## Small Angle X-Ray Scattering Measurements of Protein Crowding in The Frozen State

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Freezing is vital preservation for the long-term storage of proteins. It is essential for industries like pharmaceuticals and food science since the quality and conservation of the protein are necessary to maintain the product's efficacy. However, many proteins are known to aggregate, denature or degrade upon freezing. Understanding the behavior of proteins in frozen conditions can help develop preservation methods. Unfortunately, few techniques can directly study the structure of proteins in ice water. Previous studies have successfully applied small angle neutron scattering (SANS) to determine the structure factor of frozen proteins. While SANS is very well suited for these studies, it is beneficial to have an alternative method to validate and complement these findings and to provide broader access to these measurements. For this reason, we turn to small angle x-ray scattering (SAXS) since, unlike SANS, it can be performed using a laboratory source.

In this work, we used a laboratory SAXS source (Xenocs Xeuss 3.0) to perform measurements of model proteins frozen in ice. Our findings agree with previously published SANS measurements and demonstrate that SAXS can be used to perform these measurements. In this way, SAXS can be used complementary and supplementary to SANS measurements. Furthermore, the increased accessibility of SAXS measurements can allow studying more molecules and excipients.