Guidelines for High Pressure "Batch" BioSAXS

This guide applies to high-pressure BioSAXS conducted using the static "batch" system in which 60 microliter sample volumes are loaded into a large metal chamber with diamond windows that can be pressurized to 400 MPa or more. A separate guide is available for high-pressure chromatography SAXS.

High pressure BioSAXS is a more difficult experiment than normal ambient pressure BioSAXS. It generally consumes more time and more sample, background subtractions are less precise, and the Interpretation of data is more difficult. Despite these problems, HP-BioSAXS can yield excellent data if done with care.

We strongly recommend that you first check your samples with standard ambient SAXS/SEC-SAXS so you know if they are good enough for high-pressure work.

How much sample do I need?

Sample volume is currently 60 microliters. Because of radiation damage and other variables, you should expect to use several volumes of sample (at least 3). Precisely how much you will burn through depends on what protocol you use and how many pressure/temperature points you will need. The more sample the better. See the Recommended Protocol section for more details.

What is the minimum concentration needed?

HP-BioSAXS is slightly less sensitive and requires higher concentrations than normal BioSAXS, nonetheless, we have obtained usable Guinier plots of a glucose isomerase standard at approximately 0.5 mg/ml, which is not far from the 0.3 mg/ml used in normal BioSAXS for calibration purposes. Because sample-to-buffer contrast decreases significantly with pressure, we recommend using a concentration of at least 2-3 times greater than typical for standard BioSAXS. Thus, the rule of thumb we offer is

Minimum concentration (mg/ml) = 150/molecular weight (kDa).

Are there special sample preparation requirements?

Yes. Just as in normal BioSAXS, you need a precisely-matched buffer sample. This can be prepared by dialysis, SEC, centrifugal concentrator, or spin column. See normal BioSAXS recommendations for details.

You should also use a pressure-resistant buffer. Generally avoid PBS

How long does it take and how many samples should I bring?

If just doing pressure series at constant temperature, an experienced group can measure about 50 samples in 24 hr. Temperature changes are time-consuming. A temperature series on a single buffer at constant pressure running from 5 C to 50 C (steps of 5 C) takes just over 1 hour.

Data collection protocol

Because this high-pressure SAXS technology uses static (non-flowing) sample, *radiation damage is a very serious issue that can ruin your data interpretation and cause you problems with reviewers unless you carefully control for it.* Samples need to be exposed to the minimum dose necessary to obtain usable data.

Using concentrated samples will improve the signal for short exposures, but you will need to control for potential concentration effects. Concentration effects are known to change with pressure, so it is strongly recommended that you fully characterize the sample with standard ambient SAXS prior to attempting high-pressure work. This includes doing a concentration series.

While synchrotron X-ray beams can routinely reach 10^{13} photons per second in a 0.25 X 0.25 mm spot, a non-flowing sample will burn in a fraction of a second under these conditions. High pressure experiments at CHESS HP-Bio are conducted at 14 keV, a higher energy than normal BioSAXS. This is done to help X-rays penetrate the diamond windows in the pressure cell, but may have some added benefit in reducing sample damage. Based on experience so far, we recommend that beam be attenuated to ~ 5 X 10^{10} photons per second. Under these conditions, a static sample will survive multiple 1 sec exposures, especially if a 5-10 min wait period is introduced between exposures. Nonetheless, it is important to spot-check samples after high-pressure exposures, comparing them to undamaged ambient pressure results.