The avoidance of proteolysis during sample preparation

Factor	Comment
pH and buffers	Use a buffer with a pH either above or below the optimum for proteinase activity. There is no range of pH at which all proteolytic enzymes could be considered to be inactive, but above neutral proteolytic pH, the nonspecific and highly active enzymes of the lysosomes or vacuoles will be minimally active. If, as in yeasts, the cellular pH is normally lower than this, a high concentration of buffer should be used to ensure that the required pH is obtained when the cell contents are released. A neutral pH favors interactions between proteinases and any endogenous inhibitors that could provide some degree of protection. Some stabilizing effects have been noted with particular buffers, for example, phosphate.
Low temperature	Proteinase activity is minimized by carrying out all steps at low temperature. Extended procedures should be carried out in the cold and samples stored frozen.
Time	The shorter the preparation time, the less opportunity for proteolysis to occur. Lengthy procedures, such as overnight dialysis, should not be undertaken without the inclusion of proteinase inhibitors in the dialysis buffer. In some systems, for example, yeasts and some filamentous fungi, prolonged incubation of extracts results in proteinase activation because endogenous inhibitors are degraded.
Stabilizing agents	Protein stabilizing reagents can provide protection against proteolysis. The inclusion of glycerol (15–35% v/v) or dimethylsulfoxide (DMSO) (10% v/v) may be useful during preparation and storage. The presence of a reducing agent such as dithiothreitol (0.1–1 mM) or β-mercaptoethanol (1–10 mM) ensures that free thiol groups are not oxidized, but they will activate cysteine proteinases. The reagents could affect the activity of the protein of interest and reducing agents will diminish the effectiveness of some proteinase inhibitors.
Exogenous protein	Proteins such as bovine serum albumin (1–5 g/liter) may provide protection by offering an alternative substrate to endogenous proteinases. Additional steps in protein preparation may have to be included in order to remove this exogenous protein later.
Effectors	Low-molecular-weight effectors, such as substrates, substrate analogs, and cofactors, can help to maintain a protein in a stable conformation and prevent proteolytic attack. Not all substrates and cofactors will necessarily be effective. Each substrate and cofactor should be tested as part of a general screening process for protective agents. Any protective effect must be balanced against the cost of using expensive chemicals in a large-scale preparation.
Activators omitted	Potential proteinase activators such as divalent cations (these can activate and/or stabilize the enzyme) should be excluded from the extraction buffer.