Supporting Information

Thermodynamic and structural basis of phosphorylation-induced disorder-to-order transition in the regulatory light chain of smooth muscle myosin

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MD simulations

Peptides were placed in a box of TIP3 water with a margin of 20 Å between the peptide and the boundaries of the box. Counterions were added to neutralize the overall charge and to produce 150 mM ionic strength. The systems were minimized to remove unfavorable interactions, warmed up to a target temperature of 310 K and equilibrated for 40 ns. A nonbonded cutoff of 8 Å, periodic boundary conditions and an integration step of 2 fs were used. Long-range electrostatics were computed using the particle mesh Ewald method.

Energetic analysis

The molecular mechanics energy was evaluated every 20 picoseconds using NAMD[1] using a 8 Å cutoff and periodic boundary conditions. The solvation free energy was evaluated as the sum of the polar and non-polar components. The polar contribution to the solvation free energy was calculated with the Adaptive Poisson-Boltzman Solver.[2] The polar contribution was defined as the difference between the solvated system (solute dielectric of 1.0, solvent dielectric of 78.4 and ionic strength of 150mM) and the reference system (solute and solvent dielectric of 1.0, 0 M salt concentration). Harmonic smoothing was used to define the protein boundary. The non-polar contribution to the solvation free energy was approximated with the solvent accessible surface area (SASA), $\Delta G_{solv}^{np} = \gamma(\text{SASA}) + \beta$, where γ=0.0054kcal/(mol Å^2) and β=0.92 kcal/mol.[3] SASA was calculated using VMD.[4]

For the calculation of the relative free energy we used the end-point free energy model, where the initial (unphosphorylated) and final (phosphorylated) states are sampled around their configurational equilibrium. The equilibrium was monitored using the root-mean square deviation as a quantitative measurement of conformational stability. Such conformational stability was observed in the last 40 ns of simulation.

Configurational entropy

The configurational entropy was estimated using the quasi-harmonic approximation. This method yields an upper-bound approximation, S_{ho}, to the real configurational entropy, S, of a molecule based on the covariance matrix calculated from a MD-generated trajectory. Thus, for a N-atom system, the entropy can be approximated by:

$$S \approx S_{ho} = k \sum_{i}^{3N-6} \frac{\hbar\omega_i / kT}{e^{\hbar\omega_i / kT} - 1} - \ln\left(1 - e^{\hbar\omega_i / kT}\right)$$

The mass-weighted covariance matrix is diagonalized to obtain the eigenvalues λ_i (i=1,2,…,3N), from which the quasiharmonic frequencies $\omega_i = \sqrt{(kT)/\lambda_i}$ are obtained.[5]

The calculation of the all-atom covariance matrix of the MD trajectories and the estimation of the quasiharmonic entropy was performed with the program CARMA.[6] In order to ensure that the configurational sampling was adequate, we carried out MD simulations long enough (140 ns) that the entropy values converged; i.e., did not change significantly during the last 40 ns of the simulation. A 1-ns step was used for the calculation of the covariance matrix. The calculated entropy was observed to converge in both unphosphorylated and phosphorylated peptides. The entropic contribution to the free energy of ordering was estimated separately for backbone and sidechain atoms by

$$T\Delta S = T(S_{phosphorylated} - S_{unphosphorylated}).$$

Free Energy of Ordering

The free energy of ordering, ΔG_{ord}, was calculated by

$$\Delta G_{ord} = G_{phosphorylated} - G_{unphosphorylated} = \Delta\langle E_{MM}\rangle + \Delta\langle G_{solvation}\rangle - T\Delta S$$

References

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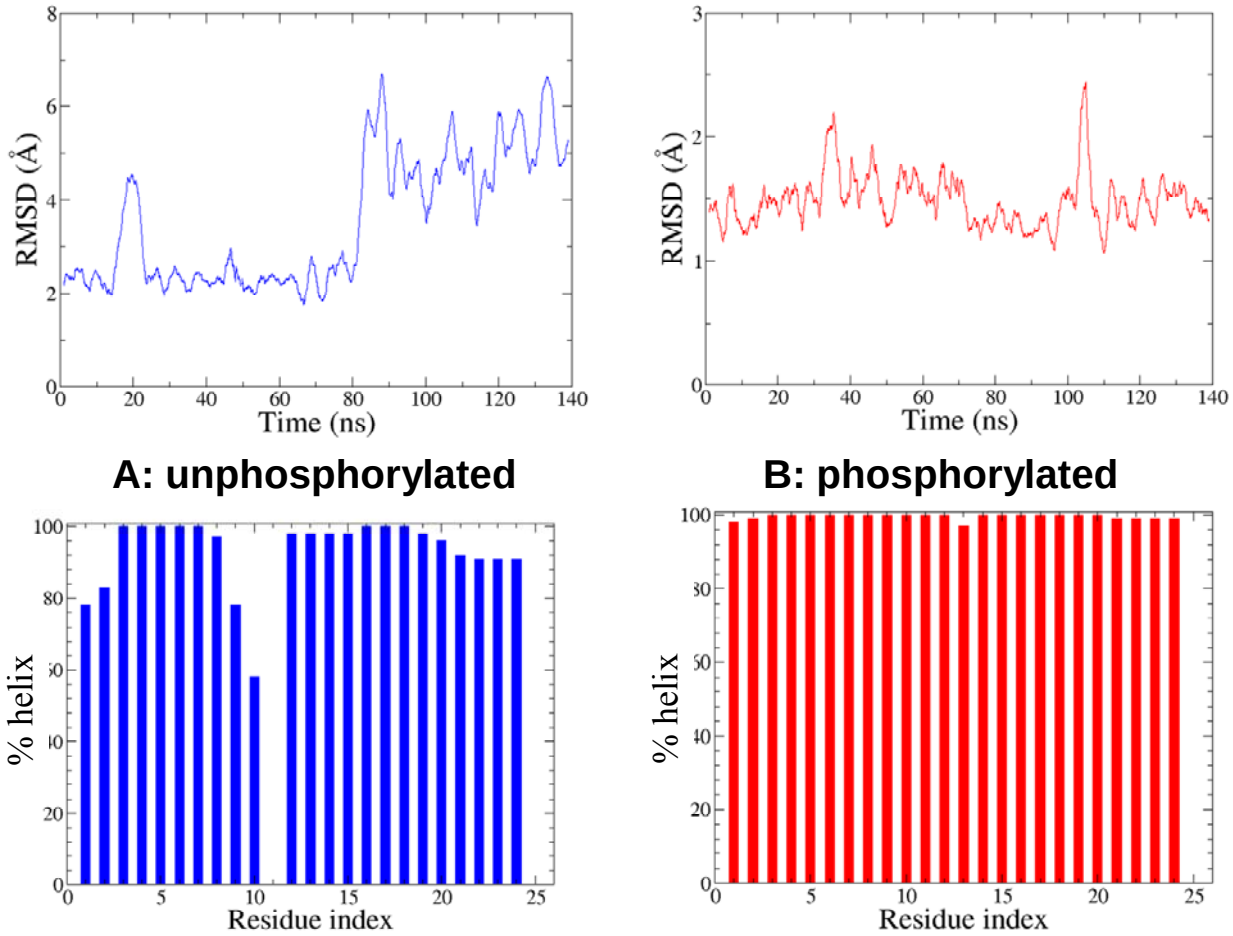

Figure S1. RMSD trajectory (top) and % of time each residue spends in α-helix (bottom) for unphosphorylated (A) and phosphorylated (B) peptide (B)..

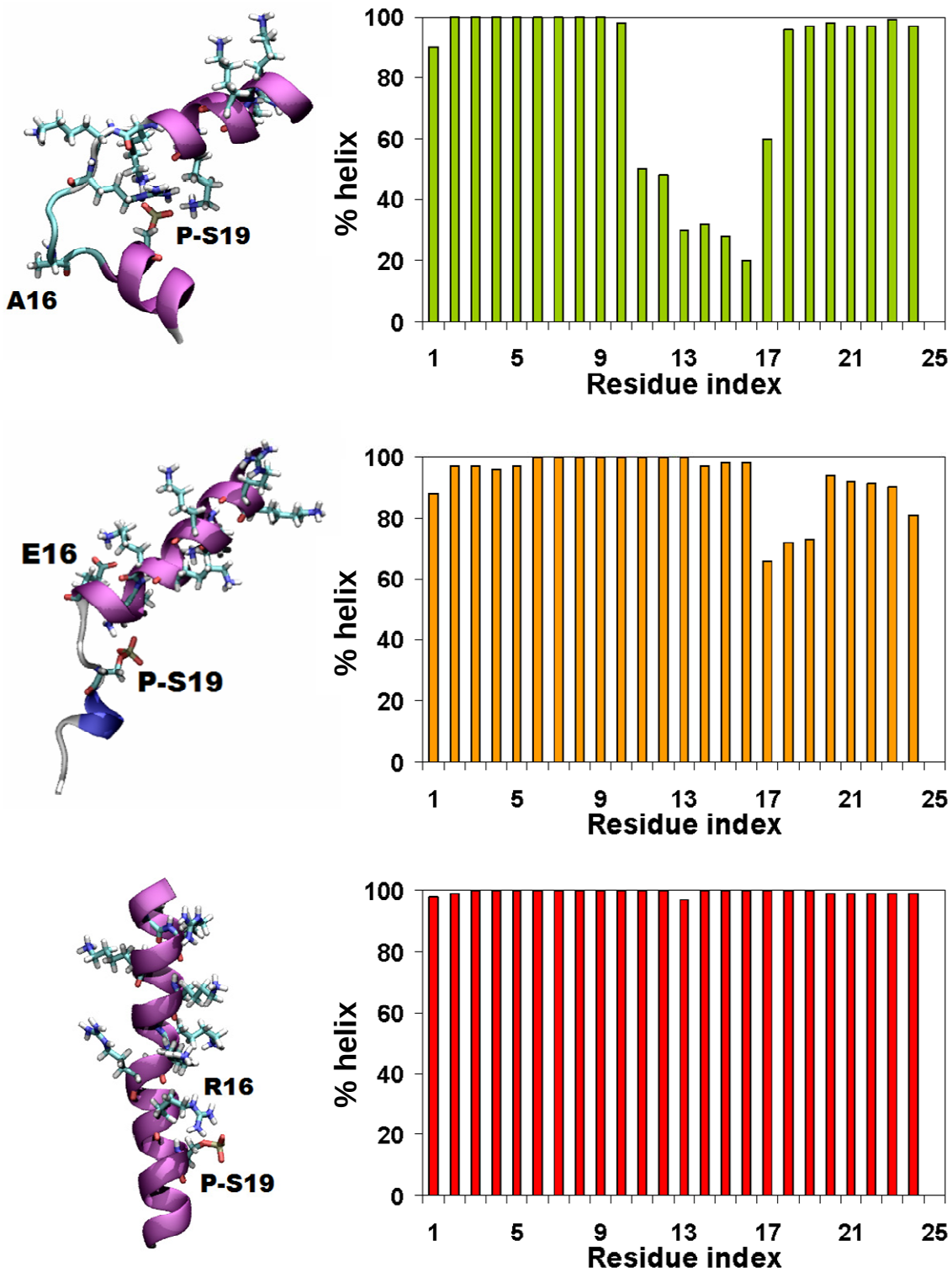

Figure S2. Structures obtained at the end of the MD simulations, and helical content of phosphorylated mutants R16A (top) and R16E (center). Phosphorylated wild type is shown for comparison (bottom). The structures are colored according to their secondary structure index (purple: α-helix; blue: 3_{10}-helix; cyan: turn; white: random coil).