

Biophysical Journal, Volume 96

Supporting Material

Atomistic Insights into Regulatory Mechanisms of the HER2 Tyrosine Kinase Domain: A Molecular Dynamics Study

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Supplementary Information

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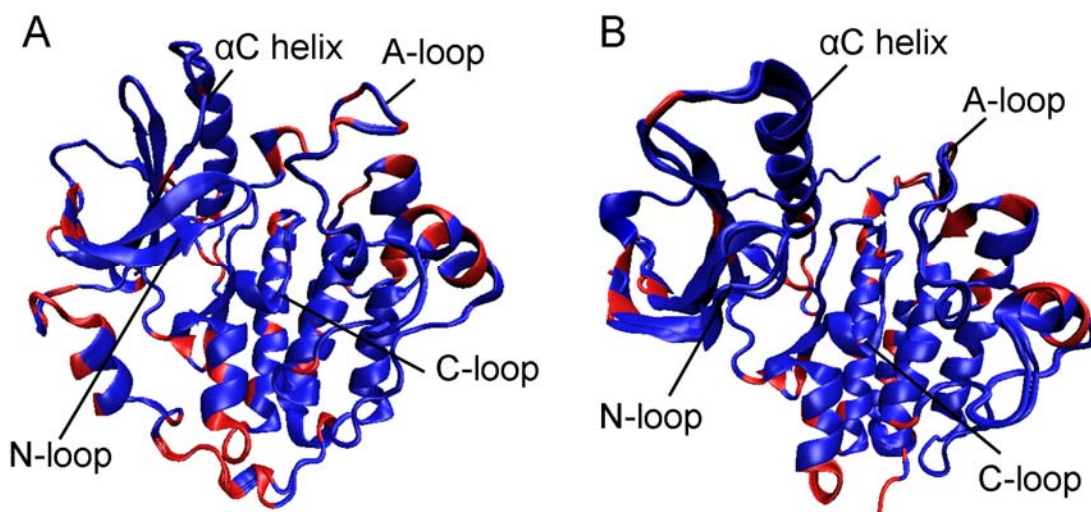


Figure S1. Alignment between EGFR and homology-modeled HER2 in (A) the inactive conformation and (B) the active conformation. The structures are colored according to sequence similarity, where blue regions indicate residue identity and red regions represent residue dissimilarity.

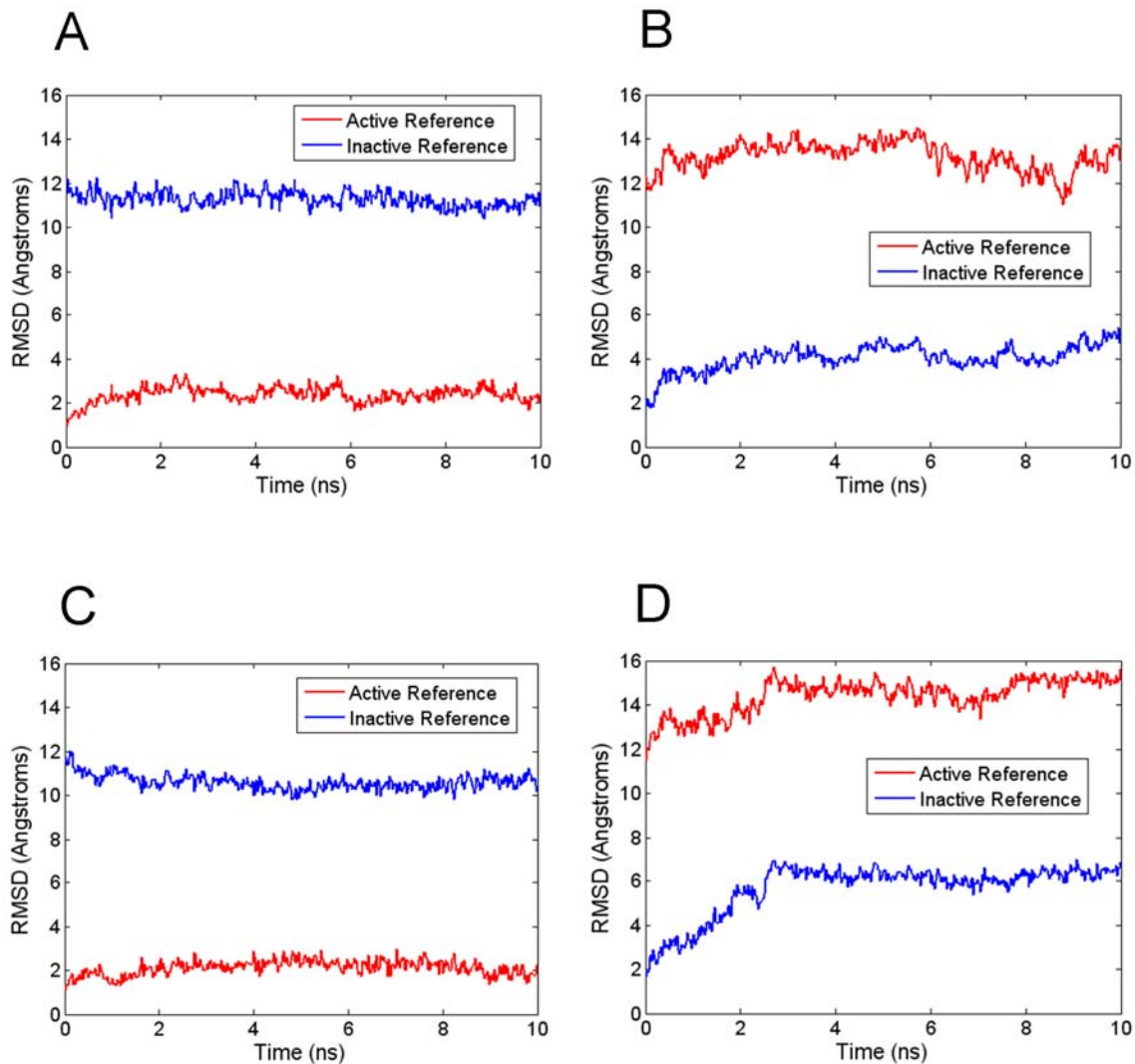


Figure S2. Time course plots of the RMSD for all backbone atoms in the A-loop of (A) the Y877-unphosphorylated active and (B) the Y877-unphosphorylated inactive trajectories, and (C) the Y877-phosphorylated active and (D) the Y877-phosphorylated inactive trajectories. The RMSD is plotted in reference to the initial active structure (*red*) and in reference to the initial inactive structure (*blue*).

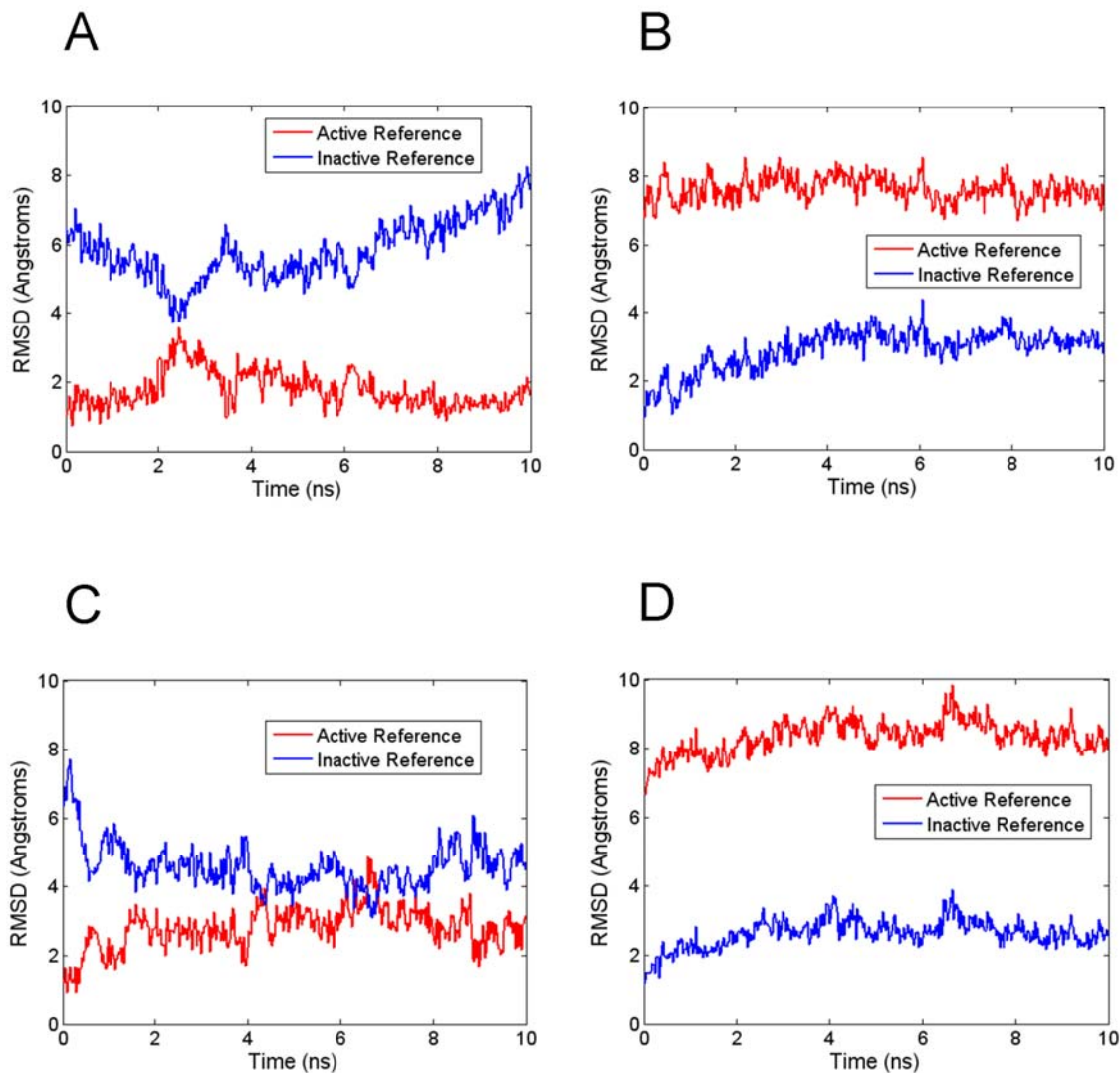


Figure S3. Time course plots of the RMSD for all backbone atoms in the α C helix of (A) the Y877-unphosphorylated active and (B) the Y877-unphosphorylated inactive trajectories, and (C) the Y877-phosphorylated active and (D) the Y877-phosphorylated inactive trajectories. The RMSD is plotted in reference to the initial active structure (*red*) and in reference to the initial inactive structure (*blue*).

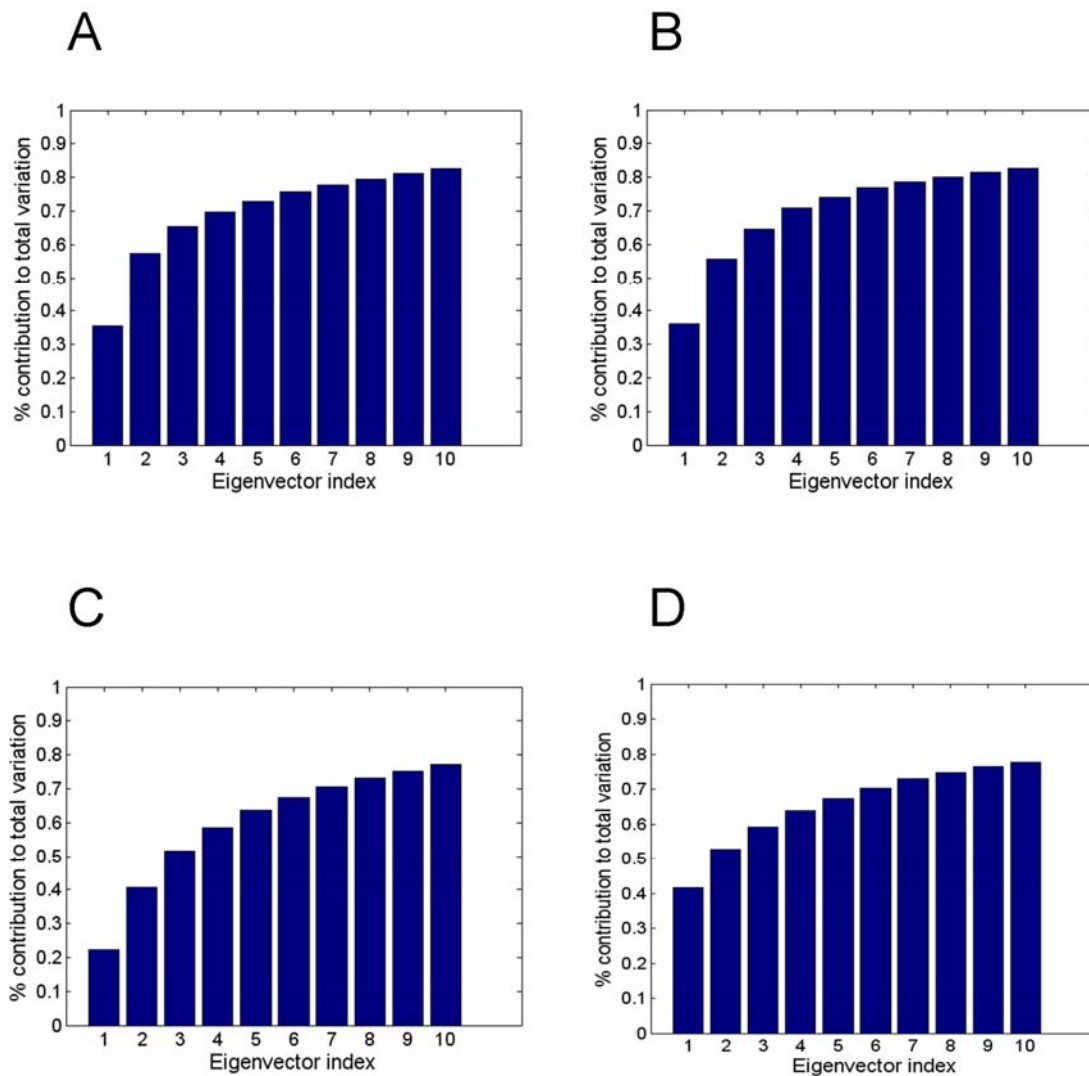


Figure S4. Cumulative percentage contribution of the top ten eigenvectors to the global motion of the protein in (A) the unphosphorylated active and (B) the unphosphorylated inactive systems, and (C) the phosphorylated active and (D) the phosphorylated inactive systems. The PCA was performed on an active site region that included the A-loop, C-loop, N-loop, and α C helix.

Movies S1-S4. Movie showing the motion along the first eigenmode for (Movie S1) the Y877-unphosphorylated active HER2 structure, (Movie S2) the Y877-unphosphorylated inactive HER2 structure, (Movie S3) the Y877-phosphorylated active HER2 structure, and (Movie S4) the Y877-phosphorylated inactive HER2 structure. The A-loop is in *blue*, α C helix in *orange*, C-loop in *purple*, and N-loop in *yellow*.

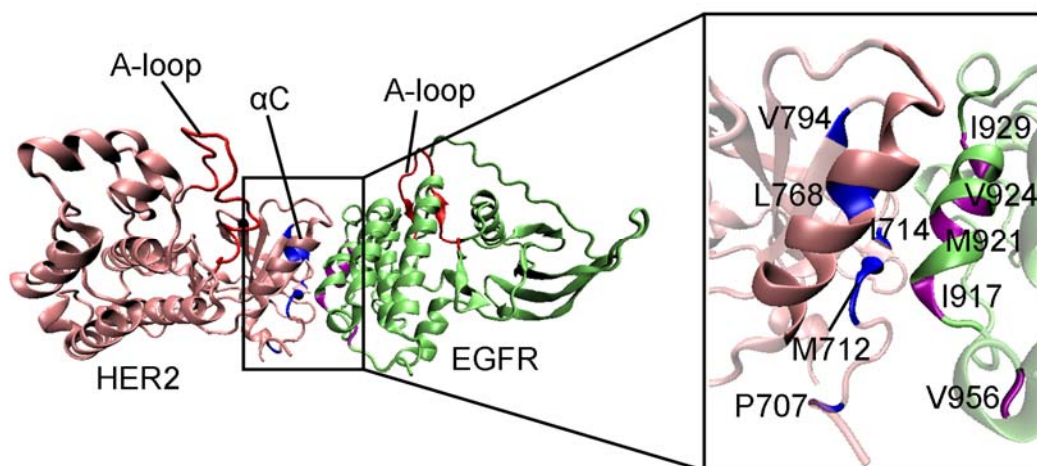


Figure S5. Snapshot of the modeled HER2-EGFR heterodimer, highlighting the residues comprising the dimeric interface for the kinase undergoing activation (HER2, *pink*) and for the activator kinase (EGFR, *green*). The residues that constitute the interface for HER2 are P707, Q711, M712, I714, L768, L790, and V794 (highlighted in *blue* in the inset). For EGFR, the interface residues are I917, M921, V924, I929, and V956 (highlighted in *purple* in the inset).

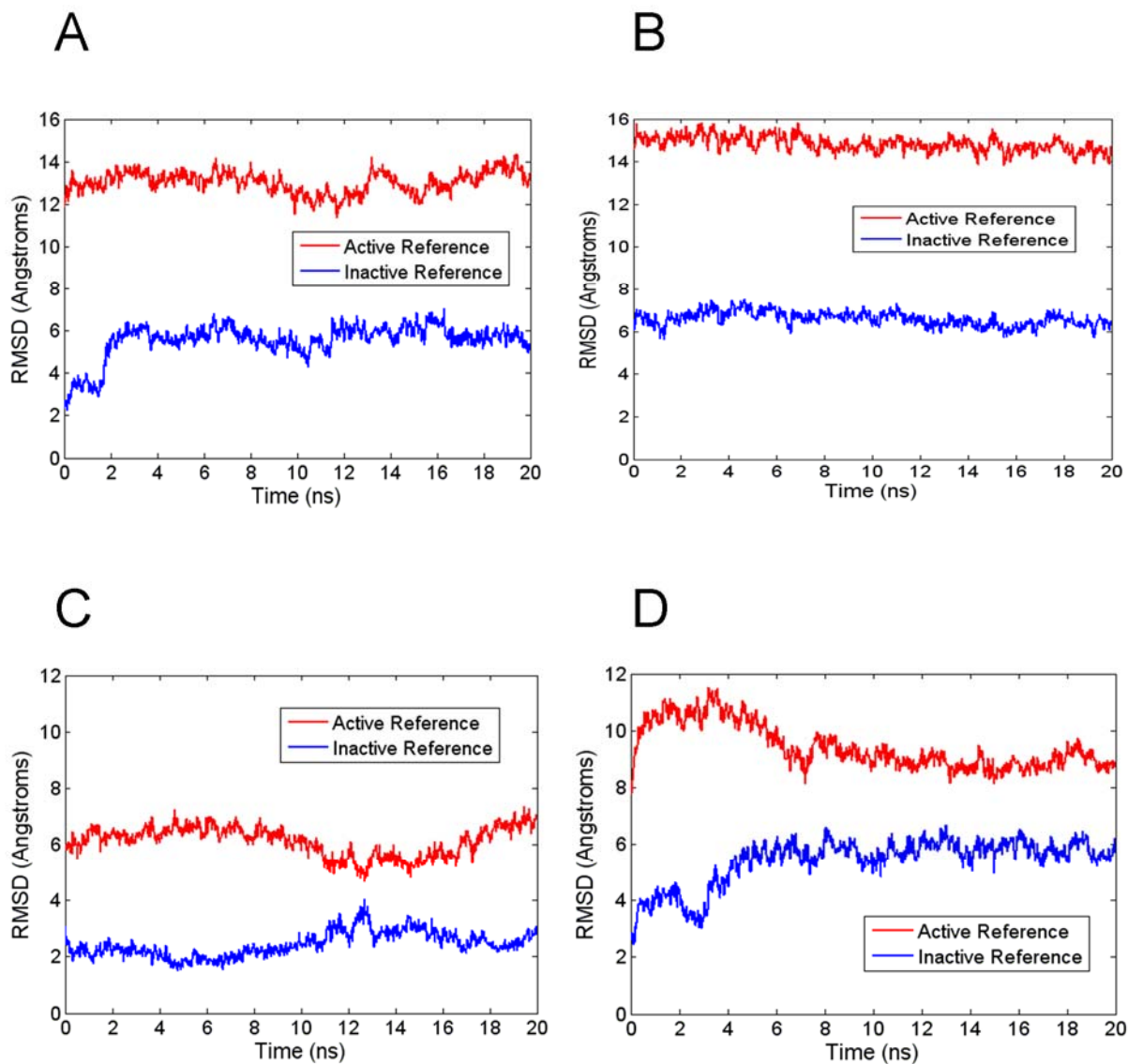


Figure S6. Time course plots of the RMSD for all backbone atoms in (A) the HER2 A-loop in the Y877-unphosphorylated dimer, (B) the HER2 A-loop in the Y877-phosphorylated dimer, (C) the HER2 α C helix in the Y877-unphosphorylated dimer, and (D) the HER2 α C helix in the Y877-phosphorylated dimer. The RMSD is plotted in reference to the active HER2 structure (*red*) and in reference to the inactive HER2 structure (*blue*).

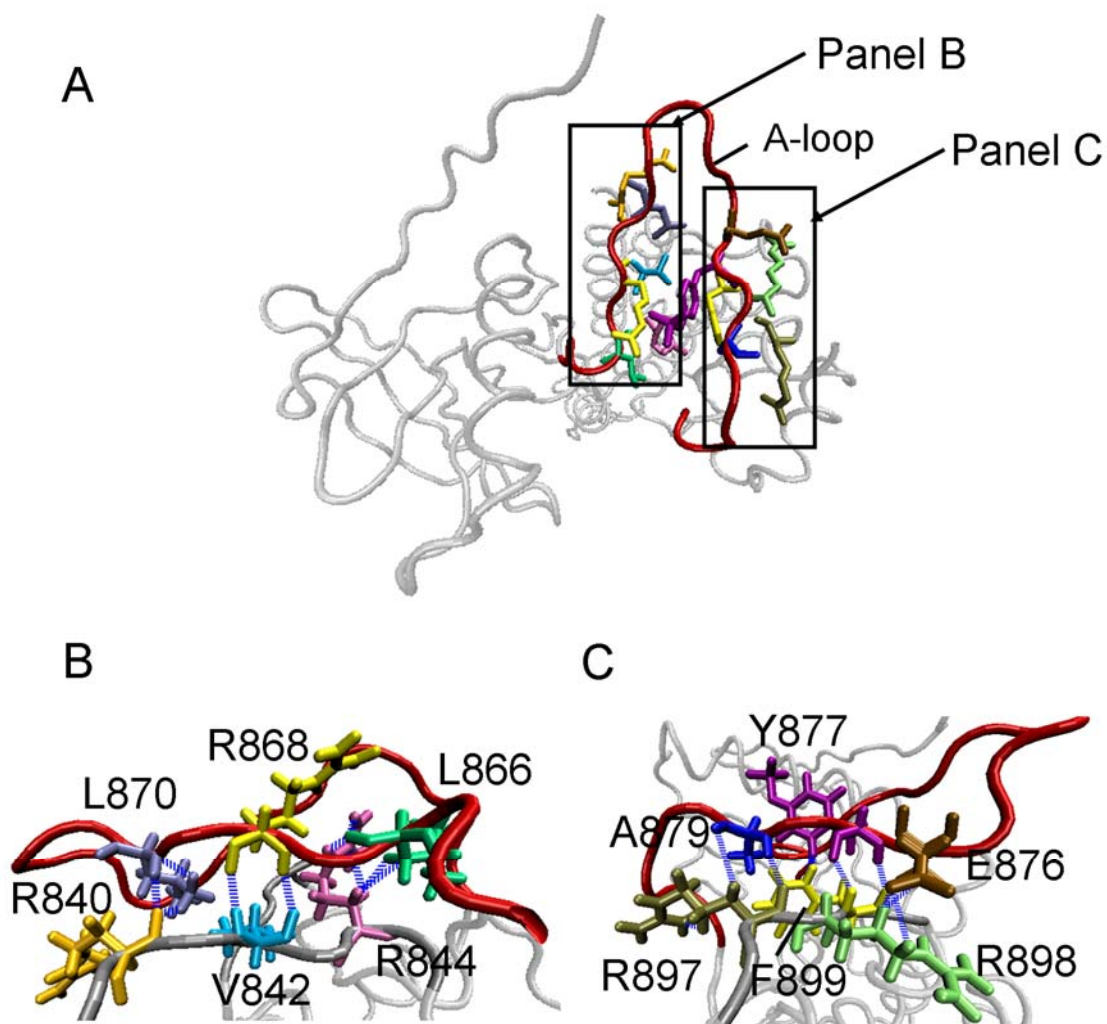


Figure S7. (A) The network of stabilizing hydrogen bonds in the A-loop of the Y877-phosphorylated active system. The structures highlight the hydrogen bonds present at (B) the N-terminal end of the A-loop and (C) the C-terminal end of the A-loop. The bonds maintain the A-loop in the active state while ensuring availability of D863 and D845 for catalysis. Hydrogen bonds are depicted as *blue dashed lines*.

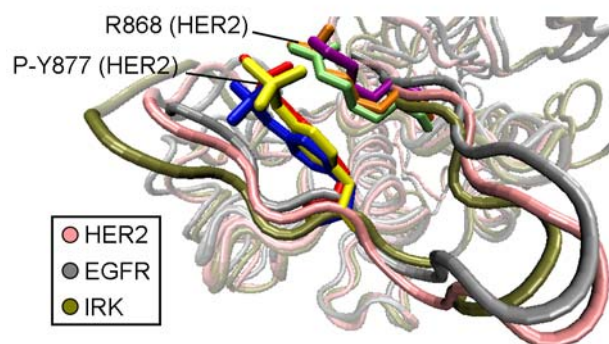


Figure S8. Alignment of the A-loop of the phosphorylated active HER2, EGFR, and IRK structures. The snapshot depicts the conserved role of the phosphorylated tyrosine residue in stabilization of the active state. The tyrosine residue is colored *yellow* (HER2), *red* (EGFR) and *blue* (IRK), while the conserved bonding residue is colored *orange* (HER2), *purple* (EGFR) and *green* (IRK).

Table S1. Comparison of the Hydrogen Bonding Network in the α C helix of HER2, EGFR, and ErbB4 Kinase in the Active and Inactive Conformations.

HER2 Active	EGFR Active	ErbB4 Active	HER2 Inactive	EGFR Inactive	ErbB4 Inactive
A763, S760*	–	–	–	–	–
N764, S760	N732, S728	N737, G733	N764, S760	–	–
E766, R756	–	–	–	–	–
E766, K883[†]	E734, K851	E739, K856	–	–	–
–	–	–	–	–	E739, R841
D769, R868	D737, K836	–	–	–	D742, R841
E770, K753	E738, K721	E743, K726	–	–	–
–	E738, F832	E743, F837	–	–	–
–	–	–	–	E738, K836	E743, R841
–	–	–	–	–	E743, R817
–	Y740, S744	L745, S749	Y772, G776	Y740, S744	L745, S749
V773, V777	V741, V745	I746, M750	–	–	–
–	–	–	M774, L785	M742, L753	M747, L758
–	A743, L679	A748, Q684	–	–	–
–	–	–	–	–	A748, R757

*Only residues, not atom names, are shown for clarity. [†]Salt bridges are highlighted in bold.

Table S2. Comparison of the Hydrogen Bonding Network in the C-loop of HER2, EGFR, and ErbB4 Kinase in the Active and Inactive Conformations.

HER2 Active	EGFR Active	ErbB4 Active	HER2 Inactive	EGFR Inactive	ErbB4 Inactive
–	–	–	V842, G865*	–	–
V842, R868	–	–	–	–	–
R844, L866	R812, L834	R817, L839	–	–	–
–	R812, E848	–	–	–	–
–	–	R817, K856	–	–	–
L846, W888	–	–	–	–	–
	A816, L775	A821, L780	A848, L807	A816, L775	A821, L780
N850, T862	N818, T830	N823, T835	–	N818, T830	N823, T835
V851, L806	–	–	V851, L806	–	–
L852, K860	–	–	L852, K860	–	–

* Bonds coupling the C-loop and A-loop are highlighted in bold.

Table S3. Comparison of the Hydrogen Bonding Network in the A-loop of HER2, EGFR, and ErbB4 Kinase in the Active and Inactive Conformations.

HER2 Active	EGFR Active	ErbB4 Active	HER2 Inactive	EGFR Inactive	ErbB4 Inactive
–	–	D836, K726*	–	–	–
–	–	D836, T835	–	–	–
–	–	–	D863, K753[†]	–	D836, K726
–	F832, E738	F837, E743	–	–	–
–	–	–	–	–	G838, R817
–	–	–	G865, V842	–	–
L866, R844	L834, R812	L839, R817	–	–	–
–	–	–	–	L834, D813	–
–	–	–	–	–	R841, E739
R868, D769	K836, D737	–	–	–	R841, D742
–	–	–	–	K836, E738	R841, E743
R868, V842	K836, V810	R841, V815	–	–	–
L870, R840	L838, R808	L843, R813	–	–	–
D871, R840	–	–	–	–	–
–	A840, G672	–	–	–	–
–	–	–	D873, R897	–	–
–	K843, D932	–	–	–	–
–	–	K848, T873	–	–	–
–	–	K848, D937	–	–	–
E876, R898	–	E849, K871	–	–	–
–	Y845, Y867	Y850, F872	–	–	–
–	–	–	–	H846, R865	–
–	–	A852, R870	–	–	–
–	E848, R812	–	–	–	–
D880, R897	–	D853, R870	D880, R897	E848, R865	D853, R870
–	–	–	K883, E757	–	K856, E730
K883, E766	K851, E734	K856, E739	–	–	–
–	–	–	–	K851, R812	–

*Only residues, not atom names, are shown for clarity. [†]Salt bridges are highlighted in bold.

Table S4. Comparison of the Hydrogen Bonding Network in the A-loop and α C helix of HER2 Kinase in the Y877-Unphosphorylated and Y877-Phosphorylated Conformations.

Inactive HER2 Y877-Unphosphorylated	Active HER2 Y877-Unphosphorylated	Inactive HER2 Y877-Phosphorylated	Active HER2 Y877-Phosphorylated
	A763, S760*		A763, S760
N764, S760	N764, S760		N764, S760
–	E766,R756	–	E766, R756 [†]
–	E766, K883	–	–
–	D769, R868	–	–
–	E770, K753	–	E770, K753
–	–	–	E770, F864
Y772, G776		Y772, G776	
–	V773 O, V777 HN	–	V773, V777
M774, L785	–	M774, L785	–
D863, K753	–	D863, K753	–
–	–	–	F864, E770
G865, V842	–	–	–
–		G865, H843	–
–	L866, R844	–	L866, R844
–	R868, D769	–	–
–		R868, R840	–
–	R868, V842	–	R868, V842
–	L870, R840	–	L870, R840
–	D871, R840	–	–
D873, R897	–	–	–
–	–	E874, T759	–
–	E876, R898	–	E876, R898
–	–	Y877, R844	Y877, R844
–	–	Y877, K883	Y877, K883
–	–	Y877, R897	–
–	–	–	Y877, R868
–	–	–	Y877, F899
–	–	–	A879, R897
D880, R897	D880, R897	–	–
K883, E757	–	–	–
–	K883, E766	–	–
–	–	V884, K887	–

*Only residues, not atom names, are shown for clarity. [†]Salt bridges are highlighted in bold.

Table S5. Comparison of the Hydrogen Bonding Network in the A-loop and α C helix of Y877-Phosphorylated HER2 and Y845-Phosphorylated EGFR Kinase in the Inactive and Active Conformations.

Inactive HER2 Y877-Phosphorylated	Inactive EGFR Y845-Phosphorylated	Active HER2 Y877-Phosphorylated	Active EGFR Y845-Phosphorylated
–	–	–	K730, E848*
–	–	A763, S760	–
–	N732, S728	N764, S760	N732, S728
–	N732, A726	–	–
–	–	–	N732, V762
–	–	–	E734, K836
–	–	E766, R756[†]	–
–	–	–	E734, K851
–	–	E770, F864	–
–	–	E770, K753	E738, K721
–	E738, K836	–	–
Y772, G776	Y740, S744	–	Y740, S744
–	V741, V745	V773, V777	V741, V745
M774, L785	–	–	–
–	A743, R752	–	–
D863, K753	–	–	D831, K721
–	D831, K721	–	–
–	D831, N818	–	–
–	F832, E738	F864, E770	–
G865, H843	–	–	–
–	–	L866, R844	L834, R812
–	L834, D813	–	–
–	–	–	K836, E734
–	K836, E738	–	–
R868, R840	–	–	–
–	–	R868, V842	K836, V810
–	–	L870, R840	L838, R808
E874, T759	–	–	–
–	–	E876, R898	–
Y877, R844	Y845, R812	Y877, R844	Y845, R812
Y877, K883	Y845, K851	Y877, K883	Y845, K851
Y877, R897	Y845, R865	–	–
–	–	Y877, R868	Y845, K836
–	–	Y877, F899	Y845, Y867
–	–	A879, R897	A847, R865
–	–	–	E848, K730
–	E848, R865	–	–
–	–	–	K851, E734
–	–	–	K851, E734
V884, K887	–	–	–

*Only residues, not atom names, are shown for clarity. [†]Salt bridges are highlighted in bold.