Structure and Dynamics of N-Glycosylated Human Ribonuclease 1

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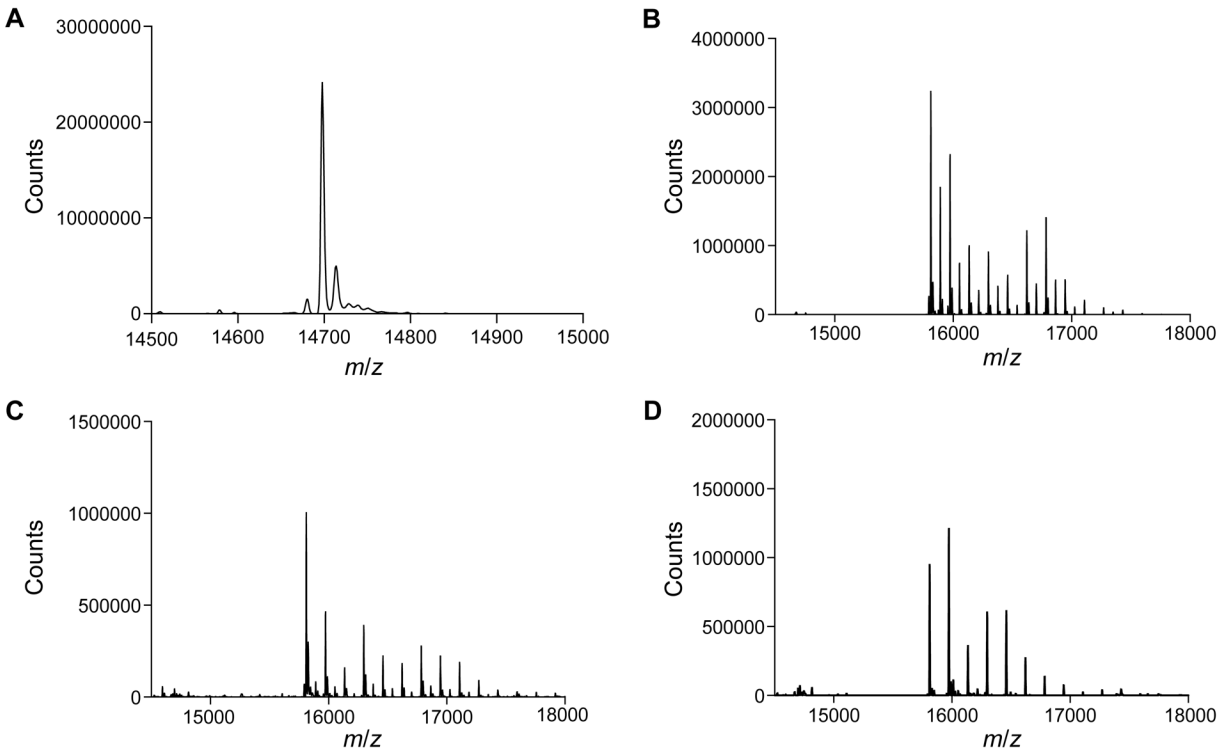

Figure S1. Deconvoluted ESI–QTOF mass spectra of unglycosylated human RNase 1 and its glycoforms. (A) Unglycosylated RNase 1 (expected, 14705.52; found, 14698.11). This protein was produced in *Escherichia coli* and has an N-terminal methionine residue. (B) NQQ glycoform (expected, 15818.38; found, 15811.56). (C) QNQ glycoform (expected, 15818.38; found 15811.83). (D) QQN glycoform (expected, 15818.38; found, 15811.75). Each additional D-mannose residue on a glycoform has a mass of 162.05 Da.

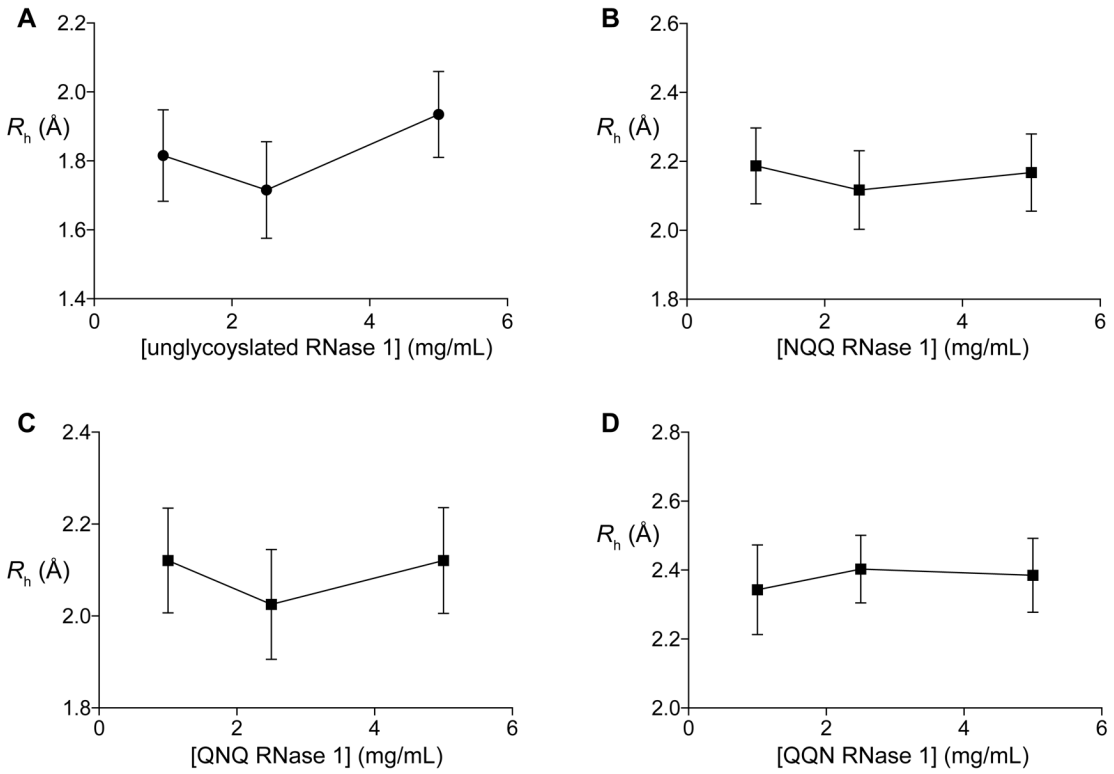

Figure S2. Concentration-dependence of R_h as evaluated with dynamic light scattering. (A) Unglycosylated human RNase 1. (B) NQQ glycoform. (C) QNQ glycoform. (D) QQN glycoform.

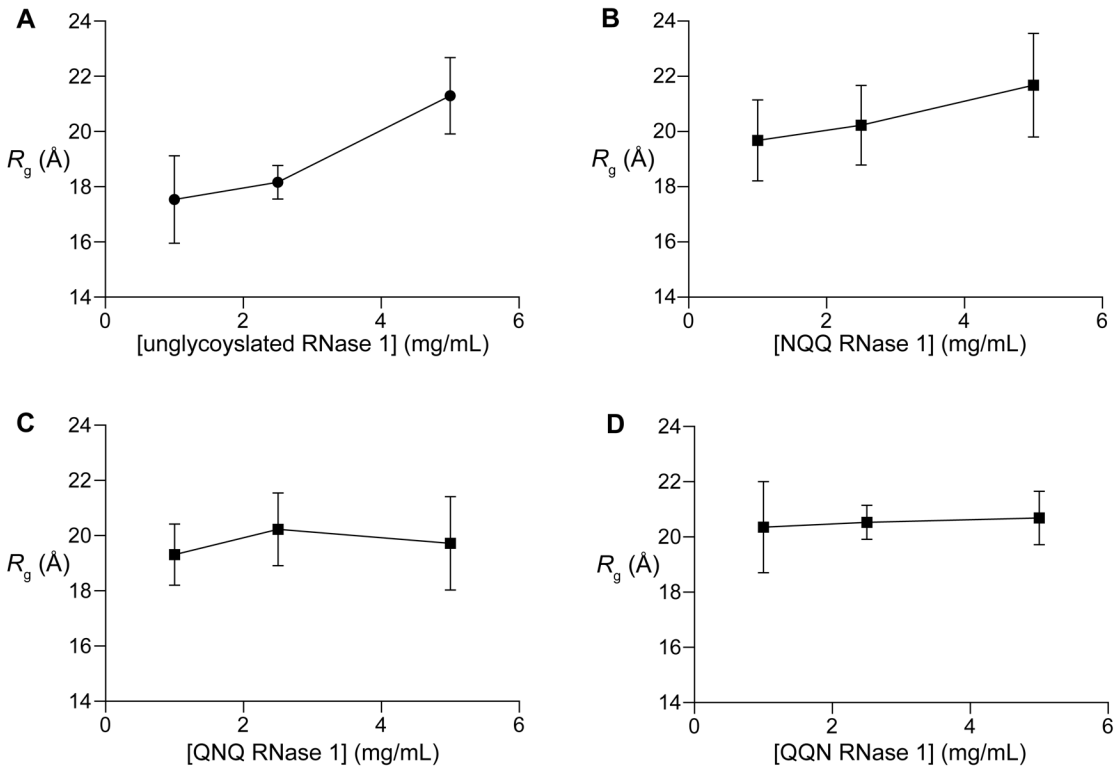

Figure S3. Concentration-dependence of R_g as evaluated with the Guinier approximation applied to unmerged SAXS curves. (A) Unglycosylated human RNase 1. (B) NQQ glycoform. (C) QNQ glycoform. (D) QQN glycoform.

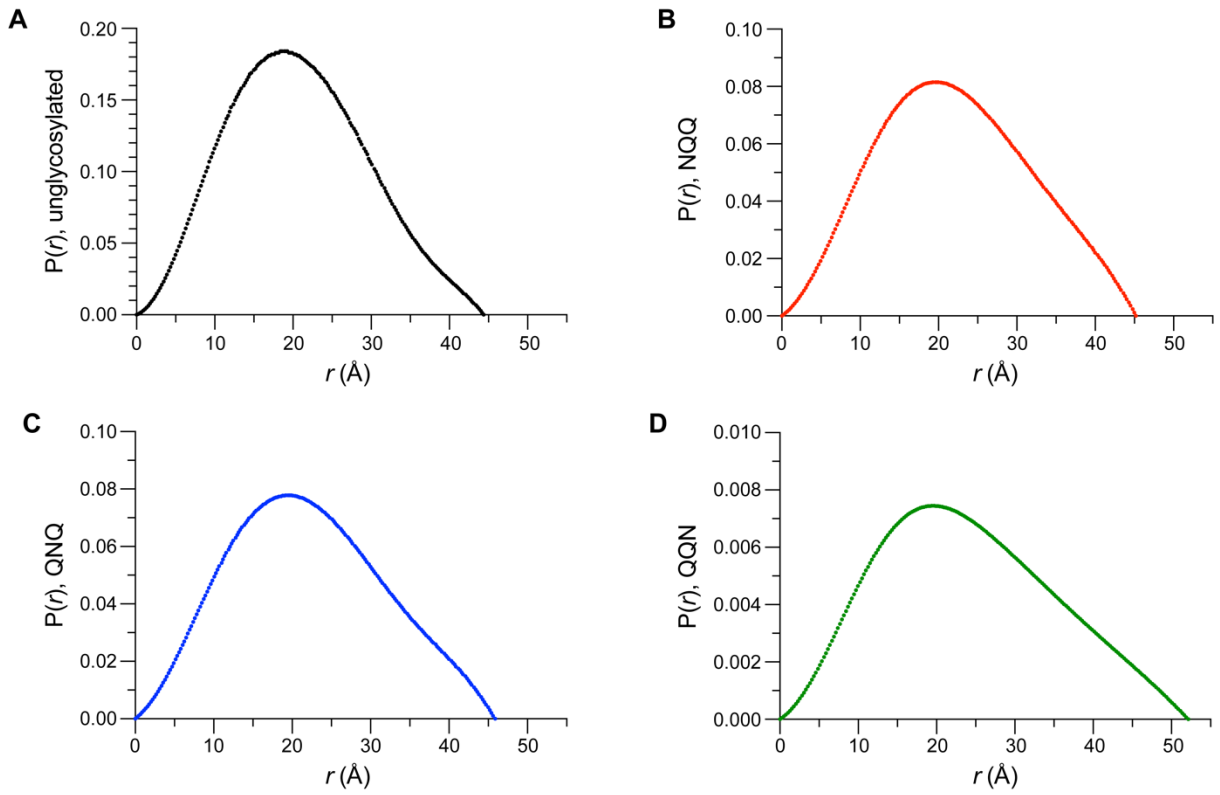

Figure S4. Distance-distribution functions of unglycosylated human RNase 1 and its glycoforms. (A) Unglycosylated RNase 1 (D_{max} = 44.44 Å). (B) NQQ glycoform (D_{max} = 45.22 Å). (C) QNQ glycoform (D_{max} = 45.85 Å). (D) QQN glycoform (D_{max} = 52.20 Å).

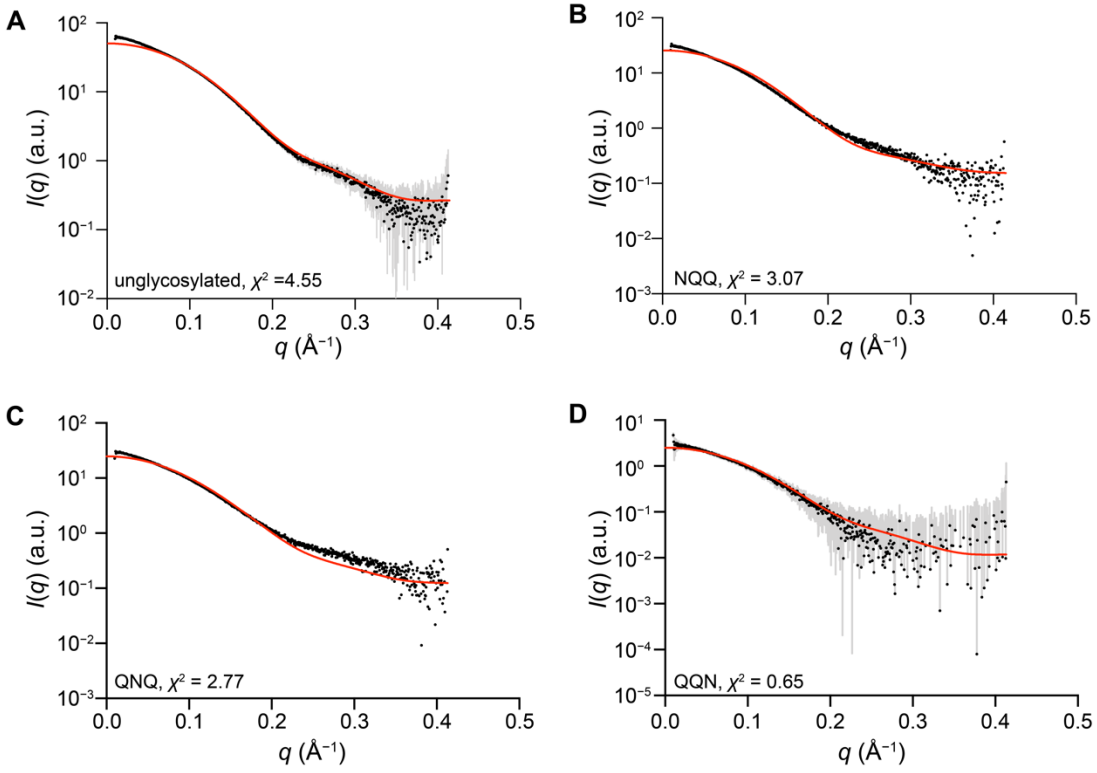

Figure S5. SAXS data for unglycosylated RNase 1 and its three glycoforms, and molecular dynamics fits to the SAXS data. For each protein, the entire trajectory was fitted with the program CRYSOL. Each panel displays a SAXS profile (● ± SE in gray), simulated best-fit structure to that profile (red line), and χ^2 value between the experimental and simulated profiles.

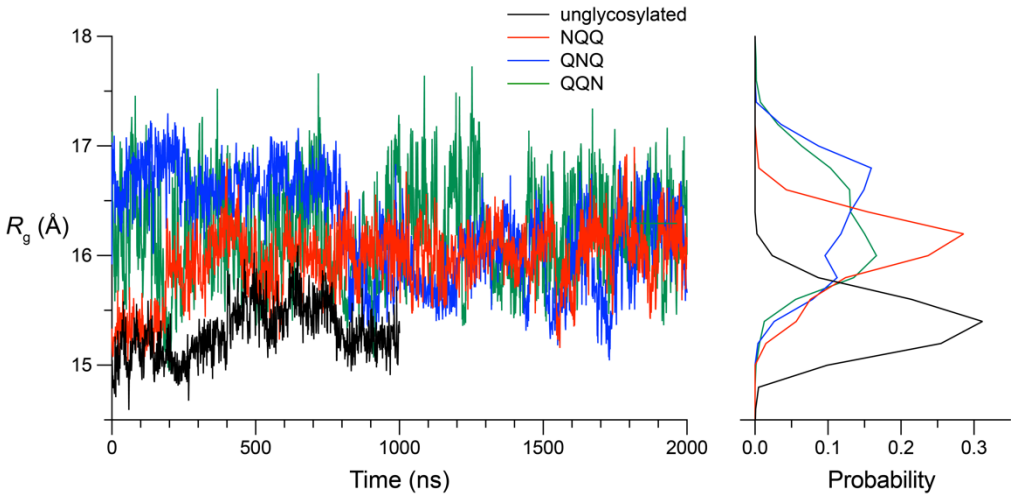

Figure S6. Simulated R_g values of RNase 1 glycoforms. The R_g value of each glycoform was tracked as a function of time (left) and by probability (right).

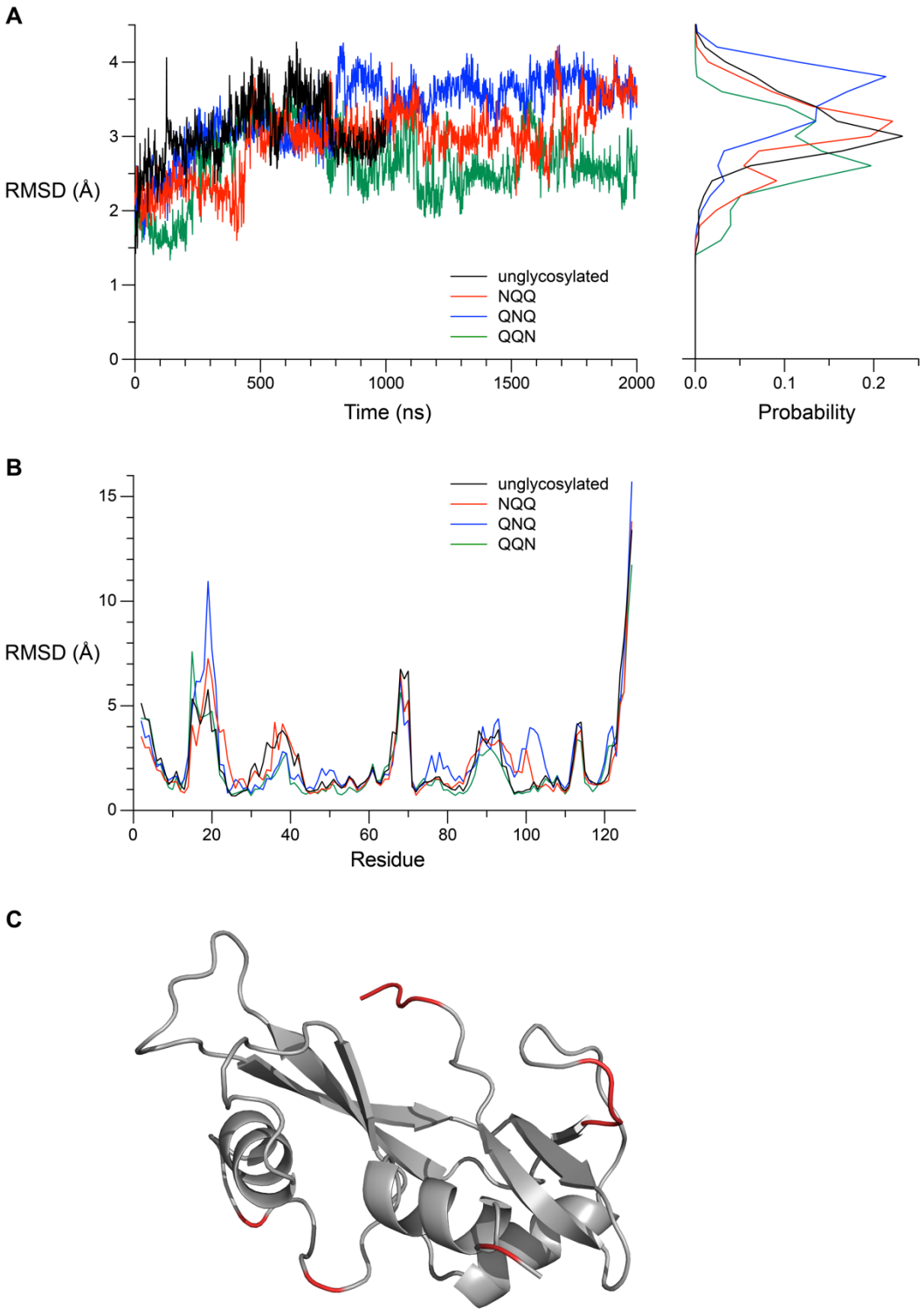

Figure S7: α-Carbon root-mean-square deviations (RMSDs) of RNase 1 glycoforms during a molecular dynamics simulation. The RMSD relative to the crystal structure of the wild-type protein (PDB entry 2q4g) was tracked as a function of time (left panel) and by probability (right panel). (B) RMSD as a function of protein residue. (C) Structure of wild-type RNase 1 with regions in which the RMSD is >5 Å are highlighted in red.

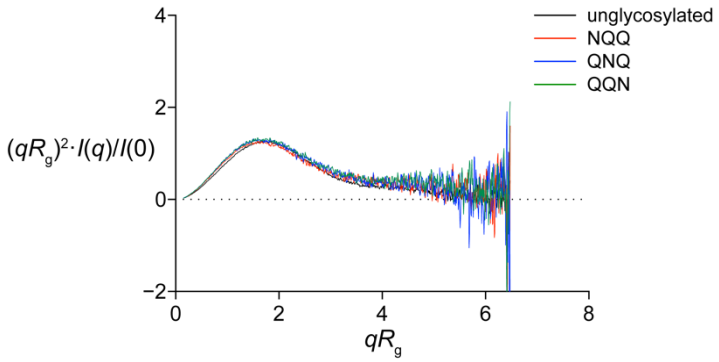

Figure S8. Normalized Kratky plot for unglycosylated human RNase 1 and its three glycoforms.

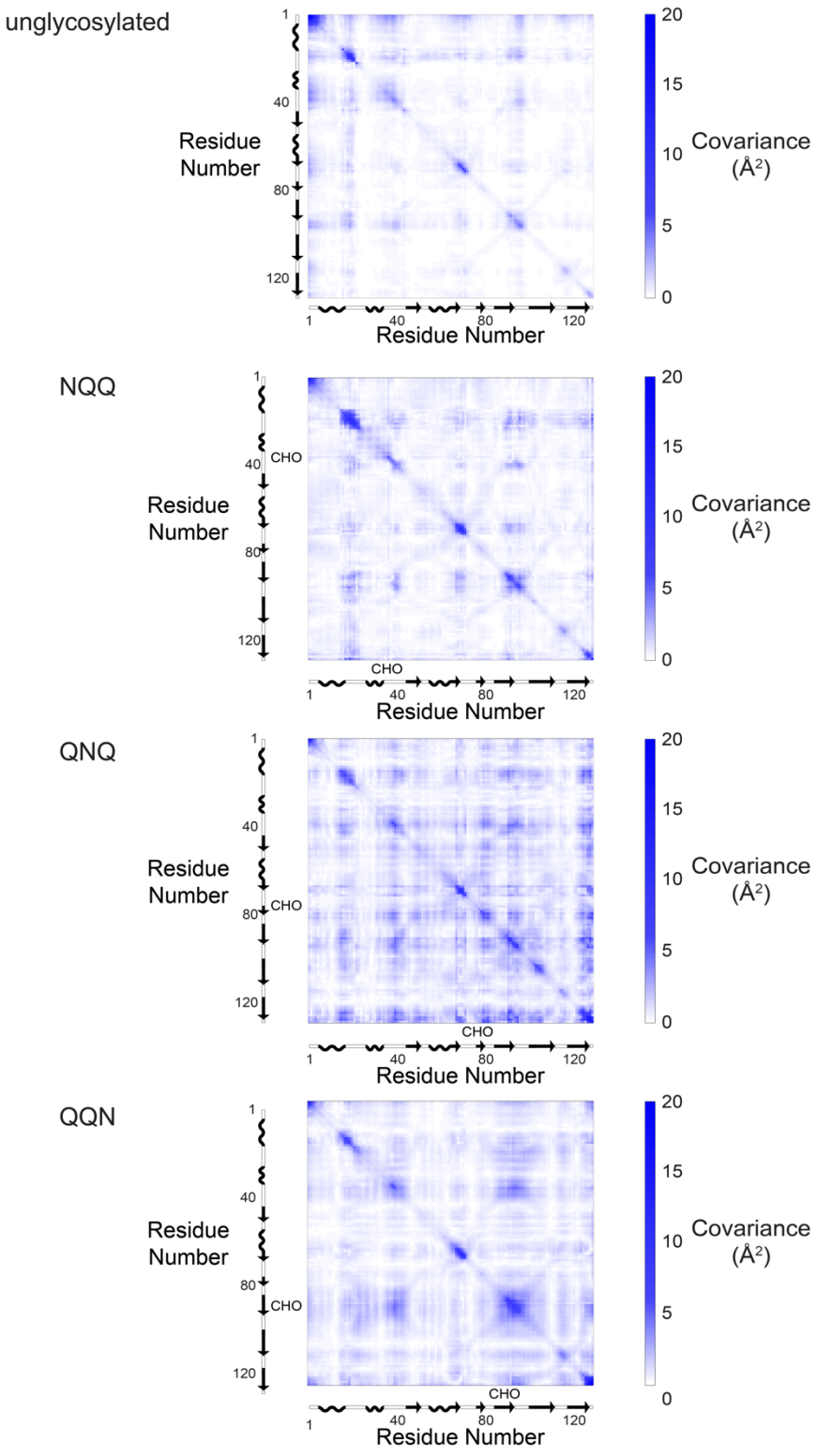

Figure S9. Covariance maps for fluctuations in unglycosylated RNase 1 and its glycoforms. Absolute values of covariance are plotted as a function of residue. α-Helices (curves), β-strands (arrows), and glycosylated residues (CHO) are indicated on each axis.

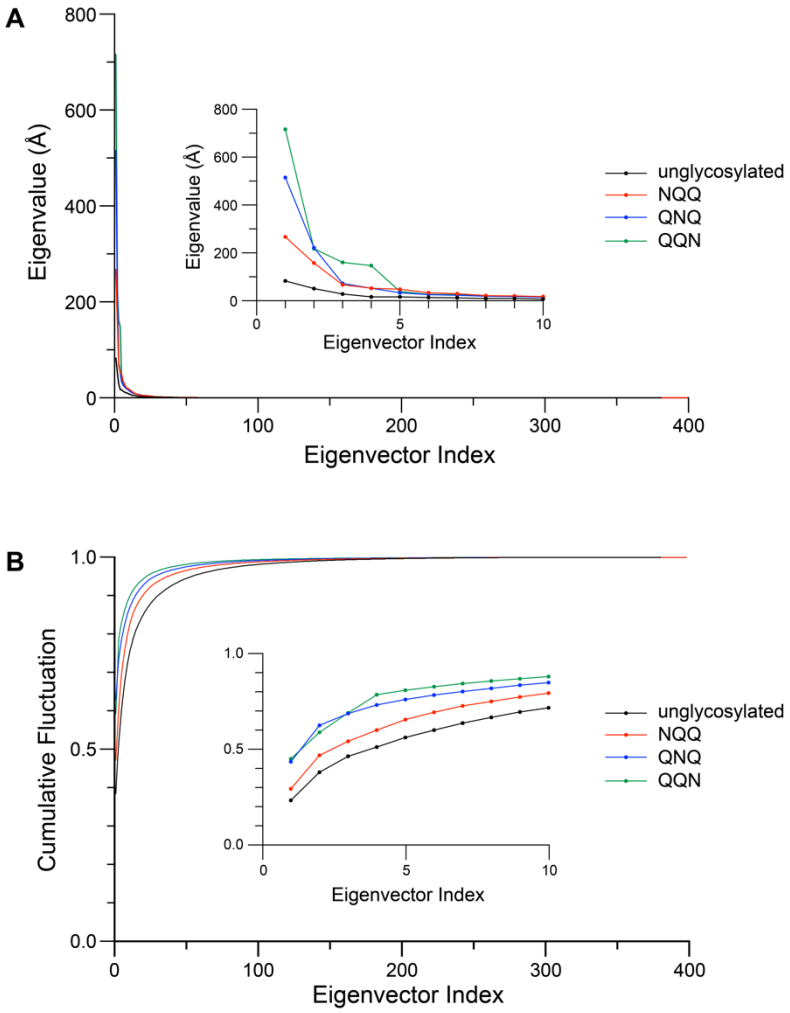

Figure S10. Scree plot and cumulative variation for principal component analysis for each RNase 1 glycoform. (A) The scree plot shows eigenvalues of the covariance matrix progressively decreasing as the principal motions of the protein are described. The insert displays the first ten eigenvectors. (B) The cumulative fluctuation shows the percentage of the protein variance described by the first *n* eigenvectors of the covariance matrix. The insert displays the first ten eigenvectors.