

# Supplementary Information

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## *Validating the CHARMM36m Protein Force Field with LJ-PME Reveals Altered Hydrogen Bonding Dynamics under Elevated Pressures*

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## Tables

**Table S1. Linear regression of average atomic RMSF with respect to the pressure.** The slopes presented have been multiplied by 10000.

$T$ (K)	$\alpha$ -helices		$\beta$ -sheets		loops	
	$10^4 a$	$R^2$	$10^4 a$	$R^2$	$10^4 a$	$R^2$
278	-0.22	0.62	0.08	0.10	0.24	0.37
293	-0.40	0.77	-0.53	0.68	-0.43	0.52
308	-0.22	0.68	-0.05	0.06	0.03	0.01
323	-0.11	0.21	-0.05	0.02	-0.08	0.04

**Table S2. Statistics of detected H-bonds in simulations with the average effective occupancy greater than 5% over all temperatures and pressures.**

Occupancy (%)	Direct						Water bridged					
	Tot.	>70	>50	>30	>10	>5	Tot.	>70	>50	>30	>10	>5
Backbones	53	37	1	5	6	4	3	1	0	1	1	0
Backbone – side chain	30	4	3	7	16	0	58	1	2	6	25	24
Side chains	17	1	2	4	6	4	44	0	4	3	28	9

**Table S3. The number of residue duet that has side chain involved H-bonds additional to backbone H-bonds.**

Occupancy (%)	Direct		Water bridged	
	Tot.	>20	Tot.	>20
Backbone – side chain	6	2	7	1
Side chains	7	4	7	5

## Figures

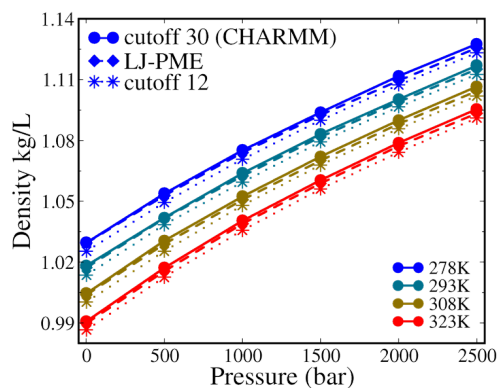


Figure S1. The TIP3P water density with respect to temperature and pressure simulated using different Lennard-Jones computational scheme.

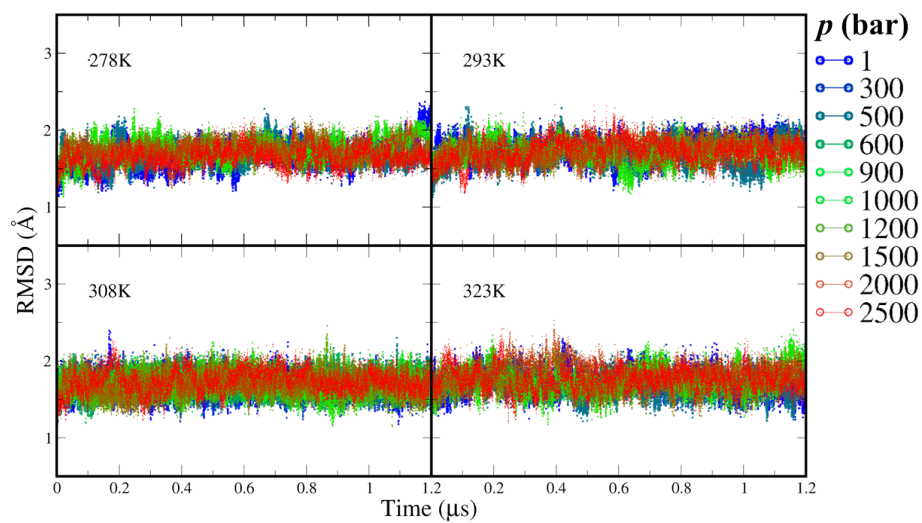
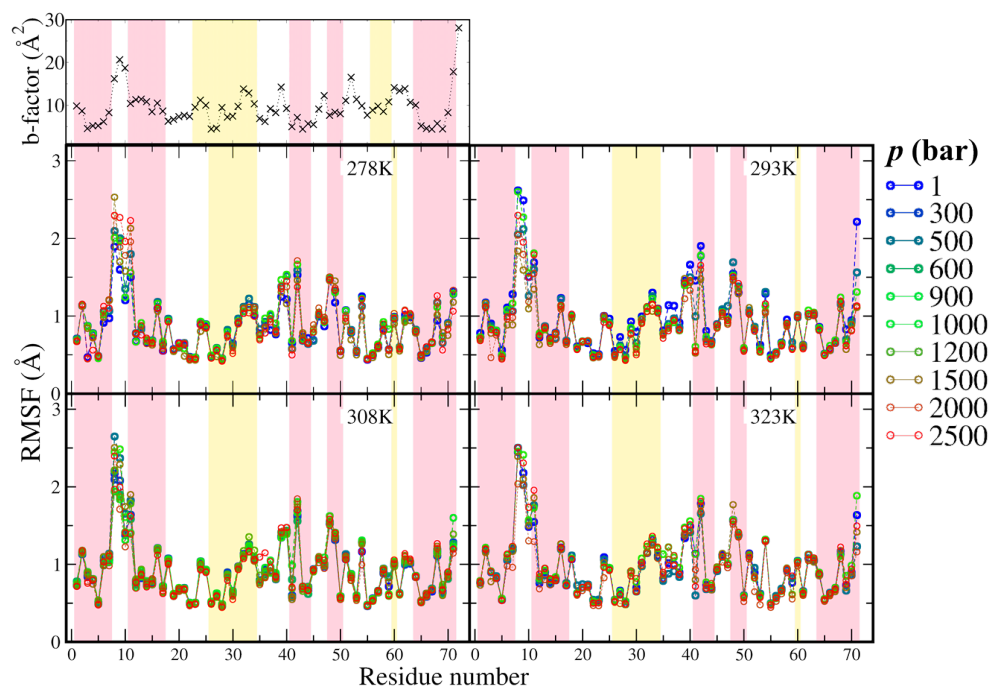
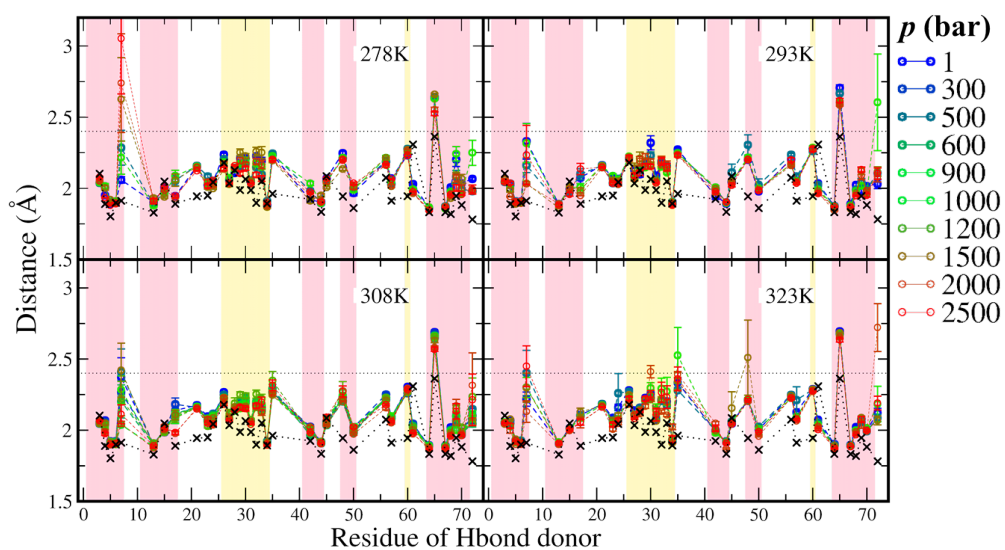


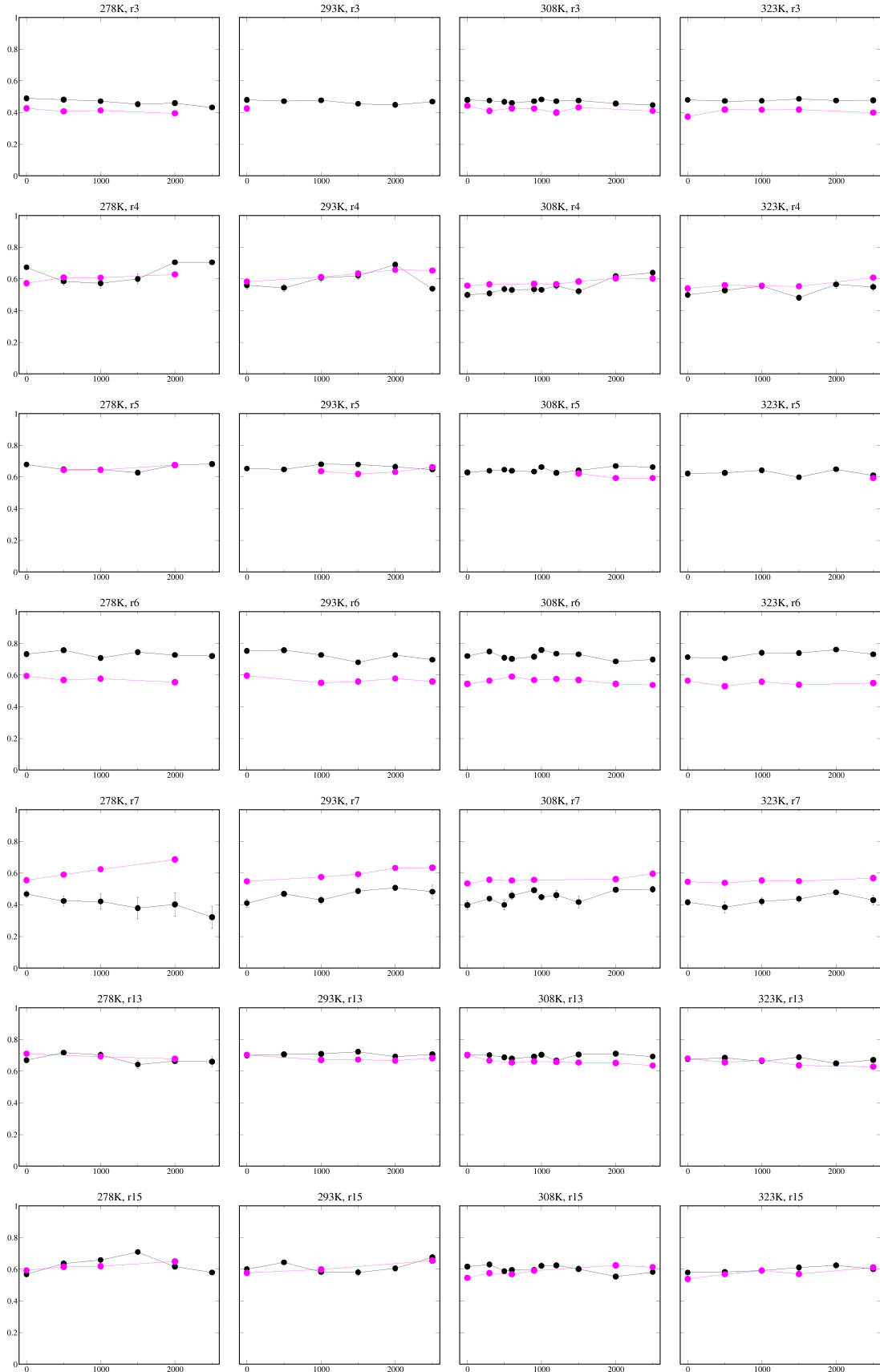
Figure S2. The coordinate RMSD of ubiquitin heavy atoms as the function of the simulation time.

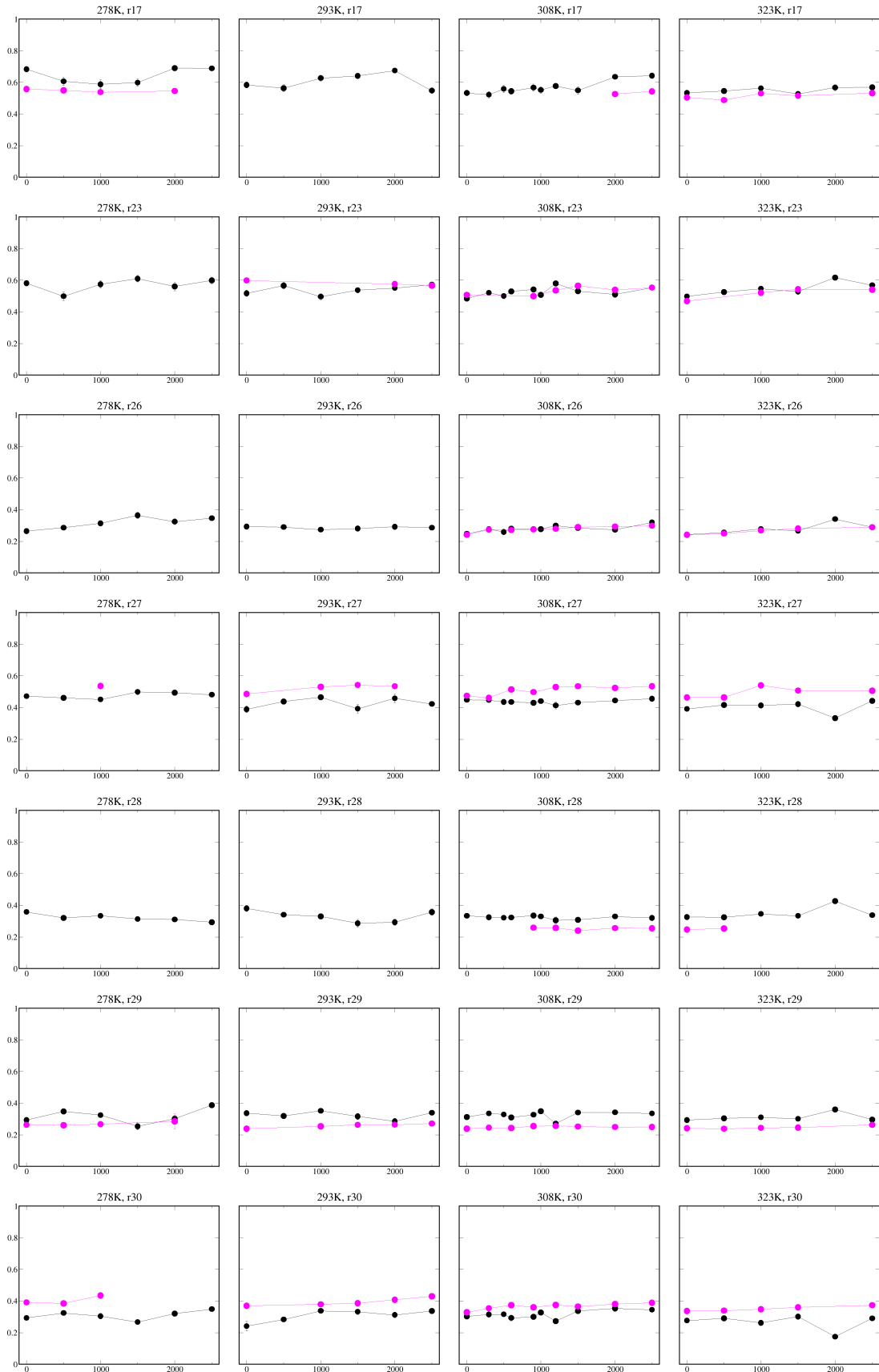


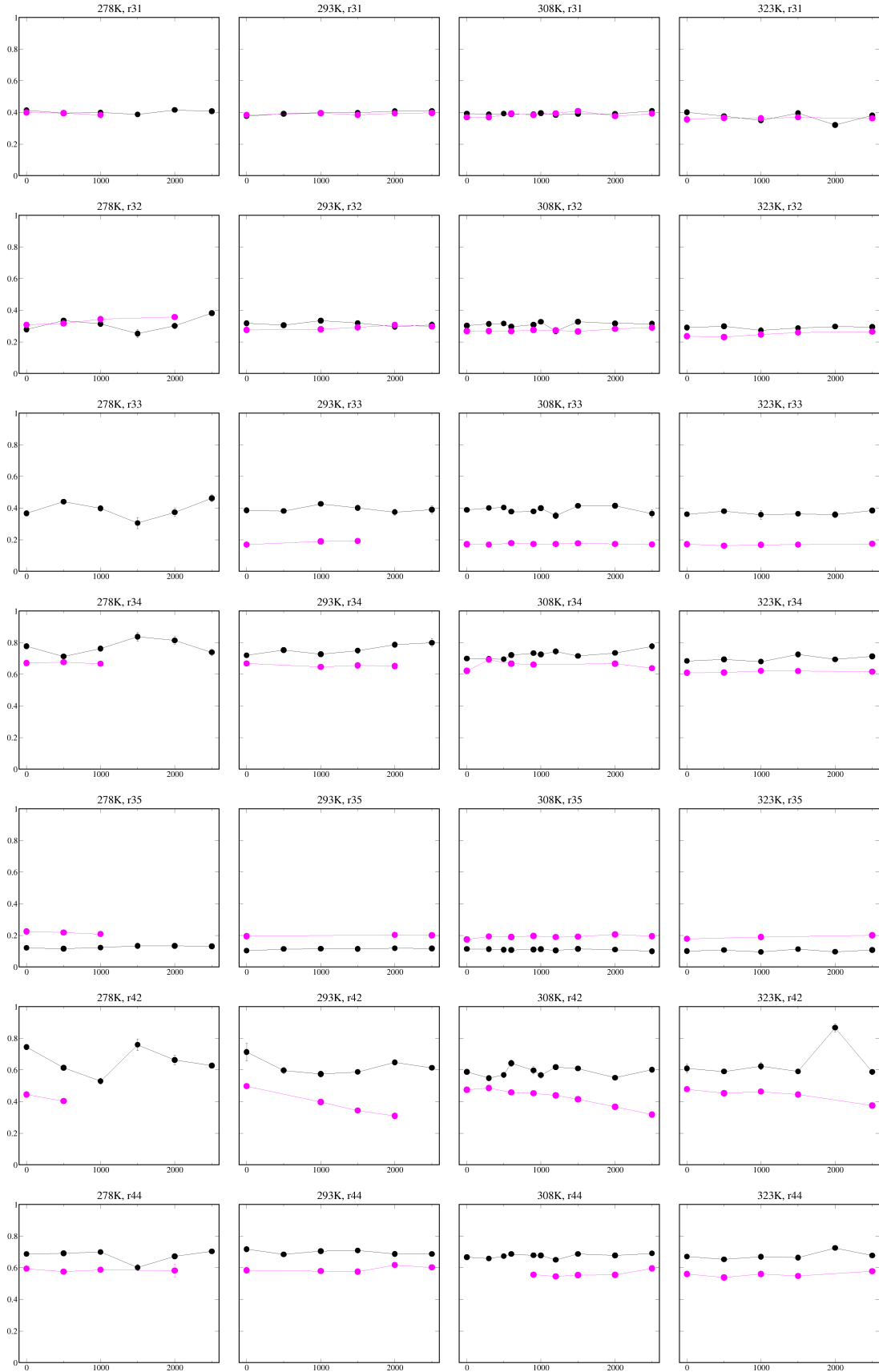
**Figure S3. The RMSF of ubiquitin residues 1—71.** The designation of secondary structure domains was colored on panel background, as helices in yellow and  $\beta$ -strand in pink. The black cross on the top-left indicates the average b-factor per residue of backbone atoms in the crystal structure 1UBQ.

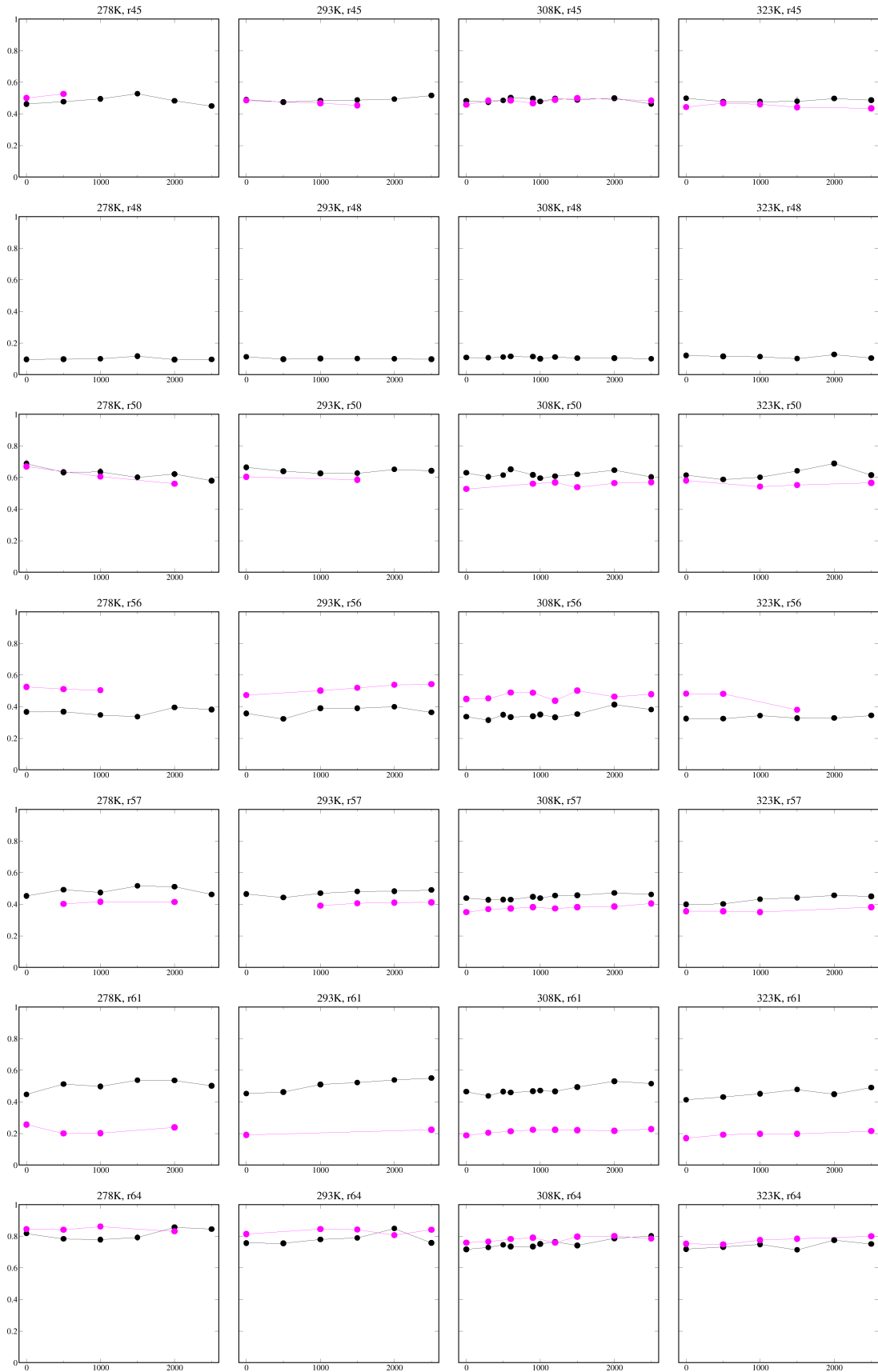


**Figure S4. The distance between donor and acceptor of backbone H-bonds in ubiquitin.** The hydrogen bonds are indexed by the donor residue. The background is colored as the secondary structure domains, *i.e.* yellow for helices and pink for sheets. The black cross indicates the  $\text{HN}\cdots\text{O}$  distance in the crystal structure 1UBQ (hydrogen atoms were added according to C36m parameters). The error bars show the averaged block standard deviation. The dotted baselines indicate 2.4 Å, under which the interaction is considered to be a strong hydrogen bond.

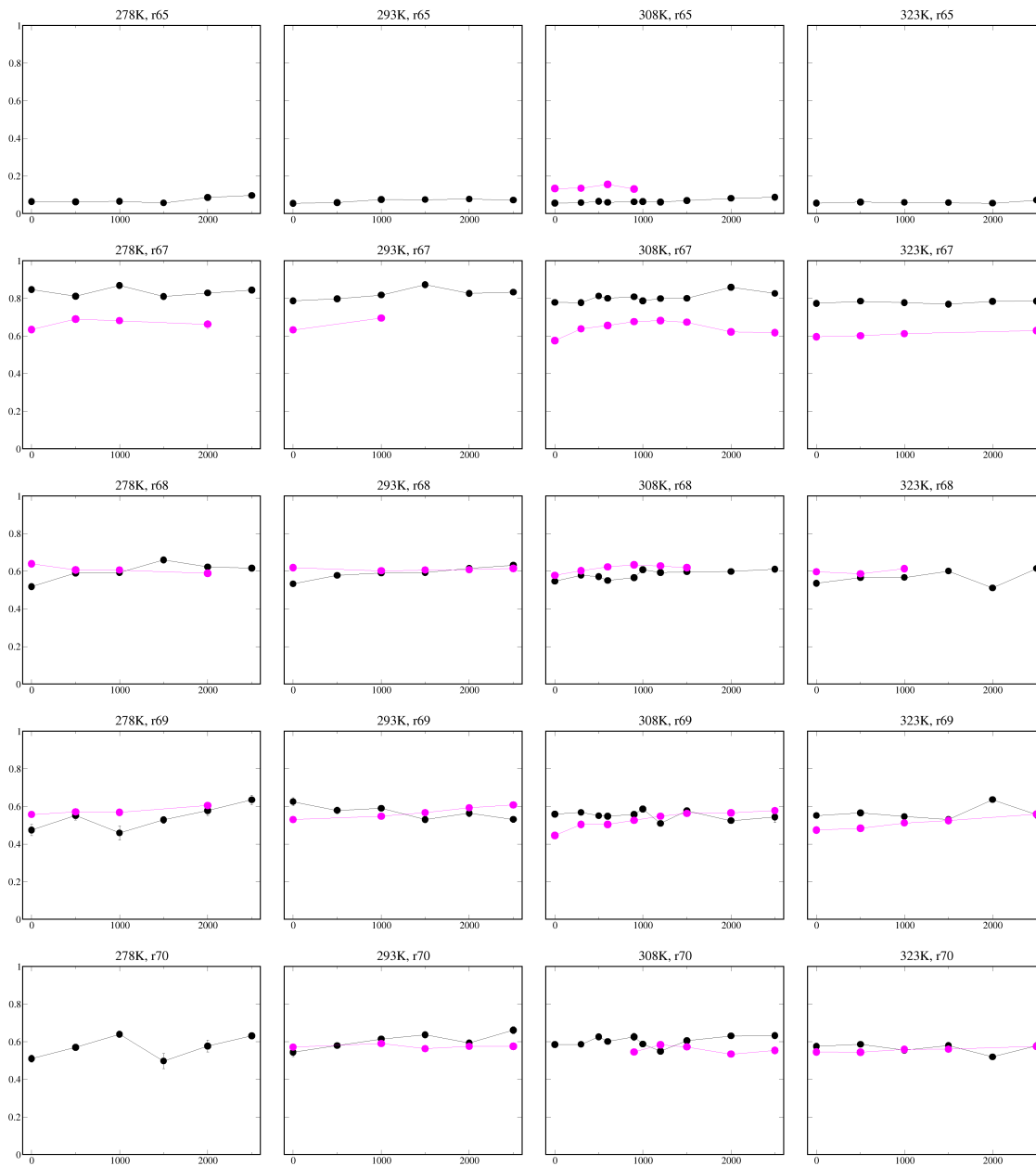




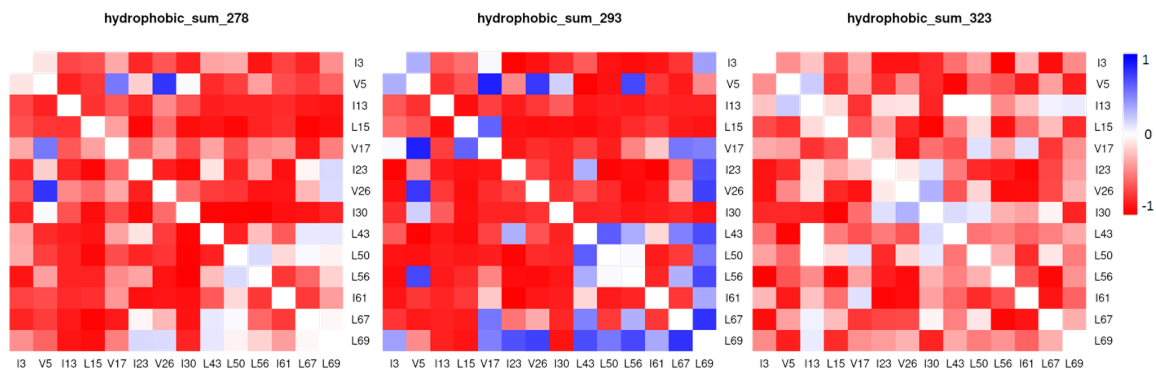




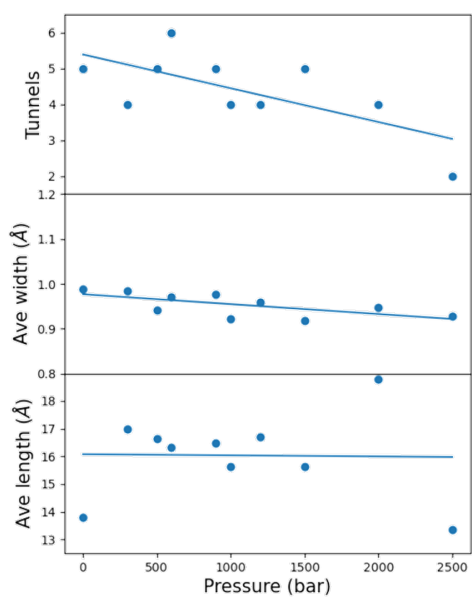




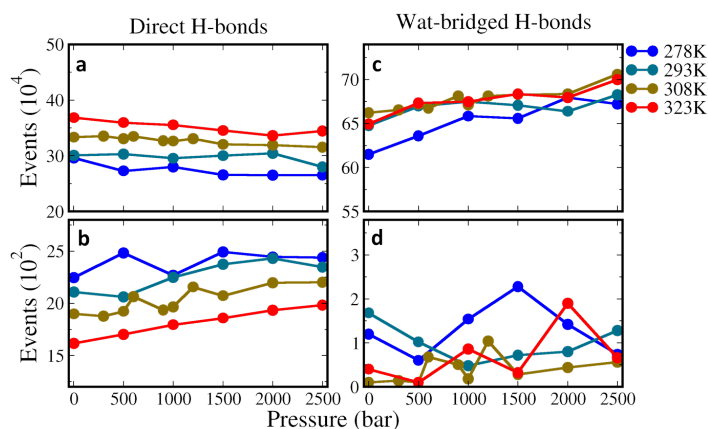
**Figure S5. The comparison of residue  $^3J_{NC}$  with respect to pressure.** The calculations are in black and experimental  $^3J_{NC}$  data<sup>1</sup> are in magenta. Each row displays four temperature systems for a hydrogen bond with  $y$ -axis showing the  $J$ -coupling and  $x$ -axis showing the pressure. Each panel is titled by temperature and donor residue identifier. The error bars of calculated data show the averaged block standard deviation.



**Figure S6. Pearson correlation coefficients between hydrophobic distances and pressure.** The pairwise distances were measured between the mass centers of 14 hydrophobic side-chains in the pocket at temperature 278 K (left), 298 K (middle) and 323 K (right). From  $\rho = 1$  (blue) to  $\rho = -1$  (red), the distances correlate with pressure from positively to negatively.



**Figure S7. The tunnel parameters for structures at 308 K in response to pressure.** The panels of scatter from top to bottom show the average tunnel number, average width of tunnel bottleneck, and average length of tunnel. The line shows the linear regression. The analysis was carried out using CAVER 3.0.<sup>2</sup>



**Figure S8. The statistics of event number for hydrogen bonds.** The total events of direct H-bond are categorized and presented as lifetime  $\leq 6$  ns (**a**) and lifetime  $> 6$  ns (**b**); the total events of water bridged H-bond are categorized as lifetime  $\leq 4$  ns (**c**) and lifetime  $> 4$  ns (**d**). Note the y-axis values of **a** and **c** were scaled by  $10^{-4}$  while the values of **b** and **d** were scaled by  $10^{-2}$ .

### Supplementary References

1. Nisius, L.; Grzesiek, S., Key stabilizing elements of protein structure identified through pressure and temperature perturbation of its hydrogen bond network. *Nature chemistry* **2012**, *4*, 711-7.
2. Chovancova, E.; Pavelka, A.; Benes, P.; Strnad, O.; Brezovsky, J.; Kozlikova, B.; Gora, A.; Sustr, V.; Klvana, M.; Medek, P.; Biedermannova, L.; Sochor, J.; Damborsky, J., CAVER 3.0: A Tool for the Analysis of Transport Pathways in Dynamic Protein Structures. *PLoS Comp. Biol.* **2012**, *8*.