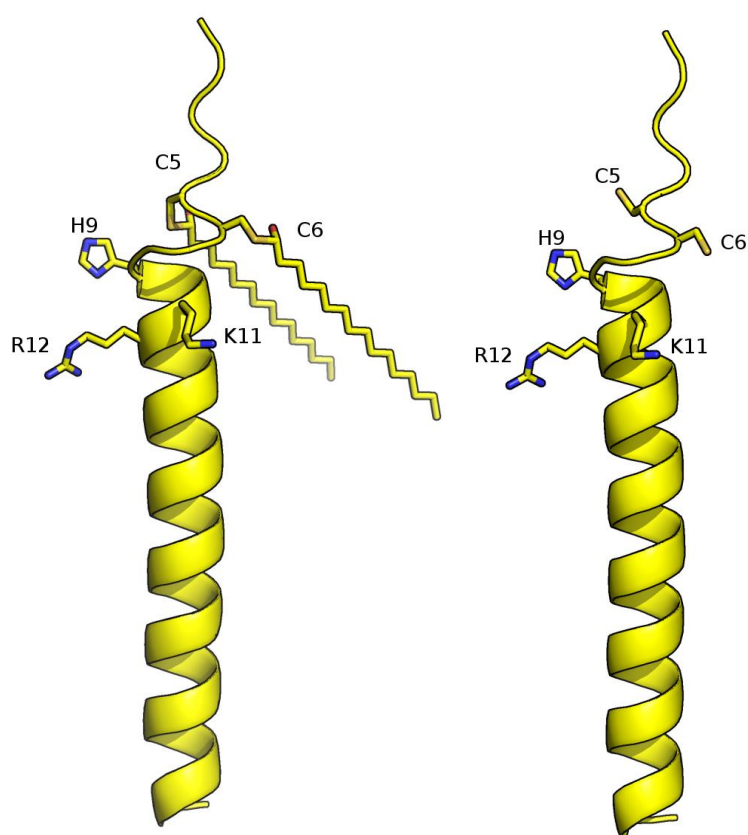# The Effect of Membrane Environment on Surfactant Protein C Stability Studied by Constant-pH MD

Catarina A. Carvalheda, [†‡] Sara R. R. Campos, [†*] and António M. Baptista[†*]

SUPPORTING INFORMATION

Figure S1. Cartoon representation of acylated (left) and deacylated (right) SP-C.

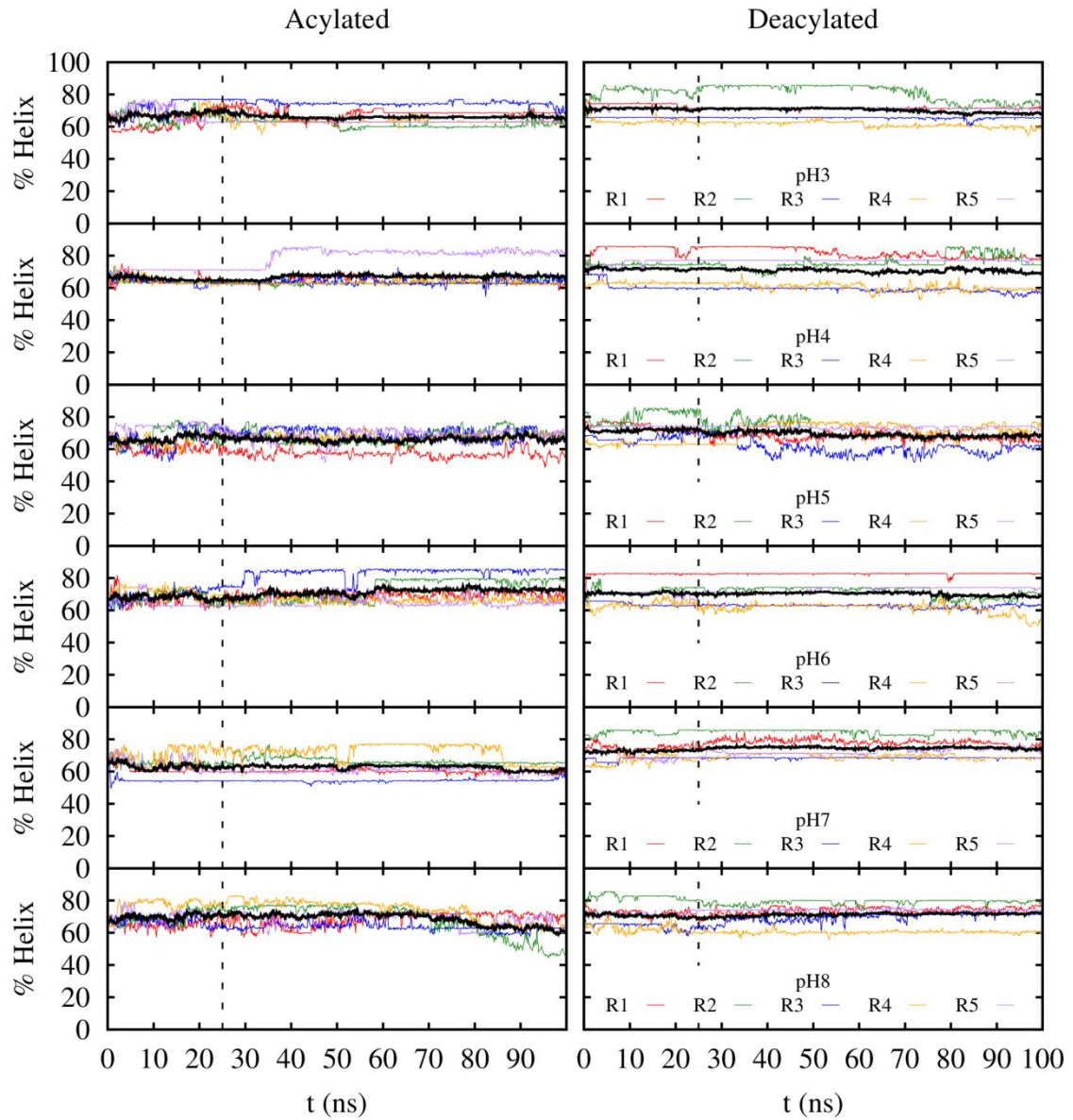[†]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal

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Helix stability

Figure S2. Percentage of residues in helix conformation during the simulation for all replicates and their average for the acylated (left) and deacylated (right) SP-C isoforms at the studied pH range averaged each 100 ps. The percentage of helix content accounts for α-helix, 3-helix and 5-helix motifs. The dashed line indicates the simulation time from which the system was considered to be equilibrated.
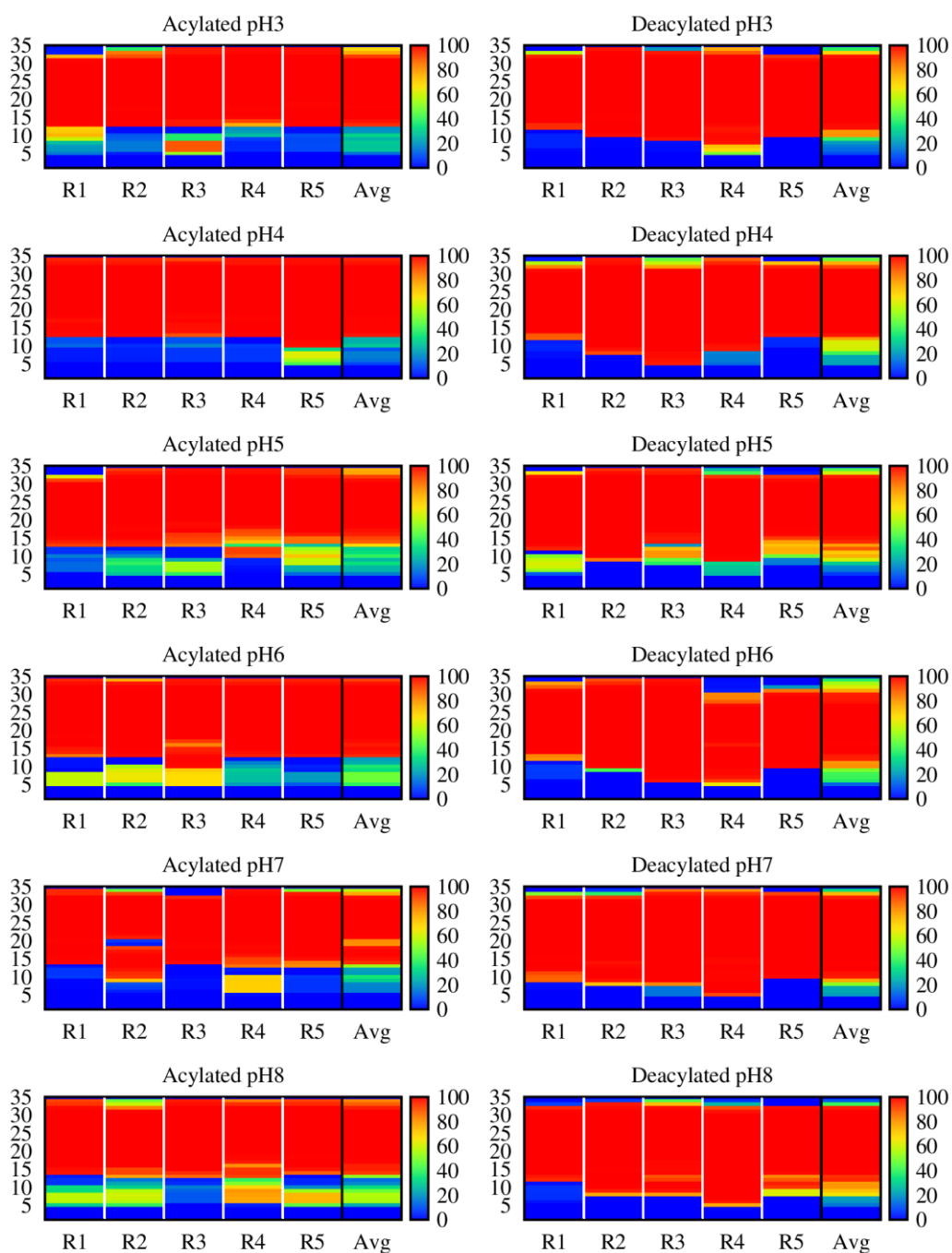
Figure S3. Percentage of time that each residue spends in the helix conformation for both the acylated (left) and deacylated (right) isoforms of SP-C at different pH values in the DPPC bilayer. The results are shown for each replicate individually (R1 to R5) together with the averaged value (Avg). Residue numbers are indicated in the vertical axis.

Hill coefficients

Table S1. Computed Hill coefficient, n, values and respective errors for the sites considered titratable in the constant-pH MD runs of both the deacylated and acylated isoforms.

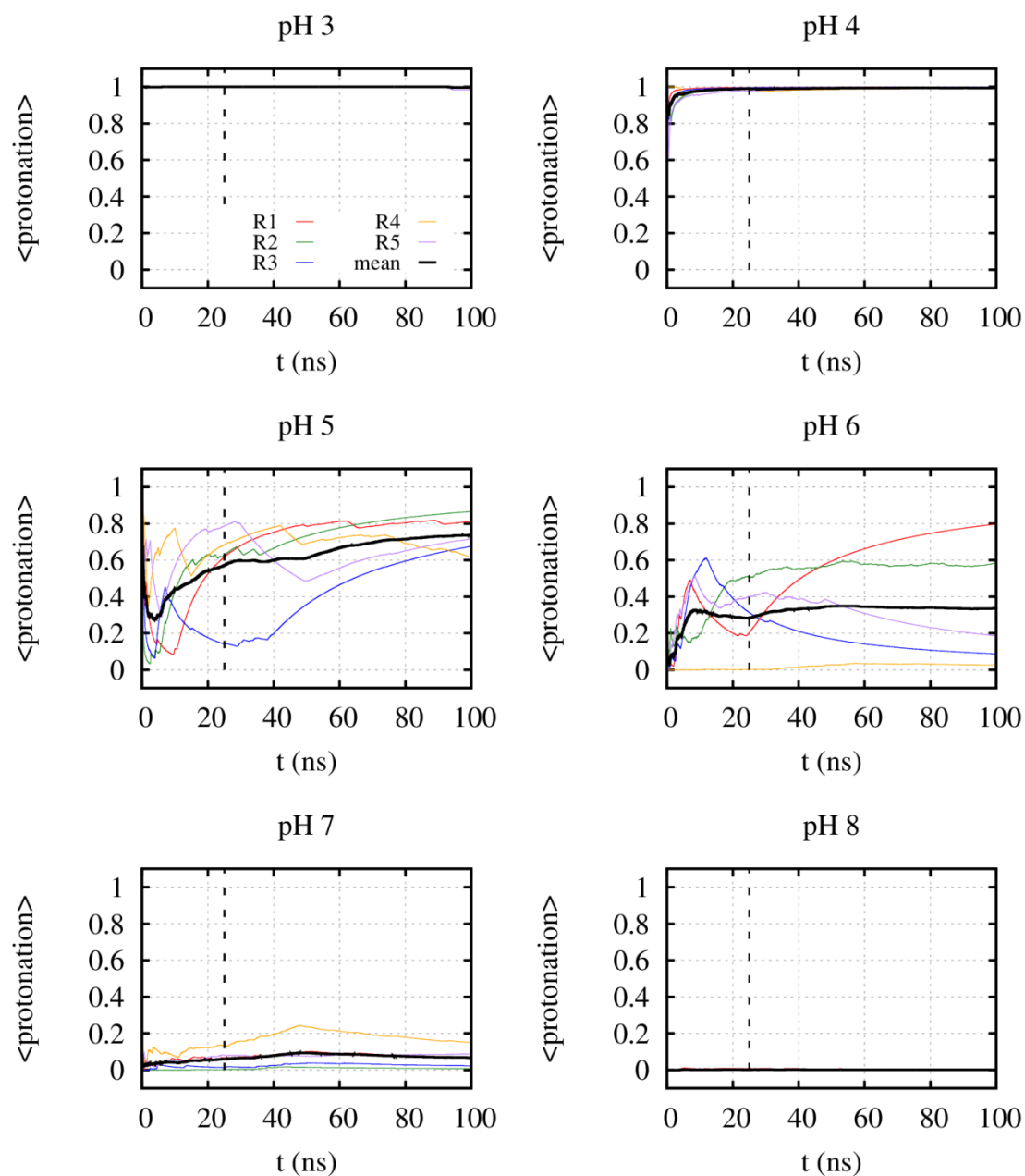| Residue	n	
	Acylated	Deacylated
Nter	0.93 ± 0.25	1.15 ± 0.18
C5	-	-
C6	-	-
H9	0.87 ± 0.31	0.71 ± 0.51
Cter	0.86 ± 0.37	0.91 ± 0.78

Cumulative protonation averages and individual Hill Curves for titratable sites

The cumulative protonation averages at each simulated pH value, considering the 5 replicates, are shown in Figures S4 to S6 for the acylated isoform and in Figures S7 to S11 for the deacylated isoform. The Hill curves fitted to the average protonation points obtained from the simulations are depicted in Figures S12 to S13 for the acylated and deacylated isoforms, respectively.
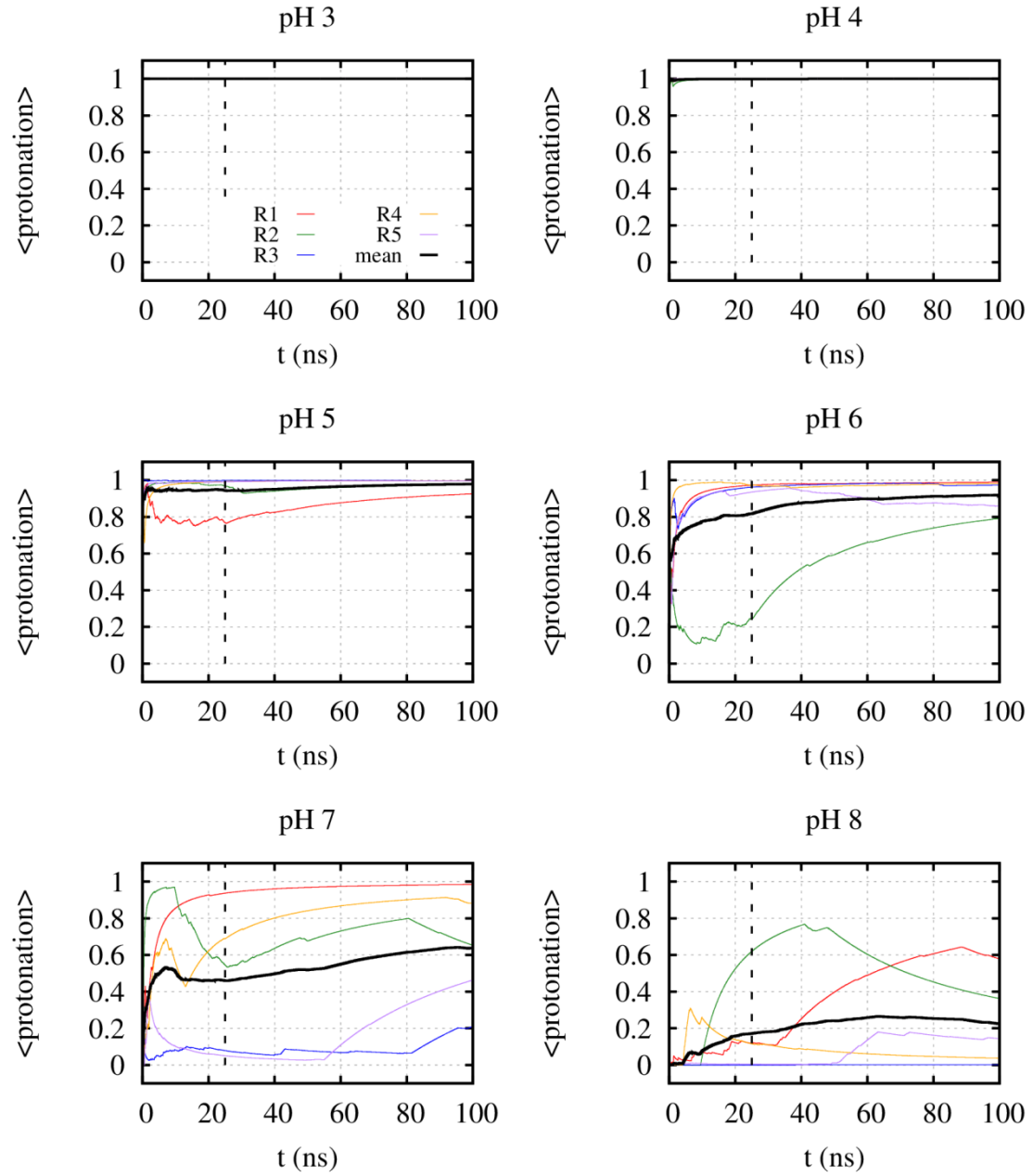
Nter

pH 3

pH 4

pH 5

pH 6

pH 7

pH 8
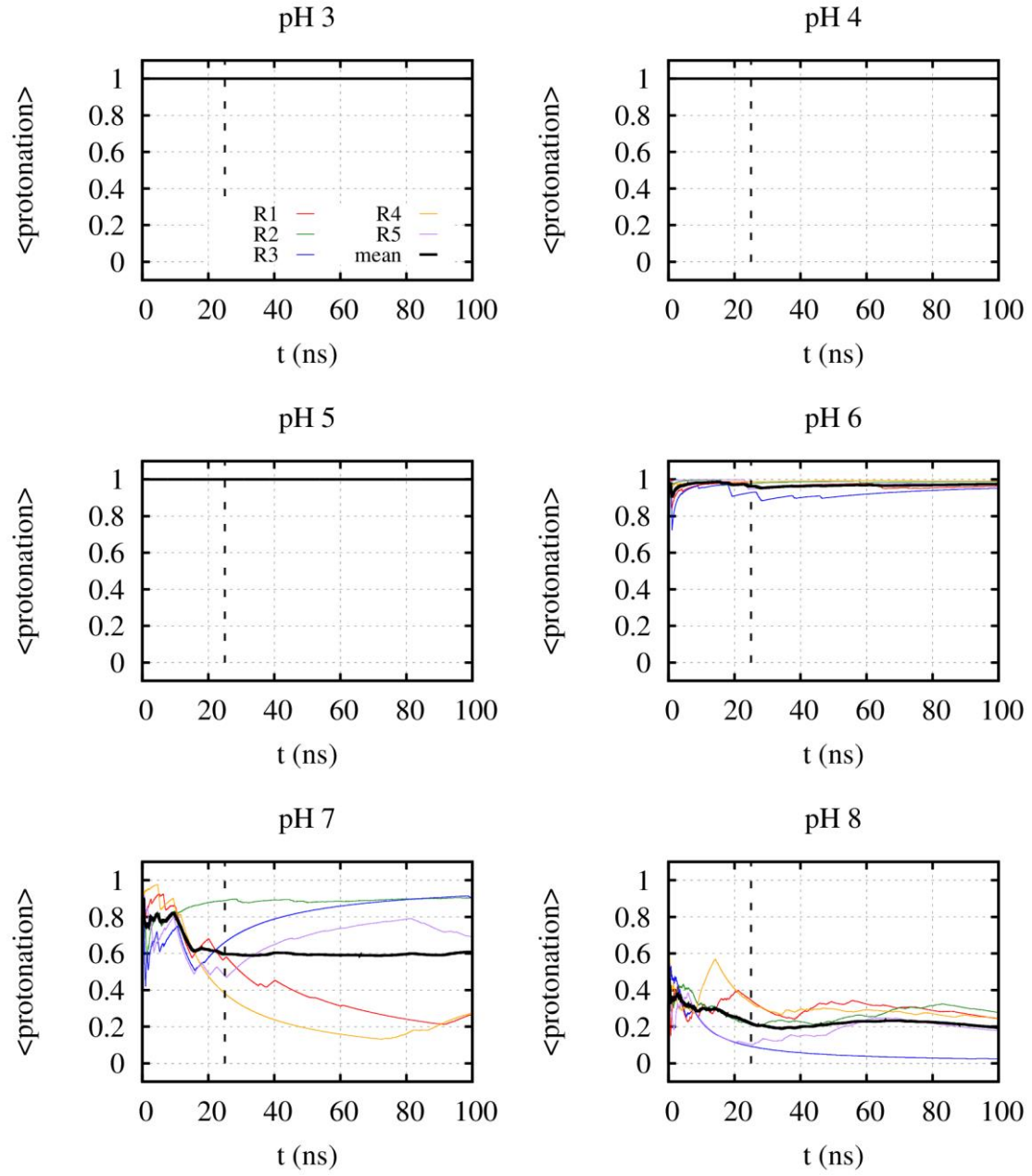
Figure S4. Cumulative protonation averages of the N-terminal residue at each simulated pH value, considering the 5 replicates and their average for the acylated isoform.
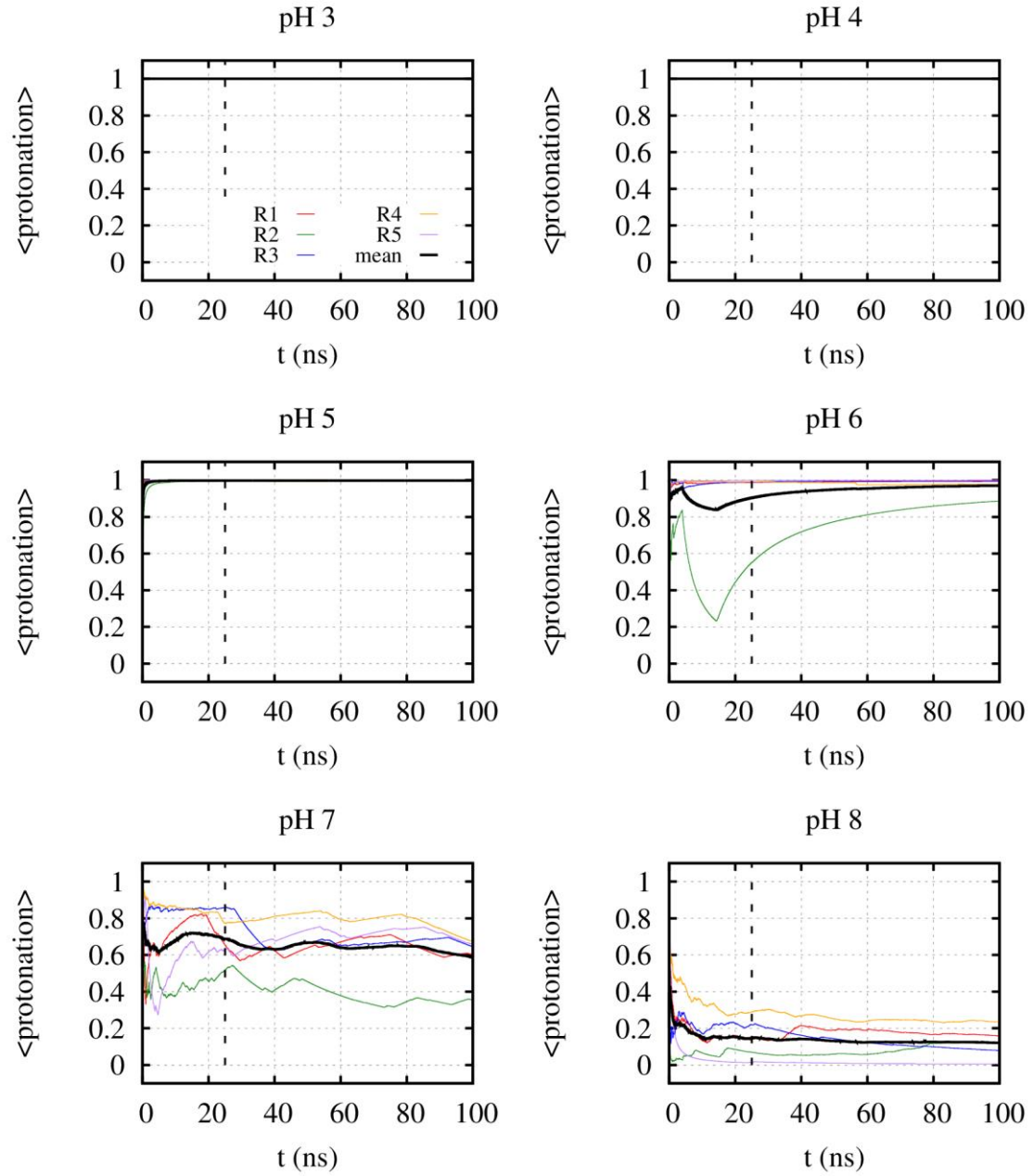
H9

Figure S5. Cumulative protonation averages of the H9 residue at each simulated pH value, considering the 5 replicates and their average for the acylated isoform.
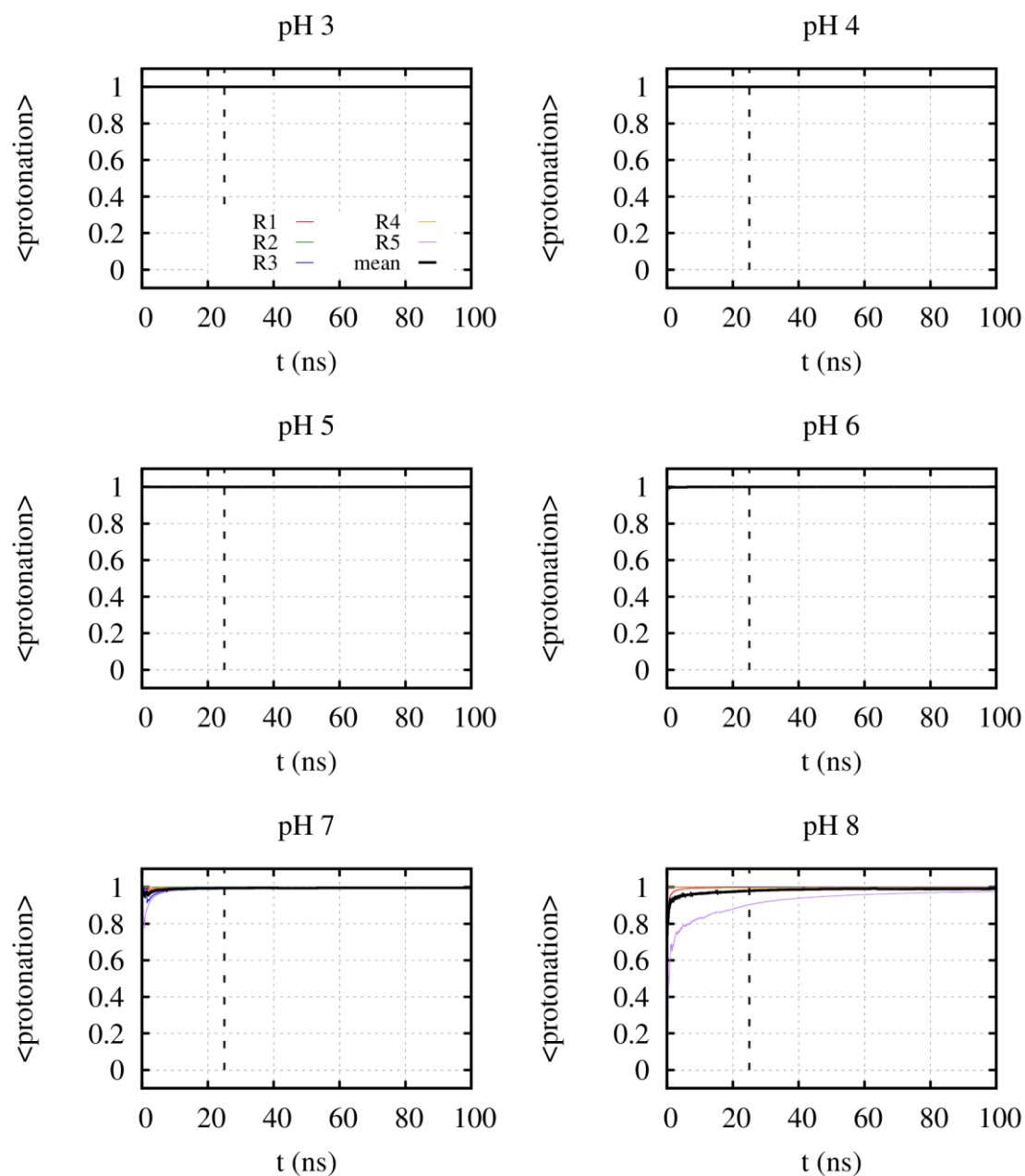
Cter

Figure S6. Cumulative protonation averages of the C-terminal residue at each simulated pH value, considering the 5 replicates and their average for the acylated isoform.
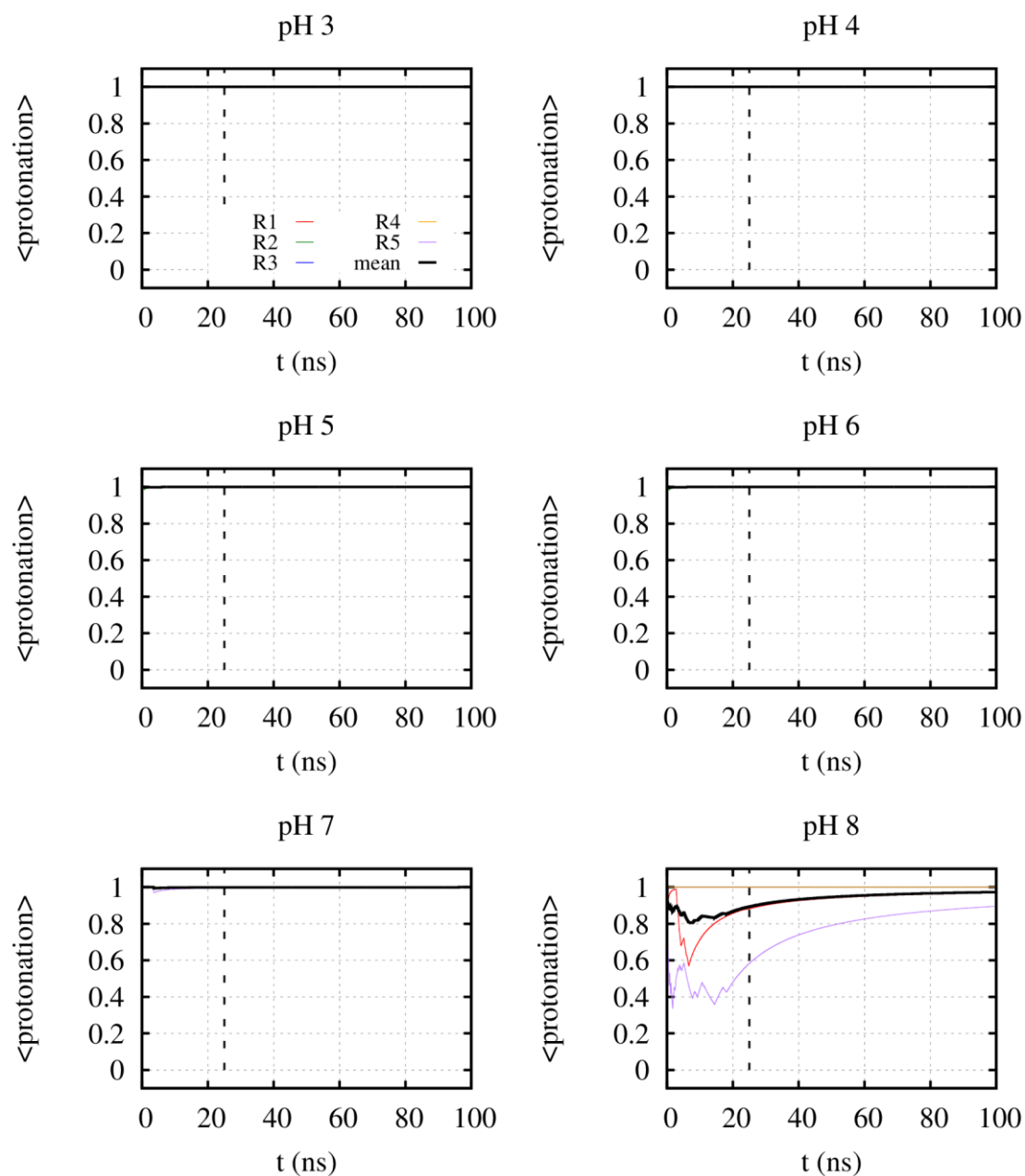
Nter

Figure S7. Cumulative protonation averages of the N-terminal residue at each simulated pH value, considering the 5 replicates and their average for the deacylated isoform.

C5

Figure S8. Cumulative protonation averages of the C5 residue at each simulated pH value, considering the 5 replicates and their average for the deacylated isoform.

C6

Figure S9. Cumulative protonation averages of the C6 residue at each simulated pH value, considering the 5 replicates and their average for the deacylated isoform.

Figure S10. Cumulative protonation averages of the H9 residue at each simulated pH value, considering the 5 replicates and their average for the deacylated isoform.

Cter

Figure S11. Cumulative protonation averages of the C-terminal residue at each simulated pH value, considering the 5 replicates and their average for the deacylated isoform.
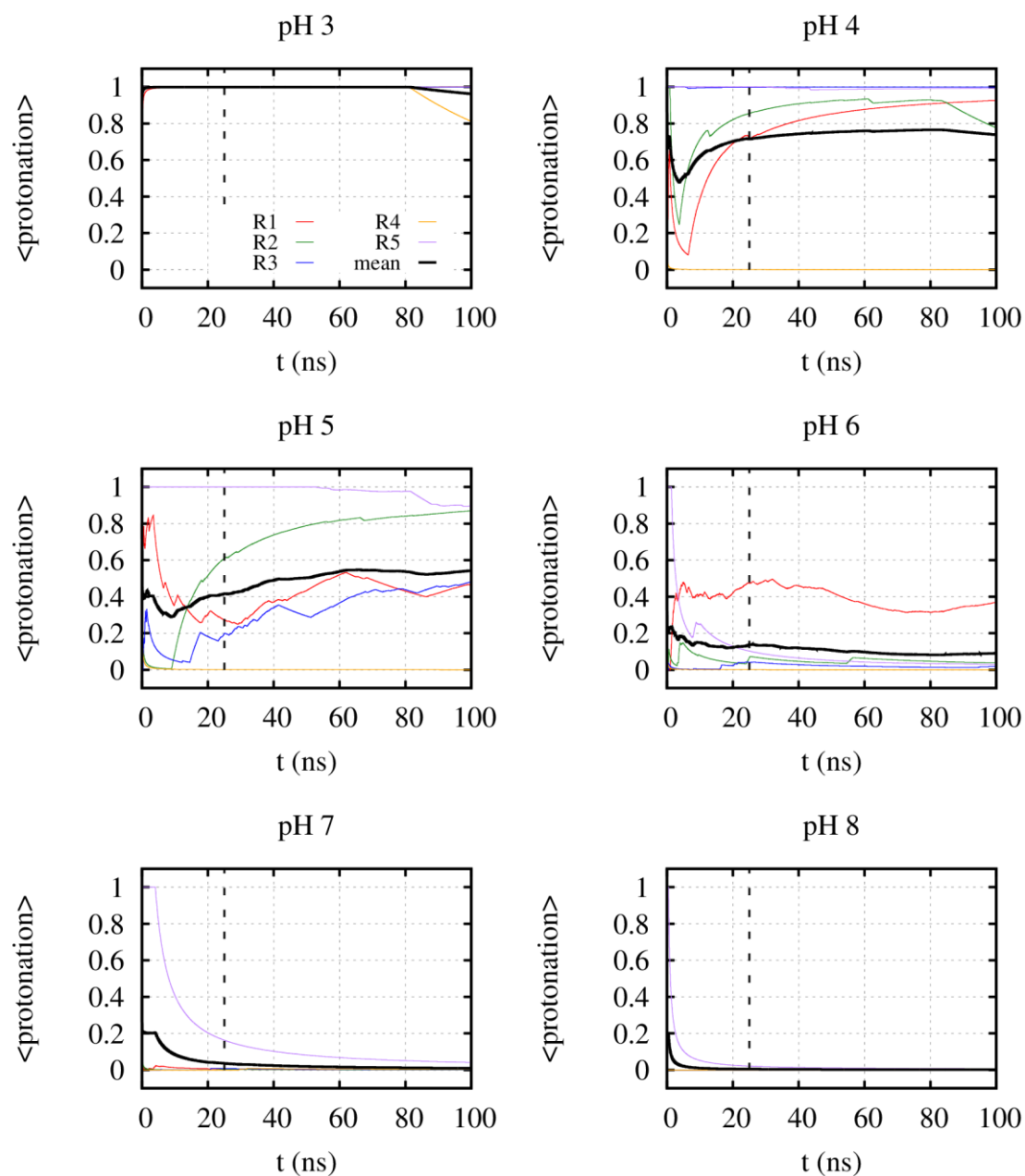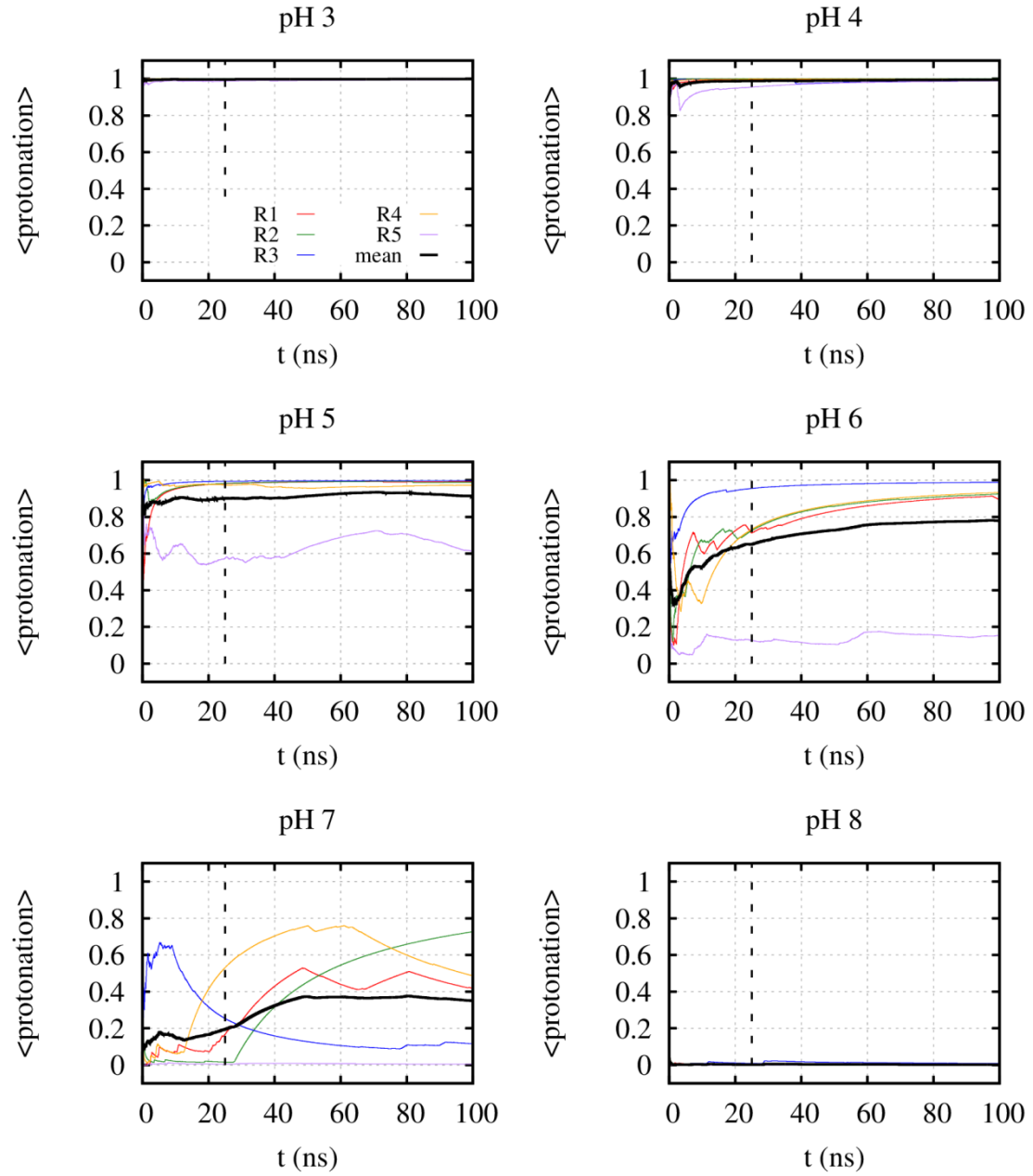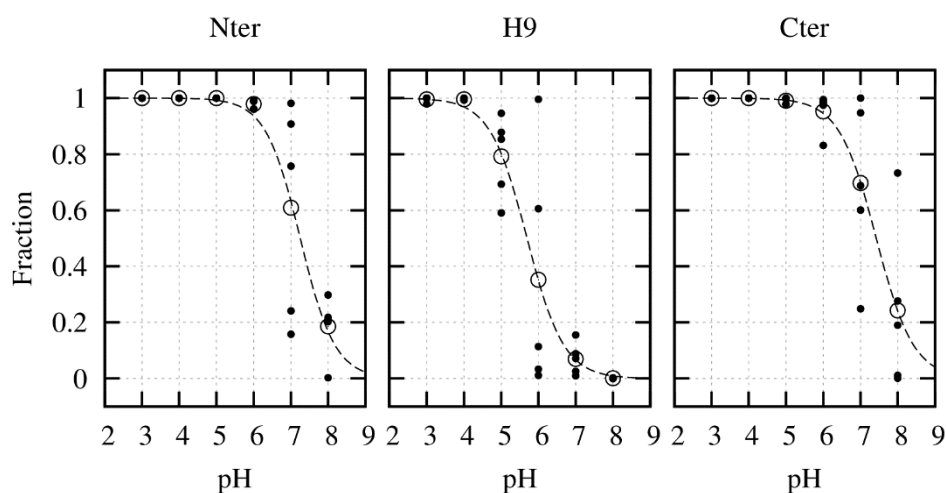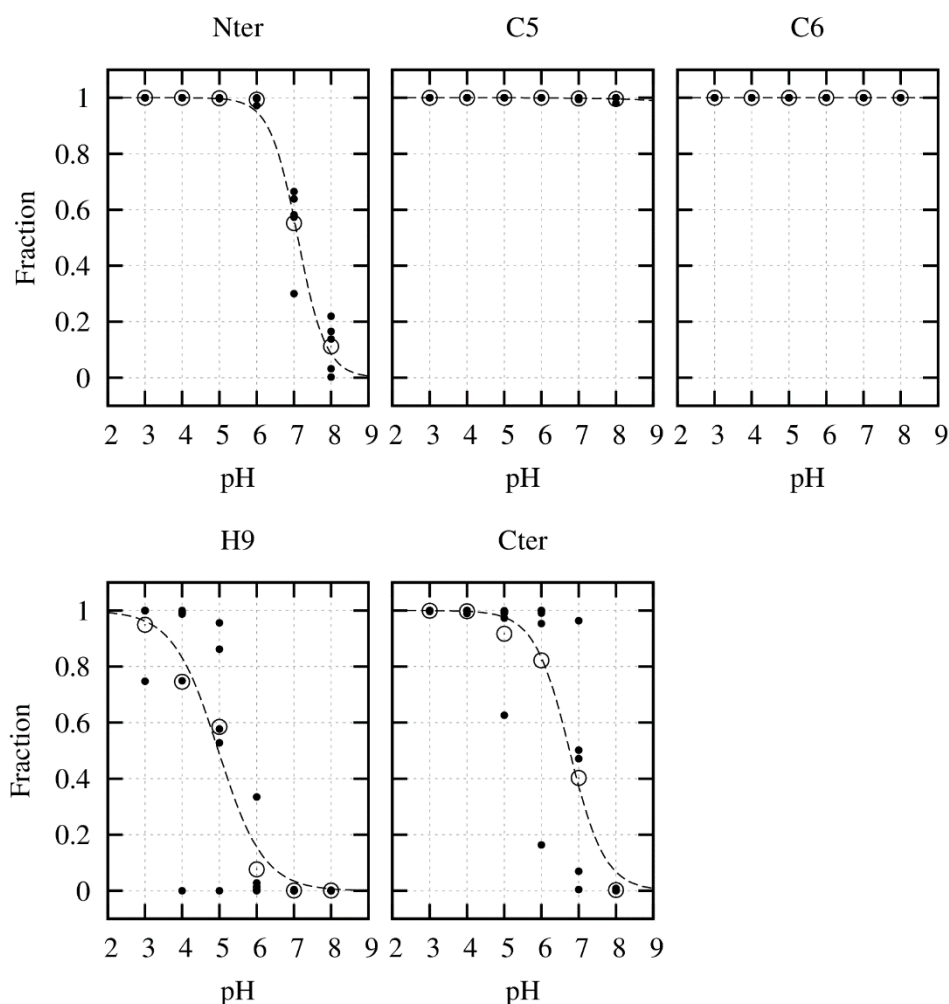
Figure S12. Average protonation points obtained from the simulations (filled circles) and their corresponding average value at each pH (empty circles) along with the respective Hill curve fits (dashed line) for the titratable sites of the acylated isoform.


Figure S13. Average protonation points obtained from the simulations (filled circles) and their corresponding average value at each pH (empty circles) along with the respective Hill curve fits (dashed line) for the titratable sites of the deacylated isoform.
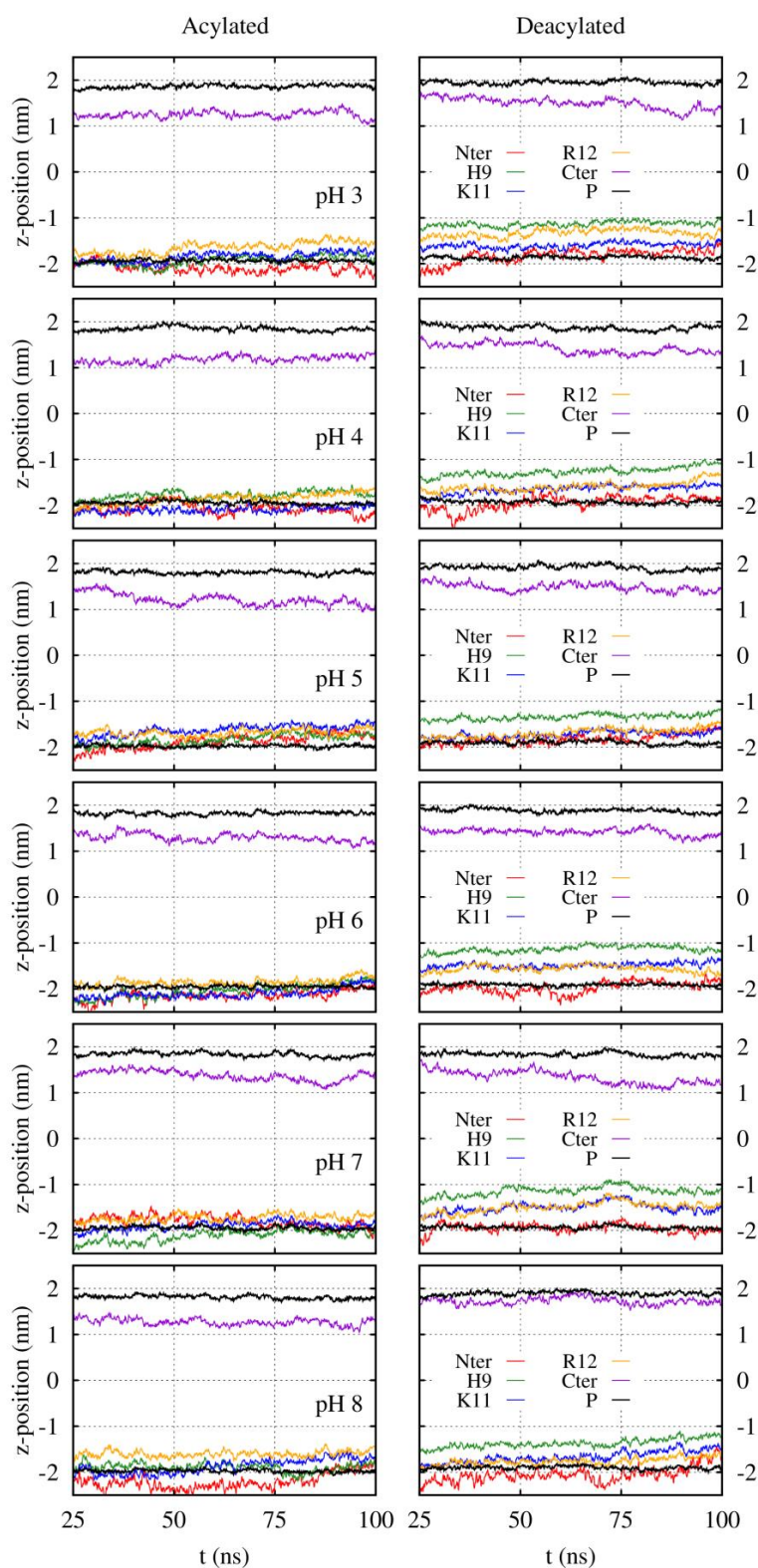
Figure S14. Average atomic coordinates of the N-terminal nitrogen atom of the amine group (red line), the center of mass of the H9 imidazole ring (green line), the nitrogen atom of the K11 amine group (blue line), the central carbon atom in the R12 guanidinium group (orange line) and the carbon atom in the C-terminal carboxyl group (violet line), compared with the average positions of the lipid phosphorous atoms (black line), in the z axis, averaged at each 100 ps, for the equilibrated segment of the acylated (left) and deacylated (right) SP-C isoforms simulations at different pH values. The value z=0 corresponds to the midpoint between the average phosphorous atomic positions.

Protein-lipid contacts

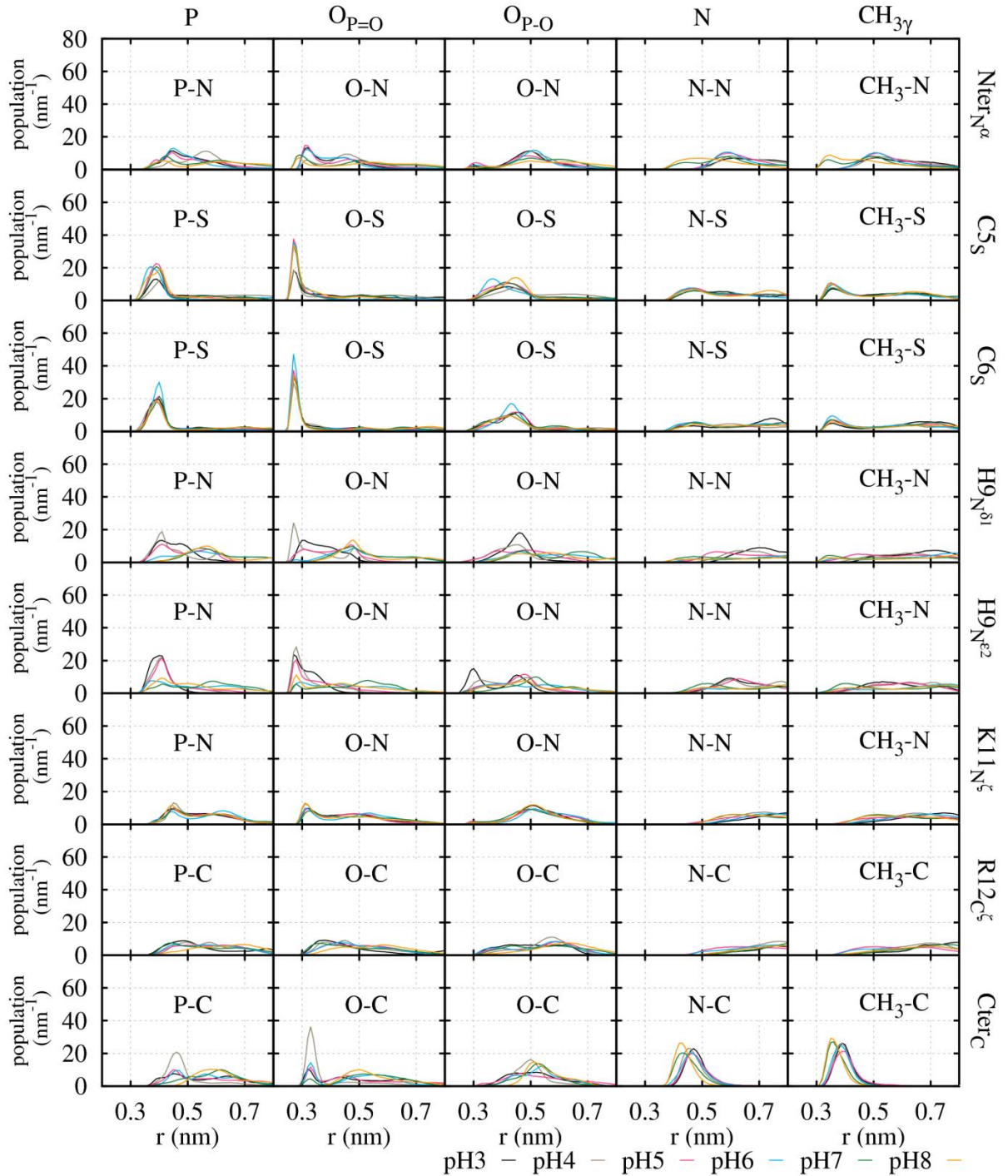

pH3 — pH4 — pH5 — pH6 — pH7 — pH8 —
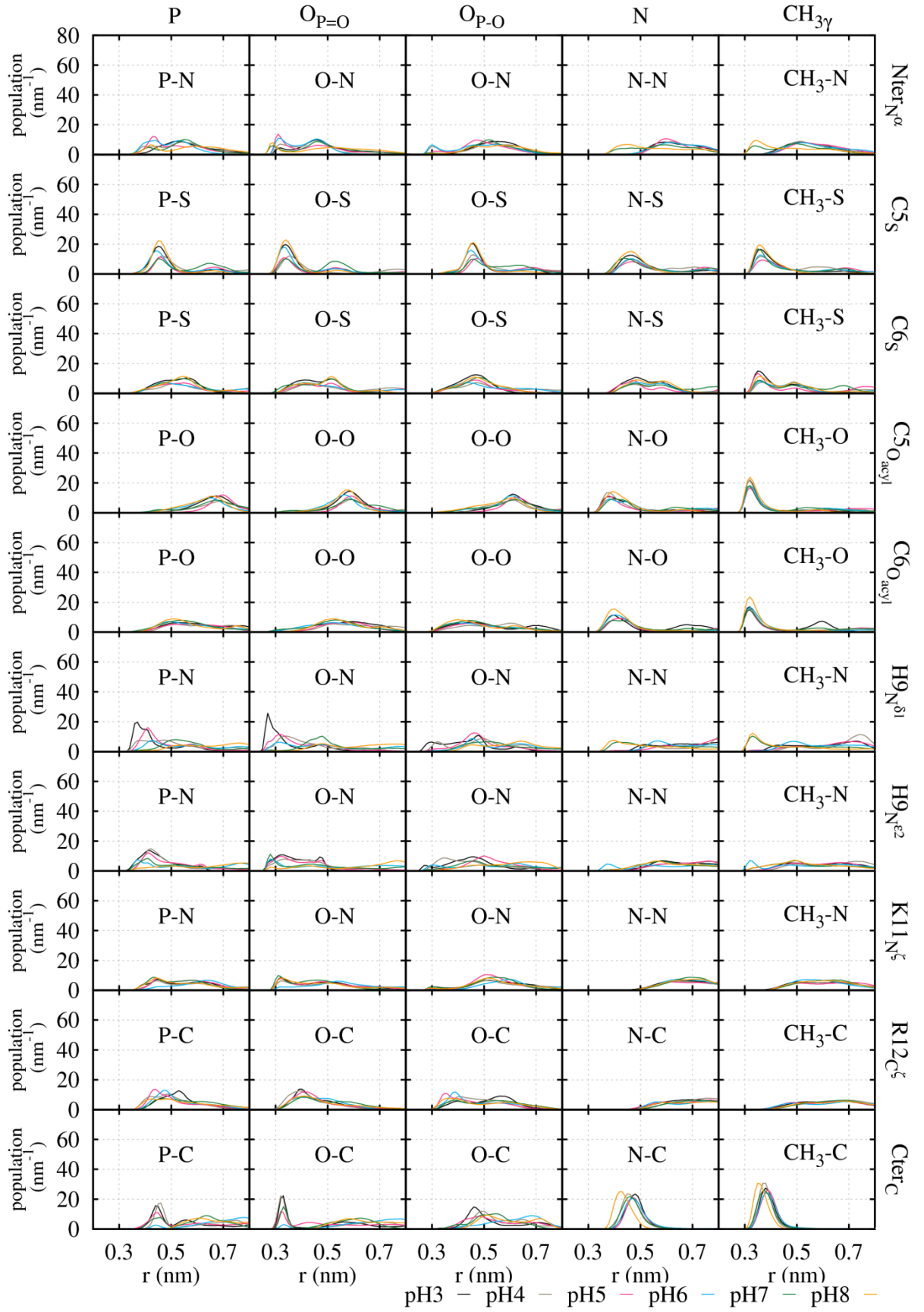
Figure S15. Probability density function of the distance of several acylated SP-C atoms to the lipid headgroup atoms in the DPPC bilayers at different pH values. Protein atom designations: the Nter N^{α} refers to the N-terminal nitrogen atom of the amine group, C5 and C6 S to the sulphur atom in the cysteine thioester group, C5 and C6 O_{acyl} to the oxygen atom of the thioester bond, H9 $N^{\delta 1}$ and $N^{\epsilon 2}$ to both histidine nitrogen atoms of the imidazole ring, K11 N^{ζ} to the nitrogen atom of the lysine amine group, R12 C^{ζ} to the central carbon atom in the arginine guanidinium group and C to the carbon atom in the C-terminal carboxyl group. Lipid atom designations: P refers to the phosphorus atom in DPPC, $O_{P=O}$ to the negatively charged oxygen atoms of the DPPC phosphate group, O_{P-O} to the phosphoester bonds oxygen atoms of DPPC, N to the nitrogen atom of the DPPC choline group and $CH_{3\gamma}$ to the methyl groups carbons in the DPPC choline group.
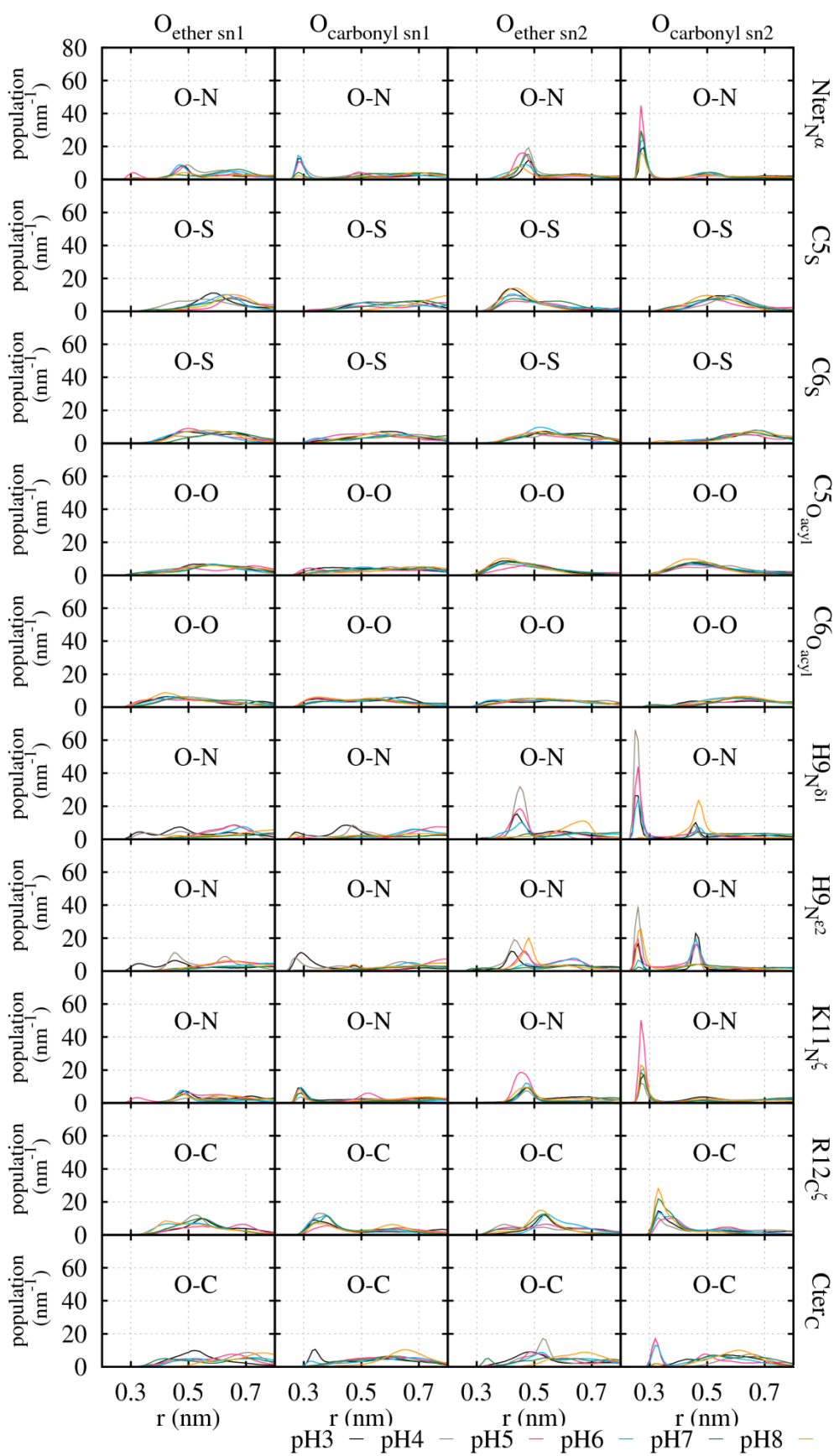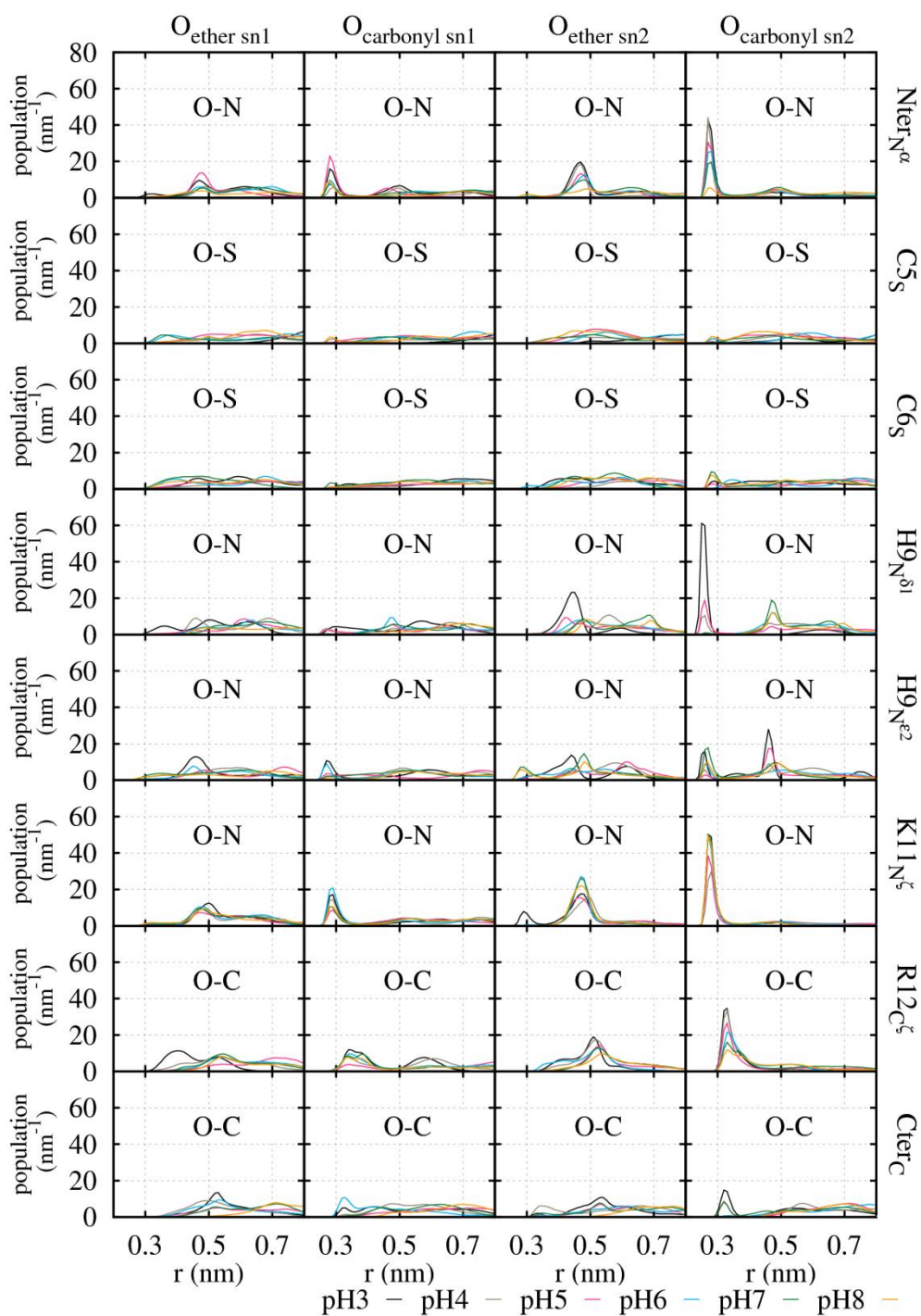
Figure S16. Probability density function of the distance of several acylated SP-C atoms to the lipid oxygen atoms in the ester bonds of the *sn*-1and *sn*-2 acyl chains in the DPPC bilayers at different pH

values. Protein atom designations according to Error! Reference source not found.. Lipid atom designations: $O_{ether\ sn1}$ to the oxygen atom in the ether (alkoxy) group of the DPPC *sn-1* chain, $O_{carbonyl\ sn1}$ to the oxygen atom in the carbonyl group of the DPPC *sn-1* chain, $O_{ether\ sn2}$ to the oxygen atom in the ether (alkoxy) group of the DPPC *sn-2* chain, $O_{carbonyl\ sn2}$ to the oxygen atom in the carbonyl group of the DPPC *sn-2*.


Figure S17. Density probability function of the distance of several deacylated SP-C atoms to the lipid headgroup atoms at different pH values in the DPPC bilayer. Protein and lipid atom designations according to Figure S15.

Figure S18. Density probability function of the distance of several deacylated SP-C atoms to the lipid oxygen atoms in the *sn-1* and *sn-2* acyl chains at different pH values in the DPPC bilayer. Protein and lipid atom designations according to Figure S16.

Membrane properties
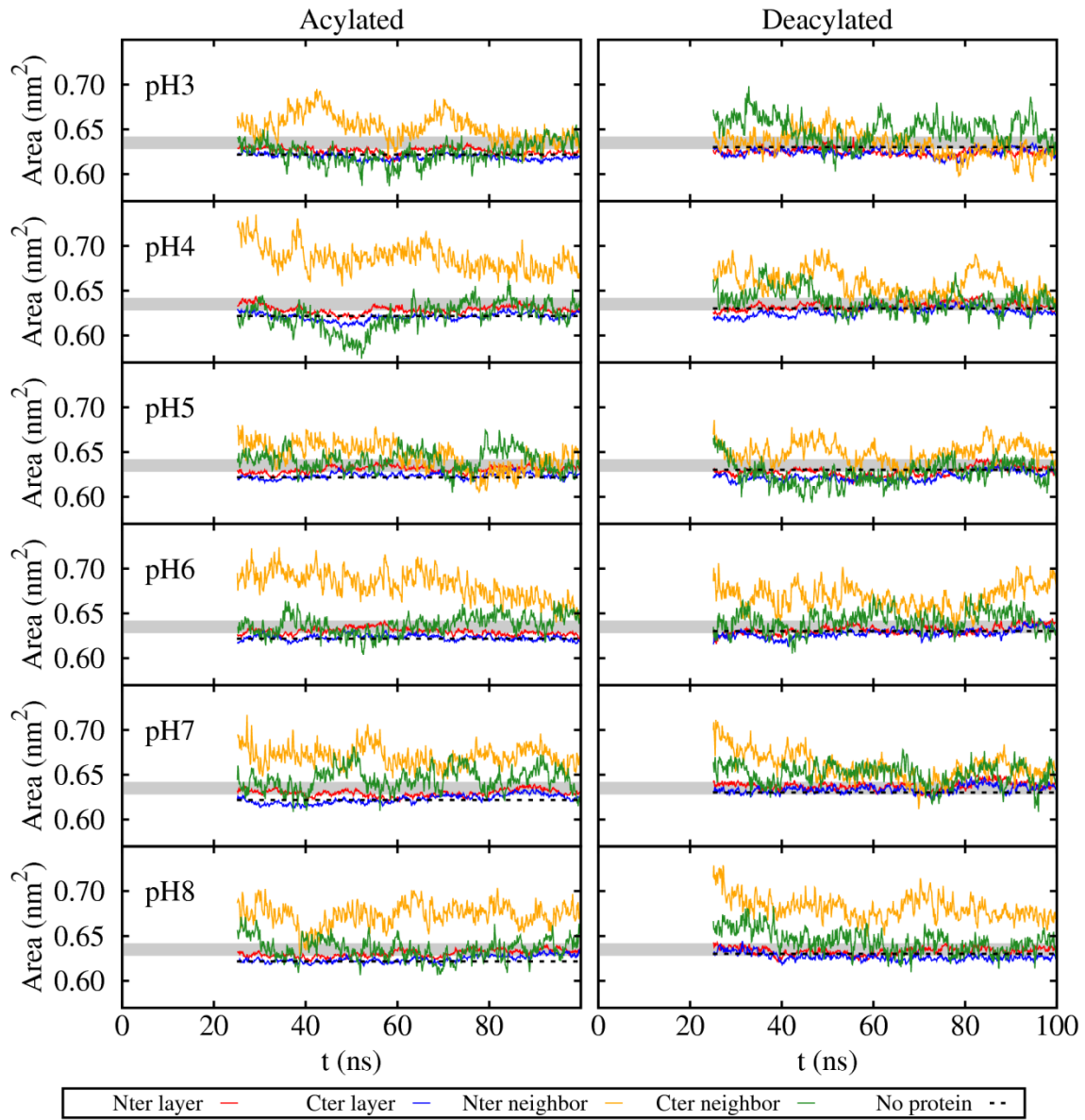
Figure S19 Average area per lipid for both the acylated (left) and deacylated (right) isoforms of SP-C at different pH values in the DPPC bilayer. The area per lipid was measured for all the lipids in the N-terminal layer (red line) and C-terminal layer (blue line) and for all the lipids within 5 Å of the protein in the N-terminal layer (orange line) and C-terminal layer (green line) separately. These are compared with the average area per lipid obtained for the equilibrated DPPC membranes used for each isoform (dashed line; see section 2.3 in the manuscript) and with the experimental area per lipid for DPPC[1] (grey area).
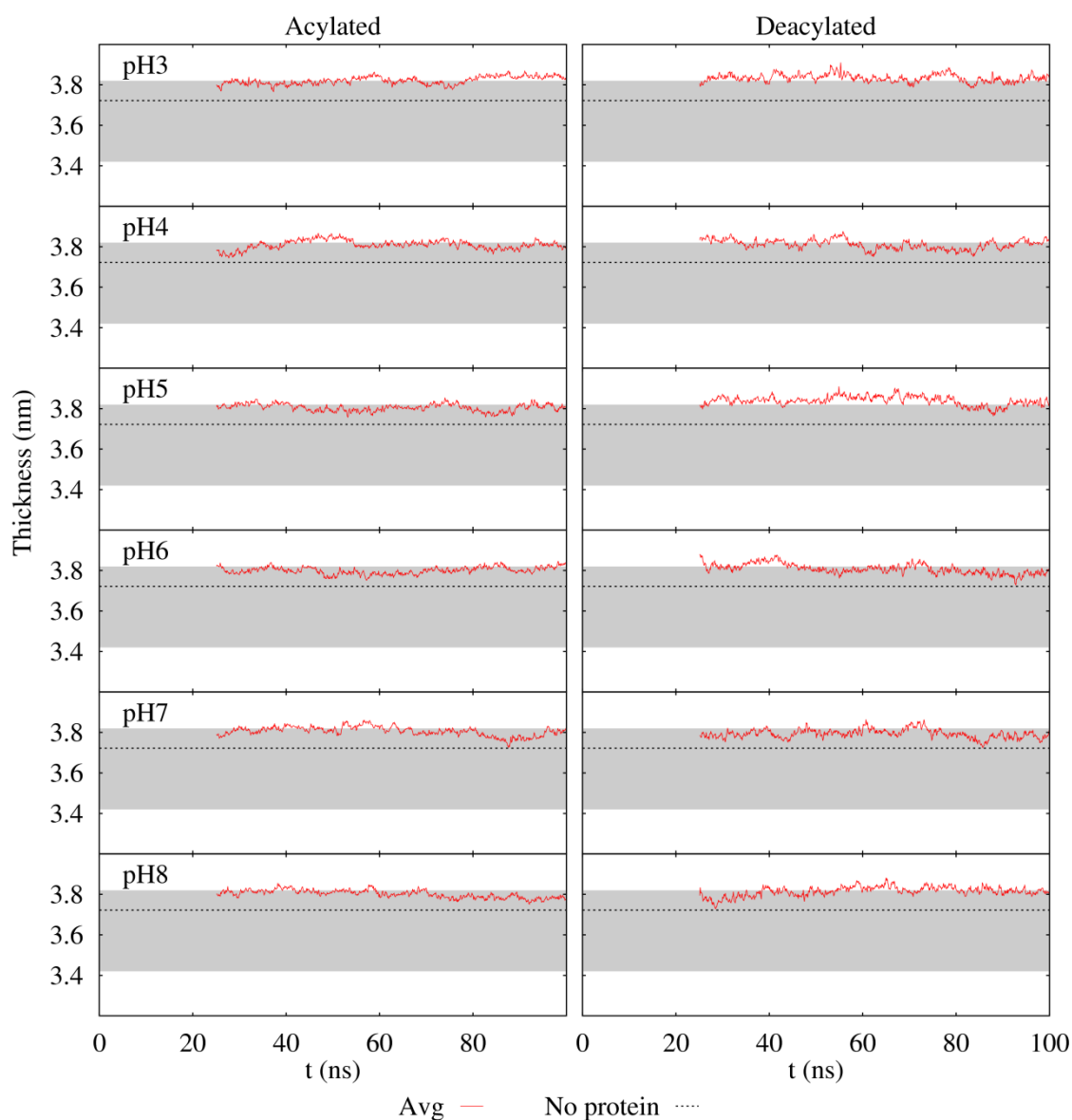
Figure S20. Average membrane thickness for both the acylated (left) and deacylated (right) isoforms of SP-C at different pH values in the DPPC bilayer. The average thickness obtained for equilibrated DPPC membranes used for each isoform (dashed line; see section 2.3 in the manuscript) and the experimental thickness for DPPC (grey area) are also shown for comparison.
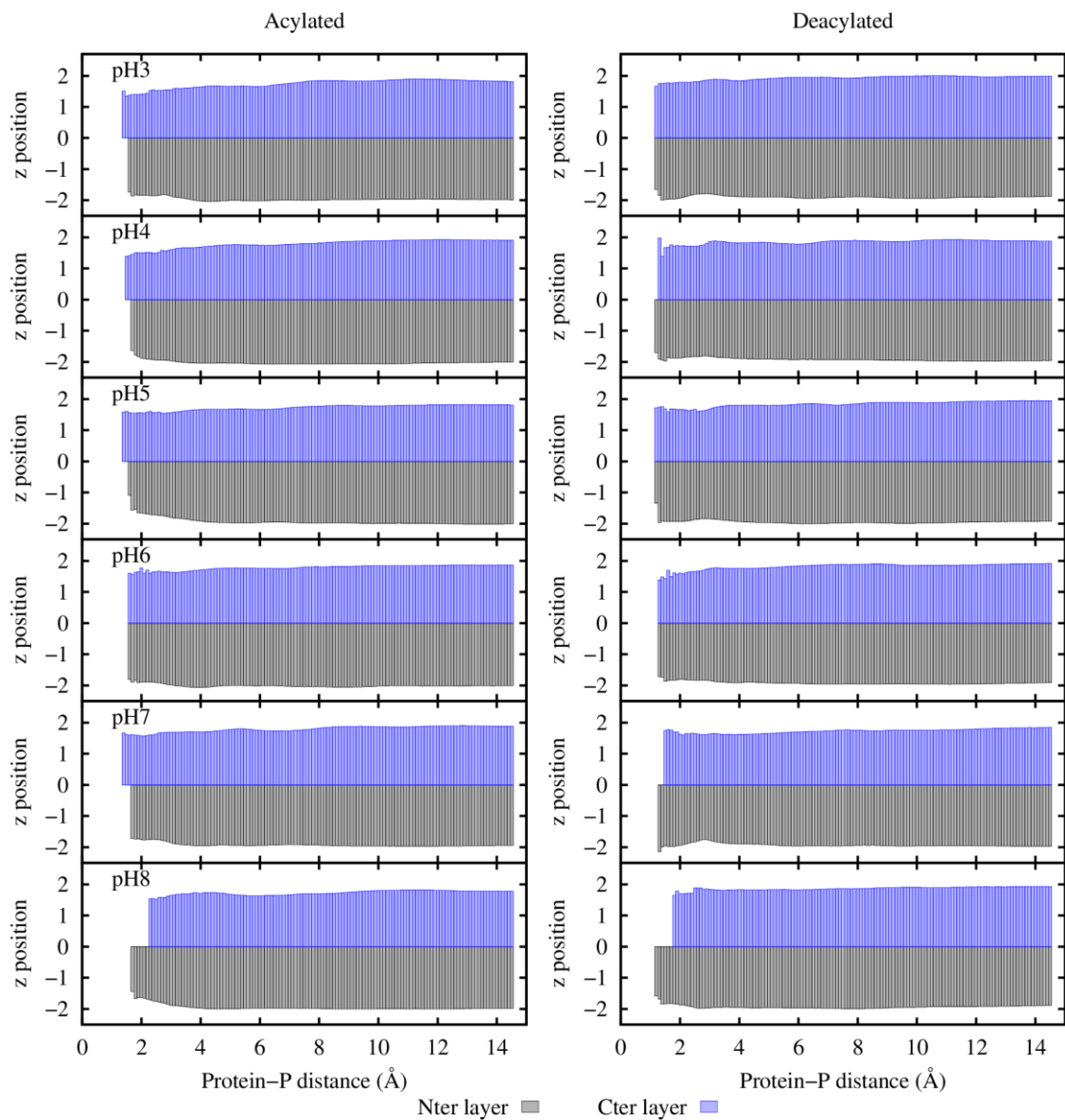
Figure S21. Average z axis position of the lipid phosphorous atoms in the N-terminal (in grey) and C-terminal (in blue) layers according to their distance to the protein for both the acylated (left) and deacylated (right) isoforms of SP-C at different pH values. z=0 corresponds to the midpoint between the average phosphorous atomic positions.
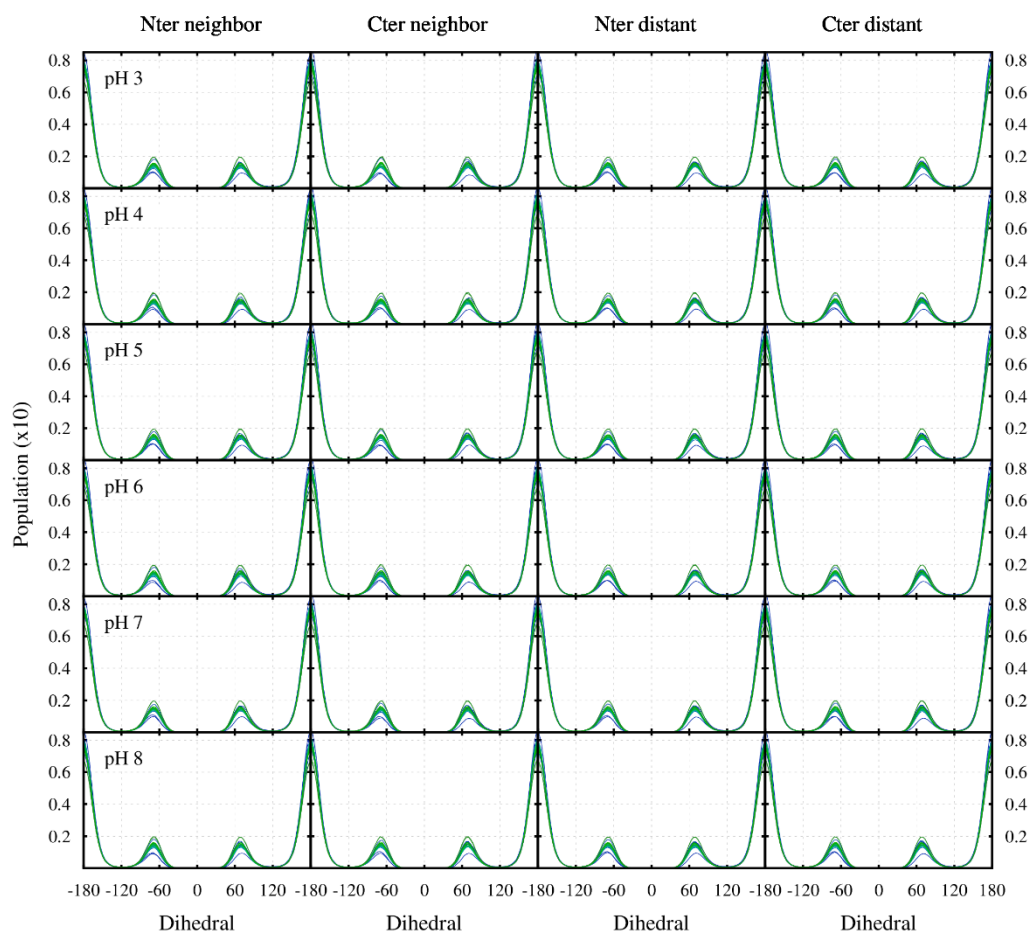
Figure S22. Acyl chain dihedral angle distribution of the neighbor (within a sphere cutoff of 0.5 nm around the protein) and distant (outside a sphere cutoff of 0.5 nm around the protein) lipid molecules in the N-terminal and C-terminal layers for the acylated SP-C isoform at different pH values. Each plot shows the 26 curves corresponding to the torsion dihedrals along the *sn*-1 and *sn*-2 chains, with the colors ranging from blue to green as one moves towards the end of the chains.

References

(1)   Poger, D.; Mark, A. E. Lipid Bilayers: The Effect of Force Field on Ordering and Dynamics. *J. Chem. Theory Comput.* **2012**, *8*, 4807–4817.