Substrate and Docking Interactions in Serine/Threonine Protein Kinases

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1. Introduction

Protein kinases have emerged as the largest family of signaling proteins in eukaryotic cells and are involved in every aspect of cellular regulation. There are over 500 protein kinases in the human genome.[1,2] The vast majority are Ser/Thr protein kinases. The Ser/Thr protein kinases interact with diverse substrates ranging from enzymes, including other kinases, to transcription factors, receptors, and other regulatory proteins. Thus, mechanisms to ensure specificity must be present. However, from emerging structural data it is becoming apparent that the ways in which protein kinases interact with their substrates local to the active site are relatively few. Instead, docking interactions, in pockets or grooves outside the active site of the kinase, are used to recognize substrates and other interacting proteins. Docking motifs in substrates bind in docking grooves within the kinase domain or adaptor protein. Docking interactions have been defined for CDKs (cyclin-dependent kinases), MAPKs (mitogen-activated protein kinases), and members of the AGC group (cAMP-dependent (PKA), cGMP-dependent (PKC)), as well as several other kinases. Further, structural data is revealing that docking interactions regulate kinase activity by unanticipated allosteric mechanisms that probably promote pathway specificity.

In this review, we outline the current structural data available on distinct Ser/Thr protein kinases. How kinases bind substrates at the active site is described, focusing on the P + 1 pocket, which is remodeled in inactive forms of several protein kinases. Substrate docking interactions, outside the active site, observed in MAP kinases, CDKs, and AGC kinases will be described. How specificity among these different families of kinases is achieved from the organization of the binding site and other factors will be discussed. Further, available data suggesting that docking interactions control kinase activity allosterically will be reviewed. Recent reviews of topics under discussion[3−10] and related topics[11−17] are available.

2. Architecture and Available Structural Data

Among eukaryotic protein kinases,[1] the Ser/Thr kinases have been classified into six large groups. These are named the AGC group, the CaMK group (for calcium−calmodulin-dependent), the CMGC group (for CDK, MAP kinase, glycogen synthase kinase, and CDK-like), the STE group (homologues of STE11 and STE20), the CK1 group (for casein kinase-1), and TKL (tyrosine kinase like). Structural data is now available for representatives of each of the well-populated groups, as well as smaller groups, such as WNKs (with no lysine),[18] revealing that the protein kinases have a common architecture.

2.1. Architecture of Protein Kinases

Protein kinases possess a two-lobe architecture that has been reviewed several times (Figure 1a).[6,7,19−21] Briefly, the N-terminal lobe is composed of a five-stranded β-sheet and a single well-conserved helix, labeled helix C based on the structure of PKA.[19] The C-terminal lobe possesses six large helices (D, E, F, G, H, and I) and two β-ribbons, β7−β8

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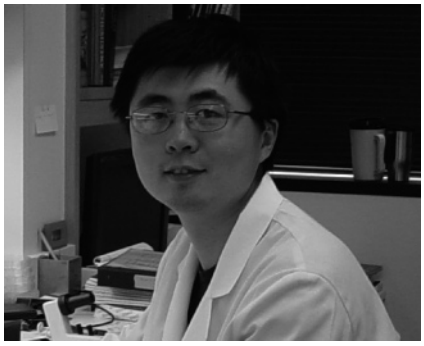

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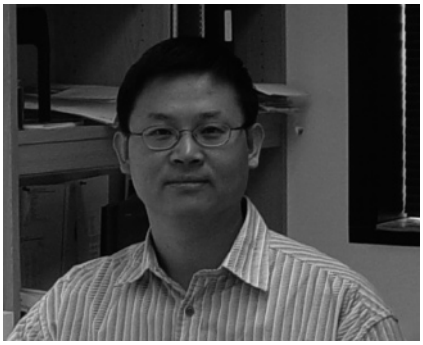
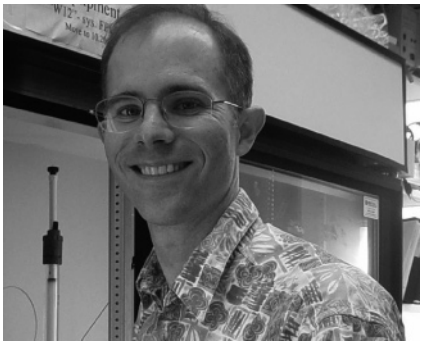


Tianjun Zhou received his Ph.D. in molecular biology from China Medical University and his postdoctoral training from UT Southwestern Medical Center. Dr. Zhou's work was focused on determining crystal structures of protein kinases in the MAP kinase pathways. He is currently employed at Ariad Pharmaceuticals, Inc., in Cambridge, Massachusetts.



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and $\beta6-\beta9$. The $\beta7-\beta8$ ribbon is present in both active and inactive protein kinases. Further, an additional β-strand interacts with $\beta7-\beta8$ forming a three-stranded β-sheet in most protein kinases, but not in PKA. The β-strand is labeled $\beta5D$ for its placement in the structure between β-strand 5 and helix D (Figure 1a). The $\beta6-\beta9$ ribbon is present only in active kinases (Figure 1a);[21] $\beta9$ is part of the activation segment. Two smaller helices, labeled P + 1 and APE (also called helix αEF)[21] in Figure 1a, are conserved in active protein kinases. The activation segment and the catalytic loop are also in the C-terminal lobe. The catalytic loop refers to a seven-residue segment (Asp166−Asn172 in PKA) that houses the catalytic aspartate (Asp166) and lysine residues (Lys168). The activation segment refers to the sequence flanked by the conserved motifs DFG (following $\beta8$; subdomain VII in the nomenclature of Hanks and Hunter[22]) and APE (subdomain VIII) (also referred to as "activation loop" or Lip). This segment is variable in size and in many kinases possesses one or more phosphorylation sites that tend to be activating.[21] The primary substrate recognition pocket, the P + 1 binding site, is adjacent to and contiguous in sequence with the activation segment (Figure 1b). Further, relatively short (~50 residue) N- and C-terminal extensions from the kinase core may pack on the core and are present for all of the Ser/Thr kinases studied crystallographically, including the smallest, CDK2.[23] Longer N- and C-terminal extensions are known to fold into a variety of separate domains (as reviewed in ref 1). Structural data for Ser/Thr kinases possessing separately folded domains (either a separate subunit or folding unit) is available for twitchin,[24] p21-activated protein kinase (PAK1),[25] CK2 (casein kinase-2),[26] G-protein-coupled receptor kinase-2 (GRK2),[27] and PKA.[28]

Protein kinases have grooves on the surface of the kinase core (Figure 1b). The grooves are a consequence of the architecture, and tend to be conserved. For example, in the structure of PKA, a groove is present between helix C and the N-terminal domain β-sheet, which is conserved in AGC

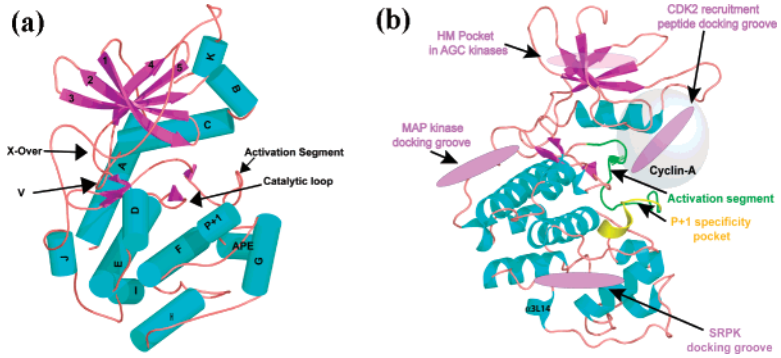

Figure 1. (a) Secondary structure of protein kinases based on PKA. Helices are cyan, β-strands magenta, and loops deep salmon. (b) The MAP kinase docking groove, the AGC hydrophobic motif (HM) pocket, and CDK2 recruitment peptide docking groove and SR docking groove are in violet. The cyclin-A binding site on CDK2 is in gray. Figures generated using PyMOL (Delano Scientific, San Carlos, CA).

kinases. The grooves serve different functions in homologous kinases, as discussed below.

2.2. Available Structural Data

Structural information is available for both active and inactive forms of at least one member of each of the major subgroups of Ser/Thr kinases[6] (Table 1). Active conformations are closely similar among all groups, such that structural signatures for active kinases have been defined.[7,23,29] The categorization of "active" versus "inactive" in Table 1 is based upon the position of a conserved threonine, discussed below, as well as other signatures. In the AGC group, PKA,[30] PDK1 (phosphoinositide-dependent protein kinase),[31] PKB,[32] and GRK2[33] have known structures. Most AGC structures are in their active conformations; however, an inactive form of PKB has been determined.[34] In the CaMK group, the structures of twitchin,[24,35] titin,[36] CaMK-I,[37] and PHK (phosphorylase kinase)[38] are available. Most of these structures are inactive conformations (Table 1), although the structure of PHK has been solved in an active, substrate-bound conformation.[39] In the CMGC group, the structures of CDK2[23] and many other CDKs (Table 1) in both inactive and active forms have also been determined.[40−42] The MAP kinases ERK2,[29,43] p38α,[44,45] JNK3,[46] and others have been solved. Both inactive and active conformations are available for rat ERK2.[29,43] In addition, glycogen synthase kinase GSK3[13] and CK2,[26,47,48] and more[49−51] of the CMGC group have been studied. In the casein kinase 1 group (CK1), CK1 from *Schizosaccharomyces pombe*[52] and rat CK1δ,[53] both in the active conformations, are available. Further, data are at hand for the CMGC group SR protein kinases (SRPKs) Sky1p[49] and SRPK1 (Table 1). In the STE family, structures are available for inactive p21-activated protein kinase (PAK1)[25] and active conformations of the MAP3K TAO2[54] and PAK1.[55] The data available in the STE group has recently doubled through the efforts of the Structural Genomics Consortium, Oxford (SGC, Table 1). In the tyrosine kinase like group (TKL), structures are available for the kinase domains of B-RAF (inactive)[56] and the TGFβ receptor.[57,58] Also, structures are available for small groups, including WNK1 and PknB.[59,60] A current comprehensive listing of protein kinase Protein Data Bank entries can be found at http://cellsignaling.lanl.gov/structure/kinase and http://www.kinasenet.org, and sequence information is available at http://www.kinase.com/.

Ser/Thr kinases have been studied structurally in complex with peptides derived from substrates and other interacting proteins (Table 2). Peptides encompassing the site of phosphorylation in substrate, which bind to the active site, have been defined structurally in relatively few kinases. These are PKA[30,61,62] and PKB[32] in the AGC group, phosphorylase kinase γ (PHK)[39] and Pim-1[12] in the CaMK group, and CDK2[63,64] in the CMGC group. The paucity of structural data may be due to poor K_m's (~0.5 mM range) of active-site directed peptides.[39,65−67] On the other hand, at this point it is usually apparent when a structure is that of an active kinase. For example, the structures of two CK1s and the STE20s TAO2 and PAK1 (Table 1) have been defined in uncomplexed but active conformations, thus providing some information about their substrate binding modes. Below we discuss interactions in the P + 1 specificity pocket.

Rather than binding substrates tightly at the active site, many kinases utilize docking interactions in grooves outside the active site to engage "docking motifs" in substrates and other molecules. Docking interactions have been defined structurally in the CMGC group and in the AGC group (Table 2, Figure 1b). The first to be published was CDK2/cyclin A in complex with a peptide derived from $p27^{Kip}$,[42] but several other cyclin A/peptide interactions have been studied.[63,67] In MAP kinases, part of the CMGC group, a docking groove is present in the C-terminal lobe, and structural data are available for p38α[68] and JNK1,[69] as well as ERK2,[70,71] and the *Saccharomyces cerevisiae* MAP kinase Fus3 in complexes with substrates[51] and other interacting proteins.[72] Further, SR protein kinases (SRPKs) are CMGC family members that phosphorylate serine/arginine-rich (SR) protein splicing factors, and a complex of SRPK1 in complex with a substrate-derived peptide has been studied.[73] In the AGC kinases, a pocket is present in the N-terminal lobe (the hydrophobic motif (HM) pocket) that is utilized by phosphoinositide-dependent protein kinase (PDK1) to bind substrates and by other AGC-group kinases for diverse purposes. Structural data are available for PDK1 lacking HM peptide[31,74] and for PKB both with[32] and without[75] HM peptide. Some of these structures have been reviewed.[4,76] In addition, data are available for Aurora kinase[77,78] and G-protein-coupled receptor kinase GRK2,[27,79,80] which also utilize the HM pocket for subunit interactions. A docking interaction has also been defined recently for the STE group kinase TAK1, in complex with its activator TAB1.[81]

3. Recognition in the P + 1 Specificity Pocket

With data available on all of the major groups of Ser/Thr protein kinases, much of the spectrum of active site substrate

Table 1. Crystal Structures of Ser/Thr Protein Kinases

group	kinase	PDB	organism	ligation state	phos state	activation state	origin of inactivity[a]	ref[b]
AGC	PKA	1ATP	mouse	MnATP and peptide	P	active		b1
		1BKX	mouse	adenosine	P	active		b2
		1CDK	pig	PKI peptide	P	inhibited		62
		1CTP	pig	inhibitor peptide	P	inhibited		29
		1FMO	mouse	PKI inhibitor and adenosine	P	inhibited		b3
		1U7E	mouse	AMPPNP, R-subunit (bovine)	P	inhibited		27
		1J3H	mouse	apo	P	open		b4
		1JLU	mouse	substrate peptide	P	active		133
		1L3R	mouse	transition state	P	active		b5
	PKB	1MRV	human	apo	U	inactive	no helix C	33
		1MRY	human	apo	U	inactive	no helix C	33
		1O6K	human	GSK3 peptide, AMPPNP	P	active		31
		1O6L	human	AMP-PNP, GSK3 peptide	P	active		31
		1GZK	human	apo	U	inactive	DFG out, Thr	75
		1GZN	human	apo	U	inactive	DFG out, Thr	75
		1GZO	human	apo	U	inactive	DFG out, Thr	b6
	PKC-ι	1ZRZ	human	BIN-1 inhibitor	P	active		b7
	PDK1	1H1W	human	ATP	P	active		28
	GRK2	1YM7	bovine	apo	U	active		29
		2BCJ	bovine	Gα ,Gβ subunits, GDP, AlF_4^-	U	active		80
	GRK6	2ACX	human	AMPPNP, Mg	U	active		79
	Aurora	2C6D	human	ADPNP	U	active		b8
		1MUO	human	apo	U	inactive	DFG out	b9
		1OL5	human	TPX-2 peptide	P	active		77
		2BFX	frog	Incenp peptide	P	active		78
	ROCK	2F2U	bovine	apo	U	active		b10
CAMK	CamK1	1A06	rat	apo	U	autoinhibited	DFG out, Thr	37
	CamK1δ	2JC6	human	inhibitor	U	inactive	Act loop, autoinhibited	*c*
	CamK1γ	2JAM	human	inhibitor	U	inactive	dimer	*c*
	ChK1	1IA8	human	apo	U	active		b11
		1ZYS	human	inhibitor	U	active		*c*
	DAPK	1IG1	human	AMPPNP, Mn	U	active		b12
		1JKS	human	Apo	U	active		b12
	DAPK2	1WMK	human	Apo	U	inactive	dimerized	*c*
	DAPK3	1YRP	human	Apo	U	inactive	dimerized	*c*
		2J90	human	JAK-Inhibitor	P	active		*c*
	Twitchin	1KOA	*C. elegans*	apo	U	autoinhibited		35
		1KOB	aplysia	apo	U	autoinhibited		b13
	MAPKAP-K2	1KWP	human	apo	U	autoinhibited		b14
		1NY3	human	ADP	U	active		b15
	PHK	1PHK	rabbit	MnATP	U	active		38
		2PHK	rabbit	phosphorylase peptide, MnATP	U	active		39
	Titin	1TKI	human	apo	U	autoinhibited		36
	MSK1	1VZO	human	apo	U	autoinhibited		b16
	Pim-1	1XR1	human	AMP-PNP	U	active		b17
		1XWS	human	inhibitor	U	active		*c*
		1YWV	human	apo	U	active		b18
		1YXT	human	AMPPNP	U	active		b18
		2BIL	human	pimtide peptide	U	active		*c*
		2BIK	human	BIM-1 inhibitor	P	active		*c*
	MARK	1ZMU	rat	apo	U	inactive	DFG out, Thr	b19
	MNK1	2HW6	human	apo	U	inactive	DFG out, Thr	b20
	MNK2	2AC3	human	apo	U	inactive	DFG out, Thr	137
	CamKII	2BDW	*C. elegans*	apo	U	autoinhibited		b21
	SNF1	2FH9	yeast	apo	U	inactive	Thr	b22
	Amp-activated	2H6D	human	apo	U	inactive	DFG out, Thr	*c*
CMGC	CDK2	1B38	human	ATP	U	inactive	Thr	40
		1B39	human	ATP	U	inactive	Thr	40
		1BUH	human	CKSHS1	U	inactive	no helix C	b23
		1CKP	human	inhibitor	U	inactive	Thr	b24
		1FIN	human	ATP	U	inactive	Act loop	b25
		1FQ1	human	KAP, ATP	P	inactive	Thr, KAP	b26
		1HCK	human	Mg ATP	U	inactive	Thr	b27
		1HCL	human	Apo	U	inactive	Thr	b28
		1JST	human	cyclin-A and ATP	P	active		42
		1PW2	human	apo	U	inactive	Thr	b29
		1H27	human	P27 peptide	P	active		63
		1H28	human	P107 peptide	P	active		63
		2CCI	human	CDC6 peptide	P	active		67

Table 1 (Continued)

group	kinase	PDB	organism	ligation state	phos state	activation state	origin of inactivity[a]	ref[b]
CMGC	CDK5	1H4L	human	fragment of p35 activator	U	active		b30
	CDK6	1BI7	human	P16INK4A	U	inactive	no helix C	b31
		1BI8	human	P19INK4D	U	inactive	no helix C	b31
		1JOW	human	viral cyclin	U	active		b32
	CDK7	1UA2	human	ATP	U	inactive	Thr	b33
	p38	1CM8	human	AMPPNP	P	active		45
		1P38	mouse	apo	U	inactive	Thr	44
		1LEW	mouse	MEF2 peptide	U	inactive	Thr	68
		1LEZ	mouse	MKK3B peptide	U	inactive	Thr	68
		1WFC	human	apo	U	inactive	Thr	b34
		1R39	human	apo	U	inactive	Thr	b35
	ERK2	1ERK	rat	apo	U	inactive	Act loop	43
		2ERK	rat	apo	P	active		28
		2GPH	rat	HePTP peptide	U	inactive	Thr	70
		2FYS	rat	MKP3 peptide	U	inactive	Thr	71
	ERK3	2I6L	human	apo	U	inactive	Thr	*c*
	JNK1	1UKH	human	JIP peptide	U	inactive	Thr	69
	JNK3	1JNK	human	ANP	U	inactive	Thr	46
	GSK3	1H8F	human	Inhibitor	U	active		13
		1O9U	human	axin peptide, inhibitor	P	active		114
		1GNG	human	FRATtide	P	active		19
	CK2	1JWH	human	apo	U	active		23
		1LP4	maize	Mg AMPPNP	U	active		b36
		1NA7	human	apo	U	active		b37
		1PJK	human	AMPPNP	U	active		b38
		1DAW	maize	MgAMPPNP	U	inactive	Thr	47
		1DAY	maize	MgGMPPNP	U	inctive	Thr	47
		1DS5	maize	AMP	U	inactive	dimerized	b39
	Sky1P	1HOW	yeast	Apo	U	active		49
		1Q8Y	yeast	ADP	U	active		b40
		1Q97	yeast	ATP	U	active		b40
	PKR	2A1A	yeast	EIF2α,	P	inactive	Thr	50
		2A19	yeast	EIF2α, AMPMPNP	P	inactive	Thr	50
	FUS3	2B9F	yeast	MgADP	U	inactive	Thr	51
		2B9J	yeast	FAR1 peptide,MgADP	U	inactive	Thr	51
		2F49	yeast	Ste5 peptide	U	inactive	Thr	72
	CLK1	1Z57	human	Hymenialdsine	U	active		*c*
	CLK3	2EU9	human	apo	U	active		*c*
		2EXE	human	apo	U	fragment		*c*
	SRPK1	1WBP	human	ASF/SF2 peptide, ADP	U	active		73
STE	PAK1	1F3M	human	apo	U	autoinhibited		24
		1YHW	human	apo K299R	U	active		55
	PAK4	2BVA	human	apo	P	active		*c*
		2J0I	human	apo	P	active		*c*
	PAK5	2F57	human	apo	P	active		*c*
	PAK6	2C30	human	apo	P	active		*c*
	MEK1	1S9J	human	MgATP, inhibitor	U	inactive	Act loop, Thr	b41
	MEK2	1S9I	human	MgATP, inhibitor	U	inactive	Act loop Thr	b41
	MEKK5	2CLQ	human	inhibitor	U	active		*c*
	TAO2	1U5Q	rat	apo	P	active		54
		1U5R	rat	Mg ATP	P	active		54
	GCN2	1ZYC	yeast	apo	U	inactive	Thr	b42
		1ZYD	yeast	ATP	U	inactive	Thr	b42
	TAK1	2EVA	human	TAB1	U	inactive	Thr, DFG out	81
	SLK	2J51	human	inhibitor	U	inactive	Thr, dimer	*c*
	SLK	2JFL	human	K0056 inhibitor	P	inactive	dimer	*c*
	STK10	2J7T	human	SU11274 inhibitor	U	inactive	dimer	*c*
CK1	CK1	2CSN	yeast	CKI7 inhibitor	U	?	Thr ?	b43
	CK1	1CSN	yeast	MgATP	U	?	Thr ?	52
	CK1γ1	2CMW	human	purine class inhibitor	U	?	?	*c*
	CK1γ2	2C47	human	inhibitor	U	inactive	Act loop, dimer	*c*
	CK1γ3	2CHL	human	inhibitor	U	?	Thr ?	*c*
	CK1γ3	2IZR	human	inhibitor	U	active ?	Thr ?	*c*
	CK1 δ	1CKI	rat	apo	U	active		b44
TKL	TGFβ TGFβ	1B6C	human	FKBP12	U	inactive	Act loop	57
		1PY5	human	inhibitor	U	inactive	Act loop	b45
	B-Raf	1UWH	human	inhibitor	U	inactive	Act loop	56
	IRAK-4	2NRY	human	staurosporine	P	active		b46

Table 1 (Concluded)

group	kinase	PDB	organism	ligation state	phos state	activation state	origin of inactivity[a]	ref[b]
other	PKnB	1MRU	bacteria	ATP-γs	U	inactive	Thr	60
		1O6Y	bacteria	MgAMPPCP	U	inactive	Thr	b47
	PknE	2H34	bacteria	apo	U	inactive	Thr, DFG-out	b48
	WNK1	1T4H	rat	apo	U	inactive	Thr, Act loop	59
	WEE1A	1X8B	human	inhibitor	U	active		b49
	STK16	2BUJ	human	staurosporine	U	?	?	*c*
	NEK2	2CL1	human	pyrrole indolinone	U	inactive	Thr, DFG-out	*c*

[a] The designations for origin of inactivity are DFG-out if the concerved DFG is displaced from the active conformation, Thr refers to displacement of Thr201 or homologous residue, and Act loop refers to other displacements of the activation loop but not Thr201. Dimer is used when dimerization has contributed to inactivity, and "?" is used where some ambiguity is present. The table is available at http://www.hhmi.swmed.edu/Labs/bg/Kinase. [b] (1) Zheng, J.; Trafny, E. A.; Knighton, D. R.; Xuong, N.-H.; Taylor, S. S.; Ten Eyck, L. F.; Sowadski, J. M. *Acta Crystallogr.* **1993**, *D49*, 362. (2) Narayana, N.; Cox, S.; Nguyen-huu, X.; Ten Eyck, L. F.; Taylor, S. S. *Structure* **1997**, *5*, 921. (3) Narayana, N.; Cox, S.; Shaltiel, S.; Taylor, S. S.; Xuong, N. *Biochemistry* **1997**, *36*, 4438. (4) Akamine, P.; Madhusudan; Wu, J.; Xuong, N. H.; Ten Eyck, L. F.; Taylor, S. S. *J. Mol. Biol.* **2003**, *327*, 159. (5) Madhusudan; Akamine, P.; Xuong, N. H.; Taylor, S. S. *Nat. Struct. 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recognition modes can be defined. This is especially tractable for the P + 1 specificity pocket. It is the only pocket constructed to accept a single amino acid from substrate and is a frequent specificity determinant.[14,82,83] Further, the P + 1 pocket is a regulatory site and is often refolded in inactive forms of protein kinases.[21] Here we review the three major P + 1 pocket recognition modes in Ser/Thr protein kinases. This site is the locus of specificity determination in tyrosine kinases as well[50,84] and binds the P0 tyrosine.

The P + 1 binding pocket of all Ser/Thr protein kinases adopts a similar architecture with respect to the backbone of the polypeptide, as typified by PKA (Figure 2a).[7,85] In PKA, Leu198−Leu205 residues directly following the activation segment phosphorylation site form the pocket. The salient features are a helical turn (from Thr201 to Leu205) that forms one wall of the pocket, the residues preceding the helix, which form the upper rim of the pocket, and a hydrophobic residue, Leu205 at the bottom. Thr201, a catalytic residue, faces the active site (discussed below). Residues on the outside of the helix, Glu203 and Tyr204, face the interior of the protein. Following this structure is the APE helix (Table 3, Figures 1a and 2b−e).

3.1. AGC, CAMK, and STE Kinases

The P + 1 sites of AGC kinase substrates are known to be hydrophobic.[83] The structure of PKA[61] revealed hydrophobic residues (Pro202, Leu198, and Leu205) lining the P + 1 pocket in the sequence ^{198}LCGTPEYL^{205} (Table 3).

Table 2. Crystal Structures of Protein Kinases in Complex with Substrate and Other Peptides

group	kinase	PDB	complex	refs
AGC	PKA	1ATP	PKI peptide	27, 61
		1CDK	PKI peptide	62
	PKB	1O6K	GSK3 peptide	31
	Aurora	1OL5	TPX-2 peptide	77
	Aurora	2BFX	Incenp peptide	78
CAMK	PHK	2PHK	phospohorylase peptide	39
	Pim-1	2BIL	pimtide peptide	to be published
CMGC	CDK2	1JST	Cyclin-A	42
		1H28	P107 peptide	63
		1H27	P27 peptide	63
		2CCI	CDC6 peptide	67
	P38	1LEW	MEF2A peptide	68
		1LEZ	MKK3b peptide	68
	ERK2	2FYS	MKP-3 peptide	71
		2GPH	HePTP peptide	70
	JNK1	1UKH	JIP peptide	69
	GSK3	1GNG	FRATtide	17
		1O9U	axin peptide	114
	FUS3	2B9J	FAR1 peptide	51
		2F49	Ste5 peptide	72
other	PKR	2A1A	eIF2	50

The substrate is recognized also by Gly200, which adopts a left-hand configuration ($\phi = 147°$, $\psi = 157°$; Figure 2a) such that the carbonyl of Gly200 points up and can accept a hydrogen bond from the substrate (Figure 2b). Tyr247 at the beginning of helix G also contributes to the pocket. Similar interactions are observed in PKB.[32] As can be seen in Figure 2b, the P + 1 pocket is situated adjacent to active site residues, such as Asp166 and Lys168, and is contiguous with the activation segment and phosphothreonine, pThr197.[86]

Two other major groups of protein kinases are hydrophobic-directed in the P + 1 site, the CaMK group and the STE group. Two structures are available of members of the CaMK group, phosphorylase kinase γ (PHK),[7,39] and Pim-1.[12] The structure of PHK, in complex with a heptapeptide possessing a phenylalanine at the P + 1 site (Figure 2c), reveals similarities to PKA. PHK has the glycine (Gly185), proline (Pro187), and other hydrophobic residues that are hallmarks of hydrophobic-directed kinases including Leu190 at the bottom of the pocket (Table 3). Pim-1 offers a slight variation, with a specificity for alanine at P + 1. The structure of Pim-1 kinase in complex with a substrate-derived peptide reveals the glycine (Gly203 in Pim-1) accepting a hydrogen bond from substrate, as in PKA. However, the proline is

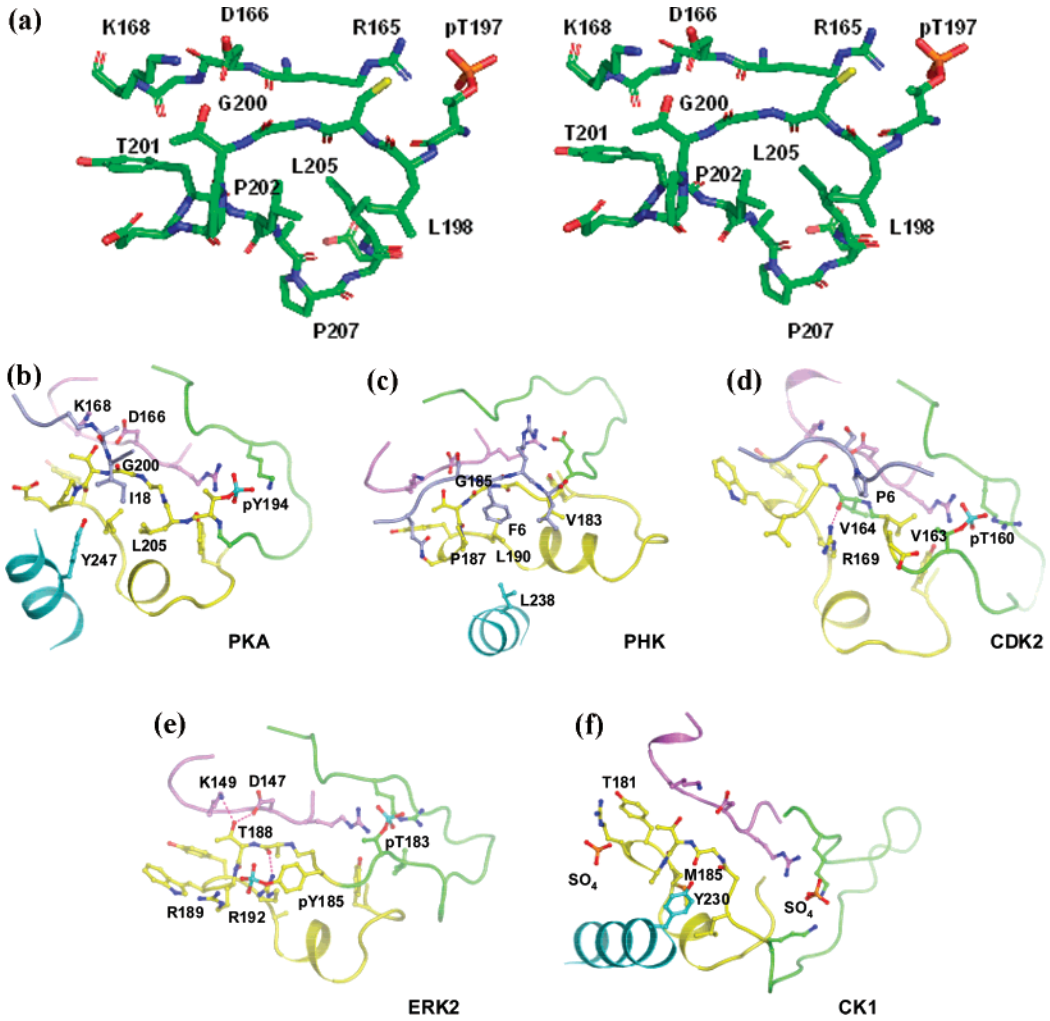
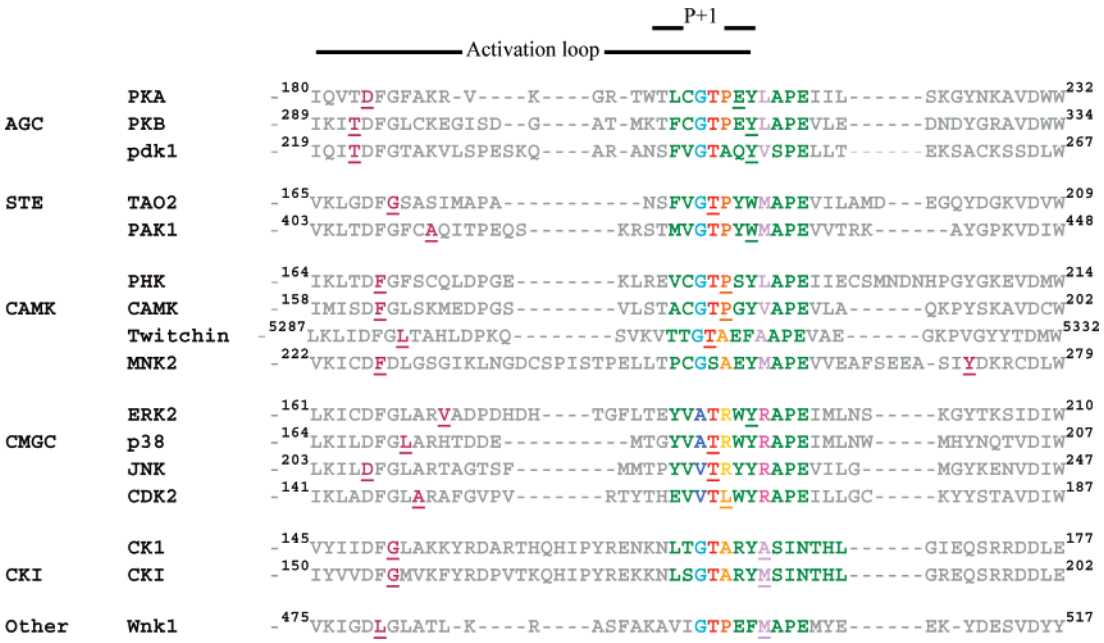


Figure 2. The P + 1 specificity pocket of Ser/Thr protein kinases: (a) all atom stereoview of the P + 1 specificity pocket of PKA (PDB file 1ATP), carbon in green, oxygen in red, nitrogen in blue, phosphorus in orange, and sulfur in yellow; P + 1 specificity pockets of (b) PKA (PDB file 1ATP), (c) PHK (2PHK), (d) CDK2 (1QMZ), (e) ERK2 (2ERK), and (f) CK1 (1CSN). In panels b−f, carbon atoms of the bound peptide are light blue, the P + 1 pocket and APE helix are yellow, the activation segment is green, the catalytic loop is magenta, and helix G is cyan. In panels b−f, only the P + 1 pocket, close by catalytic residues, and the activation segment phosphorylation site are shown rendered in ball-and-stick representation. Hydrogen bonds are shown as red dashed lines.

Table 3. Activation Loop and P + 1 Sites of Ser/Thr Protein Kinases[a]



[a] Sequences between the underlined residues are remodeled in inactive structures. Residues in the P + 1 pocket and APE helix are green, the conserved threonine is red, the conserved glycine is cyan and its replacement in CMGC kinases dark blue, and the conserved hydrophobic residue at the bottom of the P + 1 pocket is lavender and its replacement in CMGC kinases magenta.

replaced by alanine, and the P + 1 pocket is filled with the side chain of Phe201 in the activation segment, reducing the space for substrate (not shown). CaMK and AGC kinases have been shown to be especially poor enzymes toward substrates that have proline in the P + 1 site.[86]

STE group kinases are also directed toward substrates with a hydrophobic residue in the P + 1 site. This is apparent from the sequences of known substrates and from screening for kinase activity with peptide libraries.[82] The STE group is named for several of the proteins that produce sterile phenotypes in yeast,[87] STE11, STE7, and STE20.[1,88] STE11 and STE7 are STE kinases involved in three-tiered kinase signaling modules, the MAP kinase cascades (reviewed in refs 89−93). Each MAP kinase cascade activates a specific MAP kinase (which are CMGC kinases, discussed below). STE11s are MAP3Ks (or MEKK (for MAP/ERK kinase kinase), phosphorylating MAP2Ks), and STE7s are MAP2Ks (or MEKs (for MAP/ERK kinase) that phoshorylate MAPKs). STE20 phosphorylates and activates STE11 and is thus referred to as a MAP4K.[94] Close STE20 homologs such as PAK1 are putative MAP4Ks.[88,90,95,96] MAP3Ks (including STE11) phosphorylate MAP2Ks on two residues in their activation segments.[97] The MAP2K MEK2 has the sequence DS*MANS*F,[97] and both of the phosphorylation sites are followed by a hydrophobic residue. Similarly, the MAP2Ks MEK 3 and 6, which are phosphorylated, for example, by the MAP3K TAO2,[98] have the phosphorylation motif DS*VAKT*I. MAP4Ks phosphorylate MAP3Ks on a Ser/Thr followed by a hydrophobic residue (in TAO2, the sequence is PANS*F). Other MAP3Ks have related sequences. Although structures of STE group kinase substrate complexes are not available, the structures of the STE kinases TAO2[54] and PAK1[55] have been solved in active conformations. The P + 1 pockets of these proteins resemble that of PKA. In TAO2, for example, the pocket is lined by Phe182, Gly184, Pro186, Met189, and Leu234, all having homologous counterparts in PKA. On the other hand, the MAP2Ks (and STE7s), which are members of the STE group, exhibit dual specificity[99] toward Ser/Thr and tyrosine[100,101] and thus must have unique interactions in the P + 1 pocket. The mechanism for this dual specificity has yet to be elucidated.

In addition to the similarities in P + 1 site recognition discussed above, AGC, CaMK, and STE kinases have been shown recently to have common recognition modes for residues N-terminal to the P0 site of substrates.[102]

3.2. CMGC Kinases

Either the specificity of CMGC kinases in the P + 1 site is for proline, or the P + 1 site is not a strong specificity determinant. CDKs and the MAP kinases are proline directed at the P + 1 site of substrates.[14] In the CMGC kinases GSK3, CK2, and SR kinases, the P + 1 site is not a strong specificity determinant. The structure of CDK2/cyclin A in complex with substrate peptide reveals how the P + 1 pocket is arranged to bind proline in CDK2.[64] The P + 1 pocket is formed by the sequence ^{163}VVTLWYR^{169} (Table 3). The backbone of the polypeptide forms a helical turn, as in PKA (Figure 2d). However, the P + 1 pocket is filled by the side chain of Arg171 (Leu205 in PKA), so there is no room for a side chain from substrate. Further, Gly200 of PKA is replaced by Val163. Val163 also adopts a left-hand configuration, and the carbonyl accepts a hydrogen bond from Arg171, creating a flat surface with no potential for hydrogen bonding with substrate. Since the proline in substrate has no hydrogen-bonding potential, this feature probably contributes to the proline specificity of CDK2.[10,29,103]

MAPKs are CMGC kinases that phosphorylate microtubule-associated protein-2 (MAP-2) kinase in response to insulin[104] and were cloned[105] based on its activity as an S6-kinase, and the regulation of these proteins by MAP kinase has been extensively studied.[89,90,106] MAP kinases are also

proline-directed.[65,107,108] The phosphotyrosine of active ERK2 is in the P + 1 site (Figure 2e).[100] From the structure of active ERK2[29] and by homology with CDK2, it appears that the proline residue of substrate may interact directly with pTyr185, although no structural data is available for a MAP kinase with substrate bound in the active site. pTyr185 binds to Arg192, which extends out from the bottom of the P + 1 pocket, and with Arg189, which is on the edge of the pocket (Figure 2e). Phosphotyrosine 185 is required for ERK2 activity and cannot be replaced by other amino acids.[109] This may be partly explained by the remodeling of the activation segment induced by pTyr185.[29] The structure of doubly phosphorylated p38γ,[45] another MAP kinase, is similar. (The active site of ERK2 is reviewed in ref 10.)

Glycogen synthase kinase-3 (GSK3) is another CMGC group member.[110] GSK3 has recently become the object of intense study due to its involvement in pattern formation (reviewed in ref 111), neurodegeneration,[112] and insulin signaling and is a drug target for diabetes, Alzheimer's disease, and cancer.[113] Structures of GSK3 are available[13,17,114−116] and have been reviewed.[4,10] Its substrate specificity[110,115,117] and its pathway involvement[117] have also been reviewed. Many substrates of GSK3 require substrate "priming" (prior phosphorylation) at the P + 4 site of substrate,[115] and the importance of the P + 1 site appears to be diminished. The structures of GSK3 were surprising though, because GSK3 is phosphorylated on Tyr216 in the activation segment and resembles ERK2, which is proline-directed. Quite recent studies show that GSK3 phosphorylates a *bone fide* substrate, LDL-related protein-6, at positions containing proline in the P + 1 site and does not require priming.[118] This result is consistent with the similarity of GSK3 and ERK2.

SR protein kinases (SRPKs) are CMGC family members that phosphorylate serine/arginine-rich (SR) protein splicing factors[119] processively.[120] The SRPKs 1 and 2 are specific for sequences in substrates containing arginine in the P + 1 position but can accept proline,[121,122] whereas the SR kinase Sky1p[123,124] is proline directed. The structures of SRPK1[73] and Sky1p[49] have been determined. Although no active site complex is available, the structures are very similar to ERK2, with two arginines in positions homologous to Arg189 and Arg192 of ERK2 (Figure 2e). The phosphotyrosine of ERK2 is replaced by an aspartic acid in SRPK1, which may contribute to the specificity for arginine. The homologous residue in the proline-directed Sky1p is serine. Thus the SR proteins are similar to GSK3, discussed above, and can accept both proline and other residues in the P + 1 site. The SR kinases are interesting also because they build a P + 1 pocket very similar to other kinases but do not rely on activation segment phosphorlation.[21,49]

Casein kinase 2s (CK2s or CKIIs) are a subgroup of CMGC kinases for which the P + 1 site of substrates is not a strong specificity determinant but sometimes has specificity for negatively charged residues. CK2s have hundreds of putative substrates.[125] The physiological substrates and the pathways in which CK2s function are currently being defined.[126] Studies based on peptide libraries show that the P + 1 site of substrate may be proline, aspartic acid, glutamic acid, or serine.[127,128] Although no structural data is available for CK2 in complex with substrates, based on the uncomplexed structure of CK2 CKIIα,[26,47] it has been proposed[10] that a lysine residue (Lys198 in CKIIα), which replaces the Arg191 of ERK2, (Figure 2e) may explain the specificity for acidic residues.

3.3. CK1 Kinases

The CK1s (casein kinase 1) are Ser/Thr protein kinases involved in DNA repair, vesicle trafficking, cell cycle progression, and WNT signaling[129] and are common in *Caenorhabditis elegans*.[130] The P + 1 site is not a strong specificity determinant. Instead, as with GSK3, substrate priming at sites C-terminal to P0,[131,132] as well as an acidic patch N-terminal to the phosphorylation site,[14] dominates interactions. Sequences of CK1s are similar to each other but diverge from other protein kinases both at the beginning and at the end of the P + 1 pocket (Table 3). Although no substrate complex is available for CK1 family members, active conformations of two CK1s have been determined. The structure of CK1 from *S. pombe*[52] is in an active conformation in the P + 1 site (discussed below). The P + 1 site looks quite similar to PKA, except that a methionine corresponding to Leu205 of PKA completely fills the pocket, leaving a shallow depression (Figure 2f). In CK1 of *S. pombe*,[52] Thr181 in the activation segment and Tyr230 in helix G occlude the pocket (Figure 2f). In both of these CK1s, the unusual sequence SINTH following the P + 1 binding pocket mediates different interactions with helix I.

3.4. The Active Site Residue Thr201 and Remodeling of the P + 1 Pocket

Thr201 (PKA numbering) is on the upper rim of the P + 1 pocket. It is highly conserved, although replaced by Ser in CK2s. Thr201 forms hydrogen bonding interactions with the catalytic aspartate in Ser/Thr protein kinases (Asp166) and a catalytic lysine (Lys168) (Figures 2b).[7,10,85,133] Mutation of this residue abolishes catalytic activity toward peptide substrates and autophosphorylation.[85] Phosphorylation of this residue inhibits the kinase MARK/PAR-1.[134]

The placement of this conserved threonine residue is affected by activation segment phosphorylation. Activation segment phosphorylation induces conformational changes[7,29,135] that can extend from the DFG at the N-terminus[7,135,136] through the P + 1 specificity pocket at the C-terminus of the loop (the extent of remodeling is indicated in Table 3). In many kinases, the remodeling includes Thr201 (or the homologous residue). Thus the placement of Thr201 is one hallmark of the active conformation.

Several protein kinases are massively remodeled in the P + 1 site (Table 3) in inactive versus active structures. The STE20 kinase PAK1, the kinase WNK1, and the CaMK group kinase MNK-1 exhibit the greatest extent of remodeling (Table 3). In the STE20 PAK1, remodeling involves most of the P + 1 site helix, extending to Trp430 into the motif ^{429}YWMAPE^{434}.[25,54] Tyr429 and Trp430 are involved in an elaborate conformational switch between inactive and active PAK1.[25,54,55] This motif is conserved in most STE20 homologs,[54] and thus similar activation mechanisms may be present in other STE group kinases. A similar degree of remodeling is present in the low activity form of WNK1.[59] The CaMK group kinase MAP kinase interacting kinase (MNK-1) is remodeled from the DFG motif at the beginning of the activation segment through the beginning of helix F.[137] In several cases, inactive forms involve conformational changes in Thr201 (or the homologous residue). Thus, remodeling in this region affects both substrate binding and

catalytic activity. It is interesting that remodeling of the P + 1 pocket has been observed in at least one member of each of the major groups of kinases (Table 3).

To summarize the discussion of the P + 1 pocket recognition and remodeling, first, it is becoming clear that there are only a few binding modes for the P + 1 residue in substrates of Ser/Thr protein kinases. Second, the recognition modes tend to be maintained within each group. The AGC, CaMK, and STE groups recognize hydrophobic residues in the P + 1 site. Many members of the CMGC group are proline-directed. Otherwise, the P + 1 site is not a strong determinant, and the P + 1 pocket has been reduced to a surface depression. Third, it is interesting that the P + 1 specificity can be overridden. Examples include CK2 and CK1, each of which recognizes "primed" substrates. Nevertheless, the default P + 1 specificity is also used for some substrates. Fourth, structural data on uncomplexed kinases support observed P + 1 site preferences, even when structures of complexes are not available. Fifth, the P + 1 pocket is remodeled in inactive forms of at least one member of each group of protein kinases. Finally, the fact that the catalytic residue Thr201 is remodeled, along with other residues in the P + 1 pocket, implies that inactive forms are deficient in both substrate recognition and catalysis.

4. Docking Interactions

One solution to conferring specificity in the very large protein kinase family is the evolution of new binding sites for substrates and other interacting proteins. Binding sites outside the active site are referred to as docking grooves and occur on the surface of the kinase domain or in adaptor proteins. These grooves interact with contiguous peptide sequences in substrates referred to as docking motifs or docking sites. Here we will focus on five of these interactions, those of the MAP kinase ERK2, PKA, PKB, CDK2/cyclinA/p107, and SR protein kinases. Mechanisms of specificity determination will be compared. The effect of docking interactions on substrate affinity and the data suggestive that allosteric conformational changes contribute to pathway specificity will be discussed.

4.1. Docking Interactions in MAP Kinases

Twelve years ago, it was discovered that the MAP kinase c-Jun kinase (JNK) binds its substrates outside the active site, engaging substrate sequences distal from the site of phosphorylation.[138,139] Since then, a large body of data has accumulated showing that all MAP kinases studied use docking interactions to bind substrates. Further, the MAP kinases engage their activating kinases, phosphatases, and scaffolding proteins using similar docking interactions (reviewed in refs 4 and 140−147). The "D-motif" docking site, present in MAP kinase interacting proteins, is best studied. D-motif peptide complexes with target MAP kinases have been the object of six recent crystallographic studies (Table 2). The structures span the spectrum of MAP kinase binding partners. Structures were determined of p38α in complex with substrate and activating enzyme-derived peptides,[68] of JNK1 in complex with a scaffold-derived peptide,[69] of *S. cerevisiae* MAPK Fus3 in complex with substrate,[51] MAP2K,[51] and scaffold-derived[72] peptides, and of ERK2 in complex with MAP2K-derived[70] and two different phosphatase-derived peptides.[70,71] Each peptide binds to homologous loci. In this review, we focus on ERK2 in complex with a peptide derived from hematopoetic protein tyrosine phosphatase (HePTP), the structure of which we determined recently, which revealed the most extensive interactions seen to date. The structural data show that D-motif docking interactions contribute to specificity not only through unique interactions in the docking groove but through allosteric effects that remodel the activation segment and active site of the MAP kinase.

D-motifs directed toward MAP kinases encompass ~13−16 residues, $X\O_H XX(R/K)_2-X_{2-4}-\O_A X\O_B$, featuring a separation of charged (R/K) and hydrophobic elements (Ø). The docking groove for D-motifs in MAP kinases was defined by mutational analyses.[143,148−150] The docking groove was found to be in the C-terminal lobe of the kinase, near the $\beta_7-\beta_8$ reverse turn (Figure 3a) and an acidic patch in L16 (the linker at the C-terminus of the kinase lobe[43]) labeled the "CD" for common docking domain.[143] In the ERK2/pepHePTP complex, the 16-mer peptide from HePTP phosphatase forms an α-helix over the first six residues and then is extended.[70] The helix binds in a shallow depression formed where the CD domain in L16 binds atop helix F. The complex reveals the importance of the $\O_H$: it gives the helix a hydrophobic face. The helix orients the basic residues (R20′and R21′) in the peptide toward the CD domain forming an elaborate network of ionic hydrogen bonds. The extended segment of pepHePTP, including the $\O_A X\O_B$ motif, is laced perpendicularly over two helices, D and E, and under the $\beta7-\beta8$ reverse turn. The backbone of the peptide is recognized through hydrogen bonds to Gln117 and His123 (in the loop between helices D and E of ERK2). The $\O_A X\O_B$ motif contacts residues in the two helices $\O_A$ to helix E and $\O_B$ to helix D. There is considerable heterogeneity among the MAP kinases in their interactions with D-motifs, both with the CD domain and the hydrophobic docking groove,[51,70] which doubtless contributes to specificity among MAP kinases. The binding site that forms the MAP kinase docking groove is present in many protein kinases and is often utilized to bind the kinase C-terminal tail.[4,54]

4.2. HM Motifs in AGC Kinases and AGC Kinase Substrates

In the AGC group, a group-specific pocket called the HM pocket for (hydrophobic motif binding pocket) in the N-terminal lobe is formed by helix B, which prevents helix C from packing tightly on the core β-sheet (Figures 1b and 3b). This pocket serves different purposes in different AGC kinases.[3,4] In PKA, the C-terminal sequence FTEF-COOH binds in the pocket (Figure 3b). In other AGC kinases, such as PKB, the kinase C-terminal tail possesses a hydrophobic motif (HM) FXXF-S/T-F/Y, and must be phosphorylated (or replaced with a phosphorylation site mimetic) to bind to the pocket.[151] The AGC kinase phosphoinositide-dependent protein kinase (PDK1) lacks the hydrophobic motif altogether. Instead, the HM is present in PDK1 substrates. The binding of substrates both activates[152,153] and stabilizes[74,154] PDK1. This mechanism is potentially very powerful at conferring specificity, since PDK1 is not active in the absence of its substrate.

Structural data illuminating how HM peptides may interact with AGC kinases came first from the structure of PKA (Figure 3b).[155] The four residues Phe347−Phe350 form a hairpin turn that brings the two phenylalanine residues in close proximity. Phe347 packs against a valine in helix B, and Phe350 contacts the aliphatic part of the side chains of

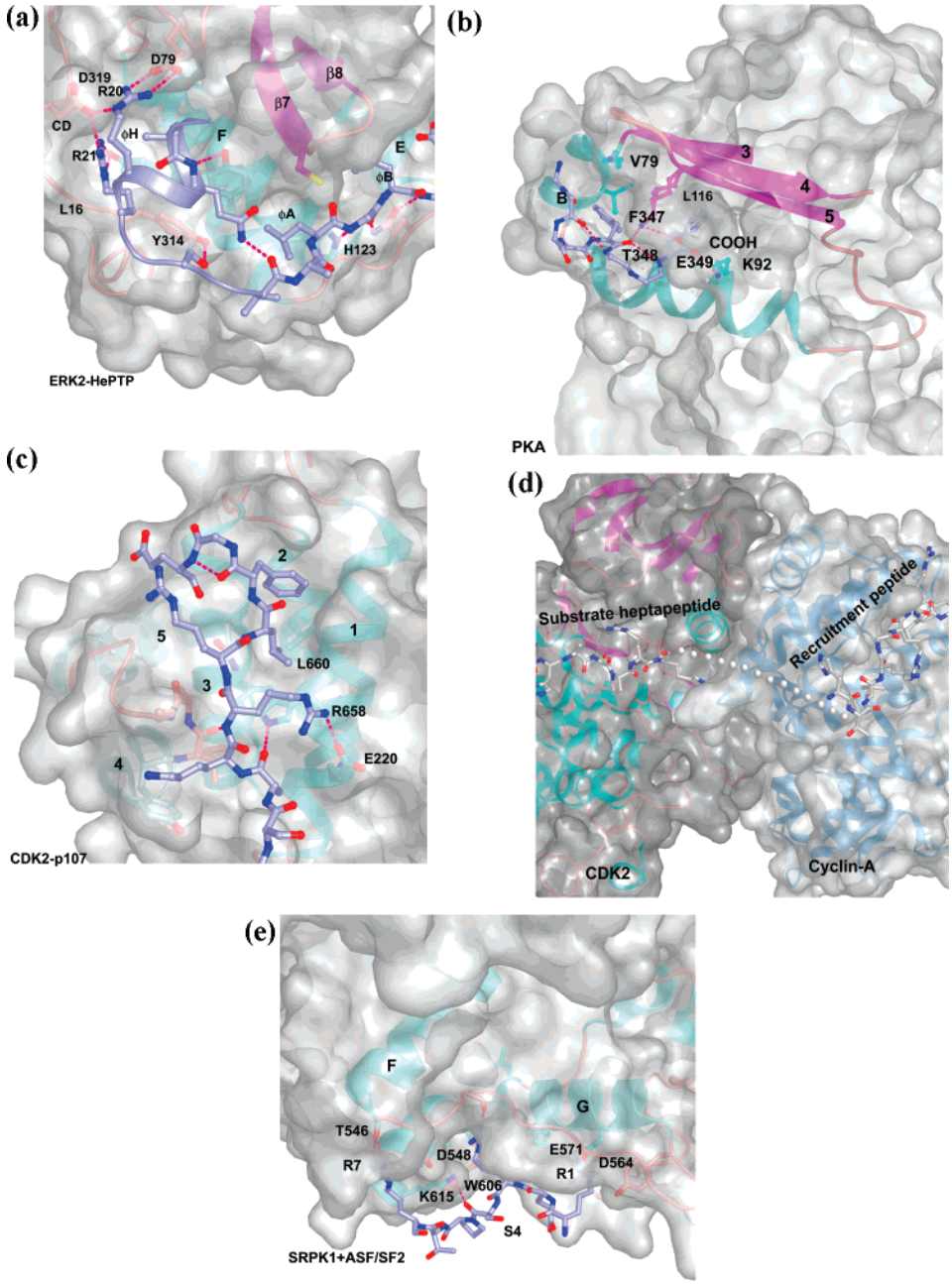


Figure 3. Docking interactions between kinase and corresponding peptides with the kinase surface rendered in gray; otherwise the coloring scheme is the same as in Figure 1: (a) ERK2 and docking peptide HePTP (2GPH). Carbon atoms of the peptide are blue. Note how the peptide helix orients the Arg20′ and Arg21′ toward the ERK2 acidic patch. (b) PKA (1ATP). The C-terminus of PKA in blue binds in the kinase HM pocket. Note how the reverse turn (at Thr348−Glu349) brings Phe347 and Phe350 close together. The side chain of Thr348, which caps the helical turn, has been eliminated for clarity. (c) CDK2 with recruitment peptide p107 (1H28). Peptide, shown in blue, is bound in the hydrophobic crevice formed by kinase helices 1 and 3. (d) Surface representation of pCDK2/cyclin-A with CDC6 bis-substrate inhibitor spanning active site and recruitment site. Visible residues shown in stick representation; disordered residues indicated by white balls. (e) SRPK1 with docking peptide ASF/SF2 in blue (1WBP) showing arginine-mediated interactions.

two lysine residues, one in helix C (Lys92) and one extending β4 (Lys111). The terminal carboxylate of PKA is also bound by the same two lysine residues. The backbone of the hairpin turn is contacted by Lys111. Thus, there is an exquisite interaction, requiring two phenylalanine residues separated by two intervening residues, accompanied by a negative charge adjacent to the second phenylalanine. Structural data on PKB HM interactions were obtained by mutating the C-terminal phosphorylation site to aspartic acid (S474D) and by making a chimera with another HM.[75] The interactions are similar but more extensive (not shown). Other AGC kinase−HM interactions have been studied structurally. The G-protein-coupled receptor kinase GRK2 uses this site intramolecularly to bind the RGS-homology domain,[27,79,80] whereas Aurora kinase uses the same groove to bind interacting proteins.[77,78] Finally, the TKL family enzyme TGFβ receptor (transforming growth factor receptor-β) also uses this groove to bind the interacting protein FKBP12.[58]

4.3. CDK2/Cyclin A Recruitment Peptide Interactions

CDKs bind substrates and inhibitors through cyclin-mediated interactions (reviewed in refs 155 and 156). The

Table 4. Docking Peptide Motifs

a) Docking site peptides for MAPK

HePTP	RLQERRGSNVALMLDV
ELK1	PQKGRKPRDLELPLSP
MEK2	MKARRKPVLPALTINP

b) Recruitment Peptide Motifs for CDKs

p27	KPSACRNLFGP
p53	STSRHKKLMFK
p107	AGSAKRRLFGE
pRB	PPKPLKKLRFD

c) Hydrophobic Motifs for AGC kinases

PKA	EKCGKE-FTEF
PKB	DSERRPHFPQFSYSASTA
PRK2	EEEQEM-FRDFDYIADWC

cyclin inhibitor p21 binds cyclin A via a recruitment peptide sequence, ACRRLFGP, and similar sequences are present in related inhibitors.[158] Substrates of CDK2 such as the tumor suppressors Rb and p107 and the transcription factor E2F also possess related recruitment sequences.[159] Only a short motif, RXLΦ, where the hydrophobic amino acid Φ is either adjacent to RXL or displaced by one residue and is conserved (Table 4). Several complexes of CDK2/cyclin A with inhibitor or substrate-derived recruitment peptides have been studied structurally (Table 2) and are also well-illustrated.[41,63] We focus on the structure the of CDK2/cyclin A/p107 peptide (Figure 3c) as an example.[63] This has two domains, the first of which binds recruitment peptides. The recruitment peptide binding domain is comprised of five helices, the first three of which resemble the first three helices of an antiparallel four-helix bundle.[160]

Two short helices pack orthogonally on one of the major helices, helix 3, such that the loop connecting them falls near the intersection of helices 1 and 3. The peptide binding groove is a shallow V-shaped hydrophobic crevice between helices 1 and 3 and the helix 4−helix 5 loop. The recruitment peptide adopts a primarily extended conformation. The conserved leucine in the motif RXLΦ is defined as P_0 by Lowe et al.[63] The leucine, as well as the Φ residue in the P_1 position, binds in the crevice between helices 1 and 3. The conserved arginine (P_{-2}) lies across the top of helix 1 and forms an ion pair with Glu220 in helix 1. Interactions with the peptide backbone are made by a glutamine in helix 3 (Gln254) and the backbone of a residue in the loop between helix 4 and helix 5 (Ile281). This simple binding groove, then, confers recognition of a positive charge separated by one residue from two hydrophobic amino acids.

The recruitment site in cyclin A is about 35 Å away from the catalytic site, and the mechanism for enhancing substrate phosphorylation was originally proposed to raise the local concentration of the protein substrate.[161] A recent elegant study[67] utilizing an ATP-mimetic bisubstrate inhibitor[162] has allowed a 30-residue peptide spanning from the active site to the recruitment site to be visualized. Indeed, several residues between the recruitment site and active site are disordered, validating the concept that recruitment interactions increase the local concentration of substrate. The authors also show that improvement in catalytic efficiency is primarily a K_m effect, which goes down 18-fold for substrate with RXL-containing substrates (see also ref 163). No conformational changes are induced in CDK2/cyclin A by the interaction with recruitment peptide[63] or bisubstrate peptide.[67]

4.4. SRPK Docking Interactions

SRPKs phosphorylate subsets of available serine phosphorylation sites within the target "RS" domains of substrates, and the exact position of phosphorylation is thought to direct the outcome of alternative splicing mediated by the SR proteins.[73,122] Ngo et al.[73] have determined the structure of a truncation of SRPK1 in the presence of substrate-derived peptide (RERSPTR) and found that the peptide binds in a docking groove, rather than at the active site. They further demonstrate the presence of this docking site in one of its substrates, the splicing factor ASF. The docking interaction appears to sequester phosphorylation sites as well as limit the phosphorylation at other sites.[73] The docking groove is formed where an insert in the connection between helices F and G unique to the SR kinases causes helix G and a two turn helix in the MAP kinase insertion (labeled $\alpha 3_{L14}$[43] (Figures 1b and 3e)) to be farther separated than in MAP kinases. Arg1 of the peptide (Figure 3e) is recognized by two residues, Asp564 and Glu571 in helix G, separated by two turns of helix. Arg3 contacts two carbonyls in the helix F/helix G connection, and Arg7 contacts the backbone of Tyr181 at the terminus of helix D and Thr546 in the helix F/G connection. The peptide backbone makes only a single contact, Lys615 at the end of helix G. The only hydrophobic interactions are with the aliphatic portion of Arg3, representing a major departure from the themes observed in other kinases. Peptide interactions do not induce any conformational changes in SRPK1 (see 1WAK.pdb). The recent structure of STE group kinase TAK1, in complex with its activator TAB1, reveals a similar locus of interactions to ASF/SF2, but the binding site is more extensive.[81]

4.5. Similarities and Differences in Docking Interactions

The docking interactions described above have some interesting parallels. First, each peptide primarily adopts an extended conformation but may have some internal hydrogen bonding. This hydrogen bonding can be significant, such as the small helix in HePTP bound to ERK2, or just a few hydrogen bonds, as in p107 bound to CDK2/cyclin A. Second, the backbone of the peptide is recognized in each case. In ERK2 and other MAP kinases, a glutamine or a glutamine and histidine make hydrogen bonds with the peptide backbone. In cyclin/p107, a histidine residue as well as the backbone of the helix 4−helix 5 loop contact the peptide. In PKA, a lysine residue (Lys111) contacts the peptide backbone. Similarly, in SRPK1, a lysine contacts the peptide backbone. A third similarity, among ERK2, PKA, and CDK2/cylcin A, is the presence of binding sites for two or more hydrophobic residues. Finally, the binding grooves invariably involve two or more helices, as well as a loop or additional secondary structure.

There are also interesting differences, which surely confer specificity among these groups of kinases. Among the groups that bind hydrophobic residues, the docking grooves are organized to bind residues separated by a different spacing:

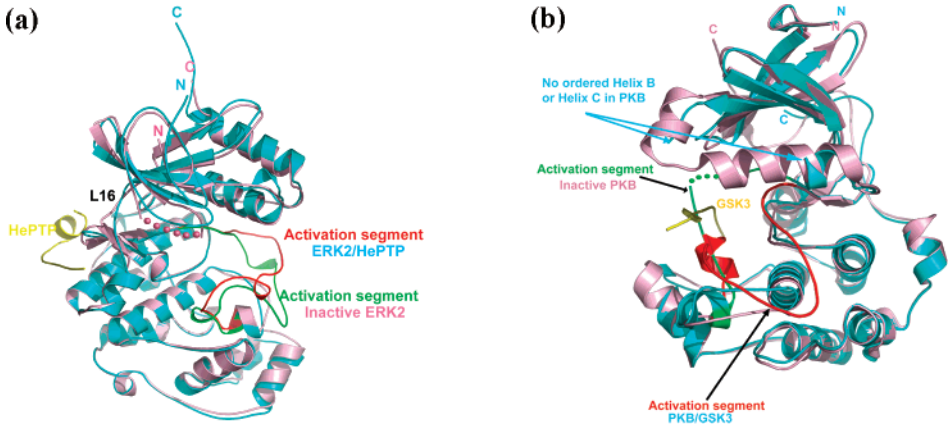


Figure 4. Peptide-induced allostery in MAP kinases and AGC kinases: (a) ERK2/pepHePTP (2GPH) superimposed with unphosphorylated unliganded ERK2 (1ERK). Note the very large changes in the activation segment and changes in the N-terminus. (b) PKB/GSK3 peptide (1O6K) superimposed with unphosphorylated PKB (1MRY). Helices B and C, as well as the activation segment, become ordered in peptide-bound PKB. Unliganded conformations are rendered in cyan and activation segment in green. Peptide-bound structures are pink, activation segment red, and peptide yellow.

Cyclins bind adjacent hydrophobic residues or residues separated by one amino acid in a depression formed by two parallel helices in contact that form the docking groove. MAP kinases recognize hydrophobic residues separated by one amino acid, through well-separated pockets on two different helices. A glutamine residue contacts the peptide backbone between the two hydrophobic residues. AGC kinases bind HM peptides that possess hydrophobes separated by two amino acids. The binding pockets are deep and close together, and an intricate network of hydrogen bonds recognizes a hairpin turn in the HM peptide. A second obvious difference among the binding grooves in MAP kinases, AGC kinases, and cyclins is the relative orientation of the hydrophobic versus charged and hydrogen-bonding functionalities, which is necessary to bind the distinctive docking motifs (Table 4).

As more kinase−substrate complexes are studied, it is becoming clear that larger surfaces of substrates can be involved in recognition. For example, the complex of PKR, a CMGC kinase, with its native substrate eukaryotic translation initiation factor 2α (eIF2α)[50] involves helix G of the kinase and an entire surface of the eIF2α β-barrel (not shown). Interestingly, the interaction of PKR with eIF2 α works allosterically on the eIF2α, inducing disorder in the loop containing the phosphorylation site. This may be important for active site recognition.

4.6. Allosteric Properties of Docking Interactions

The recent structures of MAP kinase docking peptide complexes and the AGC kinase PKB have revealed allosteric conformational changes that serve functions other than enhancing substrate recognition. In PKB, the allostery serves to activate the kinase. In MAP kinases, the allostery affects active site binding of substrates but also influences binding of processing kinases and phosphatases that act on the activation loop. These conformational changes appear to be potent methods for enhancing pathway specificity.

4.6.1 Allostery in MAP Kinase Docking Interactions

In both ERK2[70] and p38α,[68] D-motif docking peptides induce conformational changes in the activation segment (Figure 4a). In ERK2, a new conformation of the activation segment is adopted (Figure 4a). In p38α, the activation segment becomes disordered and more susceptible to proteolysis (unpublished results). Conformational changes in ERK2 occur in solution also.[164] What is the function of these conformational changes? Structural studies are available exclusively for unphosphorylated enzymes, and thus the most physiologically relevant structures involve MAP2K-derived peptides. Consider the action of MEKs, which phosphorylate MAP kinases. The phosphorylation sites of MAP kinases are sequestered or well-tethered in the low activity conformers. In unphosphorylated ERK2,[43] Tyr185, the residue phosphorylated first by MEK1/2,[101] is buried under the backbone of the activation segment. In p38α,[44,45] although the Tyr182 phosphorylation site is on the surface, it forms numerous intramolecular contacts. These contacts probably prevent other kinases from accessing MAP kinase phosphorylation sites. Thus, this allosteric mechanism may contribute to pathway specificity. A prediction is that docking peptides added in trans to docking-motif truncated kinases (or phosphatases) might facilitate activity, but this has not been reported so far. It may be that such studies must be conducted at a very high protein concentrations to account for the local concentration effect of the docking interaction, as observed for CDK2/cyclin A.[67]

The docking study in p38α showed that substrate-derived peptides also induce conformational changes. But the function of the conformational change induced by substrate is presumably different. The study of peptide-bound ERK2 provides the best insight, since structural data are available for both inactive and active ERK2. The peptide-induced structure is closer to the active form than the inactive form (rmsd 1.8 Å versus 2.4 Å). Thus, it may be that substrate peptides are complimentary to a structure closer to the active form.

The conformational changes induced by docking motif peptides are shown for ERK2 in Figure 4a. The changes occur in the activation segment, and along L16, a linker that contacts both the peptide HePTP and the activation segment. In p38α, peptides also induce changes, but the changes are different in detail. In p38α, the largest change is in the activation segment, but near the peptide binding site, the changes are in helix D and the loop between helix D and helix E (not shown). The linkage between peptide binding and activation segment disorder is unclear but may involve a relaxation process within the C-terminal lobe of p38α. Apparently, functionally similar conformational changes are

induced by different mechanisms. This difference in allosteric mechanism may contribute further to specificity determination among MAP kinases.[70]

4.6.2. Allosteric Properties of HM Motif Interactions in AGC Kinases

As discussed above, AGC kinases use the HM pocket for different purposes in different kinases: part of the active structure as in PKA, as a binding site for a regulatory segment of the same protein as in PKB or GRK2 or other subunit as in Aurora kinase, or as a substrate binding site as in PDK1. PDK1 is the most intriguing, because it has been shown that the substrate HM both binds and activates PDK1.[74,152−154] The mechanism for how this might be accomplished comes from the structure of a truncated form of PKB,[75] lacking the HM (as well as a PH domain at the N-terminus of the kinase) (Figure 4b). This structure reveals massive disorder in helices B and C. Thus, apparently the HM is required to build the active structure of the PKB. The structure of PDK1 has also been solved in the absence of a HM.[74,151] No similar disorder was observed, but a lattice contact may have stabilized the HM pocket.[151] Biochemical data (reviewed in ref 4) shows that HM interactions are required for PDK1 to have any activity. Thus, there very probably is an allosteric mechanism activating PDK1 through docking interactions, if not as dramatic as those observed in PKB. The allosteric activation in AGC kinases is very reminiscent of the allosterically induced activation loop changes in MAPKs.

No similar peptide-induced conformational changes have been observed in the CDK2/cyclin A/peptide complexes.[42,67] On the other hand, the docking interaction greatly improves the K_m, as discussed above. The activation segment phosphorylated CDK2 (on Thr160), in the absence of a cyclin, adopts an inactive structure,[40] and cyclin binding appears to be required to form the active enzyme.[41]

4.6.3. Conformational Change Energy

Conformational changes require energy. The conformational change energies (CCE)[165] cannot be measured for these docking interactions. However, CCEs have been measured for several proteins, including hemoglobin, aspartate transcarbamylase, phosphofructokinase,[166] and serpins.[167] The observed CCEs range from 3 to 6 kcal (or 3−6 orders of magnitude of affinity), with 11 kcal as an upper limit (serpins). Affinities of docking peptides have been measured for the MAP kinase ERK2[150] and for the AGC kinase PDK1.[152,168] The affinities were found to be in the micromolar range for both. If similar CCEs apply to MAP kinase and AGC kinase docking interactions, the intrinsic binding energy[169] should be better by at least 3 kcal or 3 orders of magnitude in affinity. This effect could reduce a nanomolar intrinsic affinity into the observed micromolar range. Thus, it seems probable that allostery may serve to gain high specificity at modest affinities. We think that allostery will prove to be very prevalent in signal-transducing protein−protein interactions for this reason, perhaps as important as compartmentalization[170] and scaffolding[171] in specificity determination. A further prediction can be drawn that the docking groove should bind yet-to-be-identified compounds or peptides more tightly than the native substrates and thus could be a target for drug discovery.

5. Conclusions

With structural data now available on active and inactive forms of each of the major groups of Ser/Thr kinases defined by Hunter and colleagues, several conclusions can now be drawn. First, the P + 1 specificity pocket distinguishes the kinase groups from one another. The AGC, CaMK, and Ste groups are hydrophobic-directed for P + 1 sites, the CMGC group is proline-directed (or P +1 is not a strong determinant), and in the CK1 group, the P + 1 site is not a strong determinant. The P + 1 pocket is also important because it houses a catalytic threonine, Thr201 of PKA. The P + 1 pocket and the threonine are remodeled in inactive forms of at least one member of every group of protein kinases. Second, biochemical and structural data have revealed that at least two groups of protein kinases utilize docking in grooves outside the active site to bind substrates and other molecules. Docking strategies are group specific. Third, docking interactions often involve allosteric conformational changes that affect the active site or activation segment. Allostery appears to be a powerful mechanism to confer pathway specificity by preventing reactions between inappropriate partners. In MAP kinases, one function may be to make the MAP kinase phosphorylation sites available for processing, while inappropriate kinases and phosphatases cannot access these sites. The docking interactions also promote the active structure. In PKB, the HM interaction is used to make the PKB activity dependent on phosphorylation, while in PDK1, the substrate provides the HM peptide. Further, conformational changes absorb intrinsic binding energy, reducing the affinity of substrates but perhaps not affecting the substrate specificity. For these reasons, allosteric effects may prove to be prevalent in signal-transducing machinery, as mechanisms for conferring specificity among similar proteins. We look forward to further biochemical studies to better understand how kinases are regulated and to the elucidation of larger protein−protein interactions that serve to define the specificity of protein kinases in the cellular context.

6. Abbreviations

AGC	cAMP-dependent protein kinase/protein kinase G/protein kinase C extended family
ASF/SF2	SR human splicing factor
CaMK	calcium−calmodulin dependent kinase
CCE	coformational change energies
CDKs	cyclin-dependent kinases
CK1	casein kinase-1
CK2	casein kinase-2
CMGC	CDK, MAP kinase, glycogen synthase kinase, and CDK-like
ERK	extracellular responsive kinase
GRK2	G-protein-coupled receptor kinase-2
GSK	glycogen synthase kinase
HePTP	hematopoietic protein tyrosine phosphatase
HM	hydrophobic motif
JNK	c-Jun N-terminal kinase
MAPKs	mitogen-activated protein kinases
MAP2K	mitogen-activated protein kinase kinase
MAP3K	mitogen-activated protein kinase kinase kinase
MARK	microtubule-affinity regulating kinase
MNK-1	MAP kinase interacting kinase
Npl3p	SR-like RNA binding protein
PAK1	p21-activated protein kinase
PDK1	phosphoinositide-dependent protein kinase
PepHePTP	peptide from hematopoietic protein tyrosine phosphatase
PHK	phosphorylase kinase

Pim-1	phosphatidylinositol mannoside-1
PKA	protein kinase A
PKB	protein kinase B
PKC	protein kinase C
SRPK1	serine/arginine-rich protein kinases
STE	homologs of STE11 and STE20
TAB1	TAK1 binding protein 1
TAK1	transforming growth factor-β activated kinase 1
TAO2	thousand and one kinase 2
TGFβ receptor	transforming growth factor receptor-β
TKL	tyrosine kinase like
WNKs	with no lysine

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