It is always a good idea to characterize the stability of new protein samples. You will likely notice during the purification procedure if the protein is particularly unstable or sensitive to the presence or absence of certain salts, by the formation of precipitate. Before submitting your sample for crystallization trials, you will also want to dialyze into the appropriate buffer (or water) and concentrate your sample. If during any of these steps a precipitate is formed, certain questions need to be answered:

- At what point did your sample start to precipitate—was it during a concentration step or upon addition of a ligand?
- If precipitated, can the protein be put back into solution by the addition of salt?
- Do any metals, chelators or ligands such as nucleotides need to be added to your solution?
- Should a reducing agent be present?
- Did you notice the presence of aggregates during a gel filtration step or by any biophysical analysis method (AUC, DLS, etc.)?
- Can the protein be dialysed into water or a weakly buffered solution without precipitating?
- Does your sample start to precipitate or degrade as it ages?
- At what temperature is your protein most stable?

One experiment that we recommend you perform prior to submitting your samples for crystallization trials is a room temperature time course that will test for protein degradation.

- Purify and concentrate your protein, making SDS samples throughout the course of purification and after concentration.
- Take a small aliquot (~20µL) of your final protein sample. Leave on your benchtop at room temperature.
- Every day (or every other day), take a small amount from your benchtop aliquot and make SDS samples of it (in both reducing and non-reducing conditions if possible).
- At the end of a week or two, run each of the samples on a gel alongside samples of your starting product.
- Do you see any degradation or cleavage products, or is your sample still fully intact? What does this tell you about your protein?
  - If you have enough sample to spare, it is a good idea to do this experiment with 2 aliquots—one left at room temperature (benchtop), and one at 4°C.
  - Does the 4°C sample look any better, or is your protein equally stable at room temperature?