Supporting information

Distinct Structural mechanisms determine substrate affinity and kinase activity of Protein kinase Cα

Sangbae Lee[1,#], Titu Devamani[2,#], Hyun Deok Song[1,#], Manbir Sandhu[1], Adrien Larsen[1], Ruth Sommese[2], Abhinandan Jain[3], Nagarajan Vaidehi[1,*] and Sivaraj Sivaramakrishnan[2,*]

[1] Department of Molecular Immunology, Beckman Research Institute of the City of Hope, 1500, E. Duarte Road, Duarte, CA-91010.

[2] Department of Genetics, Cell Biology, and Development, 4-130 MCB, 420 Washington Avenue SE, University of Minnesota, Twin Cities Minneapolis, MN 55455.

[3] Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Dr. Pasadena, CA-91109.

[#] contributed equally to this work.

GNEIMO-REMD Annealing Simulations.

Integration of GNEIMO with Rosetta: The use of GNEIMO simulations, and that of physical forcefields have been shown to enhance conformational sampling and protein structure refinement[1-3]. The Rosetta software suite has many functionalities that have been shown to perform well consistently, for protein structure refinement and side chain repacking[4]. We have previously combined the *GneimoSim* software[5] with the Rosetta software to (1) perform torsion MD simulations with Rosetta forcefield[6] and to (2) enhance the conformational sampling during protein structure refinement by combining the benefits torsional MD in GNEIMO with the torsional Monte Carlo sampling and side chain repacking in Rosetta using a rotamer library. The details of the software integration can be found in reference number 5.

Protocol used for performing the GNEIMO-REMD-Rosetta annealing simulations: We first repack the side chain conformations in the peptide-PKCα complex built using homology modeling method. The side chains were repacked using the *PackRotamersMover* module in Rosetta. The resulting structures were minimized using lbfgs_armijo_nonmonotone minimizer in the *CartesianMinimizer* module in Rosetta[1]. The starting structures of the 14 different peptides bound PKCα have the peptide in the extended conformation except for the p-Ser/Thr making contact with the Asp466 in the kinase. To optimize the structure of the peptide binding to PKCα, we performed 2000 to 2500 annealing cycles for each peptide-PKCα pair using GNEIMO-REMD-Rosetta torsion MD simulations using the protocol described below. The Rosetta module was used for the forcefield and side chain repacking after each annealing cycle. The side chain repacking combined with GNEIMO torsional MD enhanced the conformational sampling of the peptide substrate binding to PKCα. Each annealing cycle consists of a side chain rotamer repacking of all the residues in the peptide-kinase complex using the Rosetta *PackRotamersMover* module, an all-atom minimization using the *CartesianMinimizer*, followed by the GNEIMO-REMD-Rosetta torsion MD simulation run with 12 replicas. The GNEIMO-REMD torsion angle simulations were done using the multibody dynamic model shown in Figure S2. The range of temperature used in REMD was 200 to 300 K for a total of 12 replicas with the integration step size of 1 fs. We used the Talaris 2014 with Lazaridis-Karplus implicit solvent model with a distance dependent dielectric function, a variant of the Lobatto integrator within the GNEIMO module[3], and 0.5 ps of Nose-Hoover constant (τ) at constant temperature.

The conformation ensemble generated from all the annealing cycles for each peptide-PKCα pair was clustered using RMSD-based clustering with a cut-off of 1.5 Å. We then calculated the number of hydrogen bonds and van der Waals contacts the peptide makes with PKCα for every conformation in the most populated cluster in the ensemble. We extracted the conformation with the maximum number of hydrogen bonds and van der Waals contacts to PKCα from the most populated cluster and used this as the starting structure for performing all-atom MD simulations in explicit solvent with GROMACS module as described in the Methods section.

References

1. Larsen, A. B.; Wagner. J. R.; Jain. A.; Vaidehi N.(2014). Protein Structure Refinement of CASP target Proteins using GNEIMO torsional dynamics method, *J. Chem. Inf. Model*. **24**, 508-517.
2. Gangupomu, V. K.; Wagner, J. R.; Park, I. H.; Jain. A.; Vaidehi, N. (2013). Mapping conformational dynamics of proteins using torsional dynamics simulations, *Biophys. J*., **104**, 1999-2008.
3. Park, I. H.; Wagner, J.; Jain, A.; Vaidehi, N. (2012). Structure Refinement of Protein Low Resolution Models Using the GNEIMO Constrained Dynamics Method Folding of Small Proteins Using Constrained Molecular Dynamics, *J. Phys. Chem.* B. **116**, 2365-2375.
4. Park, H.; DiMaio, F.; Baker, D.; (2016). CASP11 refinement experiments with Rosetta, *Proteins*, Suppl. **1**, 314-322.
5. Larsen, A.; B.; Wagner, J. R.; Kandel, S.; Salomon-Ferrer, R.; Vaidehi. N, Jain, A. (2014). *GneimoSim*: A modular internal coordinates molecular dynamics simulation package, *J. Comput. Chem*. **5**, 2245-2255.
6. Alford, R. F.; Leaver-Fay, A.; Jeliazkov, J. R.; O'Meara, M.J; DiMaio, F. P.; Park, H.; Shapovalov, M. V.; Renfrew, P. D.; Mulligan, V. K.; Kappel, K.; Labonte, J. W.; Pacella, M. S.; Bonneau, R.; Bradley, P.; Dunbrack, R. L.; Jr, Das, R.; Baker, D,; Kuhlman, B.; Kortemme, T.; Gray, J. J. (2017) The Rosetta All-Atom Energy Function for Macromolecular Modeling and Design. *J. Chem.Theory. Comput.* **13**, 3031-3048.

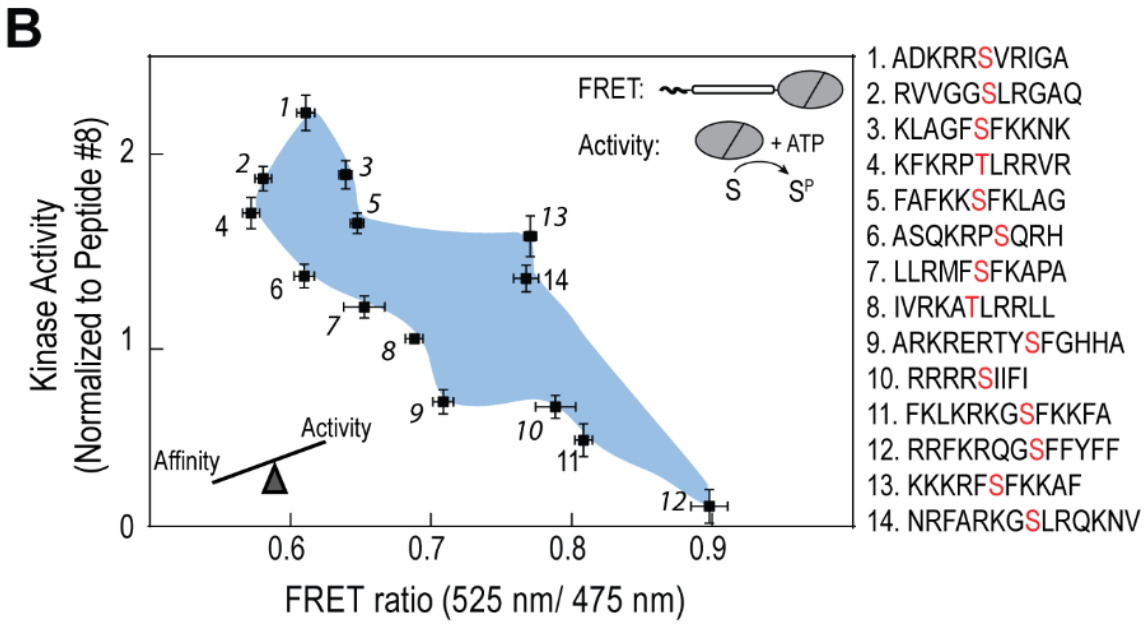

Figure S1: Measured kinase activity of PKCα for 14 different peptide substrates shown in the figure, versus the measured change in FRET intensity ratio using SPASM. This figure was taken with permission from Sommese et al J Biol Chem. 2016, 291(42):21963-21970.

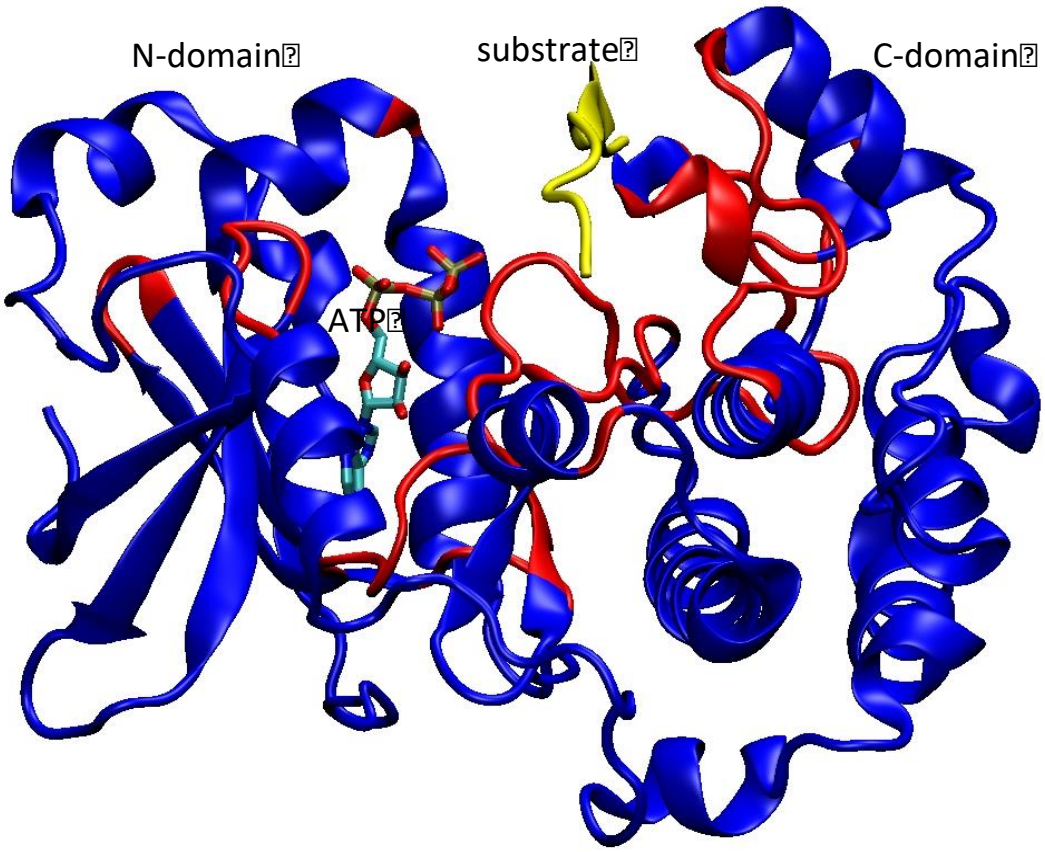

Figure S2: The structure of PKCα (cartoon representation) with the peptide substrate p4, shown in yellow, bound to it. The rigid and movable regions of the PKCα are colored in blue and red, respectively. The backbone of the secondary structures was treated as rigid bodies while the side chains of these regions were treated as flexible torsions. All the torsion angles in the red regions are treated as flexible. All the torsion angles in the peptide substrates were movable.

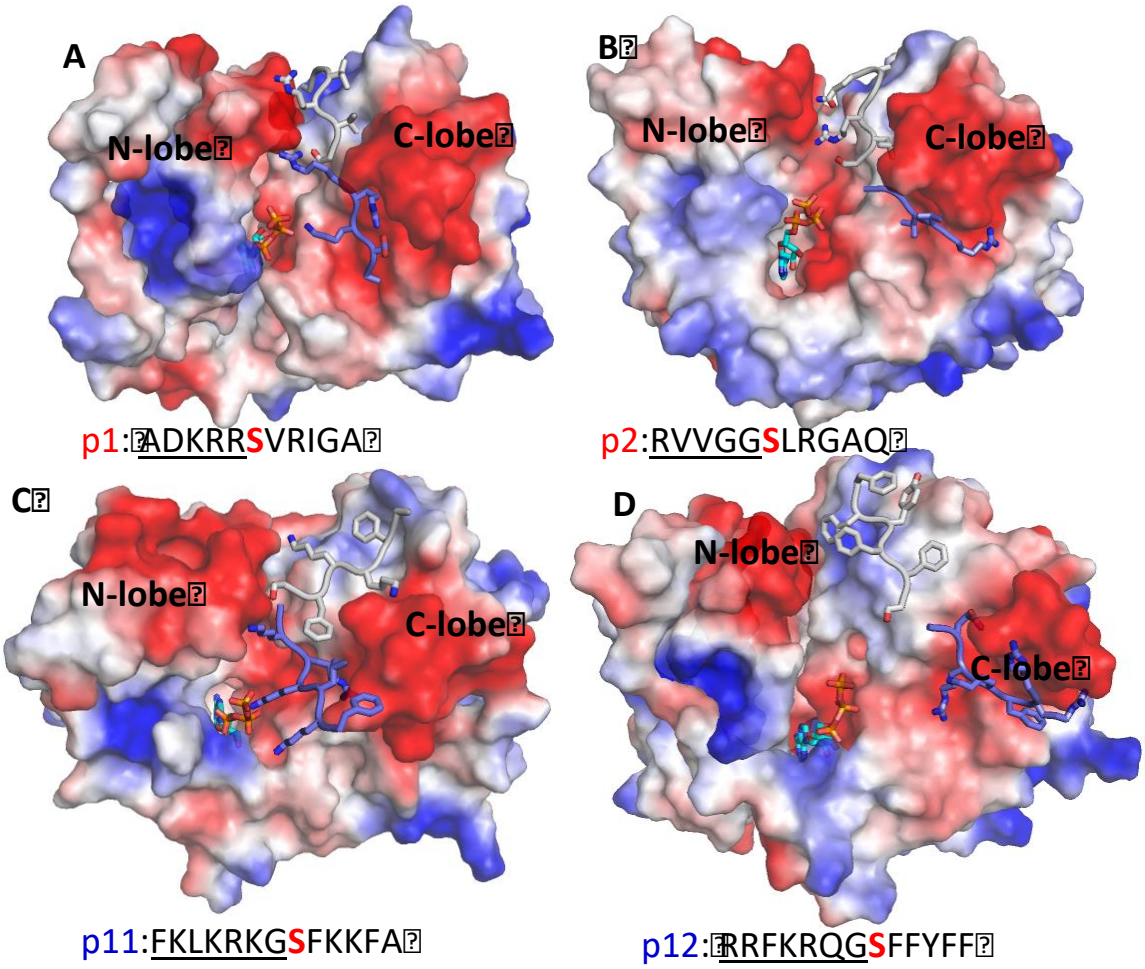

Figure S3: Electrostatic surface of the structural model of PKCα with various substrate peptides bound. The peptides p1 and p2 show high kinase activity while p11 and p12 show low kinase activity but high binding affinity (refer to Figure S1). The electrostatic surfaces were generated using Pymol. The residues N-terminus of the phosphorylated Ser/Thr are shown in blue and the ones to the C-terminus of p-Ser/Thr are shown in gray. The residues of N-terminus of the peptide underlined are embedded in a negatively charged electrostatic surface of the kinase (red).

Table S1: The amino acid sequences of the 14 peptide substrates used in this study. Position 9 contains the phosphorylated Ser or Thr.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Protein
p1				A	D	K	R	R	S	V	R	I	G	A		Kit/SCRF
p2				R	V	V	G	G	S	L	R	G	A	Q		PTP1B
p3				K	L	A	G	F	S	F	K	K	N	K		MARCKS
p4				K	F	K	R	P	T	L	R	R	V	R		Troponin
p5				F	A	F	K	K	S	F	K	L	A	G		MARCKS
p6			A	S	Q	K	R	P	S	Q	R	H				Myelin Basic protein
p7				L	L	R	M	F	T	K	A	P	A			Gaba Type A Receptor
p8				I	V	R	K	A	T	L	R	R	L	L		EGF Receptor
p9	A	R	K	R	E	R	T	Y	S	F	G	H	H	A		Optimised AKT Sub
p10					R	R	R	R	S	I	I	F	I			Optimised PKA Sub
p11		F	K	L	K	R	K	G	T	F	K	K	F	A		Optimised PKcβ Sub
p12		R	R	F	K	R	Q	G	S	F	F	Y	F	F		Optimised PKcζ Sub
p13				K	K	K	R	F	S	F	K	K	A	F		MARCKS
p14		N	R	F	A	R	K	G	T	L	R	Q	K	N	V	PKCα Pseudo Sub

Figure S4: Sequence alignment of PKA and PKC family. The residues lining the binding groove of the N-terminus of the peptide substrates are marked with arrows. These residues are either conserved or conservative replacements among the PKC isozymes.

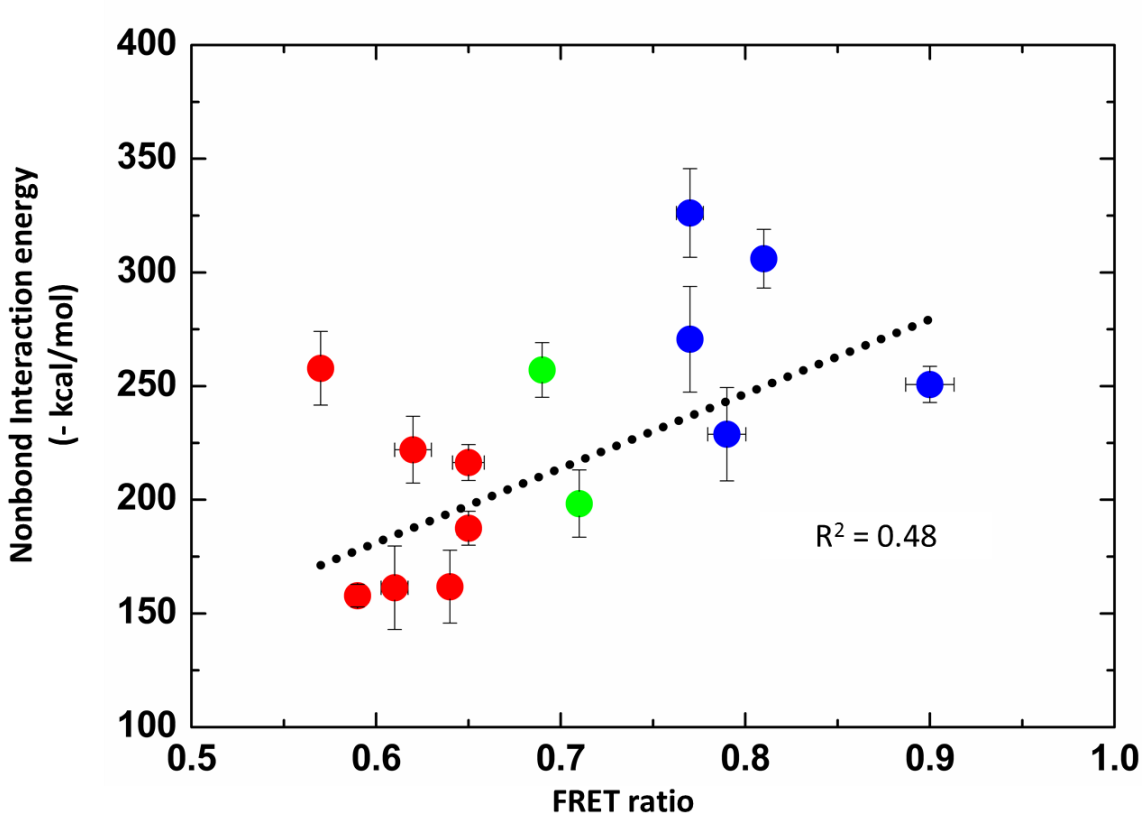

Figure S5: Calculated average interaction energy of the peptide substrates versus FRET intensity ratio. The interaction energy of the entire peptide with the entire kinase was calculated. For clarity, the weak, medium, and strong binding models are shown in red, green, and blue spheres, respectively. The error bars for FRET measurement and the nonbonded interaction energy represent the standard error of the mean (SEM) and the standard deviation, respectively.

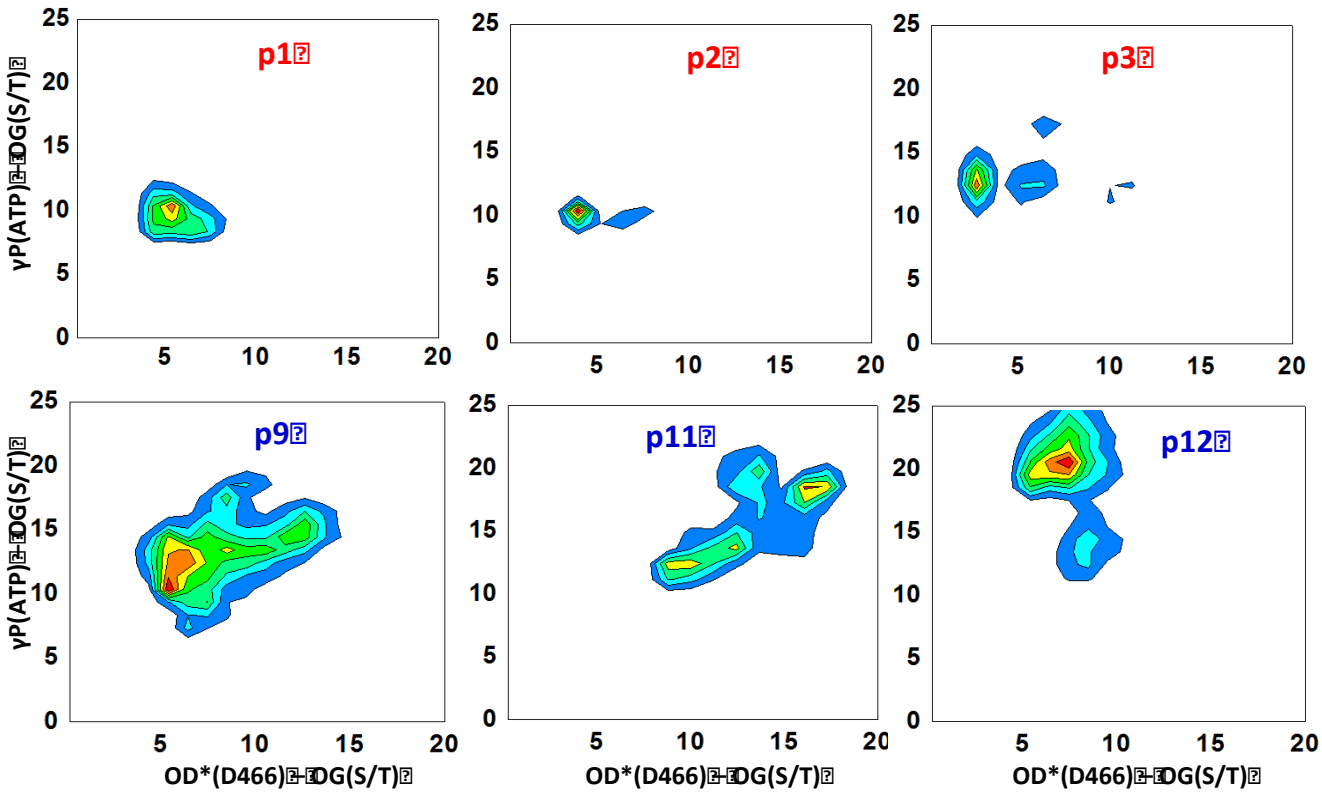

Figure S6: Population distribution of various peptide conformations from three 100ns of all-atom MD trajectories for peptide substrates p1, p2, p3, p9, p11, and p12. The population distributions are calculated with respect to two distances. The two distances are the distance between ATP(γP) and S/T(OG) of the substrate peptide and that between D466(OD) and S/T(OG).

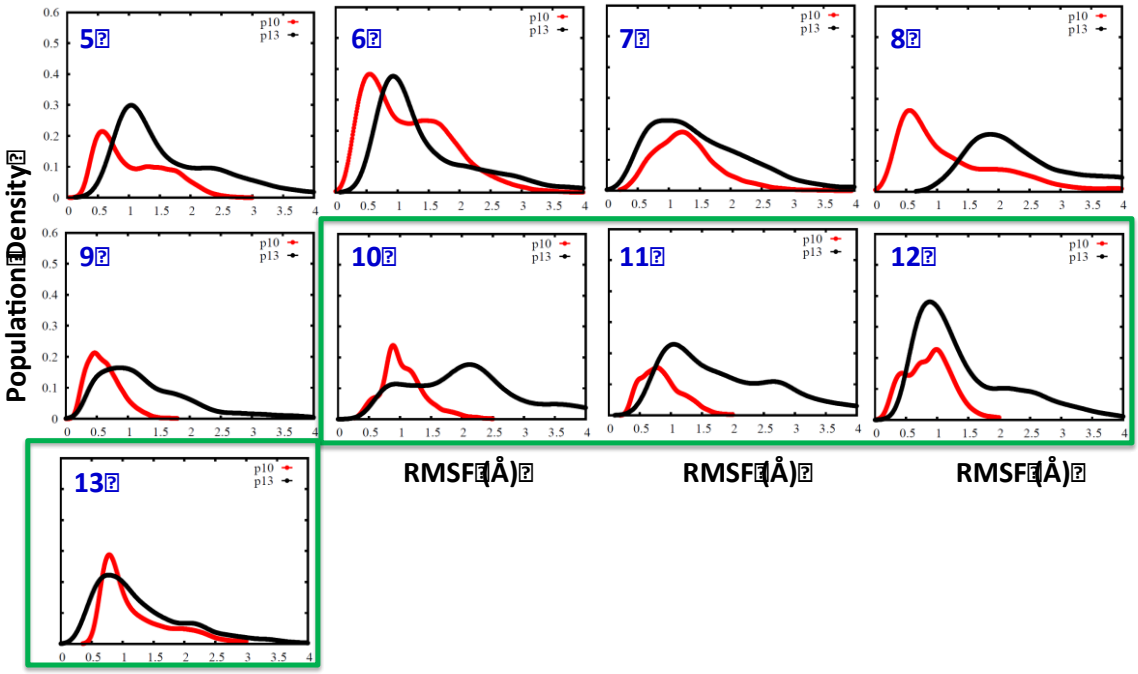

Figure S7: Population density of RMSF of heavy atoms showing the level of flexibility for every residue in peptides p10 and p13. The numbers (blue) represent the relative positions of the residue in the peptide substrate, relative to the p-Ser/Thr. number. The residues C-terminus of p-Ser9 in p13 show higher flexibility than those in p10. The residues in the C-terminus of p-Ser9 are marked with a green box.

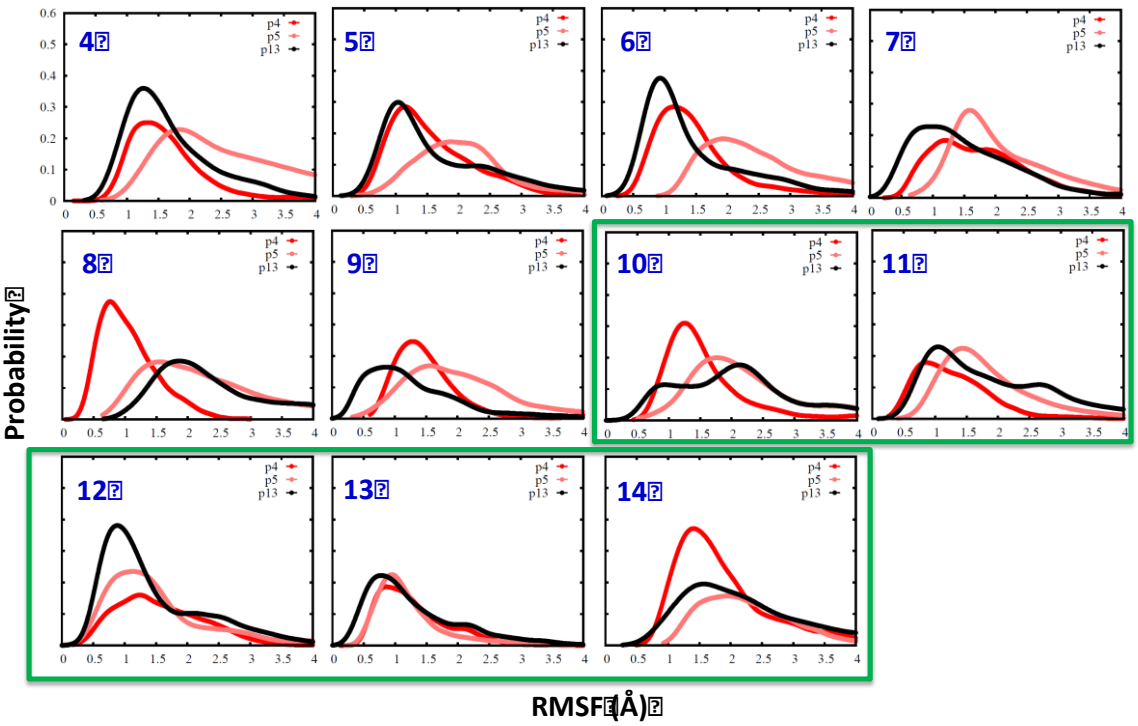

Figure S8: Population density of RMSF of heavy atoms showing the level of flexibility for every residue in peptides p4, p5 and p13. These three peptides show similar kinase activity with differences in binding affinity to PKCα. The numbers (blue) represent the positions of the residues in the peptide substrates relative to the p-Ser which is in position 9. The residues C-terminus of p-Ser9 are marked with a green box. The C-terminus residues show similar level of flexibility during the MD simulations although they have different binding affinities.

Table S2: The non-bonded interaction energies of the residues in the N-terminus (prior to the p-Ser/Thr in the peptide substrates) of the peptide substrate with the kinase. STD in the standard deviation in the interaction energies.

N - term.	Int. Energy (-kcal/mol)	STD (-kcal/mol)
p4	129.4	15.5
p5	100.0	11.8
p13	225.9	21.3

Figure S9: GNEIMO REMD Rosetta annealing Protocol. * See the beginning of the Supporting Information for the details of the workflow used for GNEIMO-REMD-Rosetta annealing simulations.