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Supporting Information

An Ensemble of Rapidly Interconverting Orientations in Electrostatic Protein–Peptide Complexes Characterized by NMR Spectroscopy

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Supporting Information

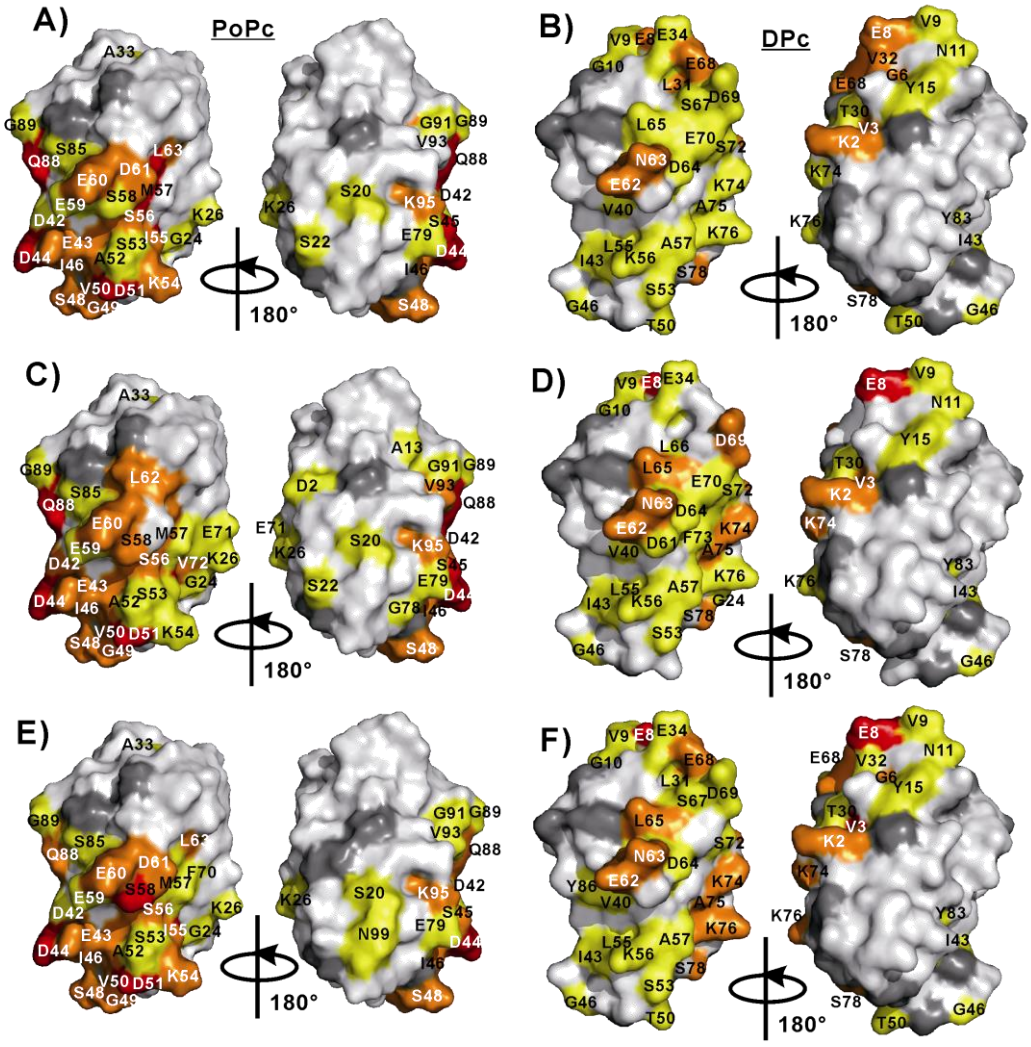

Figure S1: CSPs (extrapolated to 100% bound) mapped onto the protein surfaces from the binding of Lys_4-X (panels A and B), Lys_4-Ala (panels C and D) and Ala-Lys_4 (panels E and F) to PoPc (left, PDB entry 1TKW[1]) and DPc (right, PDB entry 1KDI[2]). Color representations: red, $\Delta\delta_{ave} \geq 0.04$ ppm; orange, $0.04 > \Delta\delta_{ave} \geq 0.02$ ppm; yellow, $0.02 > \Delta\delta_{ave} \geq 0.01$ ppm; white, $\Delta\delta_{ave} < 0.01$ ppm; grey, no data or overlapping resonances.

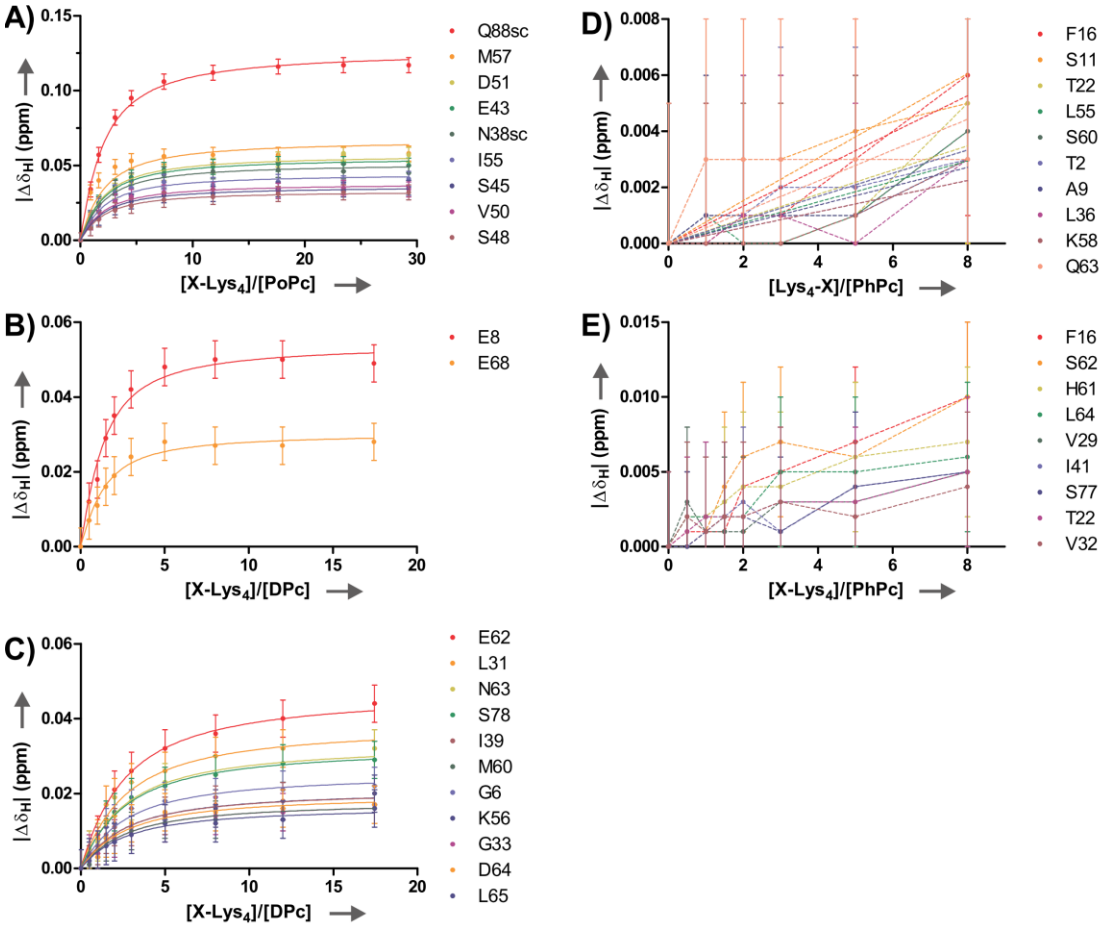

Figure S2: Chemical shift changes of Pcs resonances as a function of increasing [peptide]/[Pc] for peptides X-Lys_4 and Lys_4-X. The dissociation constants of the corresponding peptides (Table 1) were obtained by simultaneous fitting to a 1:1 binding model for PoPc (solid lines) and by simulation for 2-site binding for DPc. (A) X-Lys_4 with PoPc; (B) X-Lys_4 with DPc, strong-binding residues; (C) X-Lys_4 with DPc, weak-binding residues; (D) Lys_4-X with PhPc; (E) X-Lys_4 with PhPc. The titration points for each residue in (D) and (E) are connected with dashed lines. Error bars represent ± 0.005 ppm.

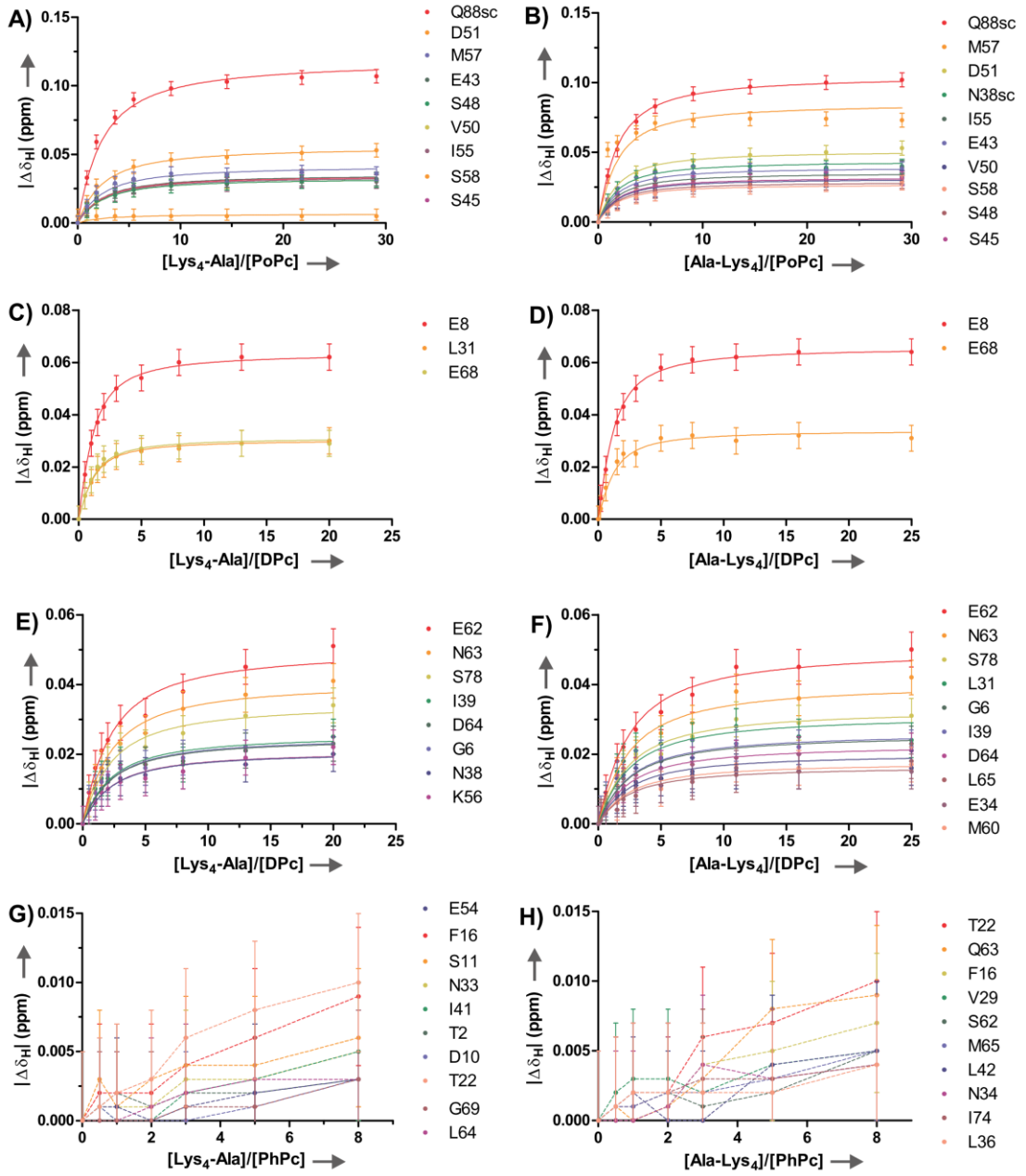

Figure S3: Chemical shift changes of Pcs resonances as a function of increasing [peptide]/[Pc] for peptides Lys4-Ala and Ala-Lys4. The residues which showed largest perturbations are shown. The dissociation constants of the corresponding peptides (Table 1) were obtained by simultaneous fitting to a 1:1 binding model for PoPc (solid lines) and by simulation for 2-site binding for DPc. (A) Lys4-Ala with PoPc; (B) Ala-Lys4 with PoPc; (C) Lys4-Ala with DPc, strong-binding residues; (D) Ala-Lys4 with DPc, strong-binding residues; (E) Lys4-Ala with DPc, weak-binding residues; (F) Ala-Lys4 with DPc, weak-binding residues; (G) Lys4-Ala with PhPc; (B) Ala-Lys4

with PhPc. The titration points for each residue in (G) and (H) are connected with dashed lines. Error bars represent ± 0.005 ppm.

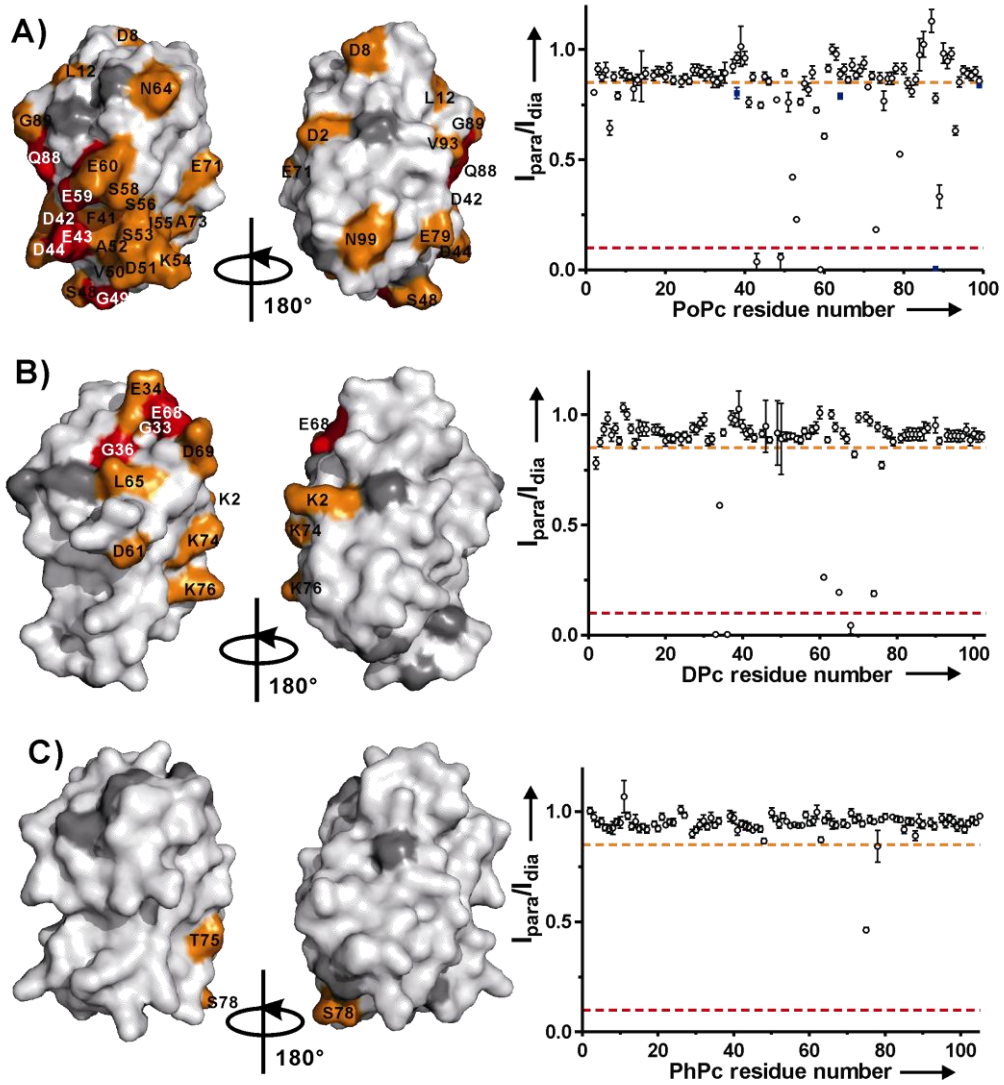

Figure S4: PRE effects in Pc- X-Lys_4 complexes. Left: PRE maps of PoPc (A, PDB entry 1TKW[1]), DPc (B, PDB entry 1KDI[2]) and PhPc (C, PDB entry 2Q5B) bound to X-Lys_4 peptide, color-coded on surface models of Pc: red, $I_{para}/I_{dia} < 0.1$; orange, $0.1 \leq I_{para}/I_{dia} < 0.85$; white, $I_{para}/I_{dia} \geq 0.85$; grey, prolines, unassigned, and overlapping resonances. Right: relative [^{1}H,^{15}N]-HSQC intensities of amides PoPc (A), DPc(B) and PhPc(C) in the complex with TOAC-containing peptides. For PoPc, the side chains are also included (blue squares). The dashed horizontal lines indicate I_{para}/I_{dia} =

0.85 (orange lines) and 0.1 (red lines). The error bars denote 2× standard deviations, derived from spectral noise levels using standard error propagation procedures.

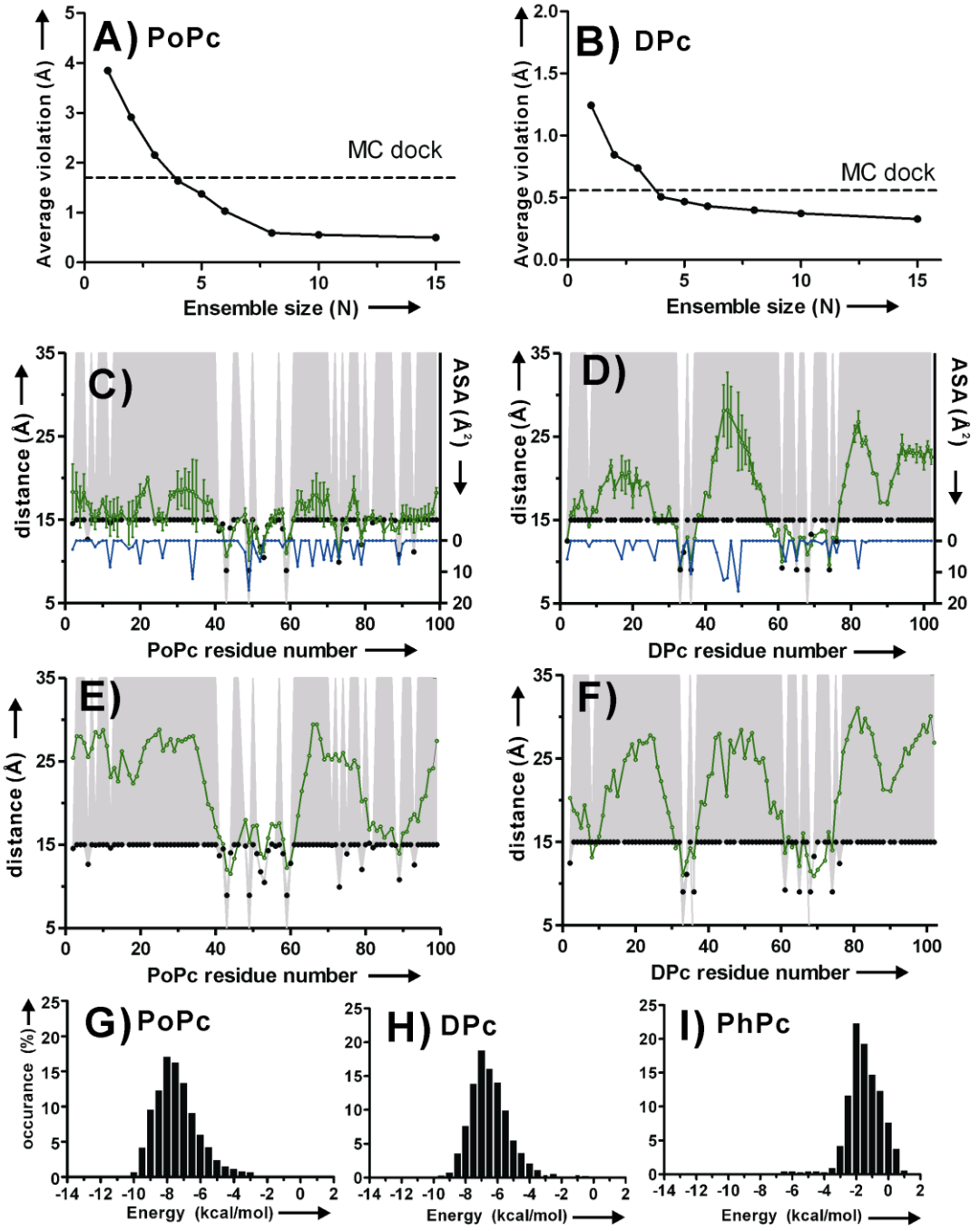

Figure S5: (A-B) Averaged distance violations against number of X-Lys_4 peptides (N=1-6,8,10,15) in the ensemble docking for PoPc (A) and DPc (B). (C-D) Correlation of experimental distances (black dots) and back-calculated average distances (green circles with connecting lines) from the ensemble docking (N=6) of

X-Lys_4 bound to PoPc (C) and DPc (D). The average distances from the 20 lowest-energy solutions of the PRE driven ensemble docking are shown as black circles connected by black lines with error bars representing the standard deviation. Right y axes show the accessible surface area (ASA) of each amide. Grey areas indicate the error margins of the experimental distances. (E-F) Comparison of experimental distances (black dots) and back-calculated average distances (green dots with connecting lines) between Pc amides and the 2000 ensembles of peptide paramagnetic oxygen atoms from MC simulations for PoPc (E) and DPc (F). Grey areas indicate the error margins of the experimental distances. (G-I) Histograms showing the energy distribution of 2000 ensembles from MC simulations: (G) PoPc- X-Lys_4, (H) DPc- X-Lys_4, (I) PhPc- X-Lys_4.

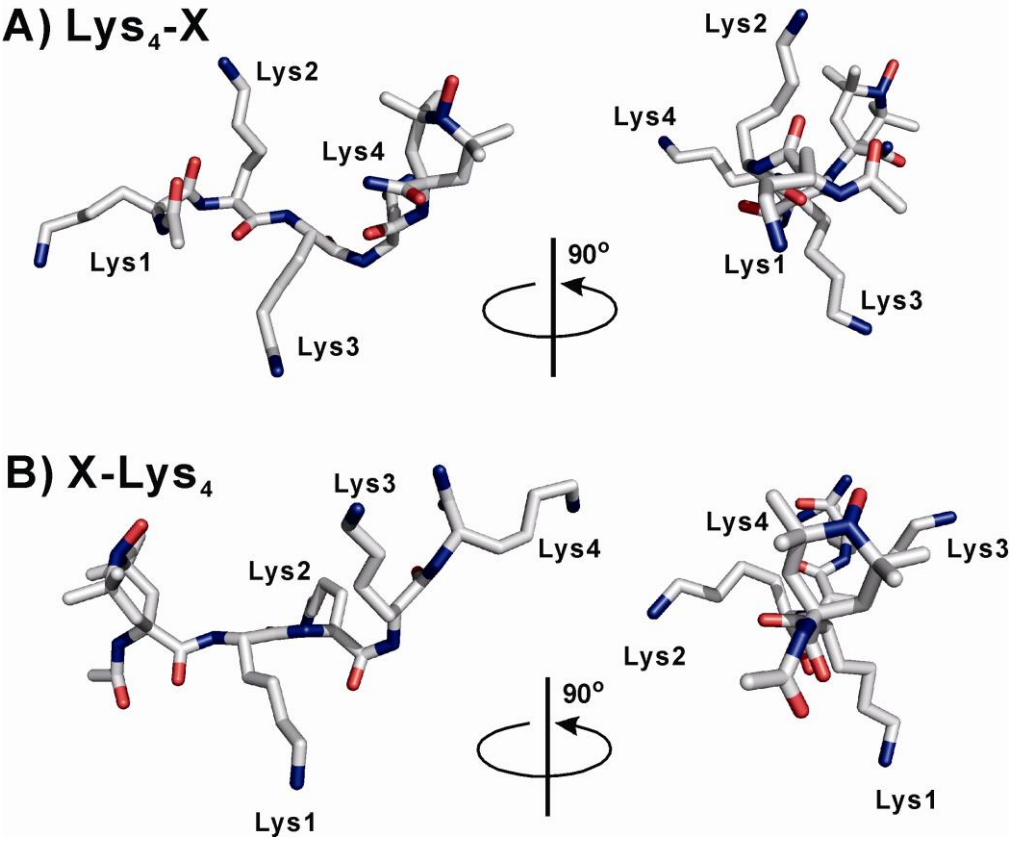

Figure S6: Modeled structures of tetralysine peptides with TOAC (X). (A) Lys_4-X; (B) X-Lys_4. The conformations were optimized in Swiss PDB-Viewer to separate the charges as far as possible. The position of each Lys residue is indicated as Lys1-Lys4.

References

[1] C. Lange, T. Cornvik, I. Díaz-Moreno, M. Ubbink, *Biochim. Biophys. Acta* **2005**, *1707*, 179–188.

[2] T. Kohzuma, T. Inoue, F. Yoshizaki, Y. Sasakawa, K. Onodera, S. Nagatomo, T. Kitagawa, S. Uzawa, Y. Isobe, Y. Sugimura, et al., *J. Biol. Chem.* **1999**, *274*, 11817−11823.