Guidelines for Sample Preparation:

1. **Oligomeric State and Homogeneity of Sample:**
   For crystallization, the protein or macromolecular complex of interest should be ~98% pure and have a single oligomeric state. There are several ways to determine sample purity, homogeneity and oligomeric state: 1.) To check purity, run an SDS denaturing gel. 2.) To check oligomeric state, run a non-denaturing gel or FPLC gel filtration column. 3.) Additionally, analyze the sample by electrospray mass spectrometry.

2. **Precipitation Point:**
   It is advantageous to know the maximum protein or complex concentration without precipitation or aggregation. To determine this, measure the $A_{280}$ of < 400 µL of the sample, concentrate briefly (< 5 min), mix and take another $A_{280}$ reading. Repeat this procedure until the volume of the sample continues to drop but the concentration of the sample remains the same. This concentration is the precipitation point of your sample. You will want to remain several mg/ml below this value for all your future work. (You can obtain a small concentrator from the CSB if needed.)

3. **Check the stability of the protein at various concentrations and temperatures**
   The CSB has controlled temperature rooms for crystallization trials at 20°C and 4°C. In order to save time and material, it is beneficial to know whether the concentrated protein is stable in solution overnight. Measure $A_{280}$ of a concentrated sample, and split it into two aliquots. Store one aliquot at 4 °C overnight and leave the other at room temperature. In the morning, spin both samples at 14,000 rpm for 10 minutes. Move supernatant to a new tube, mix and measure the $A_{280}$. At which temperature did the $A_{280}$ have the smallest (or no) change? If there was significant change in both samples, then we need to find a better storage buffer for the protein. (See Protein Stability Profile)

4. **Additional Considerations of Sample Preparation:**
   a. We may ask for the sequence of the expressed protein, if you are having stability problems or are requesting the CSB provide all services. The CSB staff will keep all information confidential regardless of type of service requested.
   b. Does the protein have disulfide bonds? If so, how many?
   c. Many proteins cannot be crystallized after a freeze-thaw cycle, so we recommend purifying the protein without freezing at any step other than the initial freezing of the cell pellet.