Biomacrolecular sample preparation for synchrotron X-ray scattering experiments

1. Buffer Composition:

Salts are often useful to suppress long-range electrostatic interactions between solutes (structure factor). They also increase background and decrease solute/solvent contrast but these effects are often negligible up to ~500 mM salt. **High-Z elements should be avoided in the buffer**. They decrease contrast and promote radiation damage by increasing photo-electron production.

Free radical scavengers should be included in the buffer when preparing for a synchrotron data collection as they help to minimize the radiation damage. Organic buffers, such as TRIS or HEPES, are recommended, which can act as radical scavengers besides pH buffer. Typical buffer concentrations are 20-100mM. Other common choices are DTT (2-10 mM), TCEP (1-2 mM), or glycerol (~5%). **Phosphate buffer should be avoided because it promotes radiation damage.**

Detergents are best avoided unless absolutely necessary (membrane proteins). Their signal can be comparable to or exceeding the protein signal complicating data interpretation.

2. Prepare sample with matching buffer

Preparation of an **exactly matching** buffer is the most crucial step. Do not use the buffer the protein was dissolved in. Long (16-48 hr) dialysis or passing buffer by centrifugation though a proper MW-cutoff filter works for this purpose. For the latter, several cycles of buffer loading will be needed to ensure the exact match. Prior to loading the sample for dialysis or filtering, it is good to pass it through a 0.22 mm filter.

3. Sample Concentration and Amount:

Typical sample concentration that produce good scattering signal is 1-10 mg/ml for RNAs and proteins. At least three concentrations for each sample, if possible, ~1, ~3, and ~5 mg/ml, are recommended, to check particle interactions and extrapolate "interaction-free" scattering profile. If you only have enough material for one sample, make a sample with concentration of 2-5 mg/ml for proteins, or ~2mg/ml for RNAs. The minimum volume for scattering experiments is 160 ul. **At least 20 ml matching buffer is needed** for each sample for background measurement and scattering cell washing.

4. Pre-examination before X-ray scattering.

Aggregation is the most common problem that can render data uninterpretable. Up to 5% of dimer relative to the monomer in question might not affect the data too much but higher levels will. Dynamics light scattering or analytical ultracentrifugation can be used to detect aggregation/polydispersity before performing X-ray scattering experiments. Native gel (single band is required), gel filtration, or centrifugation through high-MW cutoff membrane can help to remove it. Otherwise, sample conditions will have to be optimized before performing SAXS experiments.