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Supplemental Information

Resonant Soft X-Ray Scattering Provides Protein Structure with Chemical Specificity

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Supplementary Information for

Resonant soft X-ray scattering provides protein structure with chemical specificity

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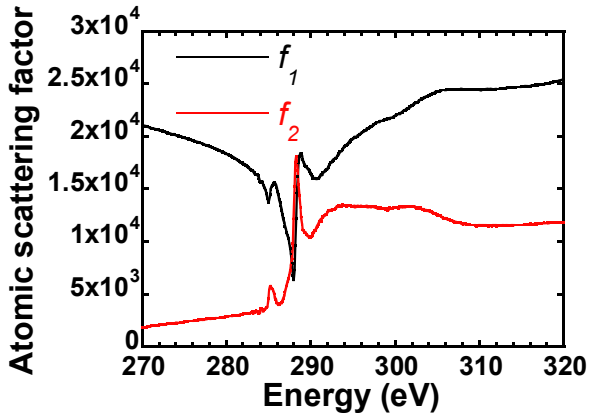

Figure S1. Related to Figure 1. Atomic scattering factors of BSA. Black curve is the sum of the real atomic scattering factors f_1 and the red curve is the sum of the imaginary atomic scattering factors f_2.

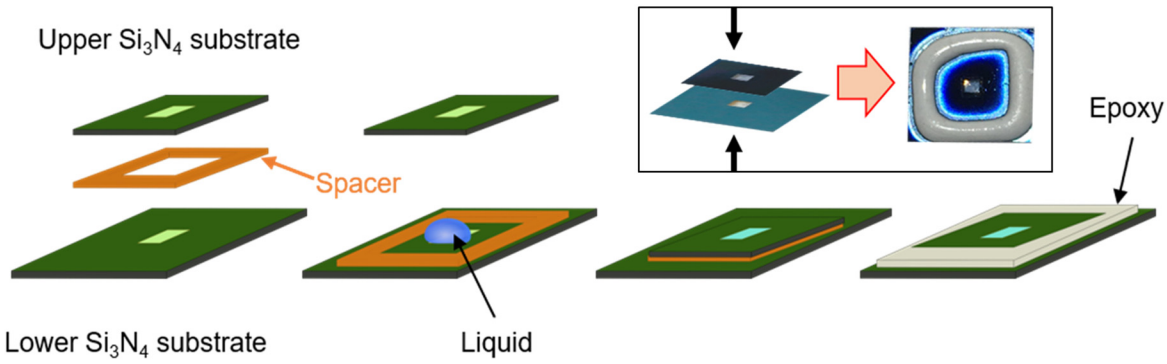

Figure S2. **Related to Figure 2. Schematic of fabrication procedure of RSoXS sample cell.** A solution of BSA was encapsulated between two commercially available Si_3N_4 substrates. Approximately 4 µL of liquid was pipetted on a 7.5 mm × 7.5 mm Si substrate with a 100 nm thick Si_3N_4 window (Norcada). The top window, which has a 1 µm spacer and a 5 mm × 5 mm outer frame (Silson), was placed on top of the liquid. Then the entire sample cell was sealed with epoxy (Loctite) and stored at room temperature for 24 hours to complete curing. Inset: photographs of constituent Si_3N_4 windows and final assembled liquid cell.

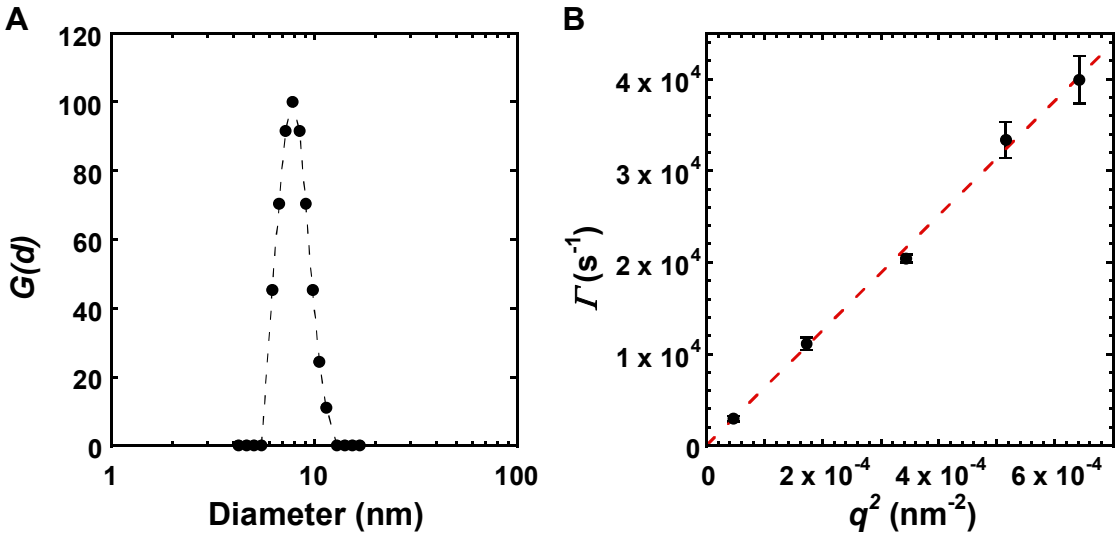

Figure S3. Related to Table 1. Size and size distribution of a solution of 10 mg/ml BSA in 1× PBS characterized by multi-angle dynamic light scattering (DLS). (A) Size distribution from data at a scattering angle of 90°. Dotted line is a guide to the eye. (B) Mean decay rate (Γ) as a function of the square of the scattering vector (q^2). The dotted line is a linear fit, where the slope is the diffusion coefficient. Error bars are standard deviations from three measurements on the same sample.

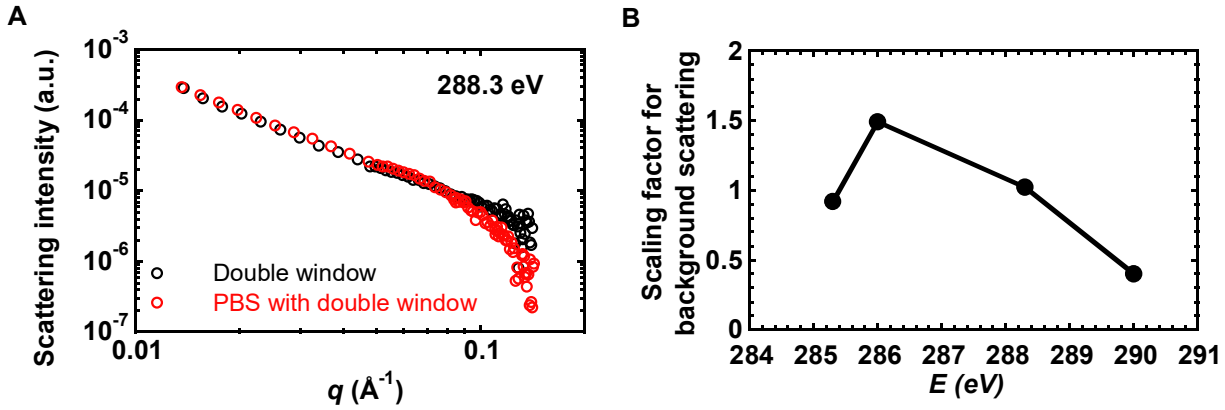

Figure S4. Related to Figure 2. Background scattering in RSoXS experiments. (A) Scattering profiles of two 100 nm thick Si_3N_4 windows (empty cell) and PBS buffer in between two 100 nm thick Si_3N_4 windows. (B) Scaling factor for background scattering that is applied prior to subtracting the background from BSA RSoXS data.

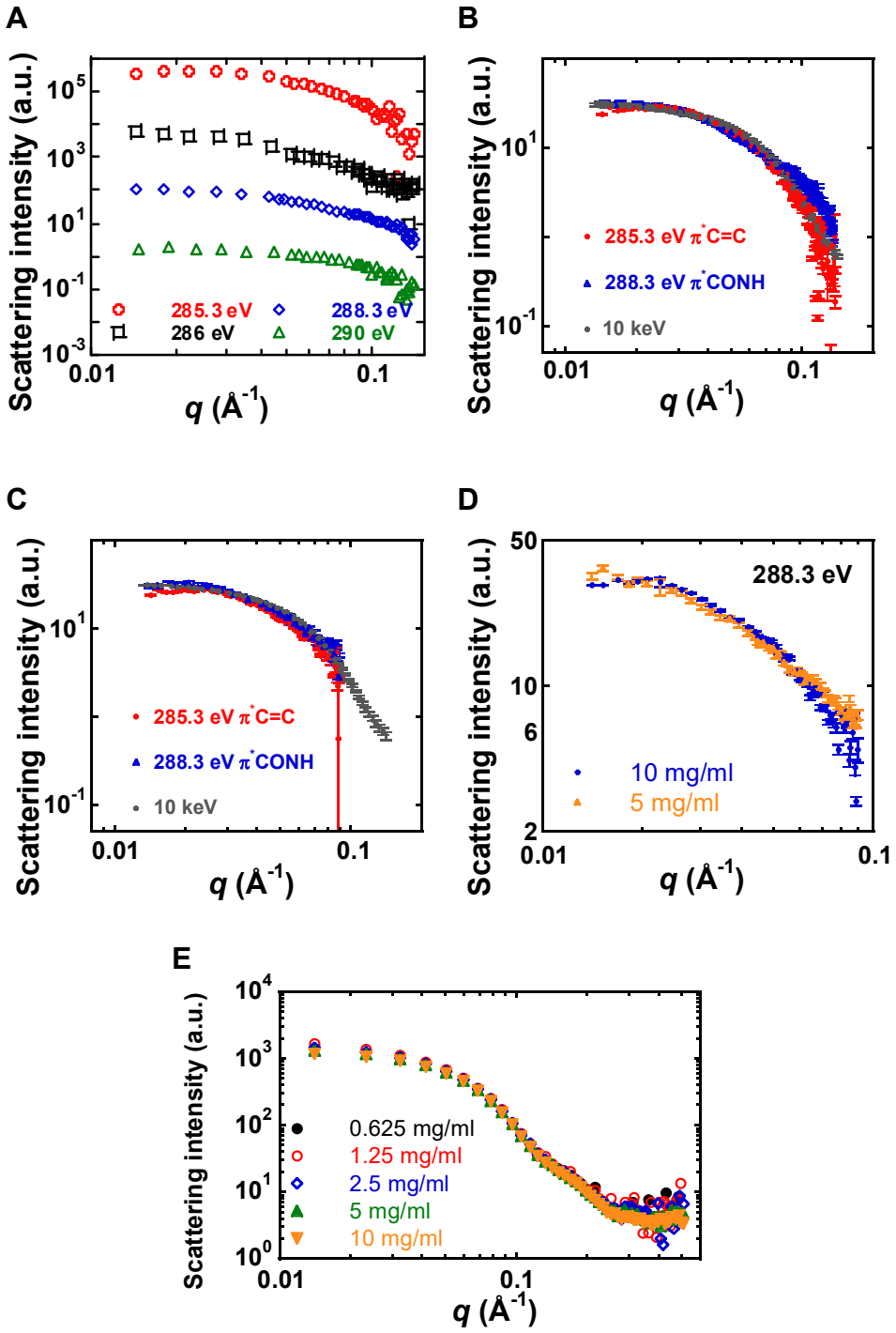

Figure S5. Related to Figure 2. Scattering profiles at different energies for BSA solutions. (A) RSoXS profiles for 10 mg/ml BSA in 1× PBS at various X-ray energies. Intensity profiles are shifted vertically for clarity. (B) Scattering data at 285.3 eV, 288.3 eV, and 10 keV for 10 mg/ml BSA in 1× PBS. Error bars represent the standard error of the mean obtained from integrating over all polar angles. (C) Average scattering profiles at 285. 3 eV (n=3), 288.3 eV (n=3) and 10 keV (n=2). Error bars represent the standard error of the mean calculated from multiple measurements. (D) Comparison of RSoXS scattering profiles of 10 mg/ml BSA and 5 mg/ml BSA in 1× PBS at 288.3 eV. (E) SAXS (11 keV) scattering profiles of BSA at different concentrations. Scattering profiles are normalized by concentration.

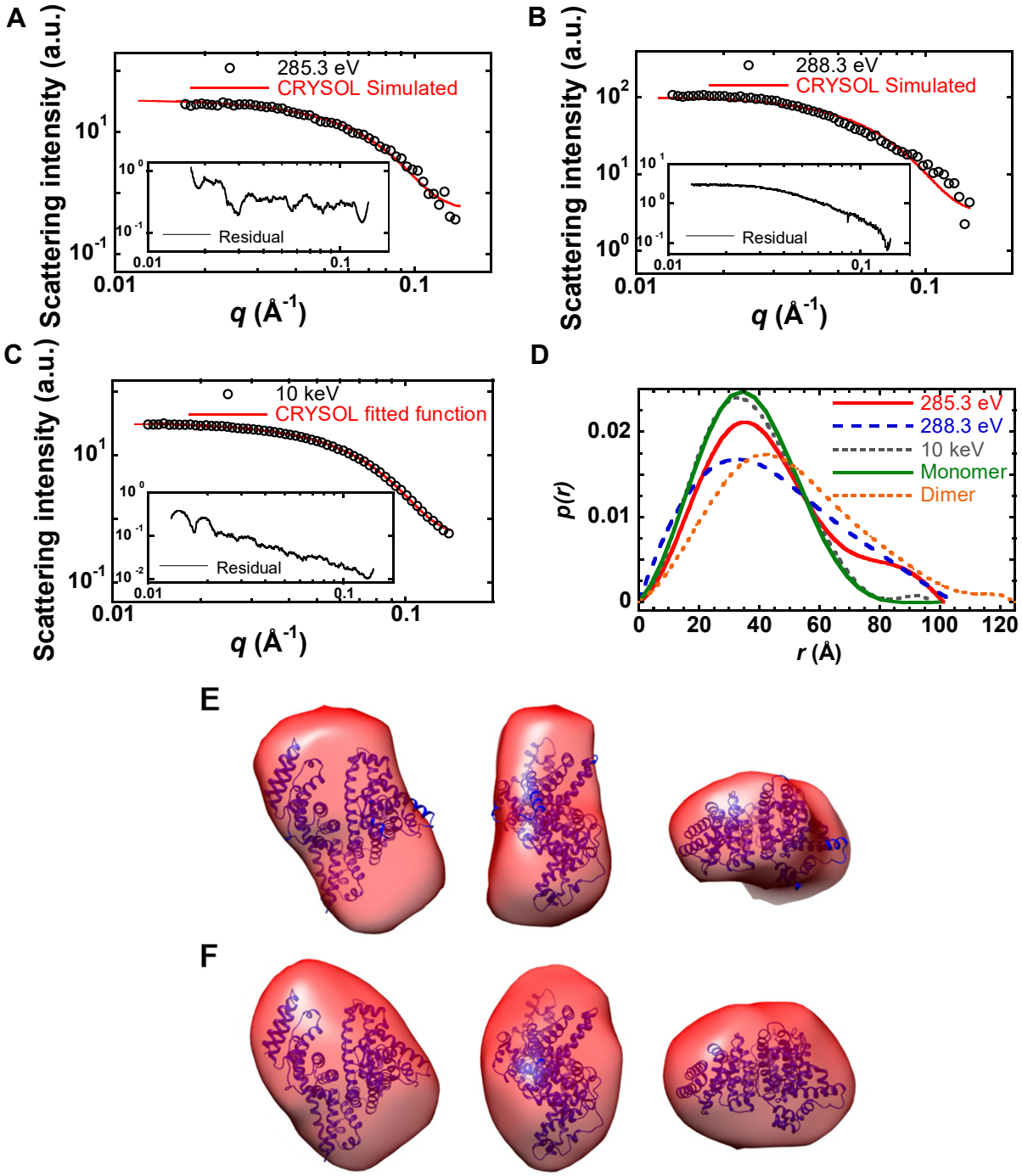

Figure S6. Related to Figure 4. Analyses of RSoXS and SAXS profiles. (A) Computed scattering curve for crystallographic BSA structure (PDB ID 3V03) obtained from CRYSOL compared to RSoXS data from 10 mg/ml BSA in 1× PBS at 285.3 eV. (B) Comparison of 288.3 eV RSoXS data and predicted scattering. (C) Comparison of 10 keV scattering (SAXS) and predicted scattering. (D) Pair distribution functions for 10 mg/ml BSA in 1× PBS at 285.3 eV and 288.3 eV (RSoXS at resonance of the carbon K edge) and 10 keV (SAXS). Also shown are pair distribution functions of BSA monomers and dimers generated from the crystallographic structure (PDB ID 3V03). Overlay of envelope generated at (E) 288.3 eV and at (F) 10 keV with the crystal structure of BSA. The red envelope was generated from (E) RSoXS or (F) SAXS data and the blue ribbons represent the BSA backbone from crystal structure data (PDB ID 3V03).

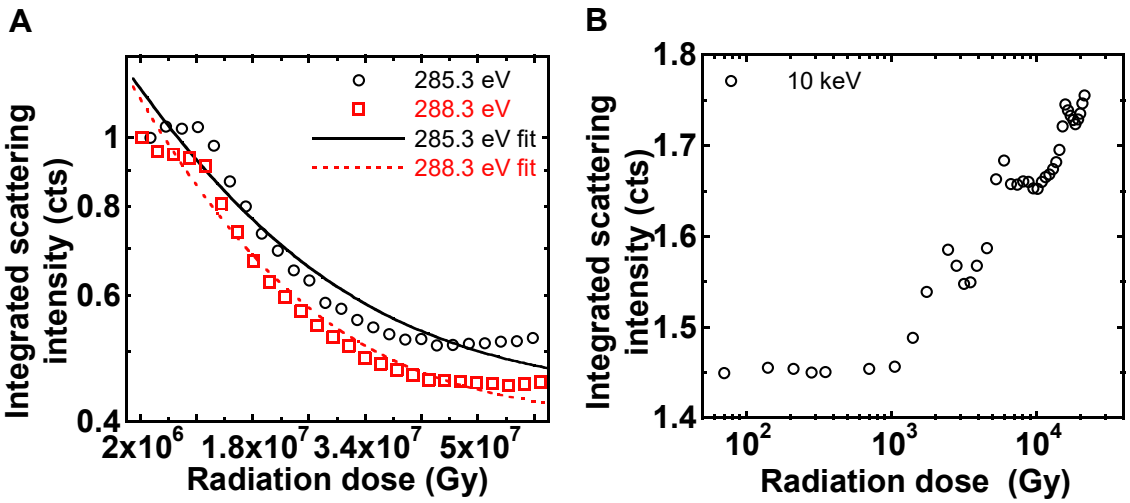

Figure S7. **Related to Figure 5. Radiation damage of a 10 mg/ml BSA in 1× PBS solution under soft and hard X-rays.** (A) Integrated scattering intensity as a function of radiation dose for BSA in 1× PBS from RSoXS experiments performed at 285.3 eV and 288.3 eV. The critical dose is found to be 2.0×10^7 Gy for 285.3 eV and 1.7×10^7 Gy for 288.3 eV. (B) Integrated scattering intensity as a function of radiation dose for BSA in 1× PBS from SAXS experiments at 10 keV.