

Supplementary Material for "The Role of High-Density and Low-Density Amorphous Ice on Biomolecules at Cryogenic Temperatures: A Case Study with Polyalanine"

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In this supplementary material, we provide additional information on (1) the structure of polyalanine at different glass and liquid states; (2) cylindrical distribution functions (CDF); and (3) thermodynamic and dynamical properties of the water-polyalanine system during isobaric and isochoric heating processes.

1 Structural Properties of Polyalanine

(i) We include in Fig. S1 additional snapshots of polyalanine in the liquid states A, A', B, B', B'', X, and Y. In these states, water is in an LDL-like (A), HDL-like (A'), LDA (B), IA (B' and B''), and HDA state (X and Y). Fig. S1 shows that the structure of polyalanine remains unchanged among all these states, independently of the liquid/amorphous ice form of water. This is consistent with Table 1 of the main manuscript that shows similar values for important structural properties of polyalanine at states A, A', B, B', B'', X, and Y.

(ii) In the main manuscript we mentioned that the structure of polyalanine remains unchanged during isobaric and isochoric heating at $T \leq 300$ K. To support this point, we calculate the radius of gyration R_g , and the length L_p of polyalanine, the number of internal hydrogen-bonds of polyalanine n_{HB} , the distance between nearest-neighbor C_α atoms $d_{C_\alpha C_\alpha}$, and the solvent accessible surface area (SASA) at $T = 80, 160, 200, 240$ K. As shown in Table S1, these properties remain practically constant when the water-polyalanine system in state B (LDA) is heated at constant pressure $P = 0.1$ MPa. Similar results are obtained when the system is heated from state B'' (IA) and Y (HDA) [see Tables S2 and S3, respectively].

The values of R_g , L_p , n_{HB} , $d_{C_\alpha C_\alpha}$, and SASA upon heating the system at constant density are included in Tables S4 and S5. Tables S4 and S5 correspond to isochoric heating runs starting with the system in states B'' and Y, respectively.

For comparison, we also show in Fig. S2 snapshots of polyalanine at $T > 300$ K where it reaches a denatured state. The structure of polyalanine in the denatured states is clearly different from the conformations shown in Fig. S1. The values of R_g , L_p , n_{HB} , $d_{C_\alpha C_\alpha}$, and SASA for polyalanine in the denatured states are included in Tables S6 and S7. The values of n_{HB} and SASA are particularly different in the folded and unfolded configurations of polyalanine.

2 Cylindrical Distribution Functions

The main manuscript includes the CDF for molecules with n nearest-neighbors (NN) where two water molecules are NN if their OO distance is smaller than $r_c = 0.34$ nm; see Figs. 6 and 7. In Figs. S3(a)-(e) and S4, we show the same CDF shown in Figs. 6 and 7 of the main manuscript but using a smaller cutoff distance $r_c = 0.32$ nm. This is important because the number of NN that a water molecule has is sensitive to the value of r_c considered; see Fig. S3(f).

In Fig. S5, we show the CDF for states B (LDA), B'' (IA), and Y (HDA), classifying water molecules as LDA or HDA based on the corresponding number of nearest neighbors a water molecule has. In Fig. S5, the water molecules are classified as LDA if its number of nearest neighbors is ≤ 4 and HDA if its number of nearest neighbors is > 4 , using a cutoff distance $r_c = 0.34$ nm. While qualitatively the CDF do not depend on r_c , the value of r_c can lead to quantitative changes in the CDF.

3 Thermodynamic and Dynamic Properties upon Heating

(i) *Isobaric heating runs.* Fig. S6 shows the enthalpy $H(T)$, isobaric heat capacity $C_P(T)$, and means-square displacement MSD of the water molecules as function of temperature upon heating the water-polyalanine system at constant pressure $P = 0.1$ MPa. Results are included for the cases where the system is originally at states B, B'', and Y. In all cases, the MSD increases sharply at $T \approx 240$ K ($q_T = 1$ K/ns) indicating that, at these temperatures, the three systems reached the liquid state. Consistent with this, we find that the values of $H(T)$ of the three systems studied practically overlap at $T > 230 - 240$ K. However, molecular motion occurs at lower temperatures in the cases where the starting state of the system is B'' (IA) and Y (HDA). Indeed, as shown in Fig. S6(c), molecular motion starts

at $T \approx 160$ K for the case where water is originally in the IA state, and at $T \approx 100$ K for the case where water is originally in the HDA state. The molecular motion at such low temperatures is due to water molecules trying to evolve to the LDA state upon heating. Note that $\text{MSD} < 0.4$ nm for $T < 240$ K. Hence at these temperatures, molecular displacements remain small (due to the fast heating rate employed). Such an aging effects of IA and HDA upon heating is consistent with the minima in $C_P(T)$.

(i) *Isochoric heating runs.* A similar picture follows from our isochoric heating runs starting from the system in states B'' ($\rho = 1.15$ g/cm³) and Y ($\rho = 1.21$ g/cm³). As shown in Fig. S7(c), both systems reached the equilibrium liquid state at $T \approx 220$ K at which the MSD of the water molecules increases sharply. However, due to the different initial density of the systems, the corresponding total energies [Fig. S7(a)] and isochoric heat capacities [Fig. S7(b)], remain slightly different at $T > 220$ K. Note that there is only a mild or negligible annealing of both glassy systems at $T < 200$ K.

Table S1: Average properties of polyalanine at selected temperatures upon heating the water-polyalanine system at constant $P = 0.1$ MPa, starting from state B [red arrow in Fig. 1(b) of the main manuscript]. R_g and L_p are the radius of gyration and length of polyalanine, respectively. n_{HB} is the number of internal hydrogen-bonds of polyalanine and $d_{C_\alpha C_\alpha}$ is the average distance between nearest-neighbor C_α carbons. SASA is the solvent accessible surface area. Number in parenthesis are the standard deviations.

T [K]	R_g [nm]	n_{HB}	L_p [nm]	$d_{C_\alpha C_\alpha}$ [nm]	SASA [nm ²]
80	0.924 (0.002)	17.857 (0.117)	3.110 (0.075)	0.3837 (0.0003)	15.869 (0.128)
160	0.924 (0.004)	17.619 (0.294)	3.118 (0.077)	0.3833 (0.0004)	15.913 (0.127)
200	0.924 (0.002)	17.381 (0.294)	3.104 (0.086)	0.3836 (0.0002)	15.945 (0.224)
240	0.928 (0.003)	17.048 (0.243)	3.199 (0.038)	0.3832 (0.0002)	15.943 (0.074)

Table S2: Same as Table S1 starting from the water-polyalanine system at state B'' [red arrow in Fig. 1(c) of the main manuscript].

T [K]	R_g [nm]	n_{HB}	L_p [nm]	$d_{C_\alpha C_\alpha}$ [nm]	SASA [nm ²]
80	0.924 (0.003)	17.500 (0.643)	3.178 (0.090)	0.3837 (0.0005)	15.870 (0.085)
160	0.930 (0.003)	17.071 (0.889)	3.195 (0.089)	0.3840 (0.0007)	15.901 (0.078)
200	0.939 (0.006)	17.000 (0.416)	3.220 (0.117)	0.3839 (0.0004)	16.090 (0.135)
240	0.933 (0.001)	17.143 (0.303)	3.228 (0.019)	0.3835 (0.0003)	16.011 (0.059)

Table S3: Same as Table S1 starting from the water-polyalanine system at state Y [red arrow in Fig. 1(d) of the main manuscript].

T [K]	R_g [nm]	n_{HB}	L_p [nm]	$d_{C_\alpha C_\alpha}$ [nm]	SASA [nm ²]
80	0.923 (0.004)	17.914 (0.114)	3.191 (0.033)	0.3835 (0.0001)	15.856 (0.351)
160	0.939 (0.009)	17.057 (0.483)	3.259 (0.037)	0.3839 (0.0003)	16.042 (0.278)
200	0.935 (0.002)	17.343 (0.368)	3.209 (0.064)	0.3837 (0.0006)	15.945 (0.199)
240	0.933 (0.003)	17.000 (0.239)	3.218 (0.031)	0.3837 (0.0006)	15.943 (0.251)

Table S4: Average properties of polyalanine at selected temperatures upon heating the water-polyalanine system at constant volume, starting from state B” [green arrow in Fig. 1(c) of the main manuscript]. R_g and L_p are the radius of gyration and length of polyalanine, respectively. n_{HB} is the number of internal hydrogen-bonds of polyalanine and $d_{C_\alpha C_\alpha}$ is the average distance between nearest-neighbor C_α carbons. SASA is the solvent accessible surface area. Number in parenthesis are the standard deviations.

T [K]	R_g [nm]	n_{HB}	L_p [nm]	$d_{C_\alpha C_\alpha}$ [nm]	SASA [nm ²]
80	0.924 (0.002)	17.857 (0.117)	3.110 (0.075)	0.3837 (0.0003)	15.869 (0.128)
160	0.924 (0.004)	17.619 (0.294)	3.118 (0.077)	0.3833 (0.0004)	15.913 (0.127)
200	0.924 (0.002)	17.381 (0.294)	3.104 (0.086)	0.3836 (0.0002)	15.945 (0.224)
240	0.928 (0.003)	17.048 (0.243)	3.199 (0.038)	0.3832 (0.0002)	15.943 (0.074)

Table S5: Same as Table S2 starting from the water-polyalanine system at state Y [green arrow in Fig. 1(d) of the main manuscript].

T [K]	R_g [nm]	n_{HB}	L_p [nm]	$d_{C_\alpha C_\alpha}$ [nm]	SASA [nm ²]
80	0.921 (0.003)	17.914 (0.070)	3.177 (0.027)	0.3836 (0.0003)	15.821 (0.295)
160	0.918 (0.003)	17.571 (0.090)	3.188 (0.031)	0.3836 (0.0006)	15.782 (0.173)
200	0.923 (0.003)	17.200 (0.630)	3.219 (0.025)	0.3829 (0.0005)	15.917 (0.226)
240	0.923 (0.001)	17.286 (0.239)	3.196 (0.024)	0.3832 (0.0002)	15.887 (0.036)

Table S6: Average properties of polyaniline in the system equilibrated at $P = 0.1$ MPa and $T > 300$ K. At these temperatures, polyaniline is in a denatured state. R_g and L_p are the polyaniline radius of gyration and length, respectively. n_{HB} is the number of internal hydrogen-bonds of polyaniline and $d_{C_\alpha C_\alpha}$ is the average distance between nearest-neighbor C_α carbons of polyaniline. SASA is the solvent accessible surface area. Number in parenthesis are the standard deviations.

T [K]	R_g [nm]	n_{HB}	L_p [nm]	$d_{C_\alpha C_\alpha}$ [nm]	SASA [nm ²]
325	1.142 (0.133)	3.967 (1.592)	3.606 (0.652)	0.3834 (0.0013)	20.583 (0.952)
350	1.007 (0.158)	1.262 (1.205)	1.714 (0.827)	0.3837 (0.0018)	20.067 (1.560)
375	0.905 (0.141)	2.117 (1.694)	1.688 (0.691)	0.3829 (0.0011)	19.293 (2.241)
400	1.133 (0.234)	1.357 (1.245)	2.732 (1.059)	0.3829 (0.0020)	20.938 (1.774)

Table S7: Same as Table S6 for the system equilibrated at $P = 400$ MPa and $T > 300$ K.

T [K]	R_g [nm]	n_{HB}	L_p [nm]	$d_{C_\alpha C_\alpha}$ [nm]	SASA [nm ²]
325	0.874 (0.087)	10.396 (1.478)	2.159 (0.625)	0.3835 (0.0018)	17.065 (1.087)
350	0.834 (0.117)	9.040 (1.560)	2.062 (0.677)	0.3821 (0.0013)	17.351 (0.912)
375	0.973 (0.094)	7.454 (1.629)	3.058 (0.660)	0.3829 (0.0014)	18.588 (0.743)
400	0.874 (0.153)	2.375 (1.527)	1.668 (0.668)	0.3821 (0.0010)	18.421 (1.720)

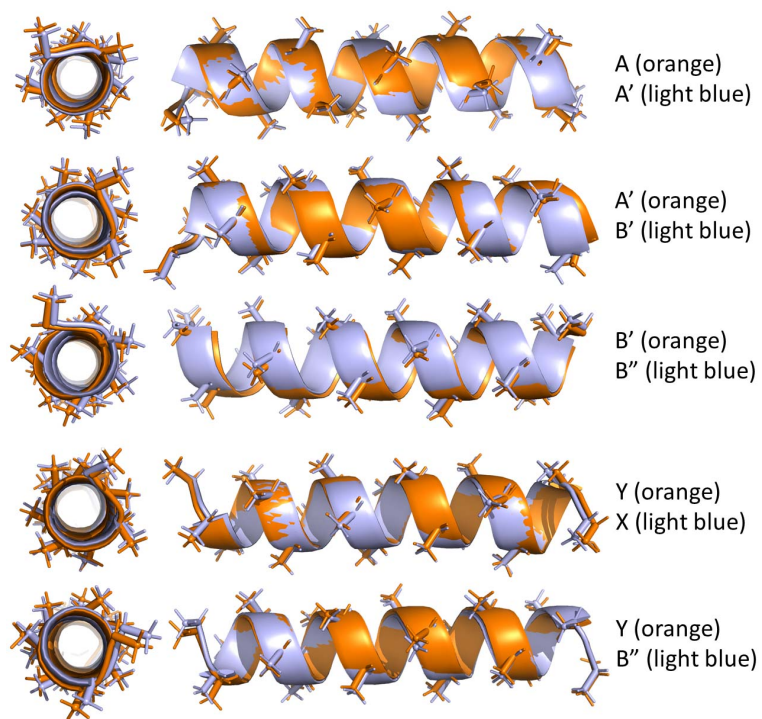


Figure S1: Superimposed configurations of polyaniline in states A-A', A'-B', B'-B'', Y-X, and Y-B''.

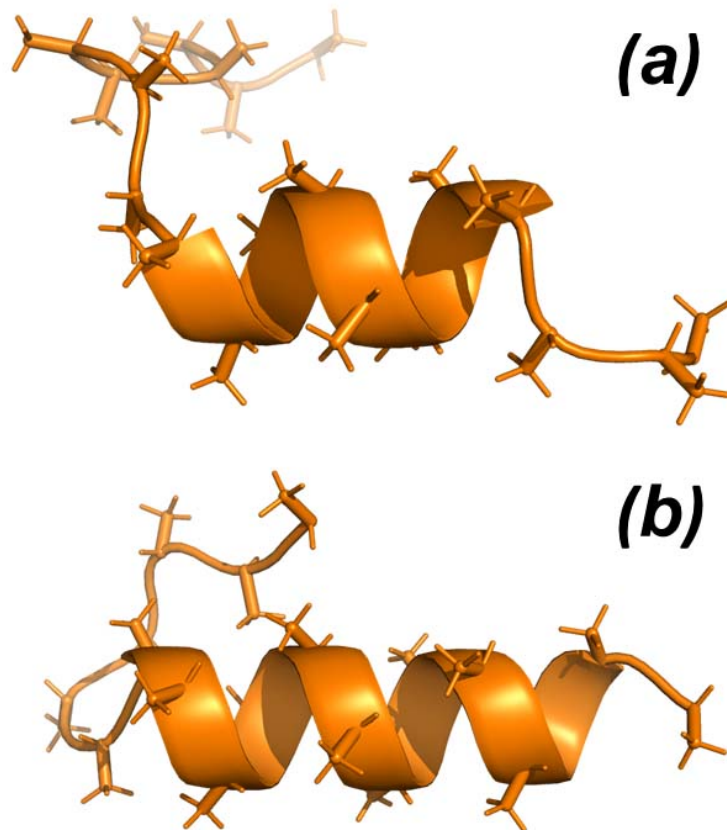


Figure S2: Snapshots of polyalanine at $T = 325$ K and (a) $P = 0.1$ MPa and (b) $P = 400$ MPa. At these conditions polyalanine is in a denatured state.

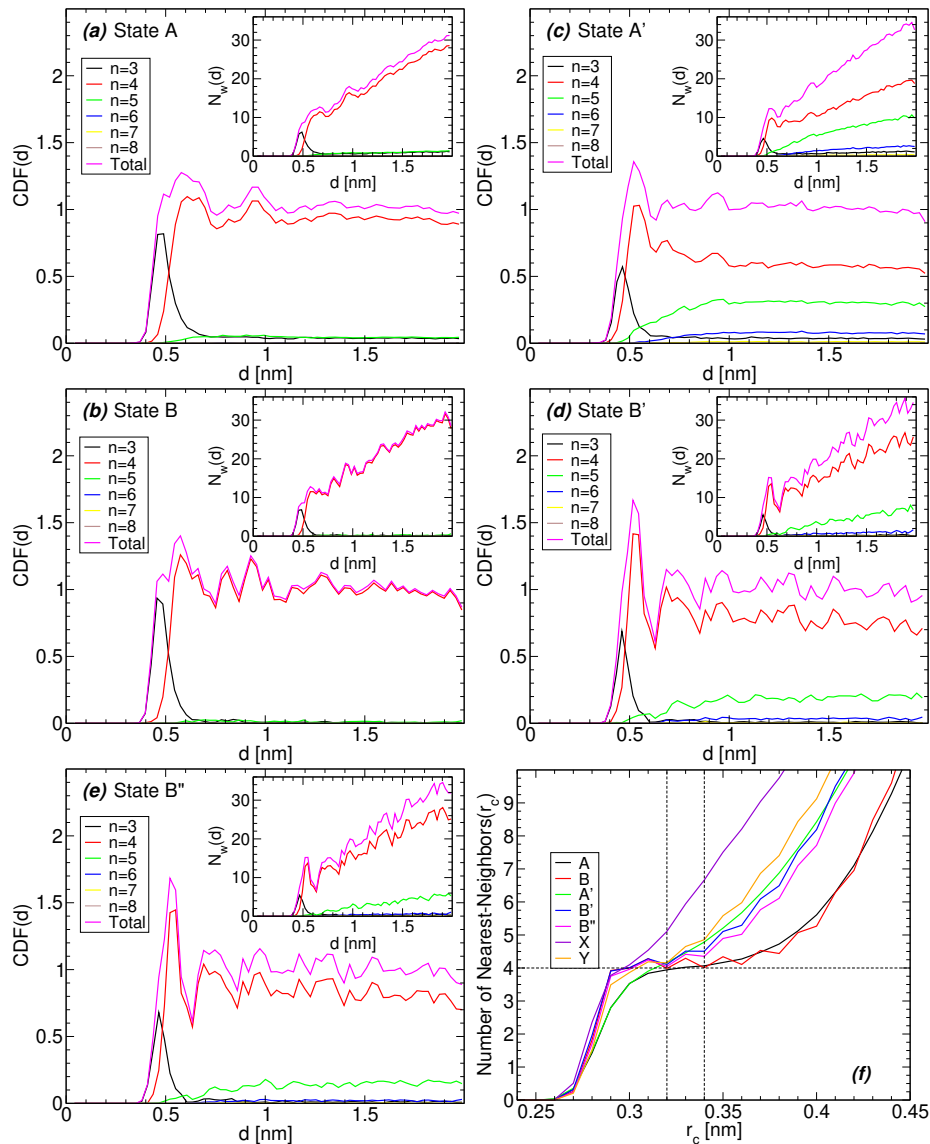


Figure S3: Same as Fig. 6 of the main manuscript for the CDF calculated using a cutoff distance $r_c = 0.32$ nm. CDF of water at states (a) A (LDL-like), (b) B (LDA), (c) A' (HDL-like), (d) B' (IA at $P = 400$ MPa), and (e) B'' (IA at $P = 0.1$ MPa) defined in Fig. 1(b)-(c) of the main manuscript. Included are the CDF for all molecules around polyaniline (magenta) as well as for molecules with $n = 3$ (black), 4 (red), 5 (green), 6 (blue), 7 (yellow), and 8 (brown). Insets in (a)-(e) are the corresponding number of molecules at a distance d from the peptide axis. (f) Average number of nearest-neighbors that water molecules have within a cutoff distance r_c from the corresponding Ow atom. In the LDL-like and LDA states, water molecules are surrounded mostly by 4 molecules; molecules with $n \geq 5$ are present in IA.

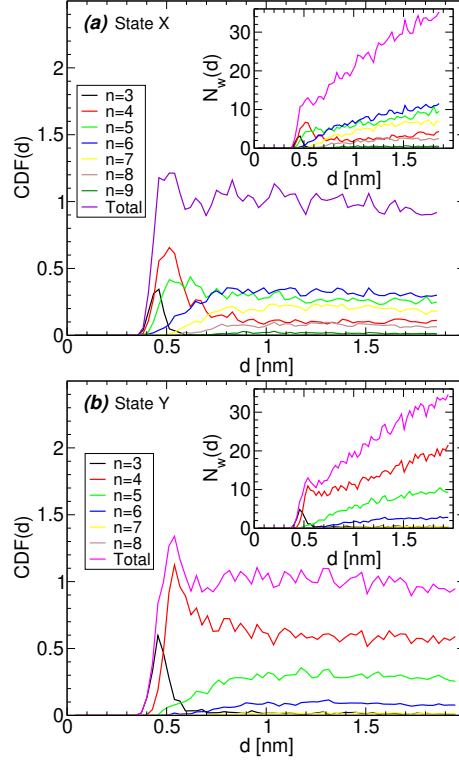


Figure S4: Same as Fig. 7 of the main manuscript for the CDF calculated using a cutoff distance $r_c = 0.32$ nm. CDF of water around polyaniline at selected states sampled during the compression/decompression cycle at $T = 80$ K indicated in Fig. 1(d) of the main manuscript. (a) CDF obtained after compression of LDA to $P = 2000$ MPa; at this state water remains in the HDA state. (b) CDF at $P = 0.1$ MPa obtained after decompressing the system from $P = 2000$ MPa. In this state, water remains in the HDA state.

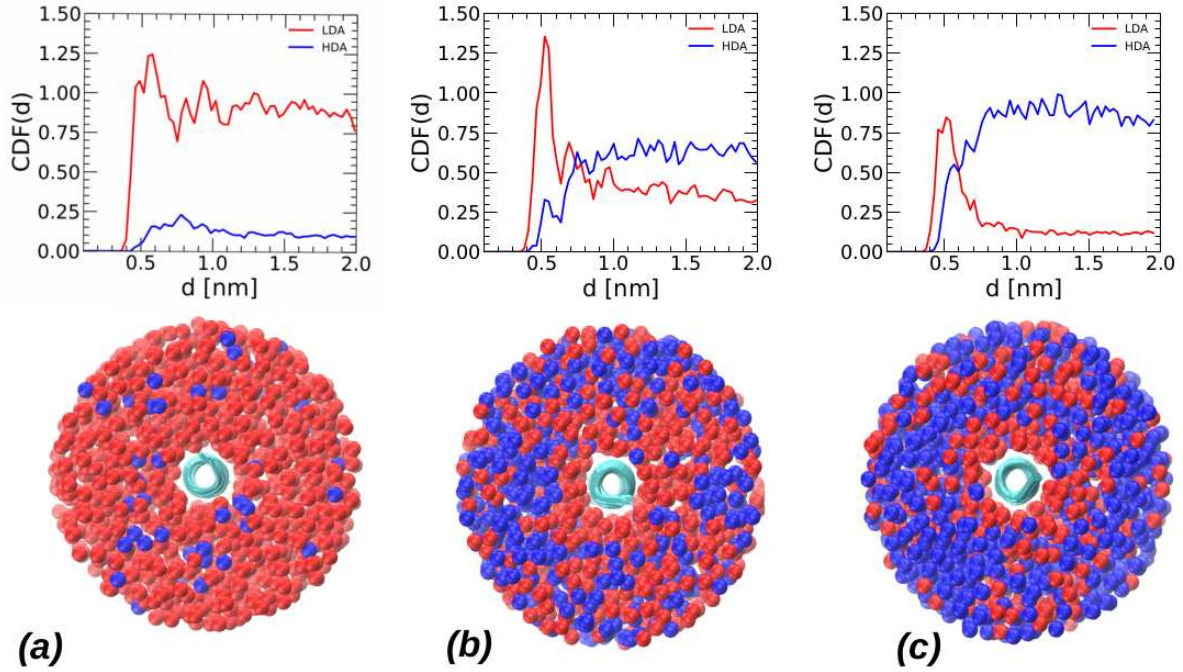


Figure S5: Cylindrical distribution functions of water at states (a) B (LDA), (b) B'' (IA), and (c) Y (HDA). In these CDFs water molecules are classified as LDA if its number of nearest neighbors is ≤ 4 and HDA if its number of nearest neighbors is > 4 , using a cutoff distance $r_c = 0.34$ nm. In the snapshots the red solid spheres correspond to water molecules that are LDA and the blue solid spheres correspond to water molecules that are HDA.

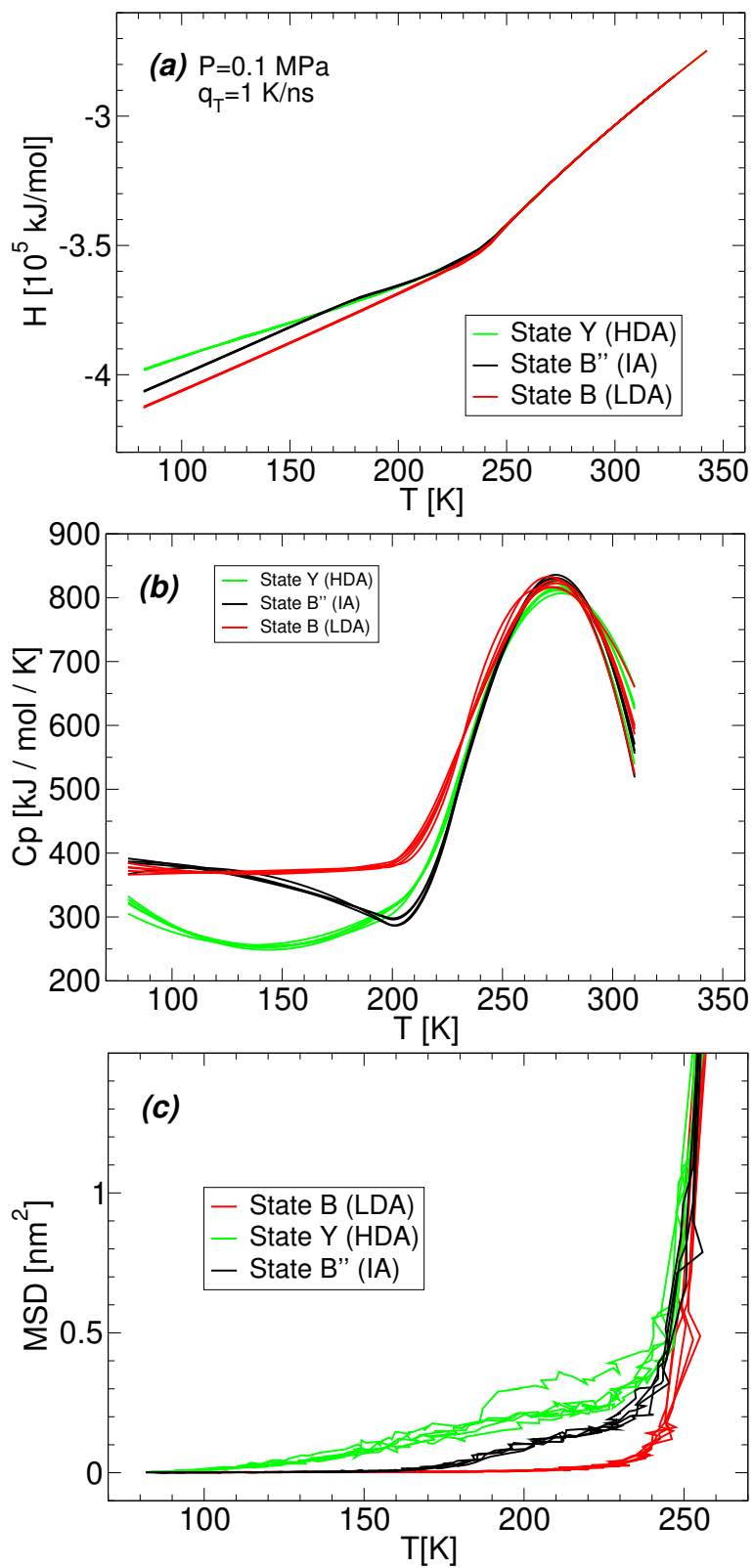


Figure S6: (a) Enthalpy H , (b) isobaric heat capacity C_p , and (c) mean-square displacement of water molecules upon heating the water-polyalanine system at $P = 0.1$ MPa ($q_P = 1$ K/ns). Black, red and green lines represent isobaric heatings of the system starting from states B'', B, and Y, respectively.

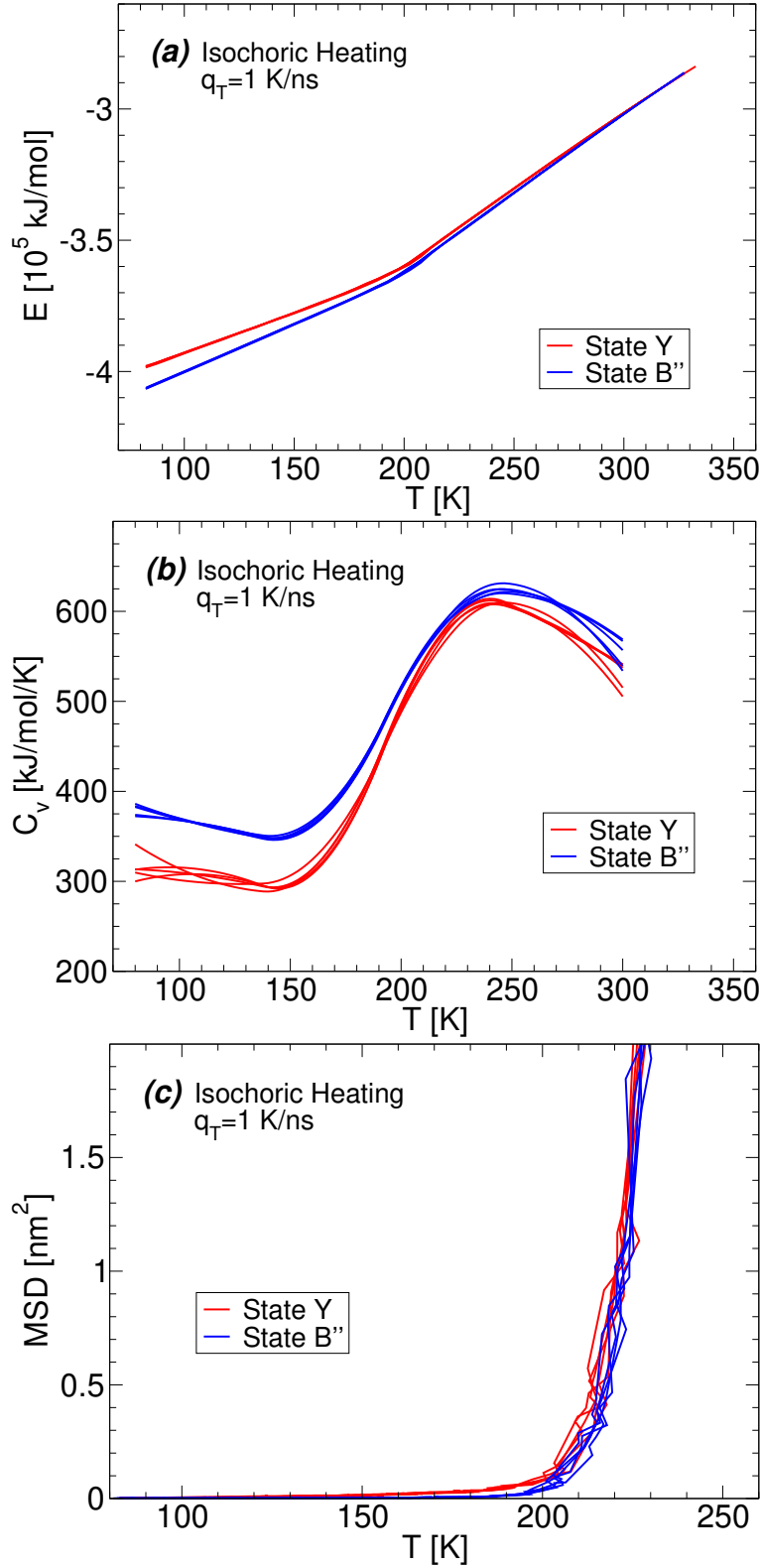


Figure S7: (a) Total energy E , (b) constant volume heat capacity C_v , and (c) mean-square displacement of water molecules upon heating the water-polyalanine system at constant volume ($q_P = 1$ K/ns). Blue and red lines represent the isochoric heating of the system starting from states B'' (IA, $\rho = 1.15$ g/cm³) and Y (HDA, $\rho = 1.21$ g/cm³), respectively.