Supporting Information

How Electrostatic Coupling Enables

Conformational Plasticity in a Tyrosine Kinase

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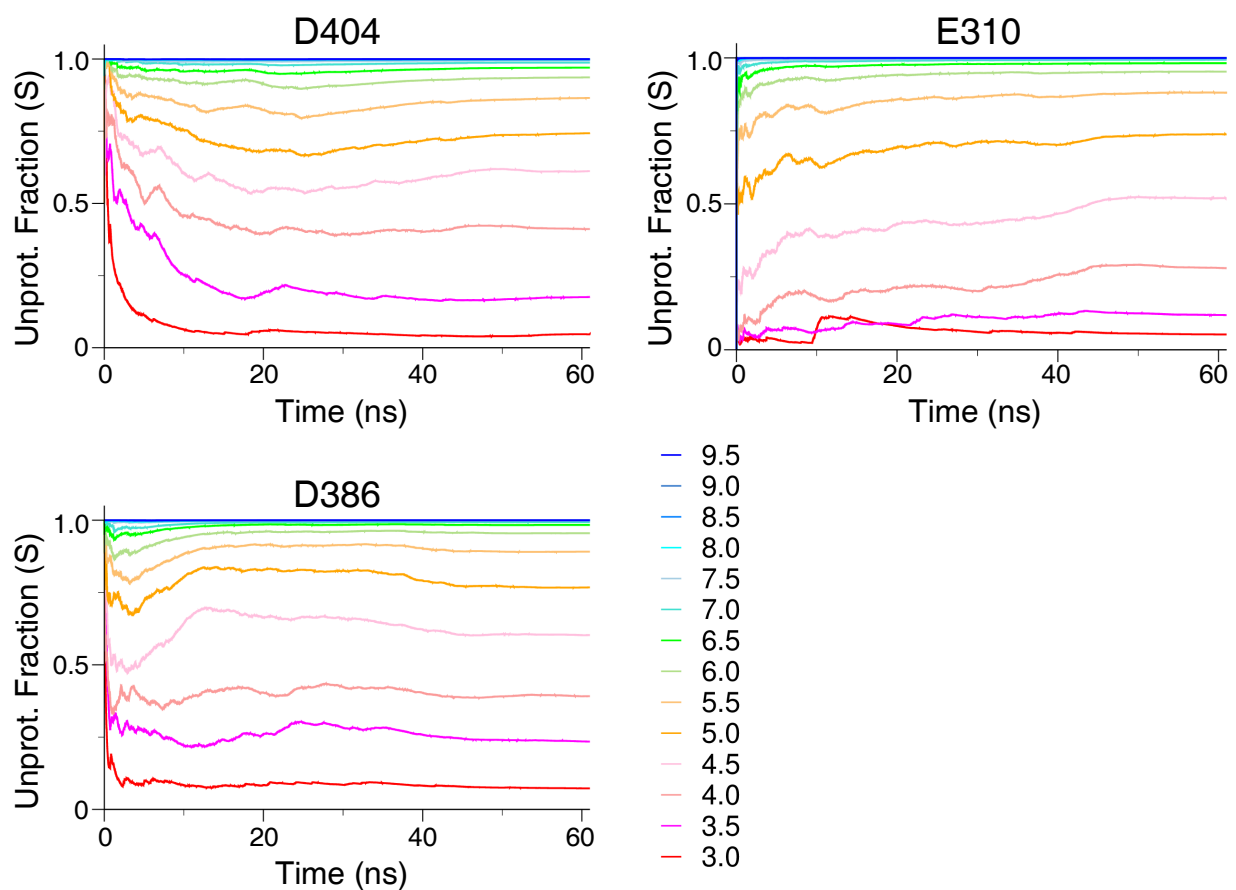
Supplemental Figures

Figure S1: **Convergence of protonation-state sampling of important residues.** The cumulatively calculated fraction of deprotonated Asp404 (top left), Glu310 (top right), and Asp386 (bottom) at different pH conditions.
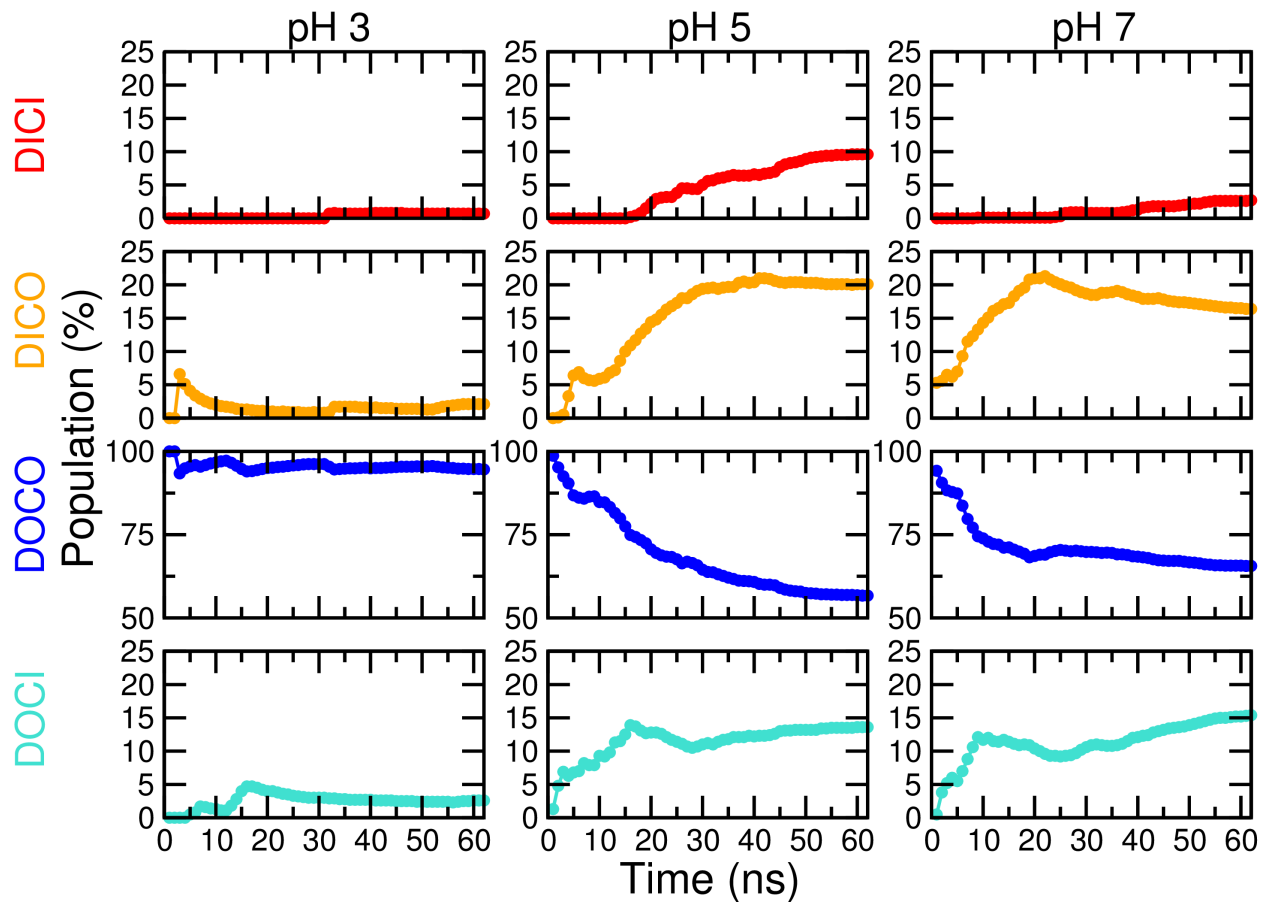
Figure S2: **Convergence of the conformational populations.** The populations of the DOCO, DOCI, DICO, and DICI states at pH 3, 5, and 7 were calculated cumulatively as a function of simulation time for all replicas.
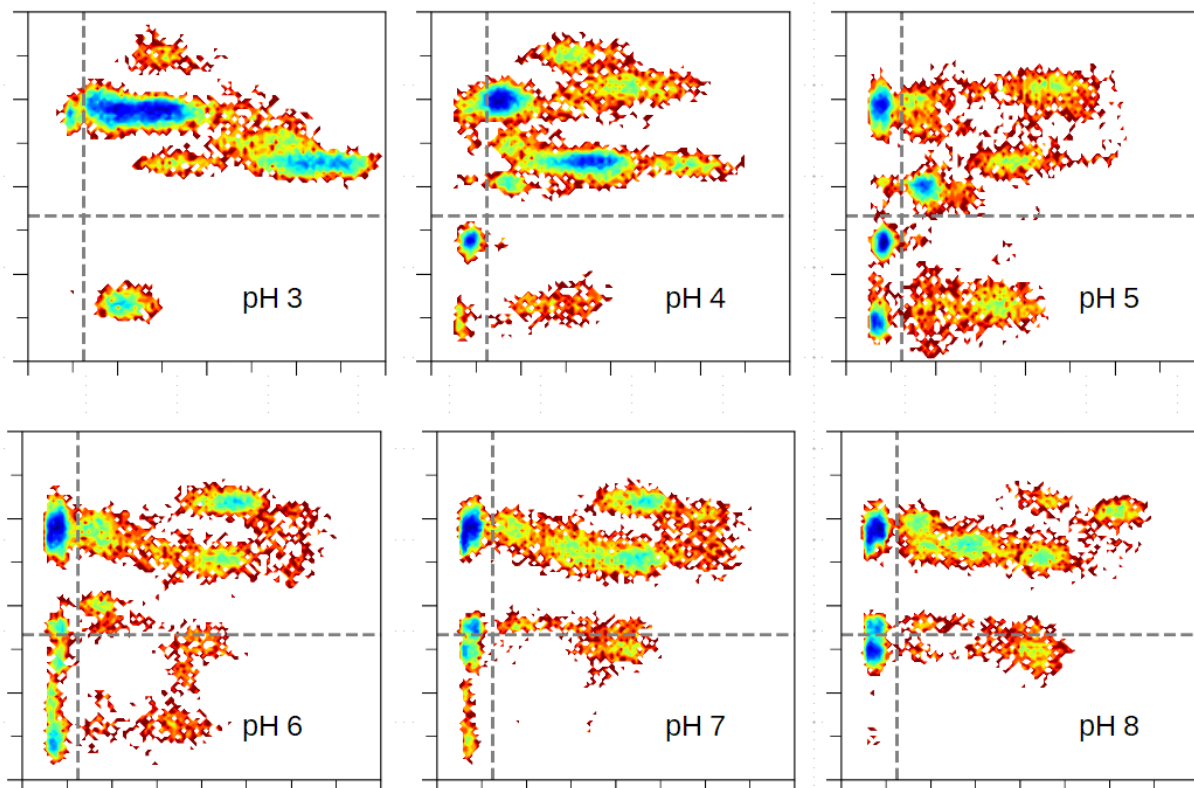
Figure S3: **Convergence of the free energy surfaces.** The free energy surfaces as a function of the pseudo torsion angle ζ(Ala403) (as y axis) and distance R between Lys295:NZ and Glu310:CD (as x axis) at different pH. These plots used the data from the last 15 ns per replica to compare with the plots shown in Fig. 2 of the main text, which used the last 20 ns per replica.
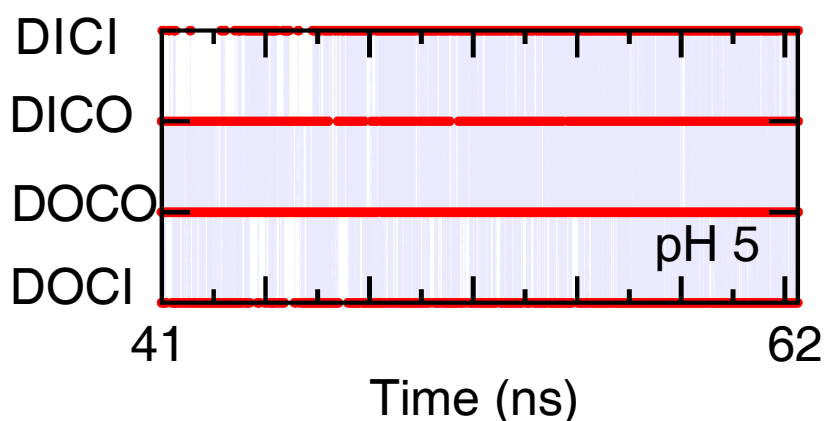

Figure S4: **Conformational transitions between four major conformational states at pH 5.** The conformational state (DICI, DICO, DODO, or DOCI) visited as a function of the simulation time (only the last 20 ns shown) from the pH 5 simulation. Red data points indicate the states visited and light blue lines indicate the transitions between states.
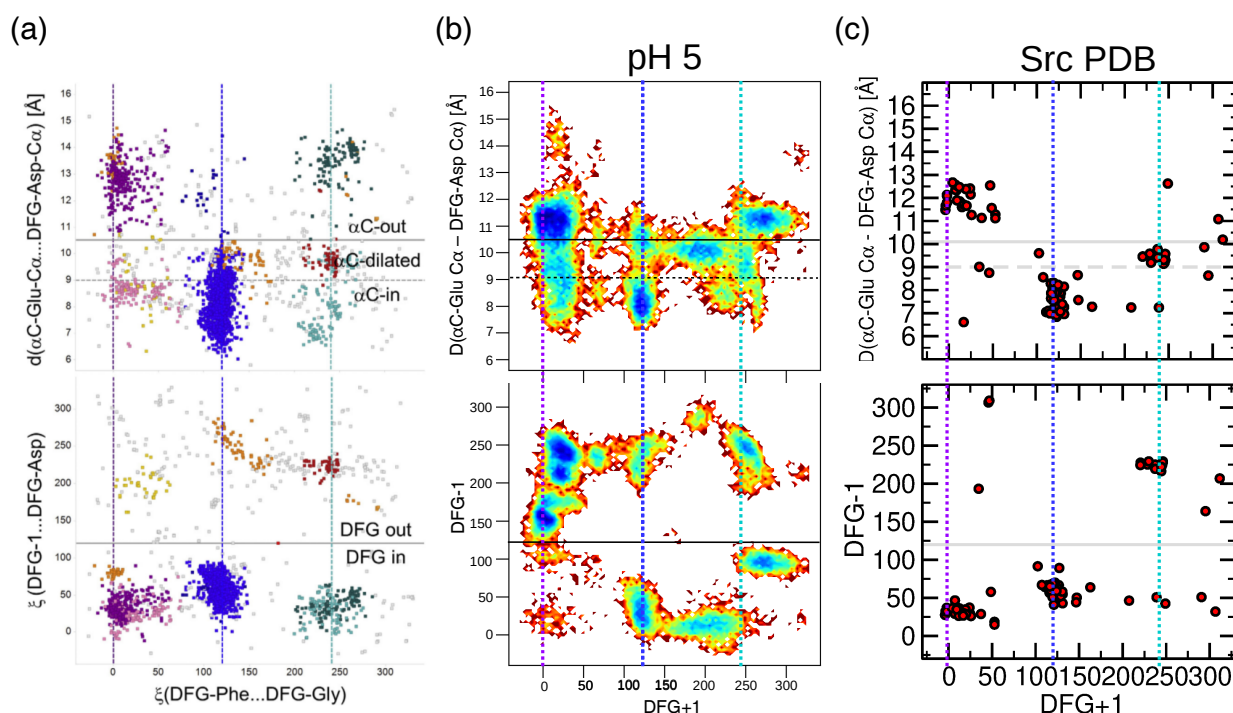
Figure S5: **The conformational landscape of the c-Src kinase calculated from our simulation is similar to the conformational landscapes of kinases from the crystal structures. (a)** The free energy surface (FES) as a function of the pseudo torsion angles ζ(DFG-Phe. . . DFG-Gly) and ζ(DFG-1. . . DFG-Asp) is shown on the bottom; the FES as a function of ζ(DFG. . . DFG-Gly) and the distance between CA atoms of αC-Glu and DFG-Asp is shown on the top. ζ(DFG-Phe. . . DFG-Gly) is defined by the dihedral angle formed by the CA atoms of four consecutive residues, DFG-Asp, DFG-Phe, DFG-Gly, and DFG+1. ζ(DFG-1. . . DFG-Asp) is defined by the dihedral angle formed by the CA atoms of DFG-2, DFG-1, DFG-Asp, and DFG-Phe. This figure is adapted from an article by Möbitz on the basis of all kinase crystal structures.[1] **(b)** The FES plots of the c-Src kinase calculated from our simulation data at pH 5. The same axes are used as in the Möbitz plots **(c)** The scatter plot made on the basis of all the Src kinase crystal structures from the protein data bank (PDB). According to Möbitz,[1] the horizontal line in the bottom FES plot classifies the DFG position as DFG-out (upper) and DFG-in (lower) states, while the two horizontal lines in the top FES plot classifies the αC position as αC-out (upper), αC-dilated (middle), and αC-in (lower). In the main text, we discussed that the αC-Glu–DFG-Asp distance is not a reliable criterion to discriminate αC-out vs. αC-in position. For example, we found that some structures with αC-Glu–DFG-Asp distances of 12 Å can still contain a salt bridge between the catalytic Lys and αC-Glu.
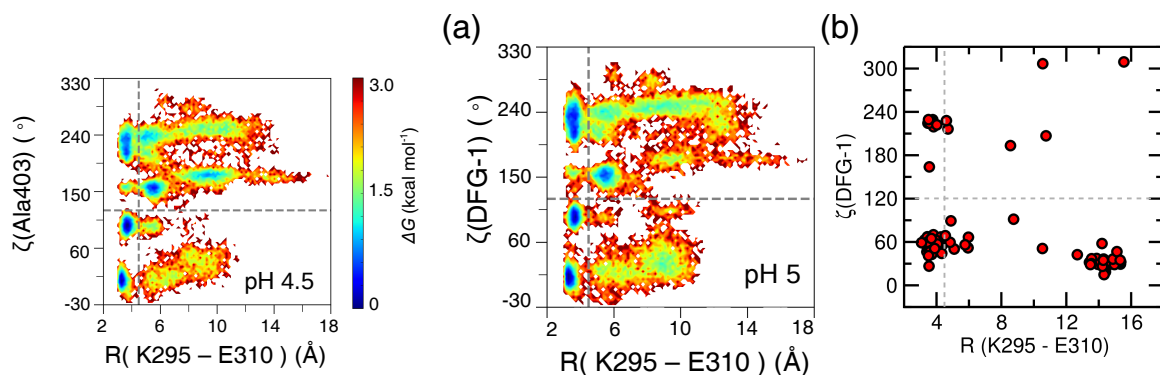
Figure S6: **Comparison between the conformational states of c-Src found in the simulation and those revealed by the crystal structures of all c-Src proteins. Left.** FES as a function of the pseudo torsional angle ζ(DFG-1) and the K-E distance R from the simulation at pH 4.5. **(a)** FES from the simulation at pH 5. ζ and R are defined in the main text. **(b)** Scatter plot using the crystal structures of all Src kinases.
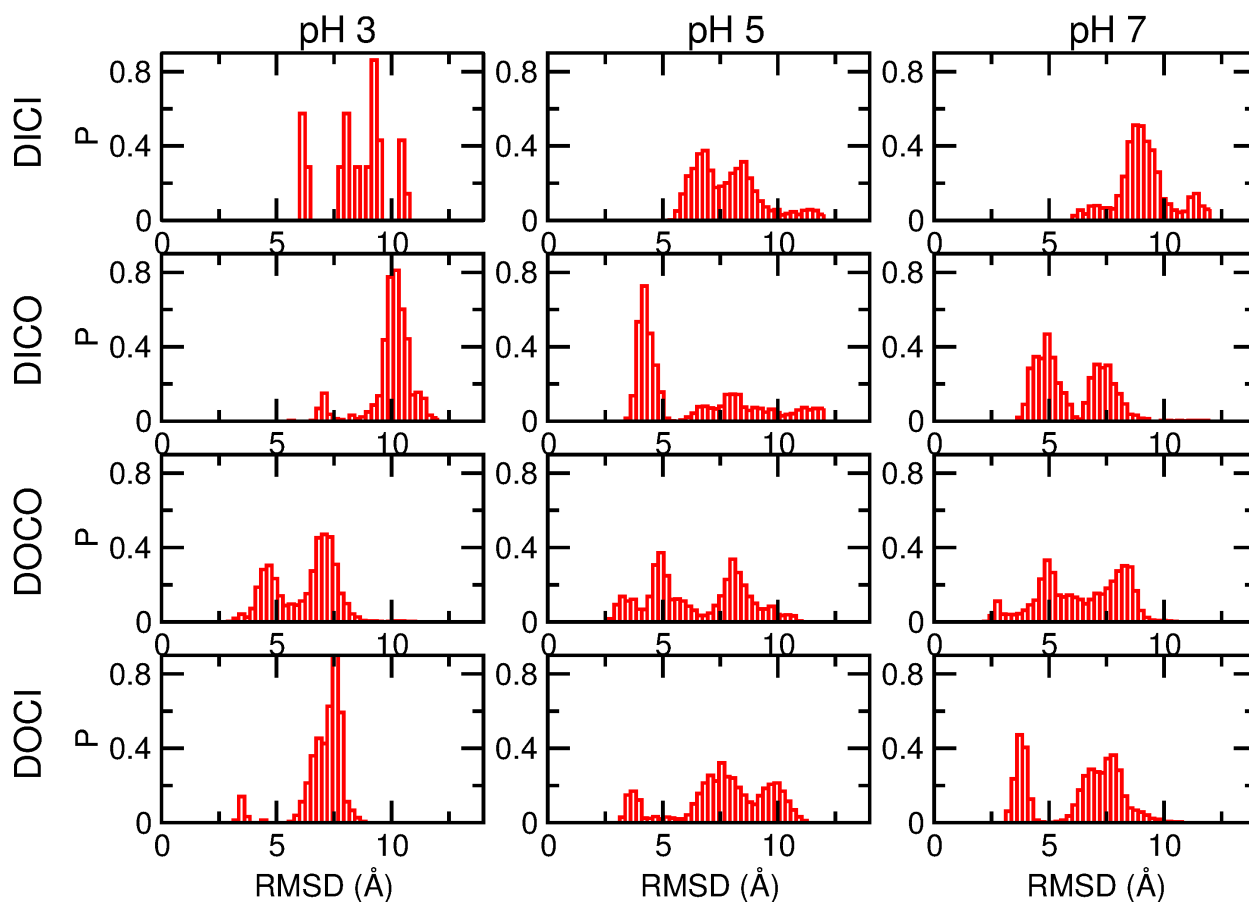

Figure S7: **Only the active DICI state exclusively samples the open A-loop conformation.** Heavy-atom RMSD of the A-loop in all four major conformational states at three pH conditions.
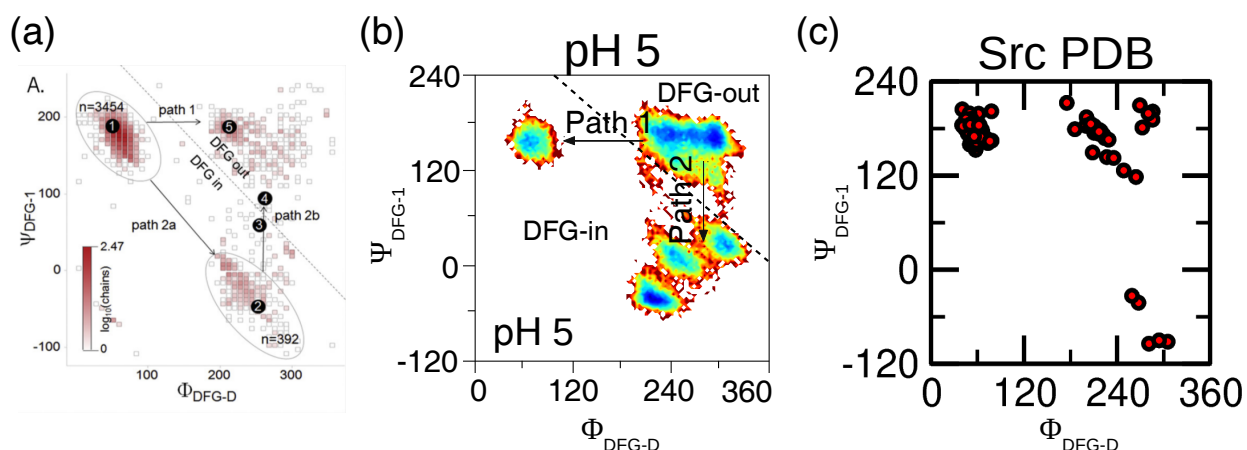
Figure S8: **The pathways of DFG-flip found by our simulation is similar to those found in the crystal structures. (a)** Scatter plot of the backbone Φ angle of DFG-Asp vs. the backbone Ψ angle of DFG-1 using all kinase crystal structures (taken from the paper by Möbitz[1]). **(b)** FES of the c-Src kinase calculated from our simulation at pH 5. **(c)** Scatter plot of Φ(DFG-Asp) vs. Ψ(DFG-1) for all Src kinases using the PDB crystal structures.
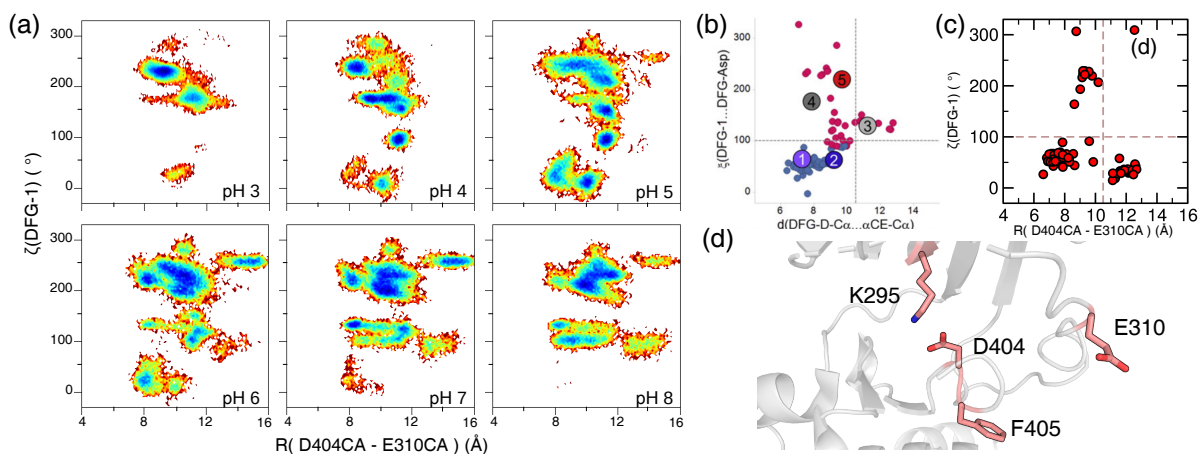

Figure S9: **Process of the DFG-flip agrees with crystal structures. (a)** FES of c-Src as a function of ζ(DFG-1) and the distance R between DFG-Asp and αC-Glu. **(b)** Scatter plot of ζ(DFG-1) vs. R(D-E) using selected kinase crystal structures (adapted from the article by [1]). **(c)** Scatter plot of ζ(DFG-1) vs. R(D-E) using all Src kinase crystal structures. **(d)** Zoomed-in view of the structure (pdb id 2QI8, chain B) with ζ of 300° and $R>$ 12Å. In this structure, DFG-Asp is rotated anti-clockwise and forms a salt bridge with the catalytic lysine.
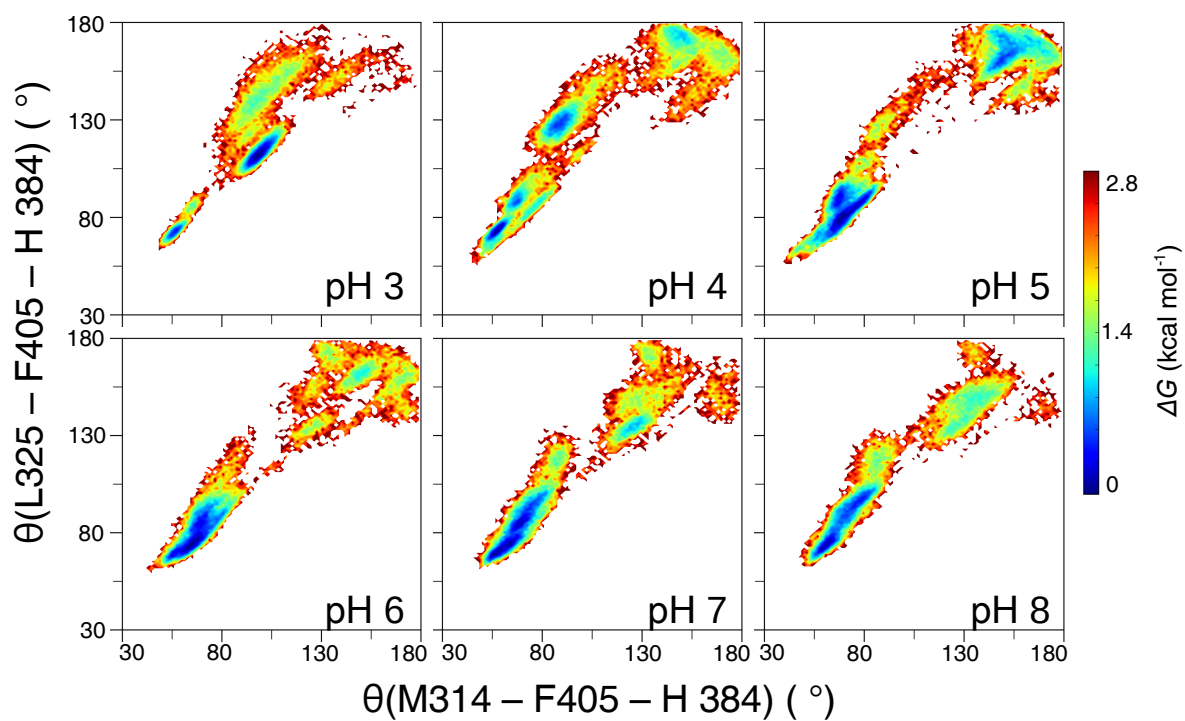
Figure S10: **R-spine formation is pH dependent.** FES of c-Src as a function of the angle between the CB atoms of Met314, Phe405, and His384 and the angle between the CB atoms of Leu325, Phe405, and His384 at different pH.

References

(1) Möbitz, H. The ABC of protein kinase conformations. *Biochim. Biophys. Acta* **2015**, *1854*, 1555–1566.