 mg/ml (0.1-0.5%), to dilute protein solutions to protect against such degradation and loss.

Many compounds may be added to protein solutions to lengthen shelf life:

- Protein stabilizing osmolytes helps to extend the shelf-life of most proteins for storage at 4°C or -20°C.
- Protease inhibitors, such as PMSF, prevent proteolytic cleavage of proteins.
- Anti-microbial agents such as sodium azide (NaN3) at a final concentration of 0.02-0.05% (w/v) or thimerosal at a final concentration of 0.01 % (w/v) inhibit microbial growth.
- Metal chelators such as EDTA at a final concentration of 1-5 mM avoid metal-induced oxidation of –SH groups and helps to maintain the protein in a reduced state.
- Reducing agents such a dithiothreitol (DTT) and 2-mercaptoethanol (2-ME) at final concentrations of 1-5 mM also help to maintain the protein in the reduced state by preventing oxidation of cysteines.

• Cryoprotectants such as glycerol or ethylene glycol to a final concentration of 25-50% help to stabilize proteins by preventing the formation of ice crystals at -20°C that destroy protein structure (Correa and Farah, 2007).

Table 1. Experimental approaches to the stability and folding of domain proteins.

Equilibrium measurements	
Compactness	Analytical ultracentrifugation, calorimetry (ΔCp), frictional parameters, H-D exchange.
Conformation	Spectroscopy: absorption, fluorescence emission, circular dichroism, NMR (combined with H-D exchange). Stability toward proteolysis and denaturants. Direct measurements of thermodynamic parameters by differential scanning calorimetry and isothermal flow-calorimetry. Ligand binding: Cofactors, group specific labels, ANS and other fluorophores.
Function (biological activity)	Enzymatic activity, ligand affinity, Michaelis constant.[a]
Kinetic measurements	
Folding	Spectroscopy (cf. conformation). Depending on the time range, methods include manual mixing, stopped flow, quenched stopped flow (double jump), relaxation techniques (T-jump, flash photolysis etc.). 2D heteronuclear NMR combined with H-D exchange and mass spectroscopy). Enzymatic activity, ligand binding.

[a]Ligand binding may cause artifacts by shifting equilibria or by stabilizing intermediates.

Table 2. Comparison of Protein Storage Conditions.

	Storage Condition			
Characteristic	Solution at 4°C	Solution in 25-50% glycerol or ethylene glycol at -20°C	Frozen at -20° to -80°C or in liquid nitrogen	Lyophilized (usually also frozen)
Typical shelf life	1 month	1 year	Years	Years
Requires sterile conditions or addition of antibacterial agent	Yes	Usually	No	No
Number of times a sample may be removed for use	Many	Many	Once; repeated freeze-thaw cycles generally degrade proteins	Once; it is impractical to lyophilize a sample multiple times

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