Supporting information

A microfabricated fixed path length silicon sample holder enables improved background subtraction for cryoSAXS

Jesse B. Hopkins[a,1], Andrea M. Katz[b,1], Steve P. Meisburger[b], Matthew A. Warkentin[a], Robert E. Thorne[a] and Lois Pollack[b]
[a]Laboratory of Atomic and Solid State Physics, Cornell University, Ithaca, New York, 14853, USA
[b]School of Applied and Engineering Physics, Cornell University, Ithaca, New York, 14853, USA

Correspondence email: jbh246@cornell.edu

S1. Efforts to mitigate fractures

A range of cryocooling conditions were tested in an attempt to eliminate fracturing. The magnitude of the elastic stresses that accumulate due to differential contraction between the sample and holder or within the sample during cooling is determined in part by the temperature range over which the sample is vitreous and "solid." This temperature range can be reduced by reducing the cryoprotectant concentration (which lowers the vitrification temperature), by increasing the final temperature, and by reducing the cooling rate (which also lowers the effective vitrification temperature) (Fahy et al., 1990; Steif et al., 2005; Yavin & Arav, 2007).

The temperature to which the sample was cooled was varied from 90 K to 190 K. Samples cooled to final temperatures above ~140 K showed no visible fractures. However, scattering from samples cooled to ~140 K to 170 K was irreproducible, and was often anisotropic. Speculatively, this irreproducibility and anisotropy is due to nonuniform elastic stresses and to microscale defects/fractures that give structure in the q range of interest. These likely arise as the sample cools through the region of extreme viscosity near the glass transition. Scattering from samples cooled to above ~170 K was isotropic, but at low q displayed a power law that in (Meisburger et al., 2013) was characteristic of ice formation. Increasing the cryoprotectant concentration in an attempt to find a temperature near 170 K at which scatter was reproducible and isotropic without the power law characteristic of ice was unsuccessful.

Two types of slow cooling were used in an attempt to reduce or eliminate fracturing. In the first, samples with larger cryoprotectant concentrations (to prevent ice formation) were cooled at 0.1

[1] Jesse B. Hopkins and Andrea M. Katz contributed equally to this work.

K/s from 250 K (to prevent initial evaporation) to the final temperature. In the second, samples with standard cryoprotectant concentrations were first cooled at ~25 K/s to an intermediate temperature such as 180 K and then cooled at 0.1 K/s to the final temperature. Both of these techniques slightly lowered the temperature at which fractures appeared, but did not alter the observed scattering behaviors.

Reducing the dimensions of the sample holder should reduce thermal gradients and peak sample stresses, but it also increases the sample cooling rate, which should increase the effective vitrification temperature and the accumulated stress at the final temperature (Yavin & Arav, 2007). Sample holders with X-ray path lengths from 0.1-1.0 mm, lateral dimensions from 0.5-2.0 mm, and thus sample volumes of ~10-1500 nL were tested. The trade-off between reduced volume and increased cooling rates caused first a lowering and then a raising of the fracturing temperature as the volume was decreased, but did not change the general scattering behaviors.

Based on these observations, for these sample holders visibly fractured samples gave the best scattering profiles, perhaps because they relieve thermal stress with large scale features that scatter at low q, minimally perturbing the q range of interest in SAXS. The conditions used in the main body of this work were the optimal conditions identified within the limited search space.

The tendency of fractures to form parallel to the walls of the sample holders suggests that the holder's geometry affects the pattern of fracture. Homogeneously stressed uniform samples can often sustain very large elastic stresses. Asymmetries in the current holders due to the design and to the presence of interior surfaces defined by {110}, {111} and {311} crystallographic planes result in nonuniform stresses in cryocooled samples. Fracture typically occurs at much lower average sample stresses due to geometry- or temperature-gradient-related stress concentration, and/or due to the presence of pre-existing defects like bubbles or inclusions (Anderson, 2004). Thus, the geometry may be chosen either to produce maximally uniform stresses or to concentrate stress and promote fracturing in non-critical regions so as to reduce fracturing in critical regions. Therefore, controlling the location and preferred orientation of fractures by sample holder geometry is a promising future research direction.

Elimination of fracturing is not a sufficient criterion to guarantee good scattering, it is likely that the overall stresses in the sample upon cooling must be greatly reduced from the current levels. Good scattering profiles have been collected from unfractured samples at 100 K (Meisburger et al., 2013). These profiles were collected from windowless, flexible, plastic sample holders, which likely minimized stresses from cooling (flexible sample holder materials also reduce fracturing in much larger samples (Rall, 1987)). Thus careful optimization of the sample holder design to

eliminate or reduce fracturing will be carried out hand-in-hand with modeling of the stress effects upon cooling to minimize overall stress in the sample.

S2. Supporting Figures

This section contains the supporting figures referenced in the text of the paper.

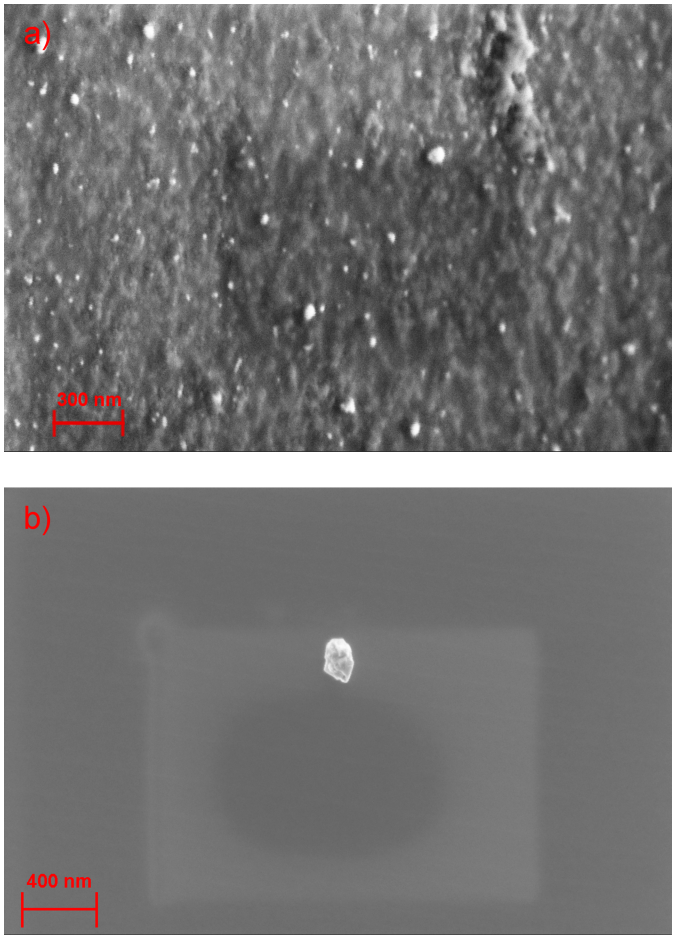

Figure S1 a) SEM image (3 keV beam energy) of a rough silicon X-ray window, before etch parameters were optimized. b) SEM image (1.5 keV beam energy) of a smooth silicon X-ray window after optimization of etch parameters. A dust particle is included for focus because windows are featureless to the SEM.

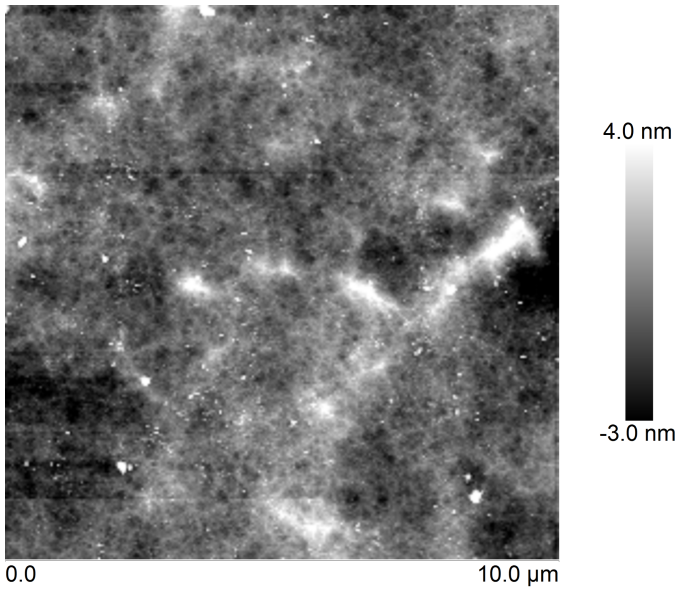

Figure S2 AFM image of a silicon X-ray window with optimized etch parameters. The height variation is on the order of nanometers over 100 μm^2. The window was cleaved from the sample holder and adhered horizontally to a substrate before AFM analysis.

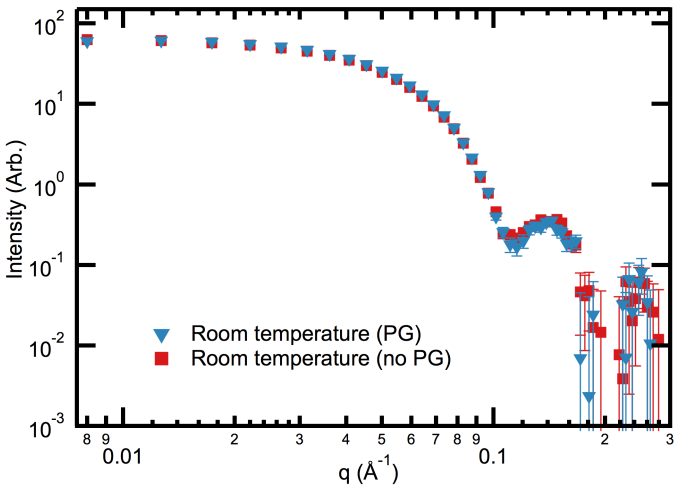

Figure S3 Comparison of glucose isomerase (2.8 mg/mL) in PG at room temperature and in the same buffer without PG at room temperature.

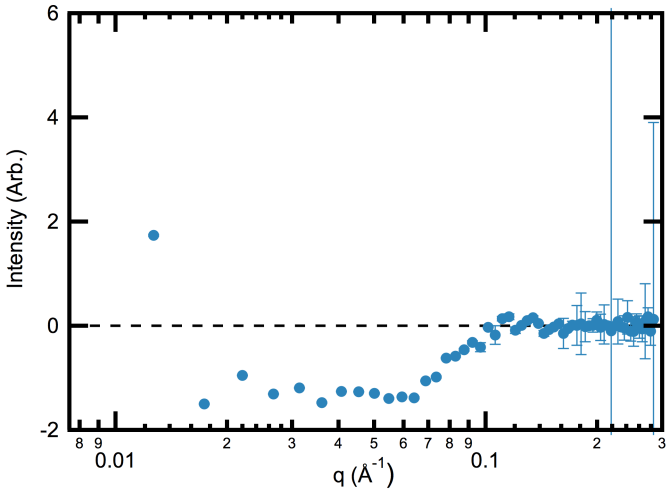

Figure S4 Residual obtained by subtracting the scattering intensity at room temperature from the scattering intensity at 100 K for glucose isomerase. The original intensity curves are shown in Figure 4. Note that due to differences in solvent structure and density between 100 K and room temperature the curves are not expected to be identical, even if the protein has identical atomic structure, so the residual should not be zero. The lowest q point has an intensity value of ~18, and is not included on the plot so residuals at high q are more visible. The q and intensity axes have the same range as Figure S7. The occasionally large error bars come from propagation of fractional errors for points near zero.

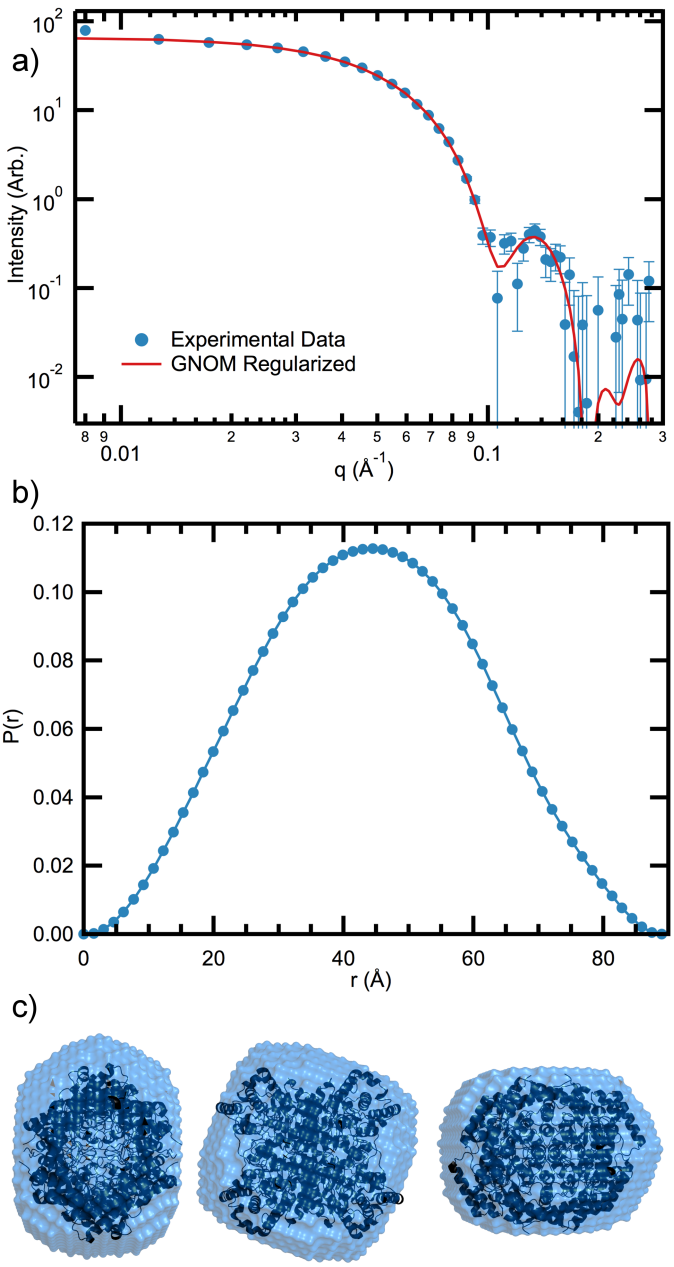

Figure S5 All data in this figure are for glucose isomerase (2.8 mg/mL) at 100 K in PG. a) Experimental data, and regularized I(q) from GNOM. χ^2 = 1.071, total estimate = 0.934. b) P(r) computed by GNOM with D_{max} = 89 Å. c) Three orthogonal views of the DAMAVER envelope aligned with the glucose isomerase crystal structure.

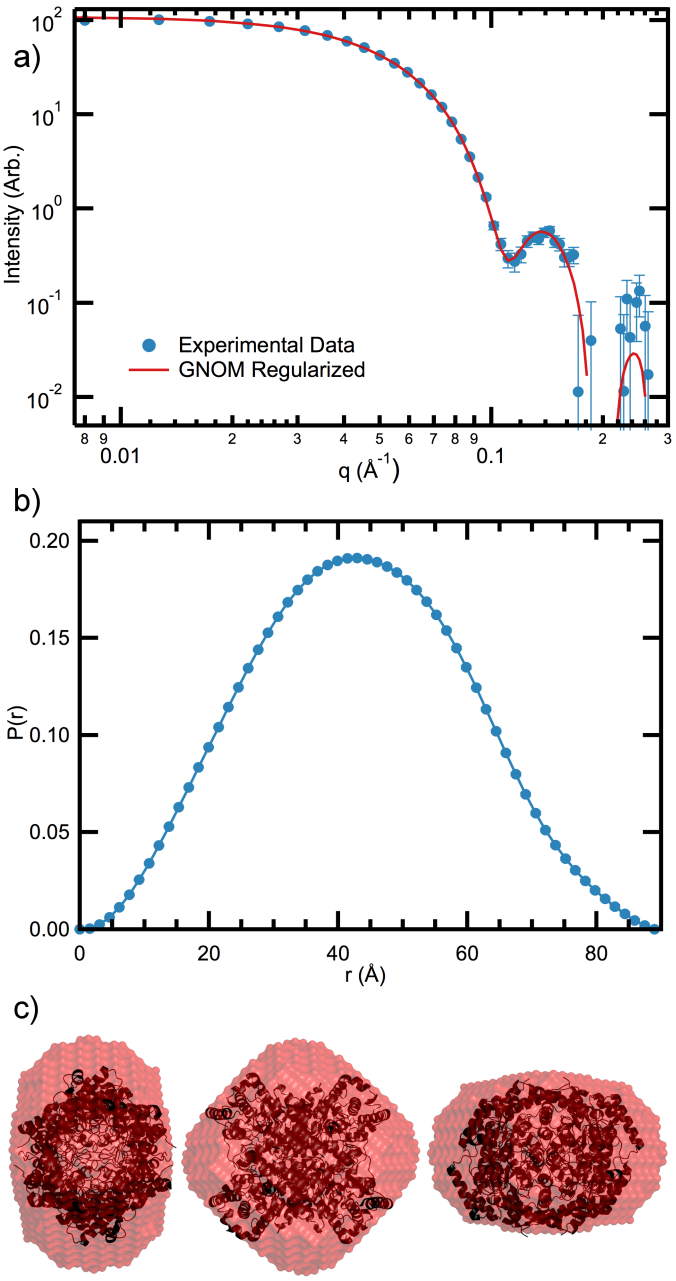

Figure S6 All data in this figure are for glucose isomerase (2.8 mg/mL) at room temperature in PG. a) Experimental data, and regularized I(q) from GNOM. χ^2 = 1.357, total estimate = 0.915. b) P(r) computed by GNOM with D_{max} = 89 Å. c) Three orthogonal views of the DAMAVER envelope aligned with the glucose isomerase crystal structure.

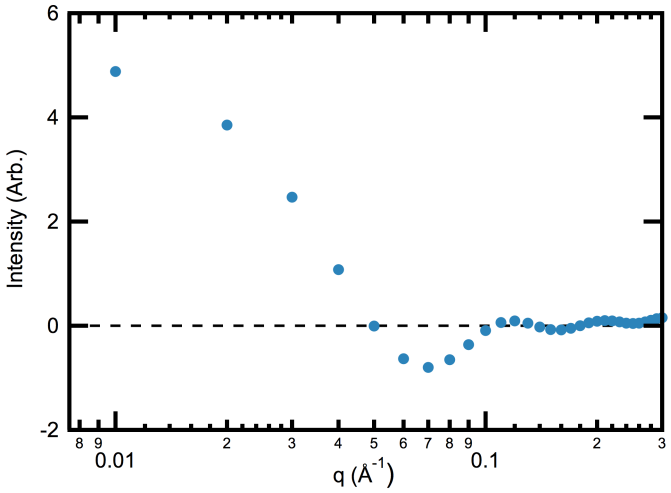

Figure S7 Residual obtained by subtracting the scattering intensity calculated for glucose isomerase using CRYSOL with default solvent parameters from that calculated with modified solvent parameters. The original intensity curves are shown in Figure 5. The modified solvent parameters mimic the change of the solvent on cooling to 100 K, so this curve should be, and is, similar to that in Figure S4. .

References

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Fahy, G. M., Saur, J., & Williams, R. J. (1990). *Cryobiology* **27**, 492–510.

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Yavin, S. & Arav, A. (2007). *Theriogenology* **67**, 81–89.