Progress towards developing an experimental platform for high-throughput CryoSAXS

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Small-angle X-ray scattering (SAXS) is a key tool for probing the structure and function of proteins, nucleic acids, and macromolecular complexes. Storage and transport of biomolecular solutions at or near room temperature where they may be unstable, large sample volume requirements per measurement, and low synchrotron data collection duty cycles are critical bottlenecks in expanding the application of BioSAXS, especially in high-throughput screening applications. CryoSAXS – SAXS performed on samples cryocooled to T ≈ 100 K, has the potential to address key issues by allowing preparation of samples in the home lab immediately after biomolecule purification, reducing radiation damage and sample consumption per measurement, and allowing the use of sample holders compatible with standard macromolecular cryocrystallography infrastructure for sample storage, shipping, and automated high-throughput data collection.

Demonstrations of CryoSAXS have shown the potential of this technique1,2, but the lack of a robust experimental platform has prevented it from becoming a routine method. We are continuing the development of sample cell arrays and methods for high throughput CryoSAXS. Using cell arrays with sample volumes per cell < 0.2 microliters, we can now obtain high quality data using T=100 K gas stream cooling, robust buffer subtraction using biomolecule and buffer scattering profiles obtained from different cells in the same array, and exposures per sample that can be increased to several minutes to maximize signal to noise. Precision cell array assembly, careful management of upstream parasitic X-ray scatter and shadowing of that scatter by the sample cell array, and performing experimental checks for cooling related artifacts are among the keys to obtaining high quality biomolecular profiles.

1. Meisburger, S. P. et al. (2013) Biophys. J. 104, 227-236.

2. Hopkins, J. B. et al. (2015) J. Appl. Cryst. 48, 227-237.