Docking interactions in protein kinase and phosphatase networks
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To achieve high biological specificity, protein kinases and phosphatases often recognize their targets through interactions that occur outside of the active site. Although the role of modular protein–protein interaction domains in kinase and phosphatase signaling has been well characterized, it is becoming clear that many kinases and phosphatases utilize docking interactions — recognition of a short peptide motif in target partners by a groove on the catalytic domain that is separate from the active site. Docking is particularly prevalent in serine/threonine kinases and phosphatases, and is a versatile organizational tool for building complex signaling networks; it confers a high degree of specificity and, in some cases, allosteric regulation.

Introduction
Biochemists dating back to Emil Fischer have traditionally assumed that the substrate specificity of an enzyme was determined primarily by stereocchemical complementarity with its active site. Although protein kinase and phosphatase active sites do possess preferred target phosphorylation or dephosphorylation sequences, these preferences are not stringent enough; often they alone cannot explain in vivo specificity. In addition to substrate target site preferences, many protein kinases and phosphatases use dedicated protein–protein interaction surfaces (Figure 1a). One common strategy is to use modular protein–protein recognition domains, such as SH2 and SH3 domains — numerous kinases contain such domains fused to their core catalytic domain. However, a second strategy is to utilize docking interactions — interactions involving binding surfaces of the catalytic domain but distinct from the catalytic active site. These docking grooves bind to short peptide docking motifs that are separate from the substrate motif that is chemically modified by the enzyme. These two supplemental recognition strategies are not mutually exclusive (Figure 1b). The development of these alternative modes of recognition provides a simple way to meet the ever-increasing evolutionary requirement for specificity in protein–protein recognition within complex networks. Related kinases and phosphatases, for example, can develop slightly different docking grooves without compromising the strict stereocchemical requirements for efficient catalysis performed at the active site (Figure 1c) [1].

In this review, we will focus on outlining our current understanding of docking interactions in both protein kinases and phosphatases. Although docking was originally discovered as a mechanism to increase enzyme–substrate specificity, docking interactions can also govern the binding of kinases and phosphatases to each other and other effectors. There are now numerous well-characterized examples of both kinases and phosphatases that utilize docking interactions. The majority are serine/threonine phosphatases, such as PP1 and calcineurin, and serine/threonine protein kinases, including CDK–cyclins, MAP kinases, PDK1 and GSK3. However, at least one protein tyrosine kinase (Csk) has recently been shown to also utilize docking.

Two solutions for increased network specificity: modular recognition domains and docking
The separation of partner recognition and partner modification in signaling circuits based on reversible phosphorylation is an evolutionarily advantageous process. It enables a high degree of transferability of protein recognition independent of catalytic function [1]. This separation appears to have taken two independent routes during the evolution of complex signaling pathways. On one hand, dedicated interaction domains separate from the catalytic domain are often used to establish links with other signaling protein partners. Examples of this include the non-receptor protein tyrosine kinases and phosphatases that contain, for example, SH3 and SH2 domains, such as the Src, Abl, Hck and Csk protein kinases and the SHP protein phosphatases [2]. On the other hand, kinase and phosphatase domains can also acquire surface grooves — referred to as docking sites — that are capable of establishing specific connections via small peptide motifs residing in interaction partners. Although domain-mediated and docking interactions are distinct, they serve a similar functional purpose in targeting catalytic domains to particular substrates, partners and cellular locations.
Comparison of interactions mediated by modular domains and docking grooves for protein kinases and phosphatases. (a) Separation of catalytic function from protein–protein recognition. A kinase or phosphatase catalytic domain, in addition to the substrate preferences of the active site, can acquire additional protein–protein recognition modes through a dedicated surface called the docking groove (orange) or through a dedicated modular protein–protein interaction domain (blue). (b) Substrates or other protein partners in turn can acquire simple peptide motifs that will interact with the docking groove or the globular protein recognition domain. (*It is also possible that substrates or kinase/phosphatase partners acquire the globular domain and the kinase/phosphatase contains the globular domain motif.*) This panel demonstrates three protein recognition interactions that have been identified for protein kinases and phosphatases. (c) Docking motifs can direct the specificity of enzymes. Protein kinase and phosphatase active sites generally have limited substrate recognition capabilities. Although a relatively large array of substrates may fit the stereochemical requirements for catalysis, those with appropriate docking motifs will be selected through interactions mediated by the docking groove. (d) Like certain modular-domain-mediated interactions, certain docking-motif-mediated interactions can be phosphorylation dependent. (e) Docking interactions can also participate in allosteric regulation of kinase or phosphatase domain catalytic activity by directly modulating the catalytic domain structure. Modular domains often allosterically regulate their catalytic domains via autoinhibitory interactions.
They can govern protein association in a phosphorylation-dependent manner, and can influence kinase and phosphatase activity through allosteric mechanisms as well (Figure 1d,e).

Interestingly, enzymes that regulate serine/threonine phosphorylation and those that regulate tyrosine phosphorylation appear to have chosen different strategies for building supplemental specificity interactions (Figure 2). Analysis of the human genome reveals that the majority of serine/threonine kinases and phosphatases do not contain any recognizable modular recognition or targeting domains (including protein–protein, protein–lipid and transmembrane motifs). Conversely, most examples of docking interactions have been identified in serine/threonine kinases and phosphatases [3]. In contrast, the majority of tyrosine kinases and phosphatases contain one or more recognizable modular targeting elements outside of the catalytic domain. Overall, these observations are consistent with a model in which serine/threonine phosphorylation, which is thought to have evolved earlier as a signaling system, has primarily utilized a docking interaction strategy for achieving higher levels of partner discrimination, whereas the later-evolving tyrosine phosphorylation systems began to utilize the strategy of recombinant with multiple alternative modular interaction domains. One possible exception to this rule is the tyrosine kinase Csk. New work suggests that the interaction of Csk with its substrates Src or Yes is mediated through a docking groove, indicating that modular domain and docking peptide interactions might co-function in some tyrosine kinases [4,5].

Identifying linear recognition elements
Most domain-mediated and docking interactions recognize short linear recognition elements rather than folded globular partners. These linear motifs are short, between three and ten amino acids, of which usually just a few residues are important for function. Linear-motif-mediated protein association tends to be more transient in nature and therefore is ideal for signaling networks. Whereas globular domains mainly arise by gene duplication, linear motifs, because of their short length, can arise convergently [6].

The small size of linear motifs makes bioinformatic identification and analysis challenging. The identification of globular domains (SH3, SH2, PTB, WW, PDZ, etc.) in signaling proteins is now straightforward using resources such as SMART and Pfam [7,8]. Efforts are also underway to catalogue linear motifs. The short length of these motifs makes them difficult to detect through sequence comparison; however, a set of proteins that all interact with a certain protein will normally share common features (Figure 3). The DILIMOT and SLiM-Disc programs, for example, can find statistically over-represented motifs in non-homologous sequences that bind to a certain kinase or phosphatase [9,10]. This approach successfully rediscovered known motifs and predicted several others using genome-scale interaction data sets [11**].

The small size of docking motifs potentially enables experimental screening of focused or randomized peptide libraries to discover novel docking peptide sequences. Docking-peptide-mediated interactions, however, are often weak and might function synergistically with interactions mediated by the active site. To overcome low-affinity binding, synthesis of multiple peptides on membranes was used to map synergistic components of weakly interacting protein–protein motifs for MAP kinases and tyrosine phosphatases [12,13].

Docking interactions in protein phosphatases
The serine/threonine phosphatase protein phosphatase 1 (PP1) has evolved effective catalytic machinery, but lacks strong substrate specificity in its active site. PPI finds its targets via a large number of regulatory subunits, which influence the activity and cellular localization of the phosphatase. To date, more than 50 PP1–protein interactions have been identified [14]. Many of the PP1-interaction partners possess one or two of the following sequence motifs: FxxRxR or RVxF [15]. Co-crystallization of PP1 with a synthetic peptide encompassing the RVSF sequence established the RVxF motif as a conserved binding sequence that associates with a hydrophobic pocket on the surface of the PP1 catalytic subunit [16,17*]. Recently, a systematic analysis of structural elements that mediate the binding specificity of PP1-interacting proteins proposed a refined consensus for high-affinity PP1 ligands [18**]. Application of the results of this study to protein sequence database searches enabled the authors to predict PP1-interaction partners with high accuracy. Furthermore, binding studies with several PP1 partners and mutational analysis have demonstrated that differences in peptide–protein interactions dictate the affinity of PP1 for cellular regulators and control the dynamic physiological regulation of PP1 functions in the cell [19].

Calcineurin (known as protein phosphatase 2B, PP2B) is a serine/threonine phosphatase whose substrate selectivity is also determined in part by docking interactions. Calcineurin plays an important role in T-cell activation by directly regulating the activity of NFAT (nuclear factor of activated T cells) transcription factors via dephosphorylation. Calcineurin–NFAT signaling depends on the transient and reversible recognition of the N-terminal regulatory domain of NFAT by calcineurin. This interaction is mediated by a conserved PxIxIT motif present in NFAT proteins and a docking groove on the surface of calcineurin [20]. Peptide cross-linking, in silico docking and experimental analysis later revealed the structural
basis of calcineurin–PxIxIT docking motif interaction, and suggested an unexpected evolutionary parallel with the PP1–RVxF motif interaction [21] (Figure 4). Finally, the importance of calcineurin–PxIxIT motif docking has also been established in other targets; the phosphatase binds to a K⁺ channel through a consensus PxIxIT motif, which is indispensable for the regulation of channel activity [22].
Docking interactions in protein kinases

In mitogen-activated protein kinase (MAPK) networks, extracellular input leads to the phosphorylation and activation of a three-tiered kinase cascade (MAPKKK-MAPKK-MAPK), which in turn stimulates a specific transcriptional response. Within the signaling cascade, the MAPKKK must first recognize the correct MAPKK and phosphorylate it, and the activated MAPKK in turn must locate and activate its cognate MAPK. Structural, biochemical, and genetic data have all shown that docking motifs from interacting proteins are necessary for MAPK to bind to and phosphorylate its targets. MAPK docking is ubiquitous, having been demonstrated in yeast and mammals [23]. The best-characterized MAPK-docking motifs are the so-called D-motifs. The consensus D-motif [(R/K)1–2-(X)2–6-F-x-F; where F denotes a hydrophobic residue] is found in activators (MAPKKs), negative regulators (phosphatases) and various substrates. MAPK docking occurs in all mammalian MAPK families (ERK, p38 and JNK) and crystal structures now exist for most of these MAPK docking complexes [24–27]. Comparison of the MAPK docking interactions from yeast to humans reveals a conserved mechanism of interaction, whereby basic residues of the D-motif bind to a negatively charged surface area (CD-site) and the hydrophobic residues bind to a hydrophobic groove on the MAPK (Φ-x-Φ groove). However, there is also a second class of MAPK docking (found only in ERK signaling) whereby a so-called DEF motif with an FxFP consensus sequence binds to a separate MAPK surface [28,29,30]. Hydrogen-exchange mass spectrometry (HXMS) data have located the DEF-docking groove near the kinase active site. FxFP motif binding to ERK2 is coupled to the positioning of its activation loop; it has been demonstrated that the phosphorylated MAPK binds this docking motif better than the inactive kinase [29]. Kinase docking also occurs at other levels in the MAPK cascade. The C-terminal region of mammalian MAPKKs (which contains a so-called DVD motif) is necessary for interacting with and discriminating between various MAPKKKs [31]. There is also evidence that MAPKK–MAPKK docking occurs in yeast [32].

Similar to the domain-mediated recognition of phosphorylated peptides (e.g. SH2, PTB, 14-3-3 and FHA domains), docking motifs can also act as regulatory elements when the docking interactions are themselves phosphorylation dependent. 3-phosphoinositide-dependent kinase-1 (PDK1) interacts with several downstream AGC kinases that contain a conserved docking motif known as the PDK1-interaction fragment (PIF) [33,34].
PIF motifs, however, must be phosphorylated before they bind effectively to the PIF pocket located on PDK1 (PIF motif: FxxFS/TTF/Y, where S/T has to be phosphorylated) [35]. Therefore, downstream AGC kinase substrates must be primed by phosphorylation before interaction with and phosphorylation by PDK1. A similar priming event is required for phosphorylation of some substrates by glyco-
gen synthase kinase-3 (GSK3), which is part of the insulin signaling pathway. GSK3 substrates must be phosphory-
ated on a residue that is C terminal to the serine/threo-
nine site to be modified by GSK3. This priming phosphorylation motif binds to a phoso-recognition docking groove adjacent to the active site of GSK3 [36]. The priming phosphorylation scheme observed in the GSK3 and PDK1 pathways provides a mechanism for making signal processing dependent on catalytically dis-
tinct phosphorylation events, thereby increasing the spe-
cificity and complexity of control.

The activities of cyclin-dependent kinases (CDKs) are
governed by the cyclins and linked to phases of the cell cycle. However, cyclins not only serve as activatory sub-
units for the kinase but also may function in substrate recognition. Many CDK substrates contain an RxL or KxL
docking motif that is remote from the site of phosphoryla-
tion. Interestingly, the RxL-docking groove is located not on the kinase but on the interacting cyclin, providing a slightly different mechanism of substrate recognition, whereby the cyclin functions as an adaptor [37,38*].

Allostery versus tethering
A fundamental question is how docking motif binding influences kinase and phosphatase activity: is it simply tethering or is allosterity also important? Overall, the primary role of docking interactions appears to be tethering. However, there are a few cases in which docking interactions play an important role in altering protein conformation and allosterically regulating activity. The binding of PIF motifs to PDK1 increases kinase activity four- to six-fold [33,34]. Studies of MAP kinases, how-
ever, suggest that the main function of D-motif docking interactions is tethering the kinase to partners; the Fus3
MAPK structure, for example, changes very little upon binding to D-motif peptides from various interaction partners [39**]. In one example, however, the activity of the same MAPK is allosterically activated by a peptide whose binding site includes the docking groove for D-
motifs [40*]. A recent structure of ERK2 in complex with a D-motif also demonstrates that peptide binding to the docking groove can have long-range effects, including reordering of the kinase activation loop [27] (Figure 5). Hydrogen-deuterium exchange data have also indicated that some flexibility is induced in the activation loop of p38 upon D-motif binding [29*].

In the case of phosphatases, binding of an RVxF motif to PP1 does not change the structure of the phosphatase domain. Similarly, allosteric effects of docking peptide binding on the catalytic activity of calcineurin have not been found. The catalytic domains of these two phosphatases may be too rigid and docking interactions apparently cannot augment structural changes at the active site. Therefore, it is likely that, in these examples, the local-
ization or tethering of the effector phosphatase domain to the protein partner is sufficient to explain the physiologi-
gical roles of docking. However, in the case of calci-
neurin–NFAT binding, it is possible that the association of the phosphatase with its PxxI/G docking peptide itself is controlled by an allosteric site. This site, bound by some small-molecule inhibitors of calcineurin–
NFAT association, is separate from both the docking groove and the active site [41].

Specificity of MAP kinase docking interactions
Although docking provides a simple way to generate new connectivity in circuits relying on kinase or phosphatase activity, the recurrent use of the same surface grooves presents a new problem: how can docking surfaces encode specific information about kinase or phosphatase interactions in the context of many related peptide motifs (Figure 6)? Signaling events regulated by MAP kinase docking interactions display a remarkable degree of fine-
tuned specificity: simple sequence variation in yeast D-motifs can influence signal flow between distinct but closely related MAPKs and their interaction partners, and it appears that induced-fit recognition allows docking peptides to achieve discrimination by exploiting subtle differences in kinase flexibility [39**].

For mammalian MAPK systems, substrates are phos-
phorylated by specific subsets of MAPKs depending on the number and sequence of the docking motifs. DEF and D-motifs form a modular system in which a different arrangement of these two docking motifs on c-Jun and JunD transcription factors governs the differential response of these two ERK2 targets upon EGF stimula-
tion [30]. In another study that highlighted the restrictive and selective nature of docking interactions, discrete ERK2 docking groove mutations differentially affected the binding and inactivation of two different tyrosine phosphatases. Importantly, some of these mutations still allowed efficient phosphorylation of ERK2 by MEK1/2 (a MAPKK) [42].

Docking and drug design
The prevalence of protein kinases and phosphatases that are involved in disease has led to intensive efforts to
develop specific inhibitors for use as therapeutics. Most kinase and phosphatase inhibitors currently target active sites. However, protein interactions, as outlined earlier, also play an essential role in linking kinases and phosphatases with their signaling partners. Thus, blocking docking interactions holds promise as an alternative
strategy for selectively inhibiting kinase and/or phosphatase signaling. For example, cell-permeable peptides containing docking motifs were shown to selectively modulate MAPK and PP1 activity in vivo [43–45].

Another approach to docking-based drug design is using high-throughput experimental or computational structure-based screens to identify small chemical compounds. The existence of a specialized substrate-targeting mechanism in calcineurin–NFAT signaling enabled the identification of small-molecule inhibitors that act by interfering with phosphatase–substrate docking rather than with the calcineurin catalytic site [46**,47]. As three-dimensional structures of docking groove surfaces on kinases and phosphatases become available, structure-based screens will be increasingly practical [48]. Taking advantage of the recently identified ERK MAPK docking surface, a computer-aided drug design study identified novel small-molecule ERK inhibitors that showed a dose-dependent reduction in the proliferation of several cancer cell lines [49]. Moreover, downstream branches of ERK signaling that are based on DEF- or D-motif-mediated...
docking can be selectively inhibited without blocking total pathway activity [28*]. These studies demonstrate that interference with docking interactions is a viable alternative to the direct catalytic inhibition of kinase or phosphatase activity.

Conclusions
A growing number of substrates as well as many regulator proteins have been shown to bind kinase or phosphatase catalytic domains through docking groove interactions. Docking-mediated interactions are particularly prevalent in serine/threonine kinases. The search for new docking peptide sequences and their corresponding protein–protein interaction surfaces on kinase/phosphatase domains is an active area of research. Computational approaches, in combination with systematic experimental analysis, will aid the identification of new examples of protein–protein interactions mediated by docking. The next challenge is to discover the molecular principles behind the specificity mediated by individual docking grooves and their corresponding peptide motifs. This new knowledge will be helpful in the design of biological tools to modulate protein–protein interactions in vivo and in elucidating the specific roles of this ubiquitous class of enzymes in diverse cellular processes. Such studies will set the stage for the selective regulation of kinase/phosphos-
phatase network activity by small-molecule inhibitors, whereby the focus would be on targeting connections rather than catalytic activity.

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


This study demonstrates that linear binding motifs can be detected using data from genome-scale interaction studies. This approach greatly facilitates the discovery of short peptide motifs that mediate protein–protein association.


The complex crystal structure between PP1 and its myosin phosphatase targeting subunit (MYPT1) revealed that interactions between the RvxF motif of MYPT1 and the PP1 docking groove represent the single most important contribution to the formation of the PP1–MYPT1 complex.


In this study, a more restrictive definition of the RvxF motif is presented based on systematic protein–protein binding analysis and computational modeling. Using this more stringent motif definition, the authors correctly predict novel PP1-binding partners with great accuracy.


If co-crystallization with docking peptides fails, alternative techniques such as cross-linking and modeling studies combined with experimental validation can still be used to map the docking interaction surface, as was demonstrated in this study of the calcineurin-NFAT system.


This study shows that the activity of the ERK2 pathway can be selectively inhibited by mutation of specific docking grooves on the MAPK. Signal flow to DEF-motif-containing substrates or to D-motif-containing substrates can be blocked by mutating the corresponding interaction sites on ERK2.


The authors demonstrate the value of HXMS in tracking conformational changes of the MAPKs p38 and ERK2. Addition of DEF- and D-motif peptides leads to conformational changes that are inferred from changes of the MAPKs p38 and ERK2. Addition of DEF- and D-motif-containing substrates or to D-motif-containing substrates can be blocked by mutating the corresponding interaction sites on ERK2.

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Structural analysis reveals that the yeast Fus3 MAPK interacts with specific and promiscuous D-motif peptides using conformationally distinct modes. The study suggests that induced-fit recognition may allow docking peptides to achieve discrimination by exploiting subtle differences in kinase flexibility. This finding provides a mechanism for how D-motif-containing interaction partners can selectively bind MAPKs through a common docking groove.


