Supporting Information for "Effects of Temperature and Salt Concentration on the Structural Stability of Human Lymphotactin: Insights from Molecular Simulations"

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I. ADDITIONAL SIMULATION RESULTS

Fig. S1

Root-mean-squared deviation (RMSD) of hLtn during the MD simulations under the four conditions summarized in Table 1 in the main text. The calculations are over residues from three different selections of the backbone atoms (N, C_α, C, O): the entire protein (1:68), residues 20 to 68 (20:68), and residues 20 to 63 (20:63). RMS best-fit of the MD structures to the averaged NMR structure is performed in all calculations with the backbone atoms of residue 20 to 68. Clearly, the N-terminal residues are rather disordered in all simulations. The C-terminal residues are more disordered in the 45°C simulations.

Fig. S2

(a) Root-mean-squared fluctuations (RMSFs) of the C_α atoms from the s10 simulation compared to those estimated based on the ensemble of NMR structures in the PDB file (1J9O)[?] . The calculated RMSF profile for the s10 condition is largely consistent with that estimated based on the ensemble of NMR structures given in the PDB file (1J9O) although the calculated RMSFs tend to be systematically larger, especially for mobile regions such as the 30's and 40's loops. (b) RMSFs of the C_α atoms calculated based on the four MD simulations under different conditions. (c) Difference between the C_α atom position in the averaged-NMR structure and the average structure from each MD simulation. The secondary structures are represented with sideways triangles for residues in β-sheets and with 'X' for residues in the α-helix. (d) Calculated NMR order parameters for the backbone N-H bond vectors for all residues, except for Pro 20 and Pro 50. For numerical reasons, the equilibrium approach for evaluating order parameters is used, in which the order parameter is given as the following,

$$S^2 = \frac{3}{2}(\langle x^2\rangle^2 + \langle y^2\rangle^2 + \langle z^2\rangle^2 + 2\langle xy\rangle^2 + 2\langle xz\rangle^2 + 2\langle yz\rangle^2) - \frac{1}{2} \tag{1}$$

where x, y, z indicate the components of the NH bond vector of interest, after the overall translation and rotation of the entire protein is removed based on RMS best-fit to the initial structure in the simulation.

Most sheet regions have low RMSF below 1 Å small deviations (<1.5 Å) from the average NMR structure and large (>0.85) N-H order parameters.

Fig. S3

Selected backbone hydrogen bonding interactions in the 30's- and 40's-loop regions during the MD simulations. (a) Backbone hydrogen-bond patterns between β1 and β2 (b1 to b5) and between β2 and β3 (b6 to b10). Distances from N to O are plotted as a function of time for each simulation for: (b) b5 plus b4 for s45 only (thick dashed line) and (c) b6.

Fig. S4

Average number of water molecules in the first solvation shell of chloride or sodium ions for 50 fs intervals during the simulation, calculated from integrating the $g(r)$ of water oxygen atoms out to 3.8 Å for chloride and 3.1 Å for sodium. For each plot, each line represents solvation number around an individual ion and all ions are plotted. When such "coordination number" is below 8 for chloride or 6 for sodium, that ion is associated with the protein (and/or another ion) so as to push one or more water molecules out of the first solvation shell. The plots vs. the minimal distances of the ions to the protein surface show many concurrent changes (data not included), which support the argument for ion-exchange during the simulations.

II. FULL REFERENCE FOR REF. 58

A. D. MacKerell, Jr. D. Bashford, M. Bellott, R. L. Jr.Dunbrack, J. D. Evenseck, M. J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. Joseph-McCarthy, L. Kuchnir, K. Kuczera, F. T. K. Lau, C. Mattos, S. Michnick, T. Ngo, D. T. Nguyen, B. Prodhom, W. E. I. Reiher, B. Roux, M. Schlenkrich, J. C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wiórkiewicz-Kuczera, D. Yin, and M. Karplus, *J. Phys. Chem.* **1998**, 102, 3586-3616.

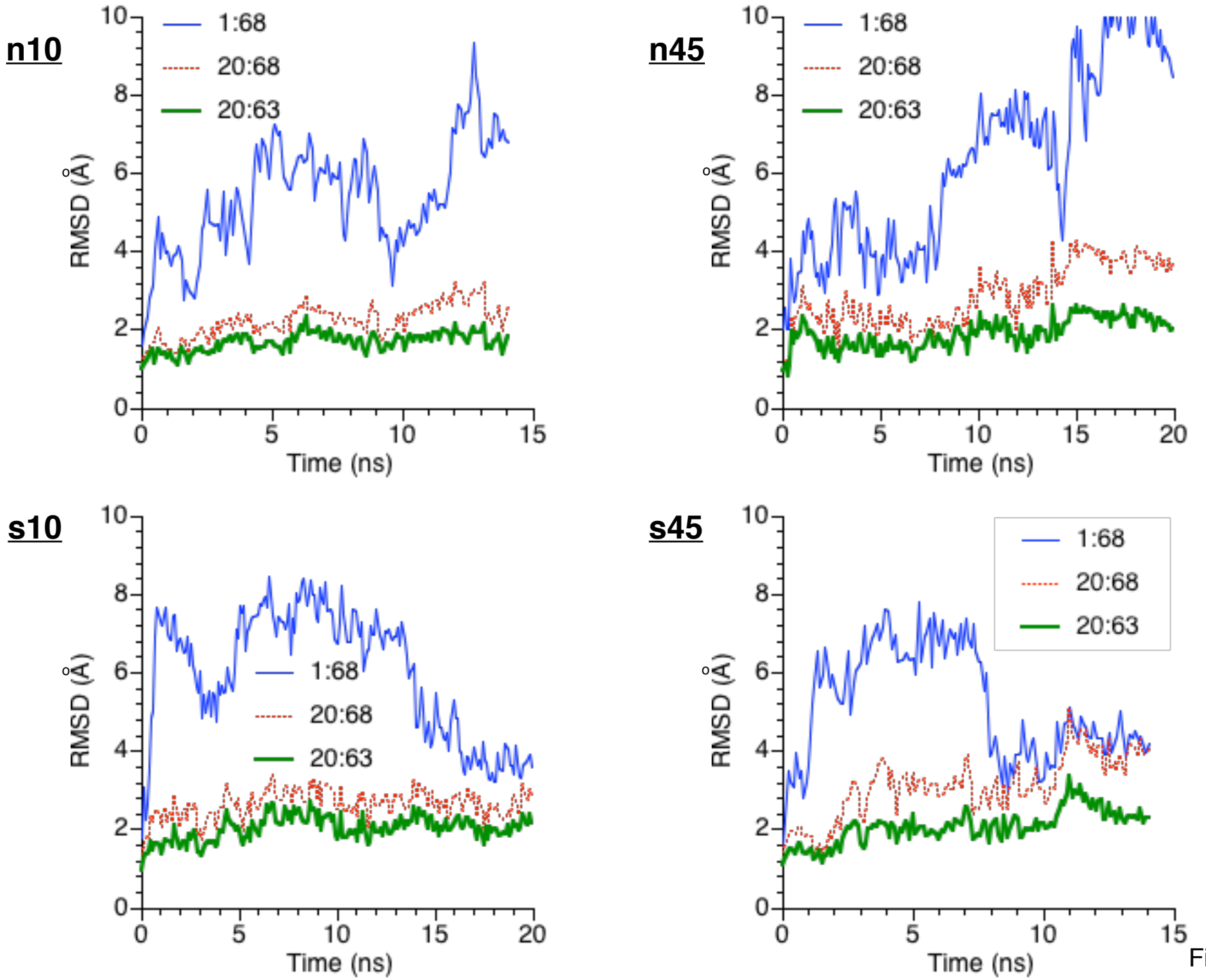
n10
1:68
20:68
20:63
RMSD (Å)
Time (ns)
n45
1:68
20:68
20:63
RMSD (Å)
Time (ns)
s10
1:68
20:68
20:63
RMSD (Å)
Time (ns)
s45
1:68
20:68
20:63
RMSD (Å)
Time (ns)

Fig S1

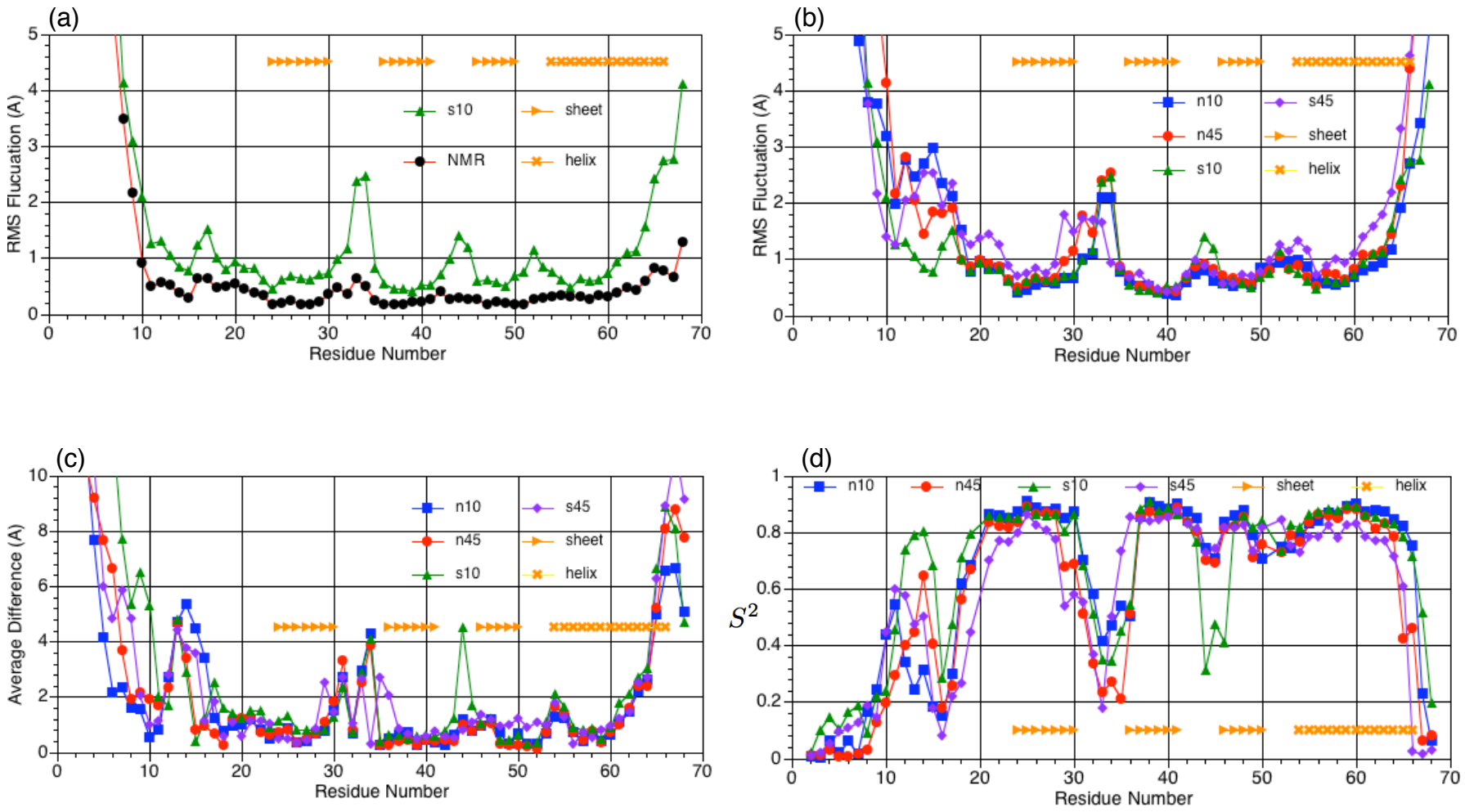
(a)
RMS Flucuation (A)
Residue Number
s10
NMR
sheet
helix
(b)
RMS Fluctuation (A)
Residue Number
n10
n45
s10
s45
sheet
helix
(c)
Average Difference (A)
Residue Number
n10
n45
s10
s45
sheet
helix
(d)
S^2
Residue Number
n10
n45
s10
s45
sheet
helix

Fig S2

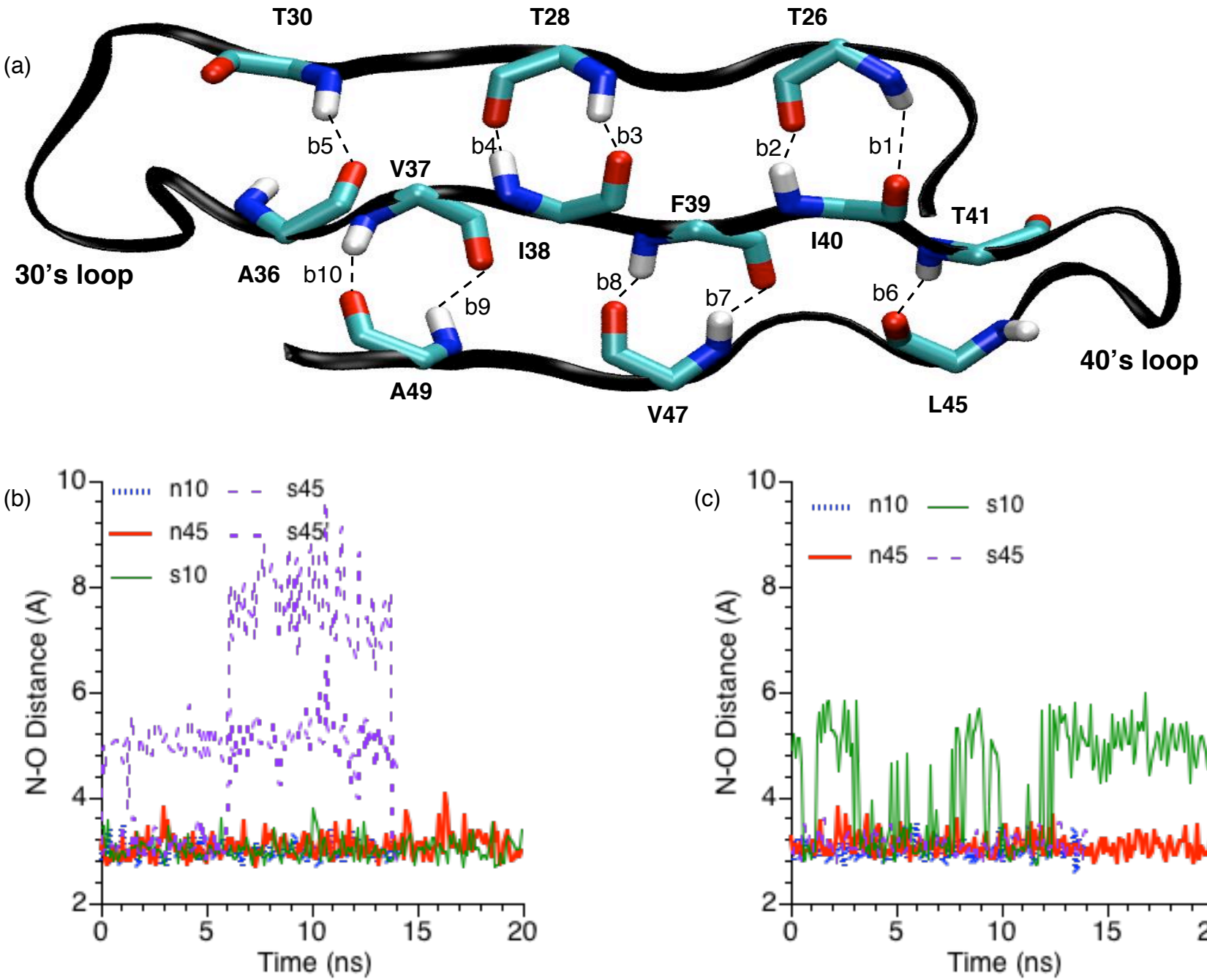
(a)
T30
T28
T26
b5
b4
b3
b2
b1
V37
F39
T41
I38
I40
30's loop
A36
b10
b9
b8
b7
b6
A49
V47
L45
40's loop
(b)
n10
s45
n45
s45'
s10
N-O Distance (A)
Time (ns)
(c)
n10
s10
n45
s45
N-O Distance (A)
Time (ns)

Fig S3

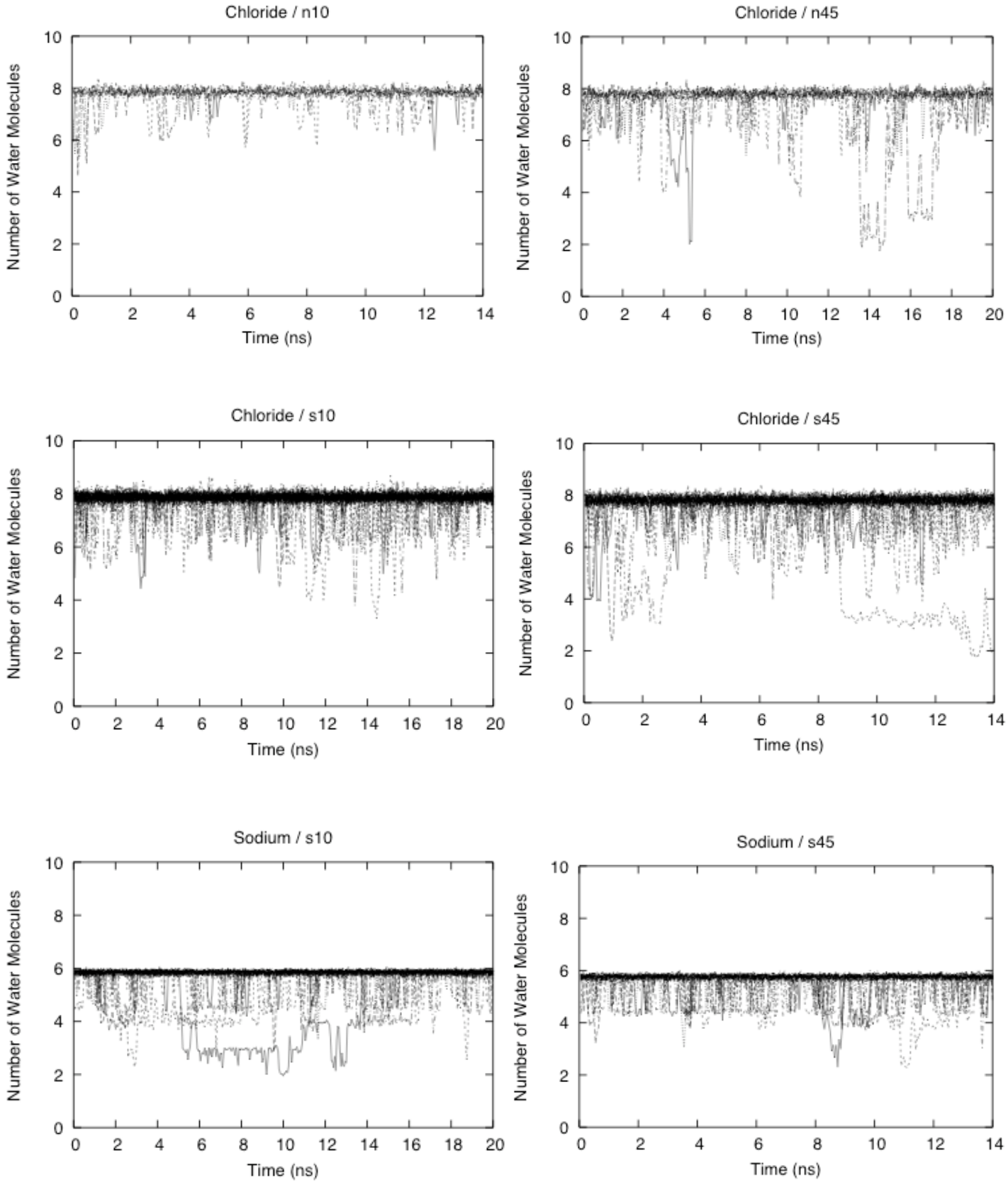
Chloride / n10
Number of Water Molecules
Time (ns)
Chloride / n45
Number of Water Molecules
Time (ns)
Chloride / s10
Number of Water Molecules
Time (ns)
Chloride / s45
Number of Water Molecules
Time (ns)
Sodium / s10
Number of Water Molecules
Time (ns)
Sodium / s45
Number of Water Molecules
Time (ns)

Fig S4