Peptide Inhibitors Targeting Protein Kinases

Hagit Eldar-Finkelman[1,*] and Miriam Eisenstein[2,*]

[1]*Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel and* [2]*Department Chemical Research Support, Weizmann Institute of Science, Rehovot 76100, Israel*

Abstract: Phosphorylation by protein kinases is a central theme in biological systems. Aberrant protein kinase activity has been implicated in a variety of human diseases, therefore, modulation of kinase activity represents an attractive therapeutic approach for the treatment of human illnesses. Development and design of specific inhibitors for protein kinases thus became a major strategy in many drug discovery programs. Inhibition of protein kinase activity may be achieved by blocking the phosphorylation activity or by disrupting protein-protein interactions. Peptides that can mimic most truly these regulatory modes are favorite choice for protein kinase-targeting. Here we focus on important motifs regulating the protein kinase signaling network and described how they may be exploited for peptide drug design.

Protein kinases are important regulators of most, if not all, biological processes. Their abnormal activity has been implicated as causal factors in many human diseases, including cancer, diabetes and neurodegenerative disorders [1-3]. Protein kinases are thus attractive targets for drug design and compounds that manipulate their cellular activity are of enormous therapeutic potential. With a target in hand, medicinal chemists can generate low molecular weight compounds that bind the target with high affinity and alter its biological behavior. In many cases, however, drugs fail as they lack appropriate pharmaceutical properties and are of limited specificity resulting in unfavorable side effects. Under these circumstances, the use of peptides, which copy 'natural' motifs that specifically influence kinase activity and/or its intracellular interactions with cognate partners, may be a promising approach for selective inhibition of protein kinases. In this review we focus on the strategies to design such peptide inhibitors, focusing mainly on the serine/threonine protein kinase family.

PROTEIN KINASE DOMAINS

The protein kinase super family accounts for nearly 2% of the genes in the human genome and codes for about 545 kinases [4, 5]. The eukaryotic protein kinases are subdivided into two major classes, the serine/threonine and the tyrosine kinases. The two classes share extensive sequence and structure homologies. The catalytic domain was initially classified into 11 conserved subdomains [6] that are architecturally folded into a small N-terminal lobe and a larger C-terminal lobe [6]. The N-treminal lobe contains a glycine-rich loop (P-loop) for ATP binding and the C-terminal domain contains a conserved activation loop (also called 'T-loop' or activation segment) which is itself regulated by phosphorylation [6-9]. Phosphorylation occurs within a cleft formed between the two lobes, and the phosphate donating ATP molecule is bound in a deep cavity next to the P-loop. The substrate binding interface is often large and includes also grooves located on the surface of the N- and C-terminal lobes, which confer kinase specificity [7].

Although all 'classical' protein kinases share a common catalytic fold, these proteins show remarkable diversity in their substrate specificity and signal transmission. This is mainly attributed to additional elements, such as distinct regions located outside the catalytic core, regulatory domains, or adaptor molecules that can directly affect the interaction with substrates or other components within the signaling network. Protein kinases are frequently controlled by protein interactions, generally involving interactions between large globular domains, evidence suggest that interactions can also occur between protein kinases and short 'linear' peptides (or linear epitopes) that bind to specific docking grooves within the protein kinase and apart from the substrate binding site (reviewed in [10-12]). As docking-mediated interactions are particularly prevalent in serine/threonine kinases (which are the main focus of this review), and are a key in their signal specificity, search for new docking peptide sequences is an active area of research, and the use of peptide-based interacting motifs has become a favorite tool for targeted disruption of kinase activity.

*Address correspondence to these authors at the Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel; E-mail: heldar@post.tau.a.c.il and Department Chemical Research Support, Weizmann Institute of Science, Rehovot 76100, Israel; E-mail: miriam.eisenstein@weizmann.ac.il

In order for peptides to act as high affinity, specific inhibitors, there are several considerations to be taken into account: the motif mimicked by the peptide must be surface exposed, its amino acid composition should not be highly conserved, finally, it is desired that the peptide will be able to adopt the 'active' conformation of the copied motif. This last requirement is a major determinant in peptides failure to produce biological response (due to loss of constrain when they are out of the context of the parent protein), and unfortunately, unlike the other two limitations, it is difficult to predict.

PEPTIDES AS COMPETITIVE INHIBITORS

The specificity of protein kinases for substrate phosphorylation typically depends on the primary amino acid sequences immediately flanking the site of phosphorylation, called the 'consensus sequence'. The numbers and types of interactions with residues surrounding the phosphorylation site vary considerably among kinases, reflecting differences in sequence specificity. This consensus sequence can serve as a template for a peptide that will act as a competitive inhibitor.

The serine/threonine protein kinase family is classified into basophilic, acedophilic and proline-directed kinases [8, 13]. The first group, which includes kinases such as cAMP dependent protein kinase (PKA), protein kinase B/Akt (PKB), and protein kinase C (PKC), prefers positively charged residues within the recognition motif. The consensus sequences of acidophilic group kinases, like casein kinase1/2, have acidic residues near the phosphorylation site. The largest family is the proline-directed kinases (Mitogen activated protein kinase (MAPK's), cyclin dependent protein kinases (CDK's), glycogen synthase kinase-3 (GSK-3)) and the recognition motifs of these kinases all include proline.

Peptides that mimic these recognition sequences can serve as substrate competitive inhibitors. For example, the natural PKA inhibitor protein, PKI, contains an RRN**A**L motif that resembles the RRX**S** consensus sequence of PKA, except that the phosphoacceptor serine is replaced by alanine. Synthetic short peptides derived from the PKI sequence (5-22 residues) are effective inhibitors [14, 15]. Another example are the peptide inhibitors derived from the unique recognition motif of GSK-3, S^1XXXS^2(p), in which the second serine (S^2) is pre-phosphorylated by a different kinase [16, 17]. Peptide inhibitors with sequence motifs of CREB (cAMP response element binding protein, KRREILARRPS(p)YR)

1381-6128/09 $55.00+.00

or HSF-1 (heat shock factor-1, KEAPPAPPQS(p)P) substrates are effective GSK-3 inhibitors *in vitro* and *in vivo* [18, 19].

Protein kinases may be auto-inhibited by a mechanism in which part of their polypeptide chain, often termed the 'pseudosubstrate', occupies the active site. Pseudosubstrates are characterized by their resemblance to sequences derived from target phosphorylation sites. Some known effective competitive inhibitors copy corresponding pseudosubstrate sequences [20, 21]. In the PKC family a pseudo-substrate sequence (of about 13 amino acids) is located in the N-terminal region of each PKC isoform [22]. Synthetic peptides derived from these sequences are highly selective inhibitors of their corresponding PKC isoforms, including α, β, η, θ and ζ [22-24]. Auto-inhibitory domains are found also in the regulatory subunit of PKA, Ca^{2+}/CaM-dependent protein kinase II (CaMK-II), myosin light chain kinase (MLCK), twitchin, and phosphorylase kinase [7, 22, 25-27]. Peptides that copy these domains inhibit protein kinase activity and bind in the catalytic binding cleft [22, 25, 27]. In GSK-3, phosphorylation at serine 9 near its N-terminus mimics the pre-phosphorylation of its substrates and results in auto-inhibition of the catalytic activity. Short phosphorylated peptides derived from this pseudosubstrate region showed moderate inhibition of the kinase [28, 29]

PEPTIDES TARGETING BINDING SITES

Mimics of Docking Sites in Binding Partners

Protein complexes represent a major mechanism for kinase-mediated signaling pathways. These can represent direct interactions with substrate proteins, or effects may be mediated by scaffold proteins. Scaffold proteins act as organizing platforms that recruit both the kinase and the substrate binding partners to the same complex. These different modes of recognition provide a simple way to meet the requirement for increased specificity of the protein kinase-substrate interactions or may increase the local concentration of the substrate near the kinase. The mitogen activated protein kinase (MAPK) family coordinates diverse signaling pathways [30-32] and contains multiple MAPK's and MAPK kinase (MEK's) isoforms that assemble the MAPK cascade [30-32]. Apparently, specific docking interactions enable their specificity in signal transmission. A high-affinity docking region, termed the D-motif, was identified in MEK, the kinase upstream of MAPK and is found in MAPK's substrates and phosphatases [33-35]. The D-site motif is typified by a cluster of basic residues followed by a hydrophobic sub-motif with some variability in the amino acid composition that allows specificity [33-35]. Peptides derived from the D-site of MEK1 were used to inhibit ERK1/2 phosphorylation [34, 36], whereas peptides derived from the D-site of MEK4 reduced JNK phosphorylation and inhibited its ability to phosphorylate respective substrates [34, 36]. D-site-like motives were found also in other binding partners of MAPK, including its direct substrate S6 ribosomal protein kinase, p90 RSK, and MAP kinase dual specific phosphatases (MKPs) [37, 38]. An interacting protein of JNK (JIP1) is a scaffold protein that facilitates the assembly of JNK-cascade components [39-42]. Synthetic peptides corresponding to the D-site of JIP1 proteins were described in several studies and showed high and selective inhibition potency toward JNKs, *in vitro* and *in vivo* [43-45]. Structures of the unbound and peptide-bound-MAPK's showed that the D-motif peptides induced different conformational changes in the different MAPK isoforms (ERK2, p38, JNK) [46, 47]. These could be well attributed to the specificity the MAPK's recognition of its substrates or upstream regulators [46, 47]. A second class of MAPK docking sites are the 'FXFP' sites found in several transcription factors substrates of ERK such as Elk-1, SAP-1, LIN-1, and Fos [48-50]. This site was shown to mediate high-affinity interactions between ERK and its substrate proteins [48-50]. Synthetic peptides patterned after the FXFP motif were shown to antagonize MAPK's ability to phosphorylate its substrates and reduce MAPK-mediated signaling [48, 51, 52].

Members of the AGC kinase family are regulated *via* phosphorylation of a hydrophobic motif ('HM') (FXXFS/TF/Y), located C-terminal to the protein kinase catalytic core [53, 54]. Phosphorylation at this site can stabilize the active conformation of the activation loop [53, 54]. Apparently the HM motif of protein kinase C-related protein kinase (PRK-2), p90 ribosomal S6 kinase (RSK), 70 S6 kinase (S6K) were shown to bind with their upstream regulator, PDK1, *via* interaction with hydrophobic binding pocket ('PIF') located within the small lobe of PDK-1 [55-57]. Interaction with PIF enhanced PDK1 catalytic activity to enable phosphorylation of the docked substrates [55-57]. Indeed, a 24 amino acid peptide derived from the hydrophobic motif in PRK-2 interacted with the PIF pocket of PDK1 and enhanced the kinase activity presumably *via* conformational changes in the catalytic core [57]. Hence peptides or small compounds that mimic the interaction of HM with the PIF pocket may function as allosteric modulators of PDK1 [58].

Self Docking Motifs

Some peptide inhibitors mimic motifs within the kinase itself. These peptides do not bind directly to the kinase; rather, they compete with the kinase for binding to its targets. The three-dimensional structures of several protein kinases have revealed distinct regions that bind downstream or upstream partners. Fukami *et al*. characterized the activation loop encompassing the DFG-APE motif, found in all protein kinases, as a potential template for inhibition. In MAPK, the activation loop is phosphorylated at the 'TEY' motif by the upstream kinase MEK. Apparently, this phosphorylation triggers a conformational rearrangement of the activation loop. This rearrangement is also triggered by the corresponding phosphorylated peptide. Interestingly, this peptide did not inhibit MEK, whereas the non-phosphorylated peptide did inhibit MEK activation of MAPK presumably due to competition with MEK [59]. It is clear from these studies that the structure changes induced by phosphorylation are important elements for recognition. In this particular case, the non-phosphorylated peptide mimicked the interaction of MEK with its substrate MAPK [59].

A recently developed technology termed KinAce identified conserved motifs common to all protein kinases as potential templates for design of peptide inhibitors. Crystal structures of kinases complexed with peptidic substrates identified two regions termed αD and HJ-αG located at the subdomains V and X, respectively, that are involved in substrate binding [60-63]. Based on these observation the KinAce technology provides a 'general recipe' for kinase-based sequence peptide inhibitors [64]. Peptides derived from these regions should compete with substrate binding, yet, the variability of the amino acid compositions within these regions suggests that specificity may be achieved [64]. The use of this technology has been demonstrated in several studies. For example, peptides derived from HJ-αG regions of PDK1 or PKB inhibited phosphorylation of the downstream targets of these kinases *in vitro* and in cells [64] and peptides derived from the αD regions of PKA and PKCε reduced substrate phosphorylation and induced anti-diabetic effects in animal models [65-67]. Notably, unlike the substrate competitive inhibitors described previously that interact with the kinase, these peptides are derived from the kinase and interact with the substrates preventing their access to the kinase.

SH2/SH3 Domains in Tyrosine Kinases

In contrast to the serine/threonine kinases, the tyrosine kinase (TK) docking motifs tend to be found in modular domains that are separate from the kinase domains. Non-receptor TKs have well-defined interaction domains, the Src homolgy domain termed SH2 and SH3, that are small modular protein motifs (about 100 and 60 amino acids, respectively). The SH2 domains interact with phospho-tyrosine residues in the context of pYEE, whereas SH3 domains recognize proline-rich motifs in the context of PXXP. As SH2 and SH3 domains are found in oncoproteins and mediate sig-

naling pathways in cancer cells, SH2 and SH3 domains interaction inhibitors are potential antitumor agents. Unlike most of the docking sites found in Ser/Thr kinases, the SH2/SH3 domains have defined structures and three-dimensional structures of peptides complexed with these domains have allowed structure-based peptide drug design. However, peptides designed to bind to these interaction sites do not show high efficacy. The SH3-directed peptides have low affinity, whereas the phosphorylated SH2-directed peptides have limited cell permeability. Numerous studies have focused on development of peptidomimetics that target SH2/SH3 domains. These peptidomimetics are chemically modified with non-natural amino acid substituents and with hydrophobic residues to improve specific binding and *in vivo* efficacy [68-70]. These inhibitors have been developed for Src, Lck, PI-3K and Zap70 [69, 71].

PEPTIDES COMPETING WITH ANCHORING AND TARGETING PROTEINS

Modular binding partners target some Ser/Thr kinases to their specific substrates *via* changes in cellular localization. Binding of a peptide to this targeting domain may prevent the interaction of the kinase with its substrates. The PKC isoforms are known to translocate from cytosol to particulate fractions in response to various stimuli. A family of adaptor molecules, termed RACKs (from Receptors for Activated C-Kinase), interact with the various PKC isozymes and facilitate their cellular translocation [72, 73]. The RACK binding domain is a short sequence located in the PKC's N-terminal-subdomain and is different in each isoform. Thus, the translocation of PKC isoforms could be disrupted selectively using peptides derived from the specific RACK docking region (64, 67). The use of such peptides has enabled analysis of the functions of individual PKC isozymes. For example, peptides derived from the C-2 region of PKC-βII prevented hormone-induced membrane translocation of PKCβII and inhibited insulin-induced *Xenopus* oocyte maturation [74]. Peptides derived from V1 region of PKCε and PKCδ selectively blocked cellular translocation in heart membranes and different cardio-protection effects were observed in ischemic hearts depending on the isozyme targeted [75, 76].

Another example is the cellular regulation of PKA, which is controlled through interactions with a family of distinct but functionally homologous proteins called AKAPs (A-Kinase Anchor Proteins) [77, 78]. Each AKAP contains an amphipathic helix binding motif that anchors the PKA-regulatory subunit (R) into cellular sites of cAMP generation and PKA substrates [77, 78]. This allows spatial regulation of PKA by c-AMP which is critically important in biological processes such as development, differentiation and apoptosis. Peptides encompassing the amphipathic helix binding motif of AKAP (also termed Ht31) were shown to be effective inhibitors of PKA through inhibiting its recruitment to AKAP. For example, treatment with AKAP-derived peptide inhibitors affected synaptic transmission [79, 80], oocyte maturation [81] and sperm motility [82]. Recently, a combination of bioinformatics and peptide array technologies were used to generate high-affinity binding peptides that were based on the AKAP binding motif. These peptides were selective toward some PKA-regulatory subunit isoforms and shown to be effective in displacing PKA from its cellular AKAP-anchoring sites [83, 84].

Schematic presentation of the peptide-targeted motifs (i.e. substrate binding site, activation loop and specific docking sites) is illustrated in Fig. (**1**).

NEW TECHNOLOGIES FOR DESIGN AND SELECTION OF PEPTIDE INHIBITORS

Historically, the consensus motif of a given protein kinase was extracted from individual substrates that were experimentally tested. The use of chemical libraries and computational analyses enhanced our ability to identify phosphorylation and interaction motifs. Originally peptide libraries were designed to identify optimal consensus sequences favored by certain protein kinases [4, 85]. In these experiments, a degenerate library of peptides with a fixed serine, threonine or tyrosine residue was subjected to phosphorylation *in vitro* and the subset of the phosphorylated peptides was retrieved from the peptide mixture and sequenced. The sequences obtained were then scored for preferred amino acids at each position. In many instances, the optimal peptide sequence motifs determined by this method closely matched the known phosphorylation sites, additionally, new motifs were identified [85, 86]. For example, an optimal peptide substrate sequence for Akt (PKB), ARKRERTYSFGHHA, determined using a screen of a peptide library, had high affinity for the kinase [87]. The peptide has been recently used in the development of small peptide inhibitors of Akt [88].

Large-scale screening made possible through the development of phosphoproteomics analysis based on mass spectroscopy (MS) is being used to catalog thousands of *in vivo* phosphorylation sites that can be screened for important and new phosphorylation motifs [89, 90]. In a recent bioinformatics analysis of a large database of phospho-peptides obtained from HeLa cells protein extracts hundreds of phosphorylation motifs were found; some were novel and some matched known consensus sequences of protein kinases [91]. This large-scale analysis increases our data base of phosphorylated sequences that can be further implicated in rational drug design.

A different approach was recently described to screen motifs for protein-protein interactions. In this technique, a combinatorial library of conformationally constrained peptides is attached to a platform protein such as thioredoxin A (termed 'peptide aptamer') and utilized in a yeast two hybrid screen [92, 93]. This approach can be used to identify peptides that interact with a specific motif within a kinase. In the studies of Colas *et al.* [94], a set of aptamers that recognized different epitopes at the CDK-2 (cyclin dependent protein kinase) surface were identified using a peptide library and the yeast two-hybrid strategy. One of the selected peptide aptamer, termed pep8, was shown to bind at the active site of Cdk2 and to inhibit its catalytic activity. Treatment with pep8 resulted in inhibition of cell cycle progression in human cells [95] and defected embryogenesis of *Drosophilae* [94, 96]. Moreover peptide aptamers containing signals that alter cellular localization may be created that change the target's compartmentalization and manipulate its activity [94]. Altogether, peptide aptamer strategy can identify important motifs within the proteins and may also serve as a tool for development of high affinity peptide-based drugs. This new knowledge is helpful in the design of biological tools to modulate protein–protein interactions *in vivo*.

COMPUTATIONAL STRUCTURE-BASED DRUG DESIGN

The growing number of sequences and three-dimensional structures of protein kinases provides an important starting point for bioinformatic analyses, such as the identification of targeting motifs and specific docking sites (reviewed in [12, 97-100]), followed by design and screening of putative peptide inhibitors. Prediction of the interaction geometry between proteins and peptides is a difficult task due to the high flexibility of the peptide ligand. Nevertheless, successful interface design studies have been presented, which modified and improved the specificity of protein-peptide interfaces [101-103]. The interactions of peptides with protein kinases are further complicated by the conformation changes that many kinases undergo upon ligand binding, such as domain movements and local changes in the glycine rich P-loop, the C helix and the activation loop [104]. The computational study by Helms and McCammon indicated that binding of the inhibitory PKI peptide 5-24 to the c-AMP dependent protein kinase stabilizes a closed (active) conformation of the kinase, whereas in the absence of the peptide the open state or an intermediate ATP bound state are favored [105]. Brinkworth *et al.* used the active conformations of two protein kinases and showed that the calculated enthalpy of kinase/peptide binding

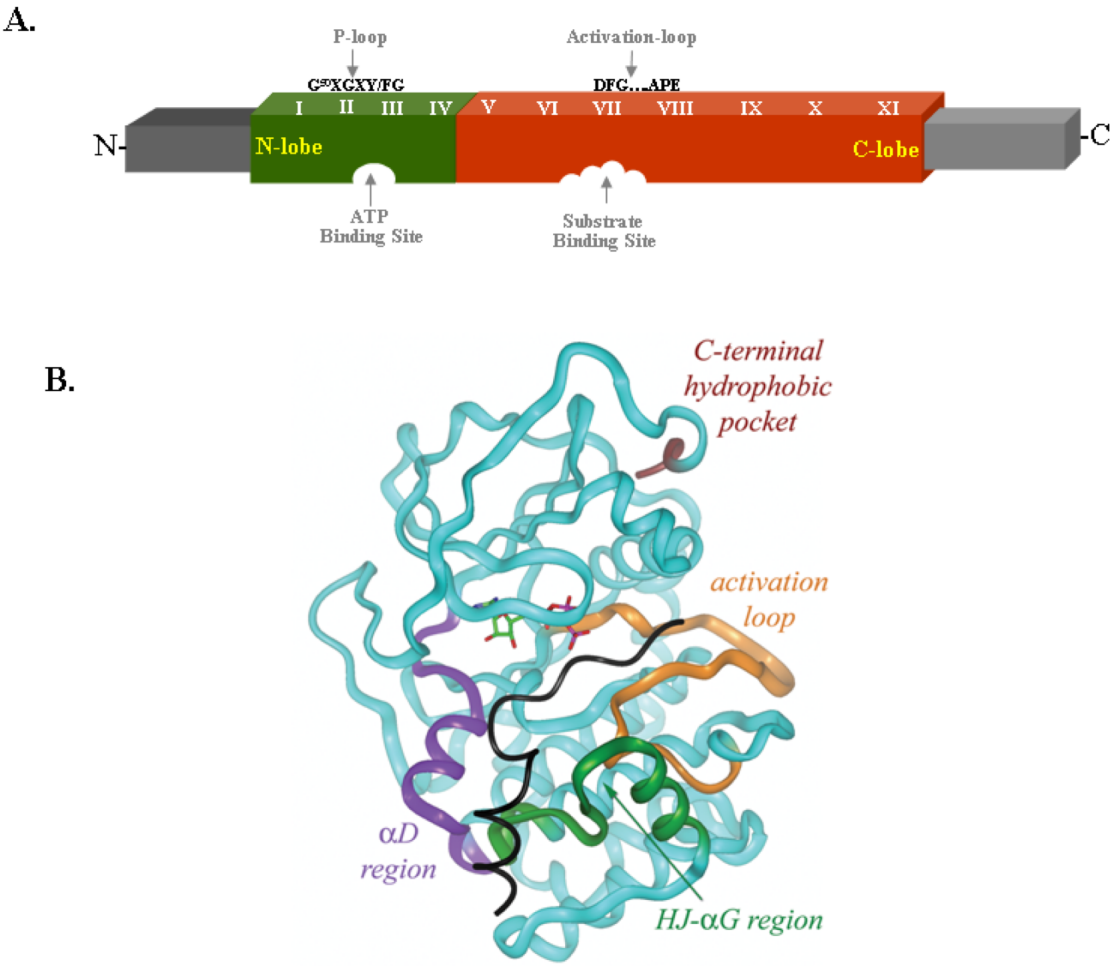

Fig. (1). Protein kinase domains and targeted motifs. A Schematic presentation of domain structure of protein kinase with conserved bilobal catalytic domains, N-lobe (green) and C-lobe (red), composed of 11 conserved subdomains. Conserved domains including **P-loop** involved in ATP binding, and **activation loop** with the DFG….APE motif are marked. **B.** Motifs targeted by peptide inhibitors are mapped onto the ribbon diagram of PKA (PDB entry 1atp). Shown are the positions of the activation loop (orange), the αD region (purple), the HJ-αG region (dark green) and the C-terminal hydrophobic pocket (brown). The substrate binding position is represented by the inhibitory peptide PKI (the black ribbon). ATP is shown as a stick diagram colored by the atoms (green for C, red for O, blue for N and magenta for P).

can distinguish good peptide substrates from non-substrates, although the catalytic efficiencies for the tested cases were not predicted well [106]. Both studies relied on experimentally resolved structures of kinase/peptide complexes and their results emphasize the difficulty in predicting the structure of such complexes.

Protein kinase/peptide interaction geometry was predicted for the complex GSK-3β/pCREB [107]. The unphosphorylated GSK-3β adopts the active form and upon phosphorylation of Y216 undergoes only a local side chain conformation change. Computational docking of pCREB to GSK-3β identified several docking sites on the surface of the kinase, which are separate though spatially close to the active site. Residues Phe 67, Gln 89, Asn 95 of GSK-3β were shown to be involved in pCREB and other substrates binding by numerous experimental assays (Fig. **2**) [107]. Identification of such specific interactions is of high importance in drug design because they provide a docking template for peptides or small molecules designed to confer specific GSK-3 inhibition. Recently, a general computational procedure has been described for predicting protein kinase peptide specificity, namely prediction of putative docking sites in kinases and preferred peptide substrates [108]. These recent modeling studies will hopefully lead to development of specific peptide inhibitors.

The SH2 domain of tyrosine kinases is relatively rigid, as it does not undergo significant conformation changes upon ligand binding. This led to several modeling studies in which the interaction energies of SH2 with phosphorylated and non-phosphorylated peptides were calculated [109-113]. The computed binding energies in most of these studies show good agreement with experimental data suggesting that computations can be used for predicting protein-protein interactions mediated *via* SH2 domains and that such data can be used to improve and extend experimentally derived cellular signaling and protein interaction maps. Similar computations were used to evaluate the interaction energy between peptides and the Abl-SH3 domain, whereby putative binding partners were predicted in good agreement with experiment. Notably, predictions of interaction geometries are rare. Reasonably accurate binding geometries to SH2 domains were predicted for several short peptides by Zhou *et al*., with minimal restraints, such as the location of the phosphate group [109].

The studies discussed above aimed at elucidating the interaction energies and/or interaction geometries of peptides to the catalytic domains or to regulatory domains of protein kinases. These data can be used for identifying specific protein/peptide interactions that can subsequently be used for designing specific peptide or peptide-

mimicking inhibitors. An alternative computational method to elucidate inhibitors is to design peptides that bind to the kinase substrates or to other modulators of their activity [64, 114].

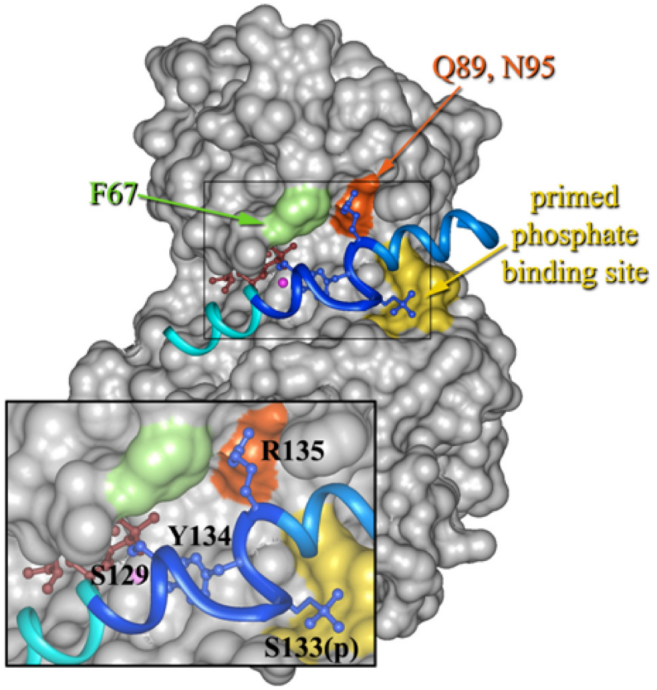

Fig. (2). Docking model of GSK-3β with peptide substrate pCREB. The solvent accessible surface of GSK-3β is shown in grey. Residues Q^{89} and N^{95} are emphasized in orange, residue F^{67} is colored in light green and the positive primed phosphate binding site is depicted in yellow. The ribbon diagram represents pCREB. The p9CREB fragment, which was shown to bind to GSK-3β [99], is colored in dark blue and the N- and C- terminal helices are colored in cyan and blue, respectively. The insert zooms in on the interaction between GSK-3β and pCREB. The side chains of residues S^{129} (phosphorylation site), S^{133}(p) (primed phosphorylation site), Y^{134} and R^{135}, (which interact with F^{67} or Q^{89} and N^{95}, respectively) are shown. ATP is shown as brown ball and stick model, and the Mg^{+2} ion is depicted by the magenta sphere.

PROSPECTIVES

Selective inhibition of protein kinases is an extremely challenging goal of many drug discovery programs. Here we highlighted the main strategies for the design of peptide inhibitors of protein kinases and suggest that peptides offer a number of advantages relative to other types of drugs such as ease of discovery, specificity, and safety. Traditionally, peptides have been considered less attractive than 'small molecule' drugs, due to several limitations of peptides. These include metabolic instability, inability to readily cross cell membranes, potential immunogenicity, and the inability to be administered orally. However, several new strategies were shown to improve the bioavailability and stability of peptides. Chemical modifications can make the peptide more stable towards an enzymatic attack and various formulation techniques can be used to protect the peptide from proteosomal degradation. Formulations evaluated include liposomes, nanoparticles, microparticles and matrix tablets comprising various auxiliary agents such as enzyme inhibitors and multifunctional polymers [115-118]. In addition, Pumps, buccal sprays, intra-nasal delivery, and transdermal patches offer attractive alternatives to injections [117, 119-121].

Hence, it is a worthwhile cause to re-examine the opportunities that peptides provide as potential drug compounds with appropriate technology and design.

ACKNOWLEDGMENT

We would like to acknowledge Israel Science Foundation grant 755/04 (H. E-F) and the Kimmelman Center for Macromolecular Structure and Dynamic at the Weizmann Institute of Science (M. E).

ABBREVIATIONS

PKA	=	cAMP Dependent protein kinase
GSK-3	=	Glycogen synthase kinase-3
FRAT	=	Frequently rearranged in advanced T-cell lymphoma
GBP	=	GSK-3-Binding protein
GID	=	GSK-3-Interacting domain
CDK	=	Cyclin dependent kinase
JNK	=	c-Jun N-terminal kinase
AKAP	=	A-Kinase anchoring proteins
PKC	=	Protein kinase C
P90 RSK	=	p90 Ribosomal S6 kinase
MLCK	=	Myosin light chain kinase
MAPK	=	Mitogen-activated protein kinase
MEK	=	MAPK kinase
JIP	=	JNK-Interacting protein
ERK (MAPK)	=	Extracellular signal-regulated kinase
PRK	=	Protein kinase C-related protein kinase
PDK-1	=	Phosphoinositide-dependent kinase
PKB	=	Protein kinase B
PIF	=	PDK-1 Interacting fragment
CAMK	=	Calcium/calmodulin-dependent protein kinase
HIV-TAT	=	TAT protein from human immunodeficiency virus
CaM	=	Calmodulin
RACK	=	Receptor for activated C-kinase
CREB cAMP	=	Response element binding protein

REFERENCES

[1] Blume-Jensen P, Hunter T. Oncogenic kinase signalling. Nature 2001; 411(6835): 355-65.
[2] Cohen P. Protein kinases--the major drug targets of the twenty-first century? Nat. Rev. Drug Discov 2002; 1(4): 309-15.
[3] Saltiel AR, Pessin JE. Insulin signaling pathways in time and space. Trends Cell Biol 2002; 12(2): 65-71.
[4] Manning BD, Cantley LC. Hitting the target: emerging technologies in the search for kinase substrates. Sci STKE 2002; 2002(162): PE49.
[5] Manning G, Plowman GD, Hunter T, Sudarsanam S. Evolution of protein kinase signaling from yeast to man. Trends Biochem Sci 2002; 27(10): 514-20.
[6] Hanks SK, Quinn AM, Hunter T. The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science 1988; 241(4861): 42-52.
[7] Taylor SS, Knighton DR, Zheng J, Sowadski JM, Gibbs CS, Zoller MJ. A template for the protein kinase family. Trends Biochem Sci 1993; 18(3): 84-9.
[8] Hanks SK, Hunter T. Protein kinases. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. Faseb J 1995; 9(8): 576-96.
[9] Taylor SS, Radzio-Andzelm E, Hunter T. How do protein kinases discriminate between serine/threonine and tyrosine? Structural insights from the insulin receptor protein-tyrosine kinase. FASEB. J 1995; 9(13): 1255-66.
[10] Neduva V, Russell RB. Peptides mediating interaction networks: new leads at last. Curr. Opin. Biotechnol 2006; 17(5): 465-71.

[11] 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(6): 951-62.

[12] Remenyi A, Good MC, Lim WA. Docking interactions in protein kinase and phosphatase networks. Curr. Opin. Struct. Biol 2006; 16(6): 676-85.

[13] Pinna LA, Ruzzene M. How do protein kinases recognize their substrates? Biochim. Biophys. Acta 1996; 1314(3): 191-225.

[14] Cheng HC, Kemp BE, Pearson RB, Smith AJ, Misconi L, Van Patten SM, *et al.* A potent synthetic peptide inhibitor of the cAMP-dependent protein kinase. J Biol Chem 1986; 261(3): 989-92.

[15] Glass DB, Cheng HC, Kemp BE, Walsh DA. Differential and common recognition of the catalytic sites of the cGMP-dependent and cAMP-dependent protein kinases by inhibitory peptides derived from the heat-stable inhibitor protein. J Biol Chem 1986; 261(26): 12166-71.

[16] Woodgett JR. A common denominator linking glycogen metabolism, nuclear oncogenes and development. Trends. Biochem Sci 1991; 16(5): 177-81.

[17] Fiol CJ, Williams JS, Chou CH, Wang QM, Roach PJ, Andrisani OM. A secondary phosphorylation of CREB341 at Ser129 is required for the cAMP-mediated control of gene expression. A role for glycogen synthase kinase-3 in the control of gene expression. J. Biol. Chem 1994; 269(51): 32187-93.

[18] Plotkin B, Kaidanovich O, Talior I, Eldar-Finkelman H. Insulin mimetic action of synthetic phosphorylated peptide inhibitors of glycogen synthase kinase-3. J Pharmacol Exp Ther 2003(305): 974-980.

[19] Kaidanovich-Beilien O, Eldar-Finkelman H. Long-Term Treatment with Novel Glycogen Synthase Kinase-3 Inhibitor Improves Glucose Homeostasis in Ob/Ob Mice: Molecular Characterization In Liver And Muscle. J Pharmacol Exp Ther In press 2005.

[20] Kemp BE, Pearson RB. Protein kinase recognition sequence motifs. Trends Biochem Sci 1990; 15(9): 342-6.

[21] Soderling TR. Protein kinases. Regulation by autoinhibitory domains. J Biol Chem 1990; 265(4): 1823-6.

[22] Kemp BE, Pearson RB, House CM. Pseudosubstrate-based peptide inhibitors. Methods Enzymol 1991; 201: 287-304.

[23] Eichholtz T, de Bont DB, de Widt J, Liskamp RM, Ploegh HL. A myristoylated pseudosubstrate peptide, a novel protein kinase C inhibitor. J Biol Chem 1993; 268(3): 1982-6.

[24] Theodore L, Derossi D, Chassaing G, Llirbat B, Kubes M, Jordan P, *et al.* Intraneuronal delivery of protein kinase C pseudosubstrate leads to growth cone collapse. J Neurosci 1995; 15(11): 7158-67.

[25] Brickey DA, Bann JG, Fong YL, Perrino L, Brennan RG, Soderling TR. Mutational analysis of the autoinhibitory domain of calmodulin kinase II. J Biol Chem 1994; 269(46): 29047-54.

[26] Hu SH, Parker MW, Lei JY, Wilce MC, Benian GM, Kemp BE. Insights into autoregulation from the crystal structure of twitchin kinase. Nature 1994; 369(6481): 581-4.

[27] Bagchi IC, Kemp BE, Means AR. Intrasteric regulation of myosin light chain kinase: the pseudosubstrate prototope binds to the active site. Mol Endocrinol 1992; 6(4): 621-6.

[28] Dajani R, Fraser E, Roe SM, Young N, Good V, Dale TC, *et al.* Crystal structure of glycogen synthase kinase 3 beta: structural basis for phosphate-primed substrate specificity and autoinhibition. Cell 2001; 105(6): 721-32.

[29] Ilouz R, Pietrokovsky S, Eisenstein M, Eldar-Finkelman H. New Insights into the Autoinhibition Mechanism of Glycogen Synthase Kinase-3 beta. J. Mol. Biol 2008; in press.

[30] Seger R, Krebs EG. The MAPK signaling cascade. Faseb J 1995; 9(9): 726-35.

[31] Raman M, Cobb MH. MAP kinase modules: many roads home. Curr Biol 2003; 13(22): R886-8.

[32] Chang L, Karin M. Mammalian MAP kinase signalling cascades. Nature 2001; 410(6824): 37-40.

[33] Enslen H, Davis RJ. Regulation of MAP kinases by docking domains. Biol Cell 2001; 93(1-2): 5-14.

[34] Bardwell AJ, Flatauer LJ, Matsukuma K, Thorner J, Bardwell L. A conserved docking site in MEKs mediates high-affinity binding to MAP kinases and cooperates with a scaffold protein to enhance signal transmission. J Biol Chem 2001; 276(13): 10374-86.

[35] Xu B, Wilsbacher JL, Collisson T, Cobb MH. The N-terminal ERK-binding site of MEK1 is required for efficient feedback phosphorylation by ERK2 *in vitro* and ERK activation *in vivo*. J Biol Chem 1999; 274(48): 34029-35.

[36] Ho DT, Bardwell AJ, Abdollahi M, Bardwell L. A docking site in MKK4 mediates high affinity binding to JNK MAPKs and competes with similar docking sites in JNK substrates. J Biol Chem 2003; 278(35): 32662-72.

[37] Roux PP, Richards SA, Blenis J. Phosphorylation of p90 ribosomal S6 kinase (RSK) regulates extracellular signal-regulated kinase docking and RSK activity. Mol Cell Biol 2003; 23(14): 4796-804.

[38] 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(9): 6506-15.

[39] Dickens M, Rogers JS, Cavanagh J, Raitano A, Xia Z, Halpern JR, *et al.* A cytoplasmic inhibitor of the JNK signal transduction pathway. Science 1997; 277(5326): 693-6.

[40] Whitmarsh AJ, Cavanagh J, Tournier C, Yasuda J, Davis RJ. A mammalian scaffold complex that selectively mediates MAP kinase activation. Science 1998; 281(5383): 1671-4.

[41] Mooney LM, Whitmarsh AJ. Docking interactions in the c-Jun N-terminal kinase pathway J Biol Chem 2004; 279(12): 11843-52.

[42] Kelkar N, Standen CL, Davis RJ. Role of the JIP4 scaffold protein in the regulation of mitogen-activated protein kinase signaling pathways. Mol. Cell Biol 2005; 25(7): 2733-43.

[43] Kaneto H, Nakatani Y, Miyatsuka T, Kawamori D, Matsuoka TA, Matsuhisa M, *et al.* Possible novel therapy for diabetes with cell-permeable JNK-inhibitory peptide. Nat Med 2004; 10(10): 1128-32.

[44] Guan QH, Pei DS, Zong YY, Xu TL, Zhang GY. Neuroprotection against ischemic brain injury by a small peptide inhibitor of c-Jun N-terminal kinase (JNK) *via* nuclear and non-nuclear pathways. Neuroscience 2006; 139(2): 609-627.

[45] Colombo A, Repici M, Pesaresi M, Santambrogio S, Forloni G, Borsello T. The TAT-JNK inhibitor peptide interferes with beta amyloid protein stability. Cell Death Differ 2007; 14(10): 1845-8.

[46] Kim WY, Zhou FQ, Zhou J, Yokota Y, Wang YM, Yoshimura T, *et al.* Essential Roles for GSK-3s and GSK-3-Primed Substrates in Neurotrophin-Induced and Hippocampal Axon Growth. Neuron 2006; 52(6): 981-96.

[47] Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, *et al.* A central role for JNK in obesity and insulin resistance. Nature 2002; 420(6913): 333-6.

[48] Fantz DA, Jacobs D, Glossip D, Kornfeld K. Docking sites on substrate proteins direct extracellular signal-regulated kinase to phosphorylate specific residues. J Biol Chem 2001; 276(29): 27256-65.

[49] Galanis A, Yang SH, Sharrocks AD. Selective targeting of MAPKs to the ETS domain transcription factor SAP-1. J Biol Chem 2001; 276(2): 965-73.

[50] Jacobs D, Beitel GJ, Clark SG, Horvitz HR, Kornfeld K. Gain-of-function mutations in the Caenorhabditis elegans lin-1 ETS gene identify a C-terminal regulatory domain phosphorylated by ERK MAP kinase. Genetics 1998; 149(4): 1809-22.

[51] 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(32): 29901-12.

[52] 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(2): 163-75.

[53] Parker PJ, Parkinson SJ. AGC protein kinase phosphorylation and protein kinase C. Biochem Soc Trans 2001; 29(Pt 6): 860-3.

[54] Newton AC. Regulation of the ABC kinases by phosphorylation: protein kinase C as a paradigm. Biochem. J 2002.

[55] Frodin M, Jensen CJ, Merienne K, Gammeltoft S. A phosphoserine-regulated docking site in the protein kinase RSK2 that recruits and activates PDK1. Embo J 2000; 19(12): 2924-34.

[56] Biondi RM, Cheung PC, Casamayor A, Deak M, Currie RA, Alessi DR. Identification of a pocket in the PDK1 kinase domain that interacts with PIF and the C-terminal residues of PKA. Embo J 2000; 19(5): 979-88.

[57] Biondi RM, Kieloch A, Currie RA, Deak M, Alessi DR. The PIF-binding pocket in PDK1 is essential for activation of S6K and SGK, but not PKB. Embo J 2001; 20(16): 4380-90.

[58] Engel M, Hindie V, Lopez-Garcia LA, Stroba A, Schaeffer F, Adrian I, *et al.* Allosteric activation of the protein kinase PDK1 with low molecular weight compounds. Embo J 2006; 25(23): 5469-80.

[59] Tokmakov AA, Sato K, Konaka K, Fukami Y. Inhibition of MAPK pathway by a synthetic peptide corresponding to the activation segment of MAPK. Biochem Biophys Res Commun 1998; 252(1): 214-9.

[60] Goldberg J, Nairn AC, Kuriyan J. Structural basis for the autoinhibition of calcium/calmodulin-dependent protein kinase I. Cell 1996; 84(6): 875-87.

[61] Yang J, Cron P, Good VM, Thompson V, Hemmings BA, Barford D. Crystal structure of an activated Akt/protein kinase B ternary complex with GSK3-peptide and AMP-PNP. Nat Struct Biol 2002; 9(12): 940-4.

[62] Lowe ED, Noble ME, Skamnaki VT, Oikonomakos NG, Owen DJ, Johnson LN. The crystal structure of a phosphorylase kinase peptide substrate complex: kinase substrate recognition. Embo J 1997; 16(22): 6646-58.

[63] Brown NR, Noble ME, Endicott JA, Johnson LN. The structural basis for specificity of substrate and recruitment peptides for cyclin-dependent kinases. Nat Cell Biol 1999; 1(7): 438-43.

[64] Niv MY, Rubin H, Cohen J, Tsirulnikov L, Licht T, Peretzman-Shemer A, *et al.* Sequence-based design of kinase inhibitors applicable for therapeutics and target identification. J Biol Chem 2004; 279(2): 1242-55.

[65] Licht T, Tsirulnikov L, Reuveni H, Yarnitzky T, Ben-Sasson SA. Induction of pro-angiogenic signaling by a synthetic peptide derived from the second intracellular loop of S1P3 (EDG3). Blood 2003; 102(6): 2099-107.

[66] Anis Y, Leshem O, Reuveni H, Wexler I, Ben Sasson R, Yahalom B, *et al.* Antidiabetic effect of novel modulating peptides of G-protein-coupled kinase in experimental models of diabetes. Diabetologia 2004; 47(7): 1232-44.

[67] Mack E, Ziv E, Reuveni H, Kalman R, Niv MY, Jorns A, *et al.* Prevention of insulin resistance and beta-cell loss by abrogating PKCepsilon-induced serine phosphorylation of muscle IRS-1 in Psammomys obesus. Diabetes Metab Res Rev 2008; 24(7): 577-84.

[68] Machida K, Mayer BJ. The SH2 domain: versatile signaling module and pharmaceutical target. Biochim Biophys Acta 2005; 1747(1): 1-25.

[69] Vidal M, Gigoux V, Garbay C. SH2 and SH3 domains as targets for anti-proliferative agents. Crit. Rev. Oncol. Hematol 2001; 40(2): 175-86.

[70] Yeh RH, Lee TR, Lawrence DS. From consensus sequence peptide to high affinity ligand, a "library scan" strategy. J Biol Chem 2001; 276(15): 12235-40.

[71] Sawyer TK, Bohacek RS, Dalgarno DC, Eyermann CJ, Kawahata N, Metcalf CA, 3rd, *et al.* SRC homology-2 inhibitors: peptidomimetic and nonpeptide. Mini Rev Med Chem 2002; 2(5): 475-88.

[72] Ron D, Mochly-Rosen D. An autoregulatory region in protein kinase C: the pseudoanchoring site. Proc Natl Acad Sci USA 1995; 92(2): 492-6.

[73] Schechtman D, Mochly-Rosen D. Adaptor proteins in protein kinase C-mediated signal transduction. Oncogene 2001; 20(44): 6339-47.

[74] Ron D, Luo J, Mochly-Rosen D. C2 region-derived peptides inhibit translocation and function of beta protein kinase C *in vivo*. J Biol Chem 1995; 270(41): 24180-7.

[75] Johnson JA, Gray MO, Chen CH, Mochly-Rosen D. A protein kinase C translocation inhibitor as an isozyme-selective antagonist of cardiac function. J Biol Chem 1996; 271(40): 24962-6.

[76] Liu GS, Cohen MV, Mochly-Rosen D, Downey JM. Protein kinase C-epsilon is responsible for the protection of preconditioning in rabbit cardiomyocytes. J Mol Cell Cardiol 1999; 31(10): 1937-48.

[77] Michel JJ, Scott JD. AKAP mediated signal transduction. Annu Rev Pharmacol Toxicol 2002; 42: 235-57.

[78] Beene DL, Scott JD. A-kinase anchoring proteins take shape. Curr Opin Cell Biol 2007; 19(2): 192-8.

[79] Rosenmund C, Carr DW, Bergeson SE, Nilaver G, Scott JD, Westbrook GL. Anchoring of protein kinase A is required for modulation of AMPA/kainate receptors on hippocampal neurons. Nature 1994; 368(6474): 853-6.

[80] Coghlan VM, Bergeson SE, Langeberg L, Nilaver G, Scott JD. A-kinase anchoring proteins: a key to selective activation of cAMP-responsive events? Mol Cell Biochem 1993; 127-128: 309-19.

[81] Newhall KJ, Criniti AR, Cheah CS, Smith KC, Kafer KE, Burkart AD, *et al.* Dynamic anchoring of PKA is essential during oocyte maturation. Curr Biol 2006; 16(3): 321-7.

[82] Vijayaraghavan S, Goueli SA, Davey MP, Carr DW. Protein kinase A-anchoring inhibitor peptides arrest mammalian sperm motility. J Biol Chem 1997; 272(8): 4747-52.

[83] Burns-Hamuro LL, Ma Y, Kammerer S, Reineke U, Self C, Cook C, *et al.* Designing isoform-specific peptide disruptors of protein kinase A localization. Proc Natl Acad Sci USA 2003; 100(7): 4072-7.

[84] Alto NM, Soderling SH, Hoshi N, Langeberg LK, Fayos R, Jennings PA, *et al.* Bioinformatic design of A-kinase anchoring protein-in silico: a potent and selective peptide antagonist of type II protein kinase A anchoring. Proc Natl Acad Sci USA 2003; 100(8): 4445-50.

[85] Yaffe MB, Leparc GG, Lai J, Obata T, Volinia S, Cantley LC. A motif-based profile scanning approach for genome-wide prediction of signaling pathways. Nat Biotechnol 2001; 19(4): 348-53.

[86] Songyang Z, Blechner S, Hoagland N, Hoekstra MF, Piwnica-Worms H, Cantley LC. Use of an oriented peptide library to determine the optimal substrates of protein kinases. Curr Biol 1994; 4(11): 973-82.

[87] Obata T, Yaffe MB, Leparc GG, Piro ET, Maegawa H, Kashiwagi A, *et al.* Peptide and protein library screening defines optimal substrate motifs for AKT/PKB. J Biol Chem 2000; 275(46): 36108-15.

[88] Chen G, Bower KA, Ma C, Fang S, Thiele CJ, Luo J. Glycogen synthase kinase 3beta (GSK3beta) mediates 6-hydroxydopamine-induced neuronal death. Faseb J 2004; 18(10): 1162-4.

[89] Schmelzle K, White FM. Phosphoproteomic approaches to elucidate cellular signaling networks. Curr Opin Biotechnol 2006; 17(4): 406-14.

[90] Witze ES, Old WM, Resing KA, Ahn NG. Mapping protein post-translational modifications with mass spectrometry. Nat Methods 2007; 4(10): 798-806.

[91] Schwartz D, Gygi SP. An iterative statistical approach to the identification of protein phosphorylation motifs from large-scale data sets. Nat Biotechnol 2005; 23(11): 1391-8.

[92] Colas P, Cohen B, Jessen T, Grishina I, McCoy J, Brent R. Genetic selection of peptide aptamers that recognize and inhibit cyclin-dependent kinase 2. Nature 1996; 380(6574): 548-50.

[93] Baines IC, Colas P. Peptide aptamers as guides for small-molecule drug discovery. Drug Discov. Today 2006; 11(7-8): 334-41.

[94] Colas P, Cohen B, Ko Ferrigno P, Silver PA, Brent R. Targeted modification and transportation of cellular proteins. Proc Natl Acad Sci USA 2000; 97(25): 13720-5.

[95] Cohen BA, Colas P, Brent R. An artificial cell-cycle inhibitor isolated from a combinatorial library. Proc Natl Acad Sci USA 1998; 95(24): 14272-7.

[96] Kolonin MG, Finley RL, Jr. Targeting cyclin-dependent kinases in Drosophila with peptide aptamers. Proc Natl Acad Sci USA 1998; 95(24): 14266-71.

[97] Campbell SJ, Jackson RM. Diversity in the SH2 domain family phosphotyrosyl peptide binding site. Protein Eng 2003; 16(3): 217-27.

[98] Neduva V, Linding R, Su-Angrand I, Stark A, de Masi F, Gibson TJ, *et al.* Systematic discovery of new recognition peptides mediating protein interaction networks. PLoS Biol 2005; 3(12): e405.

[99] Hou T, Chen K, McLaughlin WA, Lu B, Wang W. Computational analysis and prediction of the binding motif and protein interacting partners of the Abl SH3 domain. PLoS Comput Biol 2006; 2(1): e1.

[100] Brinkworth RI, Breinl RA, Kobe B. Structural basis and prediction of substrate specificity in protein serine/threonine kinases. Proc Natl Acad Sci USA 2003; 100(1): 74-9.

[101] Kortemme T, Baker D. Computational design of protein-protein interactions. Curr Opin Chem Biol 2004; 8(1): 91-7.

[102] Reina J, Lacroix E, Hobson SD, Fernandez-Ballester G, Rybin V, Schwab MS, *et al.* Computer-aided design of a PDZ domain to recognize new target sequences. Nat Struct Biol 2002; 9(8): 621-7.
[103] Shifman JM, Mayo SL. Modulating calmodulin binding specificity through computational protein design. J Mol Biol 2002; 323(3): 417-23.
[104] Cox S, Radzio-Andzelm E, Taylor SS. Domain movements in protein kinases. Curr Opin Struct Biol 1994; 4(6): 893-901.
[105] Helms V, McCammon JA. Kinase conformations: a computational study of the effect of ligand binding. Protein Sci 1997; 6(11): 2336-43.
[106] Brinkworth RI, Horne J, Kobe B. A computational analysis of substrate binding strength by phosphorylase kinase and protein kinase A. J Mol Recognit 2002; 15(2): 104-11.
[107] Ilouz R, Kowelsman N, Eisenstein M, Eldar-Finkelman H. Identification of novel glycogen synthase kinase-3beta substrate-interacting residues suggests a common mechanism for substrate recognition. J Biol Chem 2006; 281: 30621-30.
[108] Saunders NF, Brinkworth RI, Huber T, Kemp BE, Kobe B. Predikin and PredikinDB: a computational framework for the prediction of protein kinase peptide specificity and an associated database of phosphorylation sites. BMC Bioinformatics 2008; 9: 245.
[109] Zhou Y, Abagyan R. How and why phosphotyrosine-containing peptides bind to the SH2 and PTB domains. Fold Des 1998; 3(6): 513-22.
[110] Henriques DA, Ladbury JE, Jackson RM. Comparison of binding energies of SrcSH2-phosphotyrosyl peptides with structure-based prediction using surface area based empirical parameterization. Protein Sci 2000; 9(10): 1975-85.
[111] Suenaga A, Hatakeyama M, Ichikawa M, Yu X, Futatsugi N, Narumi T, *et al.* Molecular dynamics, free energy, and SPR analyses of the interactions between the SH2 domain of Grb2 and ErbB phosphotyrosyl peptides. Biochemistry 2003; 42(18): 5195-200.
[112] Lee JK, Moon T, Chi MW, Song JS, Choi YS, Yoon CN. An investigation of phosphopeptide binding to SH2 domain. Biochem Biophys Res Commun 2003; 306(1): 225-30.
[113] Sanchez IE, Beltrao P, Stricher F, Schymkowitz J, Ferkinghoff-Borg J, Rousseau F, *et al.* Genome-wide prediction of SH2 domain targets using structural information and the FoldX algorithm. PLoS Comput Biol 2008; 4(4): e1000052.
[114] Wexler ID, Niv MY, Reuveni H. Sequence-based protein kinase inhibition: applications for drug development. Biotechniques 2005; 39(4): 575-6.
[115] Senel S, Kremer M, Nagy K, Squier C. Delivery of Bioactive peptides and proteins across oral (buccal) mucosa. Curr. Pharm. Biotechnol 2001; 2: 175-186.
[116] Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol 2007; 2(12): 751-60.
[117] Morishita M, Peppas NA. Is the oral route possible for peptide and protein drug delivery? Drug Discov. Today 2006; 11(19-20): 905-10.
[118] Stolnik S, Shakesheff K. Formulations for delivery of therapeutic proteins. Biotechnol Lett 2009; 31(1): 1-11.
[119] Owens DR, Zinman B, Bolli G. Alternative routes of insulin delivery. Diabet Med 2003; 20(11): 886-98.
[120] Johnson PH, Quay SC. Advances in nasal drug delivery through tight junction technology. Expert Opin Drug Deliv 2005; 2(2): 281-98.
[121] Roach P. New insulin analogues and routes of delivery: pharmacodynamic and clinical considerations. Clin Pharmacokinet 2008; 47(9): 595-610.

Received: March 12, 2009 **Accepted: March 13, 2009**