Supporting Material

Breaking the radiation damage limit with cryo-SAXS

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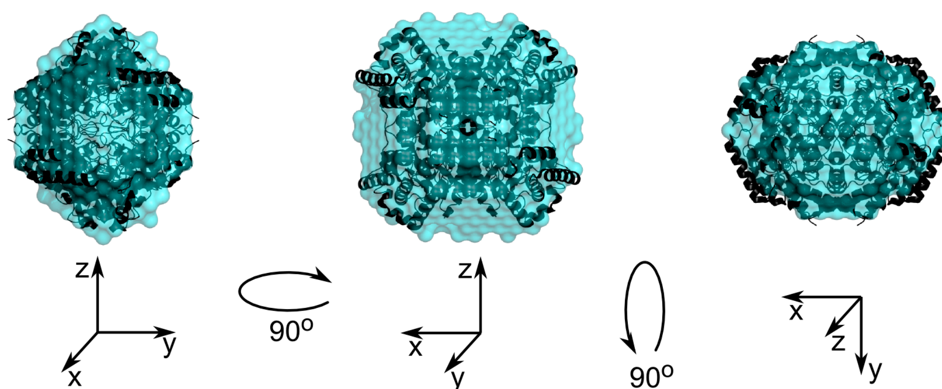

Figure S1: Improved reconstructions of glucose isomerase with symmetry constraints. Reconstructions of the low resolution envelope were performed using the cryo-SAXS curve of glucose isomerase held in the 1 μL cell over the q-range $0.01 < q < 0.2$ Å^{-1}, enforcing p42 symmetry and oblate anisometry. Ten DAMMIF reconstructions had a mean NSD of 0.531. The filtered average is shown along with the crystal structure PDB 1XIB.

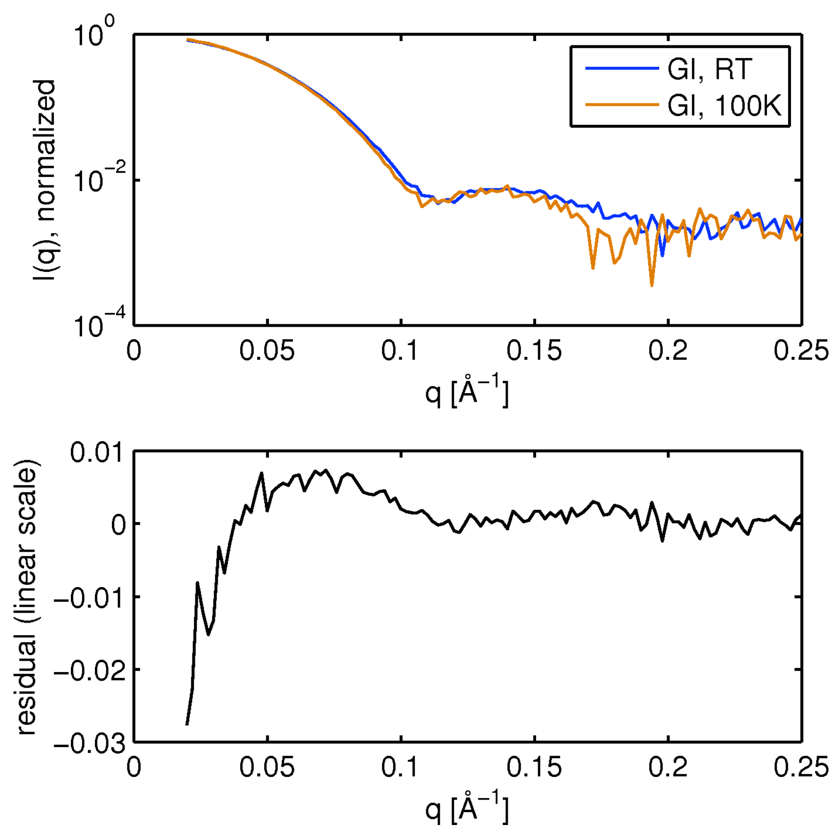

Figure S2: Direct comparison of glucose isomerase measurements at room temperature (RT, capillary flow cell) and 100 K (cryo-SAXS method, 1 μL cell). The protein concentration was 2 mg/mL, and both samples contained 45% (w/w) PEG 200.

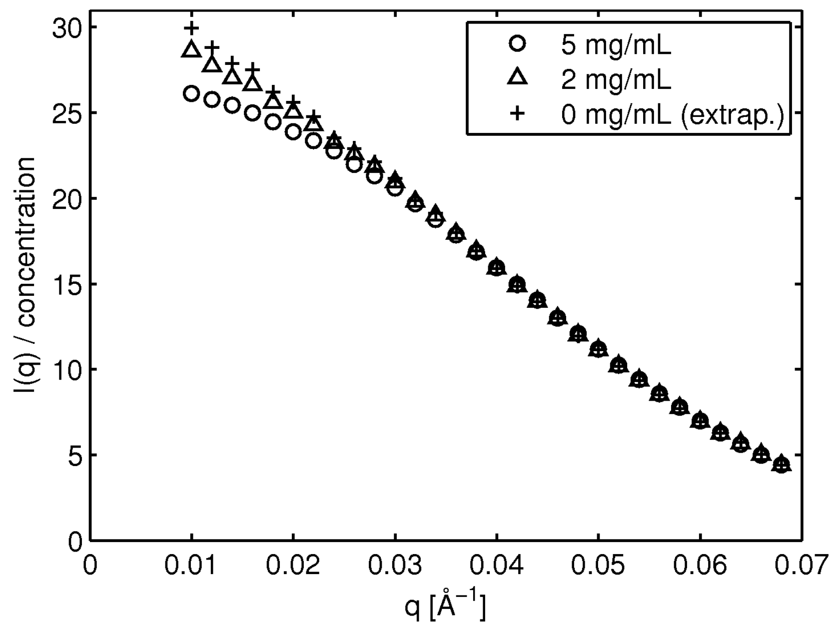

Figure S3: Observation of interparticle interference in cryocooled glucose isomerase solutions. Cryo-SAXS curves for glucose isomerase solutions at 2 mg/mL and 5 mg/mL concentration were acquired using the 1 µL cell. When the curves are scaled by concentration (matched at mid-q), the 5 mg/mL curve appears below the 2 mg/mL curve at low q, characteristic of repulsive interactions in solution. When extrapolated to zero concentration, the radius of gyration is 34.5 Å.

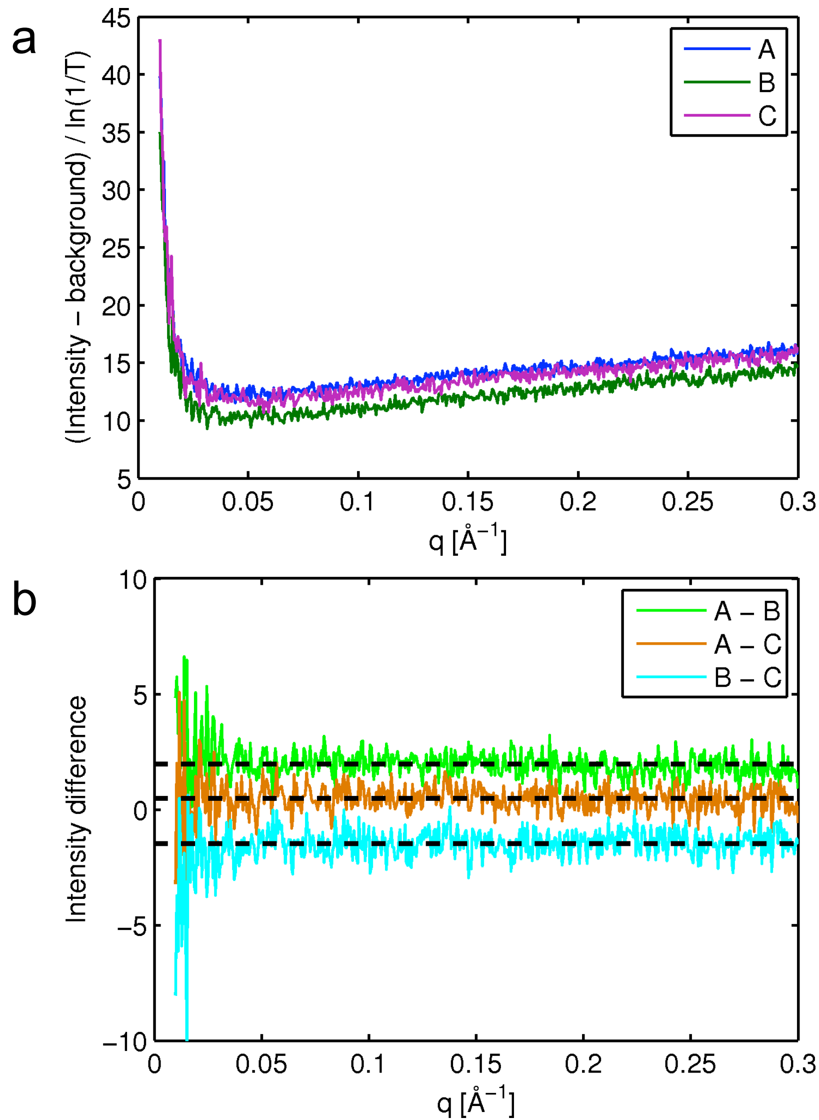

Figure S4: SAXS patterns of cryo-cooled GI buffer with 45% PEG 200 were acquired from lenticular sub-μL drops held in a 600μm crystallography loop. (a) Instrumental background scattering was subtracted from each buffer and the curves were normalized by the droplet thickness, according to the background subtraction protocol described in Materials and Methods. These drops do not have the same scattering, indicating that the background subtraction protocol does not adequately compensate for differences in sample geometry. (b) The difference between any pair of curves is well-approximated by a constant offset.

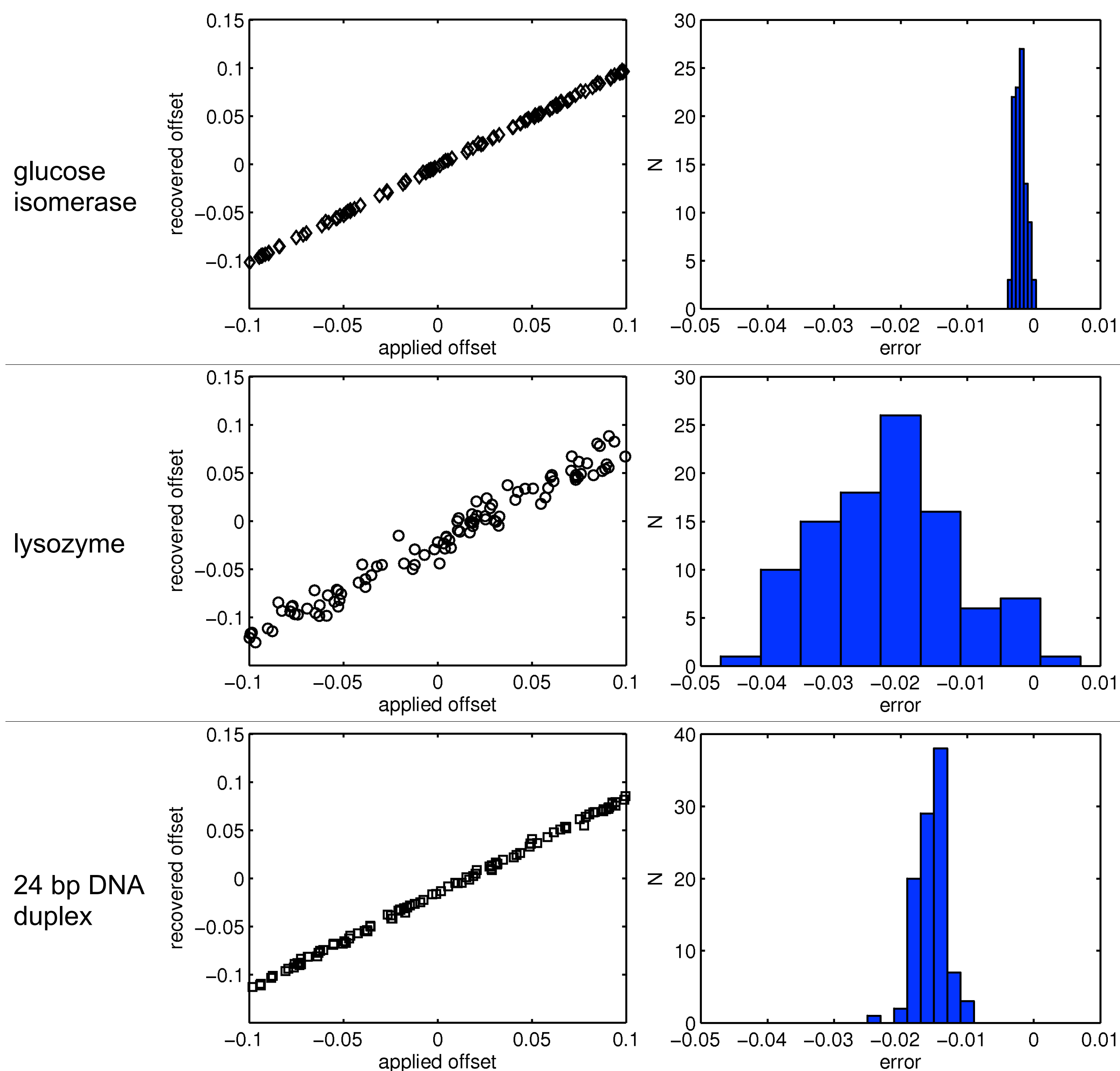

Figure S5: Performance of the BIFT method for offset correction under simulated experimental conditions. CRYSOL-generated SAXS curves for lysozyme, glucose isomerase (GI), and 24bp DNA were normalized so that I(0) = 1, re-sampled at the experimental values of q (0.02 to 0.3 in increments of 0.001 $Å^{-1}$), and Gaussian random noise corresponding to the experimental error was added. A constant offset was randomly chosen in the interval [-0.1, 0.1]. For each molecule, 100 such test curves were generated and BIFT was used to find the offset that maximized the evidence of P(r). The applied and recovered offsets are plotted on the left, and the absolute error (recovered - applied) is shown as a histogram on the right. Despite the high level of noise, the BIFT method is fairly reproducible for all simulated data. Lysozyme has the lowest precision of offset recovery; correspondingly, the data had highest relative noise because of Lysozyme's weak scattering. For GI, the SAXS curve approaches zero over the experimental q-range, making the correct offset easier to identify.