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Unique MAP Kinase Binding Sites

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Abstract

Map kinases are drug targets for autoimmune disease, cancer, and apoptosis-related diseases. Drug discovery efforts have developed MAP kinase inhibitors directed toward the ATP binding site and neighboring “DFG-out” site, both of which are targets for inhibitors of other protein kinases. On the other hand, MAP kinases have unique substrate and small molecule binding sites that could serve as inhibition sites. The substrate and processing enzyme D-motif binding site is present in all MAP kinases, and has many features of a good small molecule binding site. Further, the MAP kinase p38 α has a binding site near its C-terminus discovered in crystallographic studies. Finally, the MAP kinases ERK2 and p38 α have a second substrate binding site, the FXFP binding site that is exposed in active ERK2 and the D-motif peptide induced conformation of MAP kinases. Crystallographic evidence of these latter two binding sites is presented.

1. Introduction

MAP kinase cascades confer switch-like responses to extracellular hormonal and stress stimuli that lead to cell fate decisions such as differentiation, proliferation, apoptosis [1,2] and senescence [3]. The modules are comprised of a Ser/Thr kinase MAP3K that doubly phosphorylates a dual-specificity MAP2K, which in turn doubly phosphorylates, through a phosphotyrosine intermediate, a proline-directed Ser/Thr MAPK. The sigmoid response behavior is thought to be a consequence of the dissociative nature of these two double phosphorylation reactions [4]. Different MAP kinase modules respond to distinct extracellular stimuli. The extracellular signal regulated kinases 1 and 2 (ERK1/2) are activated by mitogens and growth hormones, while the c-Jun N-terminal kinases (JNKs) and p38 MAP kinases are activated by bacterial liposaccharides, interleukine-1, tumor necrosis factor- α , and cellular stresses such as osmotic shock and UV radiation [5]. ERK5 is activated by both classes of stimuli [6,7]. Given these central regulatory roles, it is not surprising that three out of four of these best studied MAP kinases have received very significant attention as drug targets [8].

The p38 MAP kinase pathway is a therapeutic target for inflammatory diseases such as psoriasis, rheumatoid arthritis and chronic obstructive pulmonary disease [9,10]. JNKs are drug targets for apoptosis related diseases such as Alzheimer’s disease, Parkinson’s disease, type II diabetes, hearing loss, and also for autoimmune diseases [11,12]. ERK2 pathway components are drug targets for proliferative diseases, notably MEK1 / 2 and Raf isoforms [13,14].

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Search for anti-inflammatory agents lead to the discovery of p38 α as a potential drug target [15]. A very significant effort by the drug industry has produced many more inhibitors of p38 α [16]. The vast majority of these compounds have proved to be competitive with ATP, binding to the active site (reviewed for example in [10,17]). A few inhibitors have also been found that bind to a site adjacent to the active site. This neighboring site has been termed the “DFG-out”-site, because binding at this site is always associated with conformational changes in the conserved DFG sequence (subdomain VII) [16,18] (Fig. 1). The existence of this site relies on the conformational flexibility of the activation loop of these protein kinases, and a similar site was identified in the development of the inhibitors of MAP/ERK kinase 1 and 2 (MEK1/2) [19], c-Abl [20] and other protein kinases [21,22].

MAP kinase modules utilize docking interactions to link module components and bind substrates (reviewed in [23-25]). Docking interactions may account for the pathway specificity among MAP kinase modules. Linear peptide docking motifs in substrates, MAP2Ks, and phosphatases bind to sites in MAPKs outside the kinase active site [26]. MAP2Ks have been shown recently to utilize other docking interactions with MAP3Ks [27]. The best studied docking interactions are those between MAP kinases and “D-motifs” (originally named δ -domains, but also referred to as D-sites, D-domains, DEJL, D-boxes, kinase interaction motifs, or KIMs), linear sequences with substrates and other interacting proteins. D-motifs were identified initially in JNK substrates [28], and were later found in MAP2Ks [25] and in MAP kinase phosphatases [29]. Each of the MAP kinases p38s, JNKs and ERKs [24] and ERK5 [30] bind D-motifs. In addition, transcription factor substrates and phosphatases of the MAP kinase ERK2 and p38 α have a second docking motif, named FXFP [23,31,32], for the sequence in the docking motif. The binding sites for these motifs may have potential for making specific inhibitors of MAP kinases. The idea of making substrate based inhibitors is established for protein kinases [33], but new for MAP kinases [33-36]. Here we discuss the “druggability” of the MAP kinase docking motif binding sites for D-motifs and FXFP, and present data on the existence of a novel small molecule binding site in p38 α termed the Backside site (Fig. 1).

2. D-motif binding site

The D-motifs are probably the main specificity determinant that distinguishes among different MAP kinases [25]. In D-motif swapping experiments among MAPKAP isoforms that are differentially activated by ERK2 and p38 α , the D-motif conferred pathway specificity [37]. Also, peptides derived from the D-motif of c-Jun interacting protein (JIP), which bind to the docking groove of JNK inhibit JNK activation [38] and are neuroprotective in animal models for Parkinson’s disease [39], apparently blocking apoptotic signaling through JNK. D-motifs can have different lengths. The longest have the consensus sequences X- ϕ_H -X₂-(Arg/Lys)₁₋₂-(X)₂₋₆- ϕ_A -X- ϕ_B (where ϕ_A , ϕ_B and ϕ_H are hydrophobic residues (Leu, Ile, or Val), while shorter motifs can be missing the first four residues [24]. The D-motif binding site on MAP kinases is complex, and is formed of acidic patch in the C-terminal extension from the kinase core known as the “CD domain” (for Common Docking)[26] and hydrophobic docking groove [40,41] (also referred to as “ED”[42]). The existence of two subsites may allow separate or overlapping specificities to evolve in MAP kinase substrates [43]. Crystallographic studies of p38 α [41] and JNK1 [44] revealed the hydrophobic groove to be near helices D and E. Recent data on ERK2 docking interactions [45,46] and on the yeast MAP kinase Fus3 and Kss1 [47] has revealed interactions in the CD domain. The CD domain is an electrostatic surface depression [45] to which the ϕ_H and basic residues bind.

2.1 The Hydrophobic Docking Groove

The hydrophobic docking groove appears to have the greatest potential for drug development since it has a significant hydrophobic pocket [45]. The docking groove spans across helices D and E in a crevice formed between these helices and β 7- β 8 reverse turn (Fig. 2). The

hydrophobic pocket is occupied variably by two to three hydrophobic residues in different MAP kinases. In p38 α , the ϕ_A makes van der Waals contacts with the side chains of Ile116, Leu122 in helix D, Val158 in β_7 and Cys162 in β_8 . The ϕ_B residue contacts the side chains of Ala111 and Ile116 in helix D and Val158 in β_7 . A leucine residue in the position ϕ_{A-2} is in the groove, making contacts with Phe129 and Leu130 in helix E. The interaction also involves several hydrogen bonds. In p38 α , four hydrogen bonds are made to the peptide by Gln120, His126 and the carbonyl of Glu160 (in the β_7 - β_8 reverse turn). In contrast to the docking groove, the CD domain is a surface depression that requires the D-motif to be folded into a 2-turn helix to bind [45].

2.2 Similarities and differences among MAP kinase docking grooves

The hydrophobic docking grooves are significantly different in p38 α , ERK2 and JNK's (discussed in [45]). For example, in ERK2, ERK2/Thr108 replaces p38 α /Ala111, and the larger side-chain completely blocks the site occupied by ϕ_B in p38 α . In addition, ϕ_B of ERK2-binding peptides occupy the pocket held by ϕ_A in p38 α . JNK/Ala111 binds to ϕ_A and ϕ_B like p38 α . But the site lacks ϕ_{A-2} site, which is blocked by an arginine unique to JNK (Arg127). These differences may be contributing to the success in making specific peptide-based inhibitors of JNKs [48]. Another feature of the hydrophobic docking groove is the presence of cysteine residues. p38 α has two cysteine residues that face the pocket, Cys119 and Cys162. Both ERK2 and JNK possess one cysteine, ERK2/Cys159 and JNK/Cys163. Thus, development of covalent inhibitors directed toward cysteine [49] becomes a possibility. Also, tethering, the use of mild thiol reagents to enhance affinities of compounds in fragment and small molecule libraries [50] can potentially be used to search for inhibitors targeted to the docking groove.

2.3 D-motif induced conformational changes

D-motif peptides induce conformational changes in p38 α and ERK2, and possibly in JNK. The changes involve the activation loop in each case, which becomes disordered in p38 α and adopts a new structure in ERK2. These conformational changes may promote greater access to the activation loop phosphorylation sites when activating MAP2Ks or phosphatases are bound in the D-motif binding site.

In p38 α and ERK2, the changes are known because structures are available for the unbound and peptide-bound conformations for the identical protein (rat ERK2 and murine p38 α) [41, 45]. The induced conformational changes are unique. In p38 α the changes occur near helices D and E (Fig. 2). The movement appears to be required in order to simultaneously form all of the observed hydrogen bonds made to the peptides, especially the interactions of Gln120 with residue X in (ϕ_A -X- ϕ_B) and the interaction of His126 with the docking site peptide backbone. In ERK2, the hydrophobic docking groove is preformed. Instead, conformational changes occur near the CD domain in ERK2 [45] (not shown).

The affinities for docking peptides to MAP kinases are sub-micromolar to micromolar. Can more tightly bound inhibitors be found? The peptide induced conformational changes likely absorb intrinsic binding energy [45]. Thus an inhibitor that fits in without inducing structural rearrangements may have a better binding constant. The amount of energy absorbed by conformational changes has not been measured in MAP kinases, but is 3 kcal or more in allosteric enzymes [51]. Since the D-motif induced conformational changes are different in p38 α , ERK2, and JNK, the changes probably contribute to specificity among these kinases and may be a factor to specific inhibitor binding.

3. FXFP binding site in ERK2

The MAP kinase ERK2 has a second binding site utilized by transcription factor substrates and phosphatases, the FXFP binding site [24,52]. This site was identified by changes in deuterium exchange profiles, and is near the MAP kinase insertion and helix G [32]. Mutational studies showed that Tyr231 and Tyr261, when mutated affected FXFP binding to doubly phosphorylated ERK2 (ERK2-P2) [32,53]. We have obtained crystallographic evidence of the location of this site in studies of D-motif peptides bound to ERK2 [45]. The refined structure of ERK2 in complex with a D-motif peptide derived from a tyrosine phosphatase revealed an extra tubular electron density near Tyr231 (Fig. 3).

The electron density was modeled with 3 residues derived from the D-motif peptide used in the study and is included in the PDB file 2GPH. The electron density is between the MAP kinase insertion and helix G. In low activity ERK2 (PDB file 1ERK) [54], this site is occupied with activation loop residues Phe181 and Leu182. The tyrosine phosphatase peptide and MEK2 derived peptide both induced conformational changes that caused the activation loop to exit this site. This binding site is hydrophobic, and lined by Leu198, Tyr231, Leu232, Leu235, Ala258 and Tyr261. Hydrogen bonding potential is available from Asn255 and Lys257. This site is adjacent to the active site, and could potentially be accessed by inhibitors simultaneously with the active site.

4. Backside binding pocket

In an effort to visualize protein kinase inhibitors, we soaked inhibitors for other kinases into crystals of p38 α , as a model protein kinase. We anticipated that most of the kinase inhibitors tested would bind to the ATP binding site, as was the case for the p38 inhibitors SB203580 and related compounds [7]. However, two compounds, PD98059 and sulindac sulfide, bind to a site outside the active site. The MAP2K MEK1/2 inhibitor PD98059 (1-(2'-amino-3'-methoxyphenyl)-oxanaphthalen-4-one), is a flavonoid that binds to the inactive form of MEK1 non-competitively with respect to ATP [55]. The I κ B kinase inhibitor sulindac sulfide (cis-5-fluoro-2-methyl-1-[p-(methylsulfinyl)benzylidene]indene-3-acetic acid, is a non-steroidal anti-inflammatory drug [56]. Previous studies have indicated that sulindac sulfide and PD98059 do not inhibit p38 α activity [55,56]. However, we did find that these compounds bind p38 α with IC₅₀'s of 19 μ M and 24 μ M, respectively, as measured by binding-induced changes in absorbance (at 365 nm and 329 nm, respectively). Crystals of p38 α prepared as described previously [57,58] were soaked with 1 mM sulindac sulfide or PD98059, soaked for 45 min. The structures were solved using 2.6 Å data and refined to R-factors of 21% or better (Table 1). Both sulindac sulfide and PD98059 bind p38 α near the hinge point between the two domains of the kinase, where the C-terminal helix unique to MAP kinases contacts the kinase core. We refer to this site as the Backside binding site.

4.1. Sulindac sulfide

The sulfur atom of sulindac sulfide was especially visible, with a 3.5 σ electron density pinpointing the sulfur atom, and the entire molecule is visible as contoured (Fig. 4A). The indene moiety of sulindac sulfide is held between the His107 and Lys165, with ring stacking link between the indene and the His 107 side-chain. The carboxylate group in the sulindac sulfide contacts the backbone atoms Met78O, His80N, and the carboxylate of Glu81 in the linker between helix C and β -4. The benzylidene forms van der Waals contacts with the C-terminal residues Leu353 and Asp354. The indene fluorine atom in sulindac is hydrogen bonded (2.9Å) to Lys165N ϵ . Thus there are several specific hydrogen bonds as well as hydrophobic interactions.

Although there are no known inhibitors targeted to this site, an inhibitor developed at Boehringer Ingelheim Pharmaceuticals (called CMPD1) [59] shows deuterium exchange protection profiles that include His107 and Lys165, among other residues, suggestive of potential binding to this site.

A major question of course is whether the target of sulindac sulfide binds; I κ B kinase binds the inhibitor similarly. I κ B kinase does have homology with MAP kinases in this region, including the C-terminal helix that walls the binding pocket. Thus the possibility that sulindac sulfide binds I κ B in this site cannot be ruled out.

4.2 PD98059

PD98059 binds to the same site as sulindac sulfide. The carbonyl of oxanaphthalen-4-one ring of PD98059 contacts the backbone of Met78O as with sulindac sulfide. Otherwise the interactions and the orientation are different, with PD98059 binding closer to Lys79 and Glu81 (Fig. 4b). The structure of inactive MEK1/2 is available (PDB file 1S9J) and no similar site is present [19], so PD98059 is very unlikely to bind to MEK1/2 in this region.

4.3 Proximity of the Backside site to the CD domain

It is interesting that Backside binding site is very near the CD domain. Glu81 that contacts the inhibitors is involved in an extended ionic network that includes Arg136 and Asp316, in the CD domain defined by [26] (Fig. 4A). This raises the possibility of making inhibitors that target both the Backside and docking groove. CMPD1 [59] mentioned above is an inhibitor of p38 α that affects binding and phosphorylation of MAPKAPs but not transcription factor substrates. It is intriguing that MAPKAPs have the long variety of D-motifs, making more extensive interactions near the CD domain whereas transcription factors usually have the shorter variety [24], suggestive that the inhibitor does indeed bind the Backside site.

5. Conclusions and perspectives

Although MAP kinase drug discovery so far has identified inhibitors primarily to the ATP site and DFG-out site, other substrate and small molecule binding sites may prove useful in the future for the development of novel and specific inhibitors of MAP kinases. We think the allosteric effects in MAP kinases offer an especially interesting opportunity for drug development, because of the chance of binding an inhibitor that binds more tightly than the docking peptide. Also, D-motif binding site directed inhibitors could inhibit the pathway both by substrate and MAP2K binding, and also by inhibiting conformational changes. The sites discussed here are all smaller than the ATP binding site and DFG-out site, and thus it appears likely that strategies that block the ATP site may be needed to find inhibitors to these smaller sites.

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Abbreviations

CD-domain, Common docking domain; D-Motif, Docking motif or docking site in MAP kinase substrates and MAP kinase processing enzymes; DFG-out, unusual conformation in well conserved DFG in subdomain VII; ED, hydrophobic docking groove (that is flanked by D and E residues); ERK, Extracellular response kinase; JNK, c-Jun N-terminal kinase; MAPK,

Mitogen activated protein kinase; MAP2K, Mitogen activated protein kinase kinase; MAPKAP kinase, Mitogen activated protein kinase activated protein kinase.

References

1. Chen Z, Gibson TB, Robinson F, Silvestro L, Pearson G, Xu B, Wright A, Vanderbilt C, Cobb MH. MAP kinases. *Chem. Rev* 2001;101:2449–2476. [PubMed: 11749383]
2. Johnson GL, Lapadat R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science* 2002;298:1911–1912. [PubMed: 12471242]
3. Deng Q, Liao R, Wu BL, Sun P. High intensity ras signaling induces premature senescence by activating p38 pathway in primary human fibroblasts. *J. Biol. Chem* 2004;279:1050–1059. [PubMed: 14593117]
4. Ferrell JE Jr, Bhatt RR. Mechanistic studies of the dual phosphorylation of mitogen-activated protein kinase. *J. Biol. Chem* 1997;272:19008–19016. [PubMed: 9228083]
5. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, Cobb MH. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr. Rev* 2001;22:153–183. [PubMed: 11294822]
6. Wang X, Tournier C. Regulation of cellular functions by the ERK5 signalling pathway. *Cell Signal* 2006;18:753–760. [PubMed: 16376520]
7. Wang Z, Canagarajah BJ, Boehm JC, Kassisa S, Cobb MH, Young PR, Abdel-Meguid S, Adams JL, Goldsmith EJ. Structural basis of inhibitor selectivity in MAP kinases. *Structure* 1998;6:1117–1128. [PubMed: 9753691]
8. Zhang J, Shen B, Lin A. Novel strategies for inhibition of the p38 MAPK pathway. *Trends Pharmacol. Sci* 2007;28:286–295. [PubMed: 17482683]
9. Kumar S, Boehm J, Lee JC. p38 MAP kinases: key signalling molecules as therapeutic targets for inflammatory diseases. *Nat. Rev. Drug. Discov* 2003;2:717–726. [PubMed: 12951578]
10. Kaminska B. MAPK signalling pathways as molecular targets for anti-inflammatory therapy--from molecular mechanisms to therapeutic benefits. *Biochim. Biophys. Acta* 2005;1754:253–262. [PubMed: 16198162]
11. Manning AM, Davis RJ. Targeting JNK for therapeutic benefit: from junk to gold? *Nat. Rev. Drug Discov* 2003;2:554–565. [PubMed: 12815381]
12. Resnick L, Fennell M. Targeting JNK3 for the treatment of neurodegenerative disorders. *Drug Discov. Today* 2004;9:932–939. [PubMed: 15501728]
13. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. *Oncogene* 2007;26:3291–3310. [PubMed: 17496923]
14. Sebolt-Leopold JS, Herrera R. Targeting the mitogen-activated protein kinase cascade to treat cancer. *Nat. Rev. Cancer* 2004;4:937–947. [PubMed: 15573115]
15. Lee JC, Laydon JT, McDonnell PC, Gallagher TF, Kumar S, Green D, McNulty D, Blumenthal MJ, Heys JR, Landvatter SW, et al. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* 1994;372:739–746. [PubMed: 7997261]
16. Wroblewski ST, Doweiko AM. Structural comparison of p38 inhibitor-protein complexes: a review of recent p38 inhibitors having unique binding interactions. *Curr. Top. Med. Chem* 2005;5:1005–1016. [PubMed: 16178743]
17. Adams JL, Badger AM, Kumar S, Lee JC. p38 MAP kinase: molecular target for the inhibition of pro-inflammatory cytokines. *Prog. Med. Chem* 2001;38:1–60. [PubMed: 11774793]
18. Regan J, Capolino A, Cirillo PF, Gilmore T, Graham AG, Hickey E, Kroe RR, Madwed J, Moriak M, Nelson R, Pargellis CA, Swinamer A, Torcellini C, Tsang M, Moss N. Structure-activity relationships of the p38alpha MAP kinase inhibitor 1-(5-tert-butyl-2-p-tolyl-2H-pyrazol-3-yl)-3-[4-(2-morpholin-4-yl-ethoxy)naphthalen-1-yl]urea (BIRB 796). *J. Med. Chem* 2003;46:4676–4686. [PubMed: 14561087]
19. Ohren JF, Chen H, Pavlovsky A, Whitehead C, Zhang E, Kuffa P, Yan C, McConnell P, Spessard C, Banotai C, Mueller WT, Delaney A, Omer C, Sebolt-Leopold J, Dudley DT, Leung IK, Flamme C, Warmus J, Kaufman M, Barrett S, Tecle H, Hasemann CA. Structures of human MAP kinase kinase 1 (MEK1) and MEK2 describe novel noncompetitive kinase inhibition. *Nat. Struct. Mol. Biol* 2004;11:1192–1197. [PubMed: 15543157]

20. Nagar B, Bornmann WG, Pellicena P, Schindler T, Veach DR, Miller WT, Clarkson B, Kuriyan J. Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). *Cancer Res* 2002;62:4236–4243. [PubMed: 12154025]
21. Sebolt-Leopold JS, English JM. Mechanisms of drug inhibition of signalling molecules. *Nature* 2006;441:457–462. [PubMed: 16724058]
22. Mol CD, Fabbro D, Hosfield DJ. Structural insights into the conformational selectivity of STI-571 and related kinase inhibitors. *Curr. Opin. Drug. Discov. Devel* 2004;7:639–648.
23. Jacobs D, Glossip D, Xing H, Muslin AJ, Kornfeld K. Multiple docking sites on substrate proteins form a modular system that mediates recognition by ERK MAP kinase. *Genes Dev* 1999;13:163–175. [PubMed: 9925641]
24. Sharrocks AD, Yang SH, Galanis A. Docking domains and substrate-specificity determination for MAP kinases. *Trends Biochem. Sci* 2000;25:448–453. [PubMed: 10973059]
25. Bardwell L. Mechanisms of MAPK signalling specificity. *Biochem. Soc. Trans* 2006;34:837–841. [PubMed: 17052210]
26. Tanoue T, Adachi M, Moriguchi T, Nishida E. A conserved docking motif in MAP kinases common to substrates, activators and regulators. *Nat. Cell. Biol* 2000;2:110–116. [PubMed: 10655591]
27. Takekawa M, Tatebayashi K, Saito H. Conserved docking site is essential for activation of mammalian MAP kinase kinases by specific MAP kinase kinases. *Mol. Cell* 2005;18:295–306. [PubMed: 15866172]
28. Kallunki T, Deng T, Hibi M, Karin M. c-Jun can recruit JNK to phosphorylate dimerization partners via specific docking interactions. *Cell* 1996;87:929–939. [PubMed: 8945519]
29. Zhou B, Wu L, Shen K, Zhang J, Lawrence DS, Zhang ZY. Multiple regions of MAP kinase phosphatase 3 are involved in its recognition and activation by ERK2. *J. Biol. Chem* 2001;276:6506–6515. [PubMed: 11104775]
30. Barsyte-Lovejoy D, Galanis A, Clancy A, Sharrocks AD. ERK5 is targeted to myocyte enhancer factor 2A (MEF2A) through a MAPK docking motif. *Biochem. J* 2004;381:693–699. [PubMed: 15132737]
31. Galanis A, Yang SH, Sharrocks AD. Selective targeting of MAPKs to the ETS domain transcription factor SAP-1. *J. Biol. Chem* 2001;276:965–973. [PubMed: 11029469]
32. Lee T, Hoofnagle AN, Kabuyama Y, Stroud J, Min X, Goldsmith EJ, Chen L, Resing KA, Ahn NG. Docking motif interactions in MAP kinases revealed by hydrogen exchange mass spectrometry. *Mol. Cell* 2004;14:43–55. [PubMed: 15068802]
33. Bogoyevitch MA, Barr RK, Ketterman AJ. Peptide inhibitors of protein kinases-discovery, characterisation and use. *Biochim. Biophys. Acta* 2005;1754:79–99. [PubMed: 16182621]
34. Mayor F Jr, Jurado-Pueyo M, Campos PM, Murga C. Interfering with MAP kinase docking interactions: implications and perspective for the p38 route. *Cell Cycle* 2007;6:528–533. [PubMed: 17351337]
35. Hancock CN, Macias AT, Mackerell AD Jr, Shapiro P. Mitogen activated protein (MAP) kinases: development of ATP and non-ATP dependent inhibitors. *Med. Chem* 2006;2:213–222. [PubMed: 16787369]
36. Shapiro P. Discovering new MAP kinase inhibitors. *Chem. Biol* 2006;13:807–809. [PubMed: 16931329]
37. Smith JA, Poteet-Smith CE, Malarkey K, Sturgill TW. Identification of an extracellular signal-regulated kinase (ERK) docking site in ribosomal S6 kinase, a sequence critical for activation by ERK in vivo. *J. Biol. Chem* 1999;274:2893–2898. [PubMed: 9915826]
38. Ho DT, Bardwell AJ, Grewal S, Iverson C, Bardwell L. Interacting JNK-docking sites in MKK7 promote binding and activation of JNK mitogen-activated protein kinases. *J. Biol. Chem* 2006;281:13169–13179. [PubMed: 16533805]
39. Arthur PG, Matich GP, Pang WW, Yu DY, Bogoyevitch MA. Necrotic death of neurons following an excitotoxic insult is prevented by a peptide inhibitor of c-jun N-terminal kinase. *J. Neurochem* 2007;102:65–76. [PubMed: 17490439]
40. Gum RJ, Young PR. Identification of two distinct regions of p38 MAPK required for substrate binding and phosphorylation. *Biochem. Biophys. Res. Commun* 1999;266:284–289. [PubMed: 10581204]

41. Chang CI, Xu BE, Akella R, Cobb MH, Goldsmith EJ. Crystal structures of MAP kinase p38 complexed to the docking sites on its nuclear substrate MEF2A and activator MKK3b. *Mol. Cell* 2002;9:1241–1249. [PubMed: 12086621]
42. Tanoue T, Nishida E. Molecular recognitions in the MAP kinase cascades. *Cell Signal* 2003;15:455–462. [PubMed: 12639708]
43. Barsyte-Lovejoy D, Galanis A, Sharrocks AD. Specificity determinants in MAPK signaling to transcription factors. *J. Biol. Chem* 2002;277:9896–9903. [PubMed: 11786537]
44. Heo YS, Kim SK, Seo CI, Kim YK, Sung BJ, Lee HS, Lee JI, Park SY, Kim JH, Hwang KY, Hyun YL, Jeon YH, Ro S, Cho JM, Lee TG, Yang CH. Structural basis for the selective inhibition of JNK1 by the scaffolding protein JIP1 and SP600125. *Embo J* 2004;23:2185–2195. [PubMed: 15141161]
45. Zhou T, Sun L, Humphreys J, Goldsmith EJ. Docking interactions induce exposure of activation loop in the MAP kinase ERK2. *Structure* 2006;14:1011–1019. [PubMed: 16765894]
46. Liu S, Sun JP, Zhou B, Zhang ZY. Structural basis of docking interactions between ERK2 and MAP kinase phosphatase 3. *Proc. Natl. Acad. Sci. U S A* 2006;103:5326–5331. [PubMed: 16567630]
47. Remenyi A, Good MC, Bhattacharyya RP, Lim WA. The role of docking interactions in mediating signaling input, output, and discrimination in the yeast MAPK network. *Mol. Cell* 2005;20:951–962. [PubMed: 16364919]
48. Bogoyevitch MA. Therapeutic promise of JNK ATP-noncompetitive inhibitors. *Trends Mol. Med* 2005;11:232–239. [PubMed: 15882611]
49. Powers JC, Asgian JL, Ekici OD, James KE. Irreversible inhibitors of serine, cysteine, and threonine proteases. *Chem. Rev* 2002;102:4639–4750. [PubMed: 12475205]
50. Erlanson DA, Wells JA, Braisted AC. Tethering: fragment-based drug discovery. *Annu. Rev. Biophys. Biomo. Struct* 2004;33:199–223.
51. Goldsmith EJ. Allosteric enzymes as models for chemomechanical energy transducing assemblies. *Faseb J* 1996;10:702–708. [PubMed: 8635687]
52. Zhang J, Zhou B, Zheng CF, Zhang ZY. A bipartite mechanism for ERK2 recognition by its cognate regulators and substrates. *J. Biol. Chem* 2003;278:29901–29912. [PubMed: 12754209]
53. Polychronopoulos S, Verykokakis M, Yazicioglu MN, Sakarellos-Daitsiotis M, Cobb MH, Mavrothalassitis G. The transcriptional ETS2 repressor factor associates with active and inactive Erks through distinct FXF motifs. *J. Biol. Chem* 2006;281:25601–25611. [PubMed: 16799155]
54. Zhang F, Strand A, Robbins D, Cobb MH, Goldsmith EJ. Atomic structure of the MAP kinase ERK2 at 2.3 Å resolution. *Nature* 1994;367:704–711. [PubMed: 8107865]
55. Dudley DT, Pang L, Decker SJ, Bridges AJ, Saltiel AR. A synthetic inhibitor of the mitogen-activated protein kinase cascade. *Proc. Natl. Acad. Sci. U S A* 1995;92:7686–7689. [PubMed: 7644477]
56. Yamamoto Y, Yin MJ, Lin KM, Gaynor RB. Sulindac inhibits activation of the NF-kappaB pathway. *J. Biol. Chem* 1999;274:27307–27314. [PubMed: 10480951]
57. Wang Z, Harkins PC, Ulevitch RJ, Han J, Cobb MH, Goldsmith EJ. The structure of mitogen-activated protein kinase p38 at 2.1-Å resolution. *Proc. Natl. Acad. Sci. U S A* 1997;94:2327–2332. [PubMed: 9122194]
58. Lee SJ, Zhou T, Goldsmith EJ. Crystallization of MAP kinases. *Methods* 2006;40:224–233. [PubMed: 16829129]
59. Davidson W, Frego L, Peet GW, Kroe RR, Labadia ME, Lukas SM, Snow RJ, Jakes S, Grygon CA, Pargellis C, Werneburg BG. Discovery and characterization of a substrate selective p38alpha inhibitor. *Biochemistry* 2004;43:11658–11671. [PubMed: 15362850]

Fig. 1.

The structure of inactive p38 α with substrate and small molecule binding sites indicated. The ATP binding site, the DFG-out site, CD-docking groove, FXFP binding site, and backside site are indicated as violet ellipsoids. Helices are cyan, β -strands magenta, and loops deep salmon. Figures generated using PyMOL (Delano Scientific, San Carlos, CA).

Fig. 2. Docking groove interactions between p38 α and pepMEF2A induced conformational changes. Cartoon of p38 α docking groove; residues 110-129 + 157-163. ϕ_2 through ϕ_B of pepMEF2A is shown in green and all atom colors. The p38 α /pepMEF2A is shown in cyan and all atom colors, and uncomplexed p38 α is shown in gray. The side chains of I116, C119, Q120, H126, F129, L130, V158, and C162 and backbone of E160 p38 α /pepMEF2A are displayed in ball and stick rendering. Dotted lines denote hydrogen bonds (2.6-3.2 Å). The surface of unliganded p38 α in the docking groove is shown to reveal the full size of the pocket in the absence of peptide.

Fig. 3. ERK2/pepHePTP reveals electron density near the FXFP binding site and peptide induced conformational changes. Electron density ($|2F_o - F_c|$, contoured at 0.9σ) near the helix G and the MAP kinase insertion. Although the D-motif peptide (not shown) binds in the same docking groove as MEF2A binds to p38 α [45], extra electron density is observed near helix G in ERK2. Ribbon diagram of the ERK2/pepHePTP (displayed between 176-203 +225-263 is in cyan. The side chains of L198, Y231, L232, and Y261 are shown in ball and stick. Uncomplexed ERK2 is shown in gray, and F181 and L182 are shown in ball and stick. Note that this binding pocket is available in ERK2/pepHePTP but not in ERK2.

Fig. 4.

Electron density of map with coefficients ($|2F_o - F_c|$) contoured at 0.7σ for p38 α crystals soaked in A) sulindac sulfide, and B) PD98059. The sulfur atom of sulindac sulfide is yellow, fluorine orange, oxygen red, and nitrogen blue. Residues forming contacts, K79, E81, H107, K165, P351 and L353 are displayed in ball and stick. Residues R136 and D316 in the CD domain are also displayed. Location of D-motif docking groove shown in magenta.

Table 1
X-ray Data Collection Parameters and Refinement Statistics

	p38 + Sulindac	p38 +PD98059
Diffraction data		
Space group	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁
Unit cell (Å)	a=45.59 b=85.54 c=124.26	a=45.71 b=84.57 c=125.34
Wavelength (Å)	1.5418	1.5418
Resolution (Å)	2.5	2.2
No. of measurements	275364	264392
Unique reflections	14546	25889
Completeness(%) (last shell)	89.1(78.9)	86.5(67.0)
R _{merge} (%) (last shell)	5.6(32.4)	4.8(37.5)
Refinement		
Resolution (Å)	20-2.6	20-2.4
No. of reflections (F>2σ)	12448	15196
R _{cryst} /R _{free} (%)	21.7/25.9	21.1/24.1
No. of waters	98	91
Ramachandran Plot:		
Most favoured	77.3	81.1
Additional allowed	19.2	17.0
Generously allowed	3.5	1.9
Disallowed	0	0