Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Supporting Information:

Profiling MAP Kinase Cysteines for Targeted Covalent Inhibitor Design

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Table S1: pK_{a} values of the CpHMD predicted reactive cysteines and lysines in MAP kinases in comparison to the empirical calculations[a]

Kinase	PDB	Residue	CpHMD	PROPKA	Residue	CpHMD	PROPKA
JNK1 (MAPK8)	2xrw	**C116 (FP)**[S1]	7.5 (0.0)	10.0	**C245** (αH head)	7.8 (0.1)	9.0
JNK2 (MAPK9)	3e7o	**C116 (FP)**[S1]	8.0 (0.1)	10.2	K153 (HRD+2)	8.9 (0.1)	10.1
					C177 (a.l DFG+6)[b]	< 7.0	9.1
JNK3 (MAPK10)	6emh	**C154 (FP)**[S2]	6.3 (0.1)	10.4	**C283** (αH head)	7.6 (0.0)	9.2
P38α (MAPK14)	5tbe	**C119 (EFP)**[S3]	6.9 (0.2)	9.3			
P38β (MAPK11)	3gc8				C119 (EFP)	9.4 (0.1)	9.1
P38γ (MAPK12)	1cm8						
P38δ (MAPK13)	5ekn						
ERK1 (MAPK3)	6ges	**C183 (DFG-1)**	9.1 (0.0)	12.7	C144 (αE head), C271 (C.l)	8.8 (0.0), 7.4 (0.2)	11.3, 9.3
ERK2 (MAPK1)	3w55/6g54	**C166 (DFG-1)**	9.9 (0.1)/10.0 (0.1)	11.9/13.9	C254 (C.l)	7.2 (0.1)/6.8 (0.3)	9.3/9.2
ERK5 (MAPK7)	4ic8				C208 (a.l DFG+6)	< 7.0	10.2
					C359 (CT)	8.2 (0.2)	13.0
ERK3 (MAPK6)	2i6l				C28 (g.l 5), C42 (N.l)	7.8 (0.2), 9.2 (0.1)	9.2, 9.5
ERK4 (MAPK4)	HM				K151 (HRD+2)	9.4 (0.1)	10.6
ERK7 (MAPK15)	HM				K29 (βII)	9.0 (0.0)	9.9
					K42 (catalytic)	8.4 (0.1)	10.6
					C154 (DFG-1)	9.5 (0.2)	14.0
					C249 (αI head)	< 7.0	9.3
NLK (NLK)	HM				K167 (catalytic)	7.9 (0.2)	11.7
					C179 (αC head)	8.4 (0.1)	9.6
					C281 (DFG-1)	9.7 (0.1)	12.8
					C369 (C.l)	< 7.0	8.4

[a]Residues that have been targeted by covalent inhibitors or chemical probes in the chemoproteomic screening are indicated in bold font. Errors from block analysis are given in parentheses. The block length is 5 ns per replica. In case of incomplete titration, i.e., pK_{a} < 7.0, error analysis was not meaningful and thus not performed. For those proteins without a published X-ray structure, simulations started from a homology model (HM). Two crystal structures, 3w55 and 6g54, were used to calculate pK_{a}'s of ERK2. The main difference between them is that the carboxamide group of Asn154 in 3w55 is rotated by 180° with respect to that in 6g54. The similar pK_{a}'s for the DFG-1 cysteine suggests that the sidechain conformation of Asn154 has little effect on the N-C hydrogen bonding which is a major determinant for the cysteine pK_{a}. DFG-1 pK_{a}'s for ERK2, ERK7, and NLK are listed as references. [b]Calculated using 3npc crystal structure since it is S177 in 3e7o.

Table S2: Calculated pK_{a}'s of DFG-1 cysteines in ERK1/2 and MKK7 and the local conformational environment[a]

Kinase	PDB	Conformation	Cys pK_{a}	Asp–Asn	Cys–Asn	Cys–Asp	Cys–Lys	Asp–Lys	Glu/Asp–Lys	ζ(Cys)
ERK1	6ges	DFG-in/αC-in	9.1	3.8	4.3	5.9	6.6	5.1	2.9	58.4
ERK2	6g54	DFG-in/αC-in	10.0	2.9	4.1	4.6	6.9	3.7	2.8	62.0
MKK7	6qft	DFG-in/αC-in	9.8	4.3	4.1	5.5	7.5	5.4	3.2	68.9
MKK7	6qfr[b]	DFG-outlike/αC-in	8.2	7.4	4.5	3.5	9.1[b]	3.6[b]	4.1[b]	227.7
MKK7	7cbx	DFG-outlike/αC-in	6.7[c]	7.3	4.1	3.7	7.3	2.6	5.4	231.0

[a] Column 4 lists the CpHMD calculated pK_{a}'s of the DFG-1 Cys. Column 5–10 list the various distances. Asn–Asp refers to the minimum distance between the carboxamide nitrogen or oxygen of the HRD+5 Asn and the carboxylate oxygen of DFG-Asp. Cys–Asn, Cys–Asp, and Cys–Lys refer to the distance from the sulfur of DFG-1 Cys to the carboxamide nitrogen of HRD+5 Asn, the nearest carboxylate oxygen of DFG-1 Asp, and the amine nitrogen of the catalytic Lys, respectively. Asp–Lys refers to the distance from the DFG-Asp and the catalytic Lys. Glu/Asp–Lys refers to the distance between the amine nitrogen of the catalytic Lys and the nearest carboxylate oxygen of αC-Glu in ERK1/2 or αC-Asp in MKK7. ζ(Cys) refers to the pseudo torsion angle defined by the four CA atoms of DFG-2, DFG-1 Cys, DFG-Asp, and DFG-Phe residues.[S4,S5]
[b]The amine nitrogen of the catalytic Lys is missing in the crystal structure 6qfr. The distances were taken from OpenMM homology modeling.

Supplemental figures

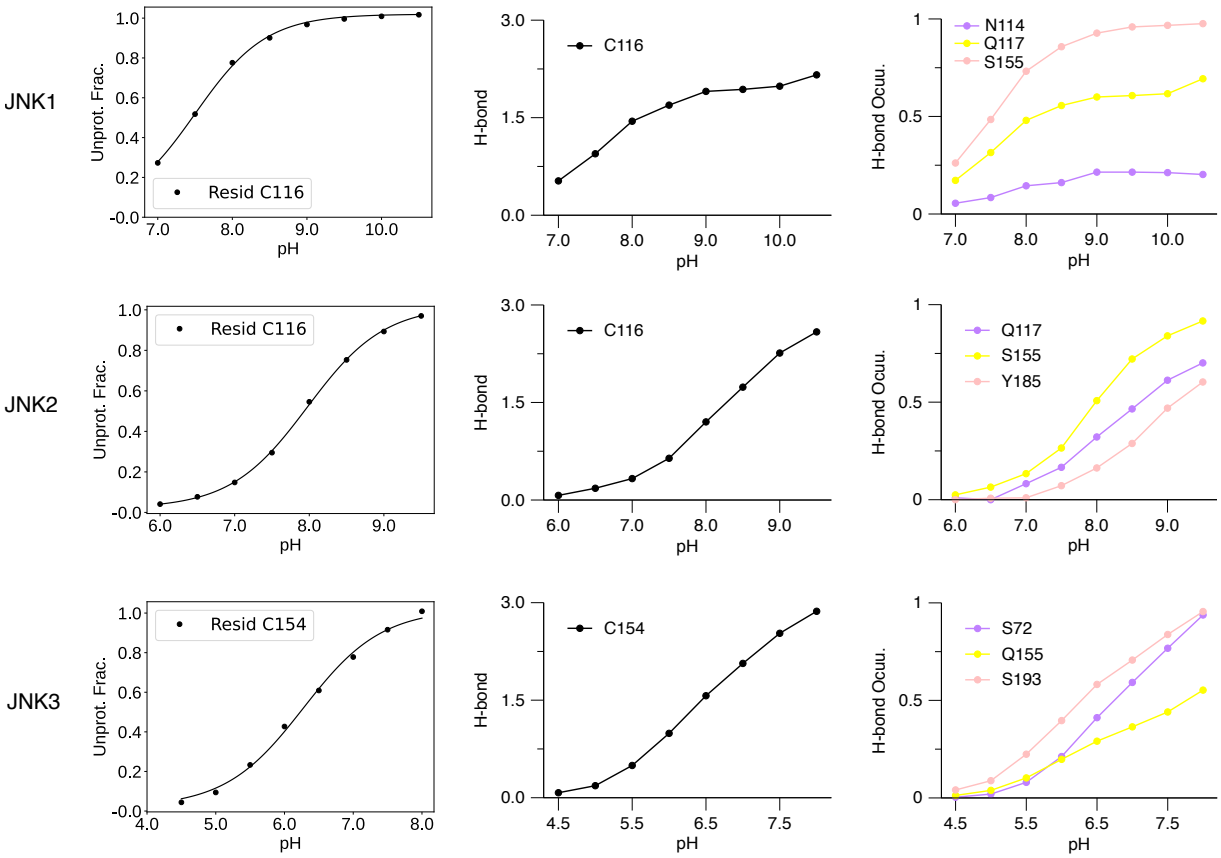

Figure S1: **Titration of the FP Cys and local hydrogen bonding interactions in JNK1, JNK2, and JNK3.** (a) pH-dependent deprotonated fractions of C116 in JNK1, C116 in JNK2, and C154 in JNK3. Curve represents the best fit to the Henderson-Hasselbalch equation. (b) pH-dependent h-bond formation of the FP Cys. (c) pH-dependent occupancy of the hydrogen bonds. A hydrogen bond is considered present if the heavy-atom donor-acceptor distance is below 3.5 Å and the donor-hydrogen-acceptor angle greater than 150 degree.

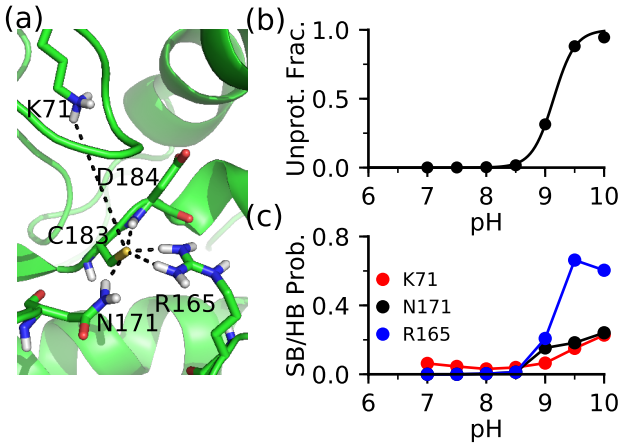

Figure S2: **Salt-bridge and h-bond formation of Cys183 in ERK1 (pdb 6ges) at high pH conditions.** (a) The DFG+1 Cys183 forms salt bridges with the catalytic Lys71 and HRD Arg165, and it accepts a hydrogen bond from the sidechain of HRD+5 Asn171. Note that the two salt bridges are exclusive. (b) Cys183 deprotonation fraction as a function of pH. (c) Salt-bridge or h-bond probabilities for Cys183 as a function of pH.

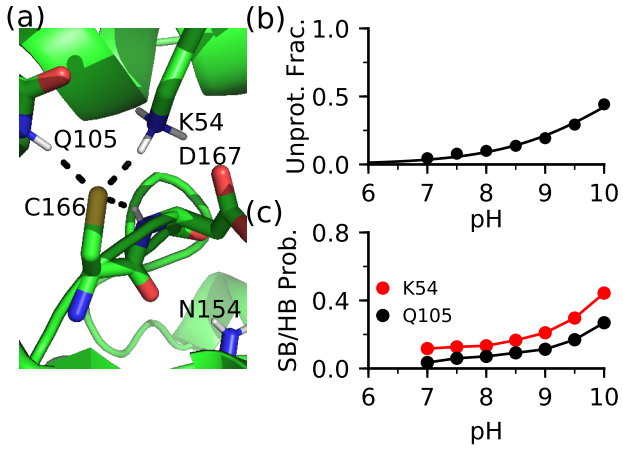

Figure S3: **Salt-bridge and h-bond formation of Cys166 in ERK2 (pdb 6g54) at high pH conditions.** (a) The DFG+1 Cys166 forms a salt bridge with the catalytic Lys54 and hydrogen bond with Gln105 backbone at the hinge region. The HRD+5 Asn has no hydrogen bond with Cys166. (b) Cys166 deprotonation fraction as a function of pH. (c) Salt bridge and hydrogen bond probabilities for Cys166 as a function of pH. Note that the HRD+6 Asn154 does not form a h-bond with Cys166.

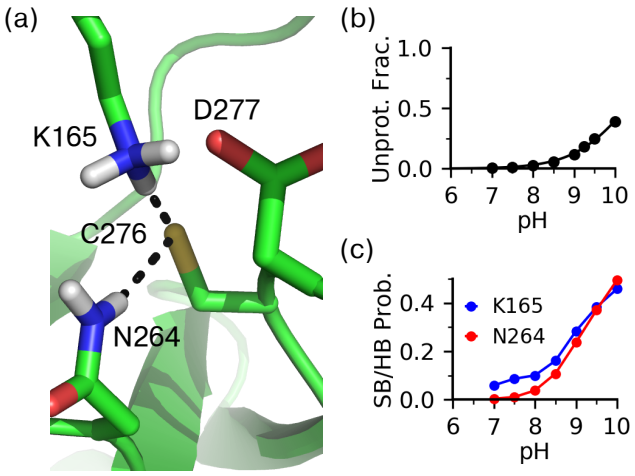

Figure S4: **Salt-bridge and h-bond formation of Cys276 in MKK7 (pdb 6qft) at high pH conditions.** (a) The DFG+1 Cys276 forms a salt bridge with the catalytic Lys165 and a hydrogen bond with the HRD+5 Asn264. Note that the DFG-Asp sidechain is in the DFG-in conformation. (b) Cys276 deprotonation fraction as a function of pH. (c) Salt-bridge and h-bond probabilities for Cys276 as a function of pH.

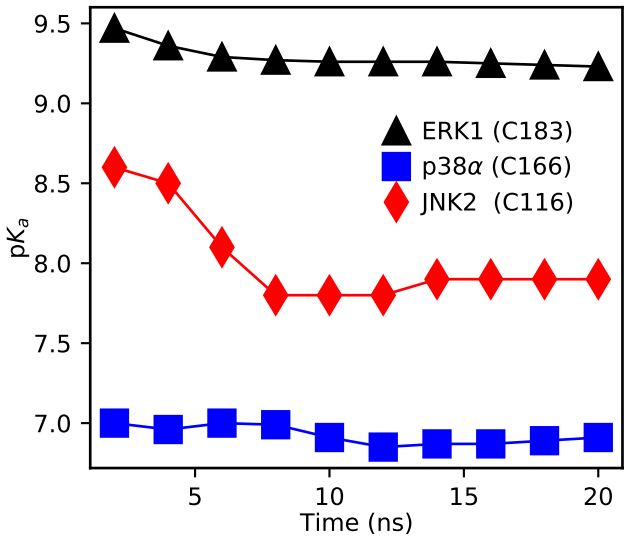

Figure S5: **Example pK_a convergence plots.** Calculated pK_a's for C116 in JNK2, C119 in p38α, and C183 in ERK1 as a function of simulation time per pH replica. Data from the last 20 ns per replica are shown. The total simulation time is 20 ns for JNK2, 50 ns for p38α, and 80 ns for ERK1. All simulations were run until the relevant Cys pK_a was converged. Errors in the calculated pK_a's for all cysteines are given in Table S1.

References

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