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Wrapping technology and the enhancement of specificity in cancer drug treatment

Ariel Fernández[1,2], Alejandro Crespo[1]

[1] Department of Bioengineering, Rice University, Houston, TX77005, [2] Department of Experimental Therapeutics, M. D. Anderson Cancer Center, Houston, TX 77030

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1. ABSTRACT

Molecularly targeted treatment of malignancy requires a careful control of drug specificity. We review recent advances in this area focusing on a particular marker for ligand-target associations: the poorly wrapped hydrogen bond or *dehydron*, a packing defect in the protein target. Dehydrons promote their own dehydration and are generally not conserved across homologs. Thus, the so-called "wrapping technology" is geared at enhancing drug specificity by developing ligands that can contribute exogenously to shield dehydrons from water attack. This type of design is guided by an analysis of protein interfaces and the assessment of environmental changes around preformed hydrogen bonds occurring upon association. Dehydron differences across *a-priori* targets have been exploited to redesign drugs in order to enhance selectivity. Tested wrapping modifications to established cancer drugs are reviewed. The rationally directed impact of the prototype compounds points to a broad applicability of the wrapping technology, ultimately leading to molecular therapies with tighter control of side effects. New perspectives on the treatment of cancer progression using the wrapping technology are outlined. In particular, we sketch a future strategy to develop highly selective inhibitors targeting a signaling complex critical to cancer metastasis.

2. INTRODUCTION

Molecularly targeted therapy is a powerful tool in the struggle against cancer. In this regard, signal-transducing molecules, the kinases, have become quintessential drug targets (1-4). However, the evolutionary relatedness of kinases makes most inhibitory drugs cross reactive, with a high likelihood of off-target associations, yielding highly uncertain and often dangerous results (4-6). The most alarming aspect of such treatments is the actual unpredictability in the extent of specificity, with the concurrent health-related risks and side-effect complications. Thus, considerable effort is currently devoted to solve this critical biomedical problem and create a translational platform to promote target specificity in drug development and drug-based imaging diagnosis. This venture has been recently sparkled by a key observation: there exists a molecular indicator for specificity hitherto unnoticed: the packing or wrapping defect not conserved across homolog proteins (7, 10). Thus, a new enabling technology has been introduced, ***the wrapping technology***, to target such packing defects and turn wrapping molecular prototypes into therapeutic and diagnostic tools. A new field, ***Molecular Theranostic Engineering,*** is likely to emerge from this synergistic endeavor and a new breed of professionals capable of translating basic research on protein associations into clinical application will likely be nourished.

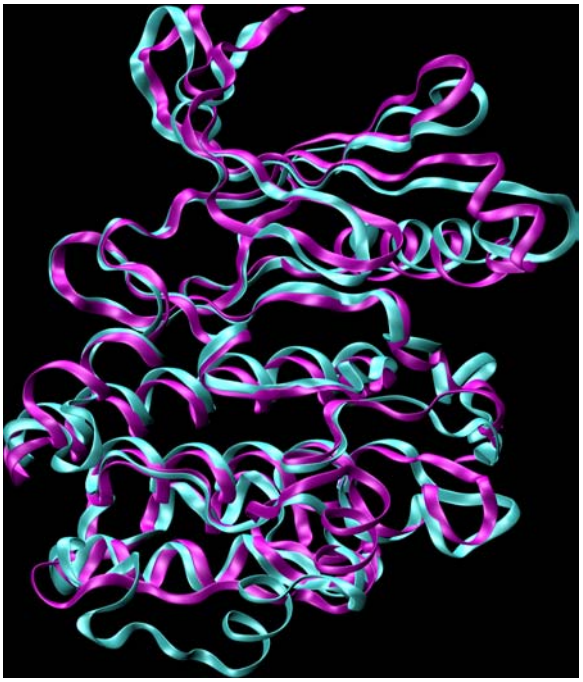

Figure 1. Structural similarity among kinases leads to cross reactivity, a likely source of side effects. Structural alignment of the phosphoinositide-dependent protein kinase 1 (Pdk1, magenta) and checkpoint kinase 1 (Chk1, verdigreen).

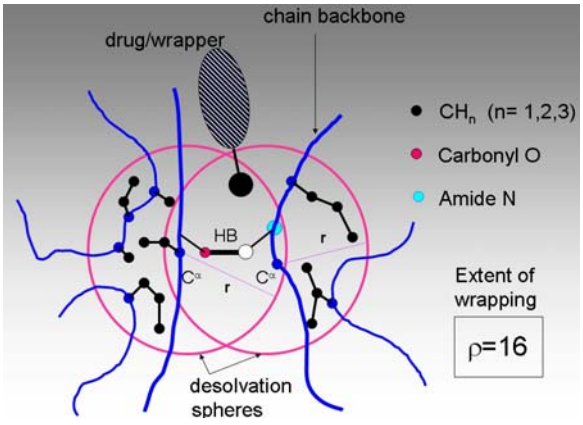

Figure 2. *The wrapping concept in drug design.* Intramolecular hydrogen bonds in soluble protein targets prevail only if they are protected from water attack. Thus, their extent of intramolecular wrapping, ρ, by nonpolar "greasy" groups (black balls) becomes central to define their stability and strength. Poorly wrapped hydrogen bonds (dehydrons) are packing defects that become strengthened from water removal. We shall design small-molecule drugs that "wrap" dehydrons upon association by contributing nonpolar groups to their desolvation spheres.

Ligand cross reactivity, amply illustrated in drug-based kinase inhibition (5, 8-10), has been identified as a major cause of side effects and of misleading or ambiguous diagnosis. The wrapping technology focuses on this problem adopting a bottom-up interdisciplinary approach that makes use of a translational platform. The main goal of this technology is to use molecular design to modulate cross reactivity within the oncokinome in order to sharpen the impact of a new generation of drugs on targets of clinical relevance for therapeutic and imaging purposes. This is a challenging problem since the extent of structural conservation of kinases, especially at the primary (ATP-) binding sites, is staggering (Figure 1). As indicated above, our starting point is the observation that there is a molecular marker for ligand specificity so far overlooked: the packing defects that are not conserved across evolutionary related proteins (11). Packing defects are functionally critical because they are indicators of protein interactivity, or markers for protein-ligand association (12, 13) and constitute a decisive factor in macromolecular recognition (14). These defects consist of intramolecular hydrogen bonds incompletely packed, or poorly protected from water attack. They are termed *dehydrons* (13, 15), because they promote their own dehydration as a means to strengthen and stabilize the electrostatic interaction. Dehydrons may be identified from protein structure by quantifying the extent of intramolecular desolvation of the hydrogen bonds. This parameter indicates the number of "***wrapping***" nonpolar groups within a microenvironment around the hydrogen bond (Figure 2). *Thus, to attain specificity, we the wrapping technology offers a strategy to engineer drugs that "wrap" packing defects that are not conserved across paralogs*.

Harnessing on this key observation, the implementation of the wrapping technology involves various interrelated components (Figure 3): (a) computational construction of a selectivity filter for the entire human kinome, (b) in-silico design and docking of drug inhibitors that serve as "wrappers" or protectors of packing defects in target kinases, (c) chemical synthesis of compound libraries based on the designed molecular prototypes, to be screened for affinity and specificity optimization, (d) molecular therapeutics, bioavailability, pharmacokinetic and imaging assays to test the specificity and efficacy of molecular wrappers on tumor cell lines, animal models and ultimately, on humans, e) X-ray characterization of the optimized protein-ligand complexes and structural dissection of the wrapping interfaces, and f) use of designed drugs as highly specific ligands to enhance the image contrast between tumor/normal tissue.

The over-all goal is to turn the foundational research on the rational in silico development of ligand/wrappers into a tool for cancer therapeutics and imaging-based diagnosis. An interdisciplinary effort will ultimately be necessary to reveal whether improved target specificity results in an enhanced therapeutic index (TI) and whether the novel structural indicator constitutes a selectivity filter useful to reduce drug toxicity and enhance the image contrast between tumor/normal tissue in imaging diagnosis.

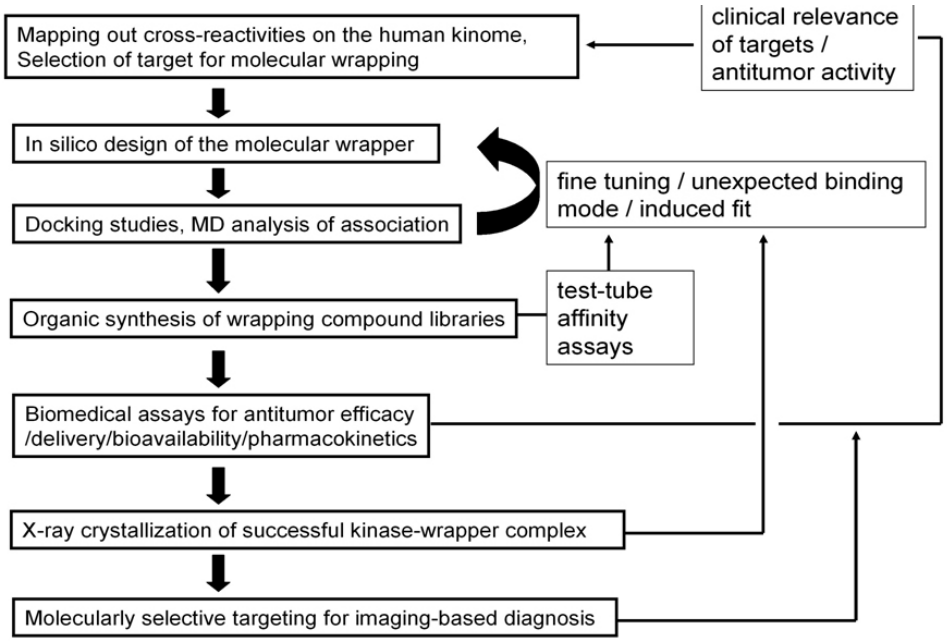

Figure 3. Flow chart of the pipelined discovery /translational components associated with the actual implementation of the wrapping technology, their interrelationships, and feedback mechanisms for adaptation and refinement needed to fine tune the design strategy.

3. BACKGROUND AND SIGNIFICANCE

Cancer remains an unsolved purge of modern society. Fundamental concepts are desperately needed, and no single institute has the resources and expertise to make the necessary breakthroughs. Molecularly targeted drug-based therapy (1-10) is regarded as one of the most valuable tools in the struggle against the disease. However, due to the cross reactivity of available protein ligands (1-5), these procedures may become very noisy and rendered useless or potentially health-threatening. On the other hand, unforeseen cross reactivity has proven to be virtually unavoidable in current combinatorial approaches to drug discovery (5).

Thus, target specificity and the modulation of selectivity towards targets of clinical relevance are critical issues in drug-based therapy and imaging diagnosis. In this regard, a fundamental problem has been addressed as the wrapping technology has been developed (7-10): What is the molecular basis for noise or cross reactivity in drug therapy and for the lack of image contrast in detection and how can we reduce them to sharpen the focus on clinical targets? To solve this problem, a novel concept in biomolecular design has been introduced and a translational platform that contains basic, applied and clinical components has been adopted.

We are very much aware that specificity might not be essential for clinical activity. The therapeutic success of Gleevec (imatinib) is a good illustration of this fact (1). On the other hand, the knowledge acquired from intense clinical use of Gleevec indicates that, although clinical activity is achieved, the nonselectivity for the target leads to side effects such as skin and hematopoietic toxicities (1, 16). The wrapping technology hinges on the well-sustained premise that controlled target selectivity directed towards a predetermined set of clinically relevant targets will lead to reduced toxicity and enhanced antitumor activity. The contention is that although specificity may not be required for clinical activity, controlled selectivity focusing the impact on targets devoid of toxicity may improve the therapeutic index of kinase inhibitors. Thus, wrapping technology introduced a novel marker for specificity and used it to re-design available drugs in order to modulate their selectivity towards targets of known clinical relevance for both diagnosis/detection and therapeutic purposes.

At the core of the problem lies the fundamental observation that human genes are evolutionary related and consequently, their expressions reveal high similarity in their 3D-structure (Figure 1). Kinases, the quintessential targets of drug therapy, illustrate this point: their homologies make drug-based inhibition cross reactive, often leading to serious side effects. Thus, it becomes critical to charter an interdisciplinary effort to design, test and clinically implement novel strategies to achieve drug specificity. This effort requires a molecular understanding of clinically relevant targets above and beyond a structural characterization.

4. WRAPPING THE PROTEIN STRUCTURE

The term wrapping indicates a clustering of nonpolar groups framing an anhydrous microenvironment for an intramolecular hydrogen bond within the structure of a soluble protein (10, 12). The extent of intramolecular hydrogen-bond desolvation, ρ, in monomeric structure

may be quantified by determining the number of nonpolar groups (carbonaceous, not covalently bonded to an electrophilic atom) contained within a desolvation domain. The desolvation domain is defined as two intersecting balls (Figure 2) of fixed radius centered at the α-carbons of the residues paired by the backbone amide-carbonyl hydrogen bond. The extent of desolvation of an intramolecular hydrogen bond within a protein-ligand or protein-protein complex requires that the count include nonpolar groups from the monomer as well as those from its binding partner(s).

The statistics of hydrogen-bond wrapping vary according to the desolvation radius adopted, but the tails of the distribution invariably single out the same "under-wrapped" hydrogen bonds in a given structure over a 6–7Å range in the adopted desolvation radius, a length scale that represents the thickness of 3 water layers, enough to define the microenvironment of a hydrogen bond.

Underwrapped hydrogen bonds constitute a type of packing defect, since there is a lack of nonpolar groups from the amino acid side chains in their spatial vicinity. A mutation which increases the number of carbonaceous groups in a side chain (e.g., glycine to leucine) corrects this defect by increasing the amount of wrapping. Wrapping of salt-bridges is similar in concept[13] to wrapping of hydrogen bonds, and indeed the concept can apply to any electrostatic interaction.

Wrapping does not need to fully exclude water molecules to enhance the electrostatic environment. By causing the water environment to be more structured, a weaker dielectric can result. A weaker dielectric results in a stronger electrostatic interaction.

Although the assessment of wrapping requires a three-dimensional structure, a disorder score, measuring the propensity for inherent disorder (12) correlates with the wrapping of individual peptides engaged in backbone hydrogen bonds. The correlation is expected, since structure stabilization is contingent on the possibility of water exclusion from backbone hydrogen bonds. Thus the disorder score, a reliable sequence-based attribute, has been used to predict wrapping for **all** tyrosine kinases, even those with unreported structure (9). The dehydron predictor based on the disorder score plot is only effective for homologs expected to possess a high degree of structure similarity with PDB reported proteins. Thus, it becomes particularly useful when focusing on the kinase superfamily, where PDB-reported kinases are evolutionarily related to those with unknown structure. This relatedness validates the homology threading that necessarily serves as framework for the disorder-based prediction.

5. DEHYDRONS

Dehydrons are defined in terms of the effect on the dielectric environment due to the approach of a nonpolar group or "wrapper" (12, 15). That is, hydrogen bonds which become strengthened and stabilized by the approach of a hydrophobic group, such as in a drug ligand that binds to a protein, are designated dehydrons. Such a change can be verified by molecular dynamics simulations[15]. Often this occurs because the environment of the bond allows the approach of water, so one can think of a dehydron as a solvent-accessible hydrogen bond. In the extreme case of thorough exposure, water can disrupt the hydrogen bond by hydrating the backbone carbonyl and amide groups.

In folds for soluble proteins at least two thirds of the backbone hydrogen bonds are wrapped on average by $\rho=26.6\pm7.5$ nonpolar groups for desolvation sphere radius 6.2Å. Dehydrons are then defined as hydrogen bonds whose extent of wrapping lies in the tails of the distribution, i.e. with 19 or fewer nonpolar groups in their desolvation domains, so their ρ-value is below the mean, minus one Gaussian dispersion.

Dehydrons constitute sticky sites with a propensity to become dehydrated (7-15). Thus, dehydrons promote protein-ligand associations that "correct" packing defects. Their stickiness arises from the charge-screening reduction resulting from bringing nonpolar groups to proximity: water exclusion enhances and stabilizes pre-formed electrostatic interactions compensating for the dehydration penalty. Thus, a sufficient number of wrappers ($\rho>19$), while making hydration thermodynamically costly, introduces a compensation by enhancing the stability of the hydrogen bond. In most PDB protein-inhibitor complexes, the ligand is in effect a wrapper of dehydrons in the protein.

Protein dehydrons are invariant across complexes of the same protein with different ligands and invariant across different crystallization forms, except in cases where the different ligands produce different induced fits of the protein. Thus, in the case of kinases, wrapping modifications of the ligand directed at modulating specificity are most reliable when they target dehydrons that occur in rigid regions of the target endowed with secondary and tertiary structure. To target dehydrons in induced fits of flexible regions (activation loop, P-loop, catalytic loop, etc.), it becomes essential to conduct a molecular dynamic study complementing the *in-silico* design in order to identify the binding mode of the perturbed ligand.

6. WRAPPING TECHNOLOGY AS A TOOL TO ACHIEVE DRUG SPECIFICITY

The wrapping technology involves a novel biomolecular perspective on protein targets needed to address a problem considered to be the graveyard of most drug-discovery or ligand design efforts: *The potential toxicity of side effects*. Side effects may be due to at least two discernible causes: a) The target protein is involved in several pathways, causing the drug/ligand to perturb off-target pathways; b) the drug/ligand is cross-reactive in the sense that its intended target possesses several paralogs which offer potential alternative binding sites because they

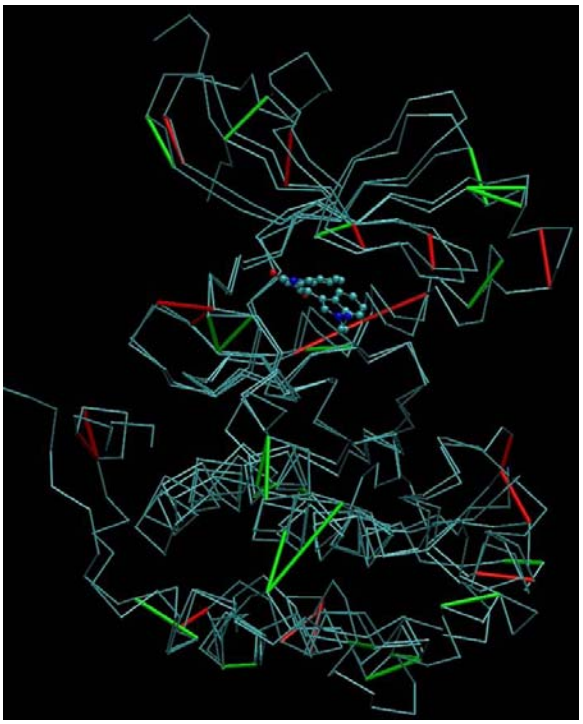

Figure 4. Aligned backbones for two paralog kinases, Chk1 and Pdk1, represented by virtual bonds joining α-carbons. The dehydrons or poorly wrapped hydrogen bonds are marked as segments joining the α-carbons of the residues contributing with the amino or carbonyl group to the backbone hydrogen bond. Dehydrons for Chk1 are marked in green and those for Pdk1 are in red. While the kinases are structurally similar (RMSD= 4.1Å), *their pattern of packing defects is different and our design strategy will take advantage of this fact*. Dehydrons may be identified from the atomic coordinates by determining the extent of intramolecular hydrogen-bond desolvation. This value is given by the number of "***wrapping***" nonpolar groups contained within a desolvation domain defined as two intersecting balls of fixed radius centered at the α-carbons of the residues paired by the hydrogen bond. The statistics of hydrogen-bond wrapping depend on the desolvation radius, but the tails of the distribution single out the same dehydrons over a 6.2-7Å range in the radius. In this proposal the value 6.4Å was used throughout. In soluble proteins at least two thirds of the backbone hydrogen bonds are wrapped on average by $\rho=26.6\pm7.5$ nonpolar groups. Dehydrons are defined as hydrogen bonds with 19 or fewer wrappers, so their p-value is below the mean minus one Gaussian dispersion.

share essentially the same fold (Figure 1). Drug discovery remains a semiempirical endeavor, essentially supplemented by structural intuition. *Thus, it is unlikely that the high levels of cross reactivity detected in high throughput screening experiments will be tempered or modulated using rational design, unless a nonstandard approach is able to discern paralogs above and beyond what a structural characterization may reveal* (5).

Tackling problem a) directly is too risky and uncertain at point in time. It demands a careful assessment of connectivity relationships among various components of the human interactome, an area still in its infancy. On the other hand, it makes perfect sense to focus on a strategy to minimize problem b) by engineering drugs based on the identification of the novel structure-based indicator of protein interactivity: the packing defect. As indicated above, such defects consist of intramolecular hydrogen bonds incompletely packed or poorly protected from water attack. Such packing defects are functionally critical because they promote protein interactivity and constitute a decisive factor in macromolecular recognition. In turn, these defects are sticky, i. e. promoters of water removal, and *not conserved across paralogs*, as clearly illustrated (Figure 4).

These properties make dehydrons ideal targets to minimize cross-reactivity in the inhibitory impact of a potential drug (7-10). Thus, dehydrons have been turned into a novel drug-design concept of enormous potential. The innovative concept of "inhibitor as wrapper of protein packing defects" has been developed (Figure 5), tested *in vitro* on specific cancer cell lines, and will ultimately tested *in vivo* combining detection/diagnosis with molecular therapeutics.

Since dehydrons are indeed markers for protein associations, the design strategies geared at targeting dehydrons with small molecules will eventually open up an avenue to tackle the problem of blocking protein-protein associations, one of the most challenging and important areas in drug discovery.

In spite of the spectacular clinical success of Gleevec, achieving selectivity by targeting kinases remains a hit-and-miss problem (1-5). However, we have identified a molecular indicator of drugability that also enables us to discriminate between these evolutionarily related targets. Figure 4 illustrates our assertion by showing the dehydron patterns for two cancer-related paralog kinases.

We have experimentally substantiated the design concept of "inhibitor as wrapper of packing defects" and thus, we are ideally positioned to tackle central therapeutic problems involving selective drug-based inhibition of cancer-related kinases (1-10). Enabling technologies are currently emerging as molecular wrappers of packing defects are turned into efficient selective inhibitors and imaging probes. *The wrapping technology appears to provide the right approach to rationally engineer specificity for at least three reasons: a) because it focuses on a novel structural feature, the dehydron, that differentiates evolutionarily related proteins representing alternative drug targets; b) because the wrapping technology is capable to effectively target dehydrons, as shown subsequently; and c) because the selectivity of commercially available drugs is in fact based on the dehydron footprint of the kinases, although the drugs were not* ***purposely*** *design to wrap dehydrons.*

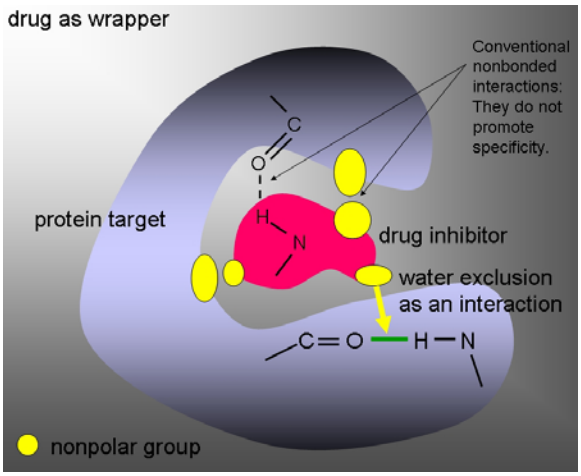

Figure 5. Standard and non-standard interactions of a drug inhibitor with its protein target. The exclusion of water from the surroundings of a packing defect (dehydron) in the protein reveals the wrapping role of the inhibitor.

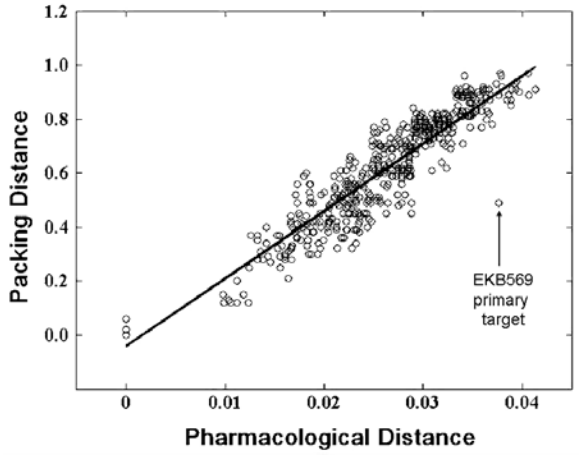

Figure 6. Correlation between packing distance and pharmacological distance for all pairs constructed from a pool of 32 PDB-reported kinases independently fingerprinted for affinity against 17 drugs. The pharmacological distance between two kinases is defined as the Euclidean distance between the normalized affinity vectors with entries corresponding to the negative logarithm of the binding constants. The pharmacological matrix (**PM**) is obtained by calculating the pharmacological distances between all kinase pairs $\mathbf{PM}_{X,Y} = \|X-Y\| = \sqrt{\sum_{n \in \text{set of inhibitors}} (X_n - Y_n)^2}$, where X_n, Y_n represent respectively the normalized values of the negative logarithm of binding constants for complexation of kinase X and kinase Y with drug n.

To demonstrate this last assertion, we first introduce a packing distance between kinases defined by comparing not the structures themselves but the packing patterns of the ATP sites. Thus, the packing distance between two kinases may be determined in four steps (9): a) alignment of their respective hydrogen-bond matrices (i,j-entry =1 if residues i,j are paired by a hydrogen bond and 0 otherwise) (cf. Figure 4); b) derivation of dehydron matrices that inherit their alignment from step a); c) restricting dehydron matrices to the ATP-binding site; and d) computing the Hamming distance between the restricted dehydron matrices. Thus, packing distances were computed across the 32 kinases reported in PDB for which drug-affinity fingerprinting was experimentally and independently obtained (5). 17 inhibitors were selected from a pool of 20 that have been independently assayed for cross reactivity against a set of 113 kinases. Three inhibitors, staurosporine, SU11248 and EKB569 were excluded from the computation since their high promiscuity is indicative of a mode of anchoring based on hydrophobic interactions with highly conserved nonpolar residues and not on dehydron wrapping, as indicated below.

The packing-distance matrix has been contrasted with a pharmacological distance matrix assessing similarities in the affinity profiling of kinases. The pharmacological matrix is thus obtained by computing the Euclidean distance between ligand-affinity vectors in $\boldsymbol{R}^{17}$ with entries given in *–ln* scale (or dimensionless ΔG/RT units, with ΔG=Gibbs free energy change associated with binding, R=universal gas constant, T=absolute temperature). By plotting packing versus pharmacological distance (Figure 6) for each pair of kinases reported in PDB and fingerprinted for affinity against the 17 drug ligands (5), we establish a strong correlation (R^2=0.9028). This correlation reveals that the pattern of packing defects is statistically an operational selectivity filter for drug design, even though individual drugs were not purposely designed to wrap packing defects in proteins!: *Pharmacological differences are essentially dictated by packing differences among targets.* Thus, future developments of the wrapping technology are expected to take advantage of this hitherto overlooked design feature to dramatically simplify the drug development effort and rationally enhance selectivity towards clinically significant targets.

7. RATIONALE FOR THE WRAPPING TECHNOLOGY

A seminal observation justifies the implementation of the wrapping technology: *homolog proteins may be distinguished based on differences in their packing defect patterns* (Figure 4)*, and packing defects are inherently sticky, thus providing ligand-anchoring sites to foster specificity*. Harnessing on this observation, the wrapping technology will ultimately translate the *in silico* design into clinically relevant therapeutic or diagnostic tools. A combined use of a sequence-based selectivity filter and novel structure-threading algorithms will enable us to ultimately focus on the entire human oncokinome.

High throughput screening techniques (5) have revealed that the inhibitor-protein interaction map for existing drugs or lead compounds tends to be diffuse or highly spread over a number of alternative targets, often

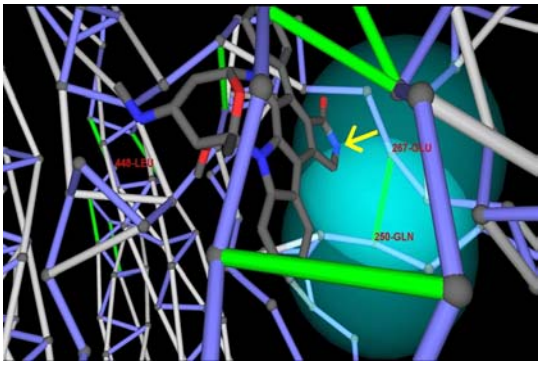

Figure 7. Relative position of kinase packing defects around the ligand indole region in a staurosporine-kinase complex. Microenvironment of dehydron Gln250-Glu267 in Src kinase framed by the desolvation spheres centered at the α-carbons of Gln250 and Glu267. Methylation at the indole N5-position (indicated by the yellow arrow) would turn the ligand into a wrapper of the nonconserved packing defect in Src kinase.

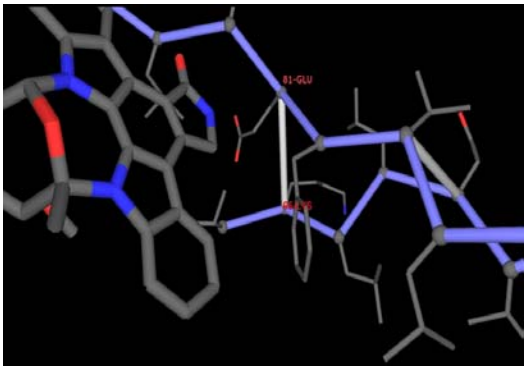

Figure 8. Wrapping environment of the intramolecularly dehydrated backbone hydrogen bond Lys65-Glu81 in CDK2 which aligns with dehydron Gln250-Glu267 in Src kinase. Only the relevant parts of the CDK2 are shown for clarity. The Lys65-Glu81 backbone hydrogen bond in CDK2 that aligns with the 250-267 dehydron in Src kinase is actually an intramolecularly well-packed hydrogen bond.

causing side effects with various levels of toxicity. Thus, by assessing packing differences across alternative targets, the wrapping technology enables the manipulation of the inhibitory impact of a new generation of drugs that exploit a novel selectivity filter and a novel selectivity switch.

In contrast with drug-design approaches based on standard structural considerations, the packing of a protein, or more precisely, its dehydron pattern, may be used as a selectivity filter to design small-molecule inhibitors. Thus, a novel form of rational design emerges to tackle the long-standing problem of avoiding side effects in drug therapy.

At this point in time, it becomes essential to carry the concept of "inhibitor as dehydron wrapper" to the next phase of development. This phase involves the higher testing ground of pharmacokinetics and possibly *in-vivo* assay, with the ultimate aim of developing a cancer therapy and detection based on wrapping compounds that selectively "correct" packing defects on target kinases.

To summarize, the drug development approach described is driven by a specific problem, the need to master drug specificity, it is highly interdisciplinary, and purports to develop a novel enabling technology with a profound translational projection into cancer therapy.

8. A STRINGENT TEST FOR WRAPPING TECHNOLOGY: FROM PROMISCUITY TO SPECIFICITY

Perhaps the most stringent test on the feasibility of engineering selectivity by designing a wrapping ligand involves modifying *staurosporine*, the most promiscuous kinase ligand available (17). The aim here is to elicit a selective inhibitory impact that distinguishes packing differences across its multiple targets. Thus, four PDB-reported staurosporine-binding kinases with significant pairwise packing distances (>0.4) and extremely low staurosporine-based pharmacological distance (<0.01) may be considered: Src kinase (PDB.1BYG), CDK2 (PDB.1AQ1), Chk1 (PDB.1NVR) and PDK1 (PDB.1OKY). Our wrapping analysis reveals that only the Src kinase possesses a nonconserved dehydron, the backbone hydrogen bond Gln250-Glu267, that may be wrapped by methylating staurosporine at the imide N6-position of the indole ring (Figure 7).

Upon structural alignment, the Src dehydron maps into well-wrapped backbone hydrogen bonds: Lys65-Glu81 in CDK2, Lys69-Glu85 in Chk1 and Lys144-Ser160 in PDK1. The lack of conservation of the Src dehydron is revealed by comparing the structural alignments of the Src kinase (Figure 7) with CDK2 (Figure 8), Chk1 or PDK1. Thus, we predict that selectivity for Src kinase may be achieved by redesigning staurosporine to turn it into a wrapper of the Gln250-Glu267 dehydron, a packing defect *not* conserved in the alternative targets CDK2, Chk1 and PDK1, of the parental compound.

The chemical modification of staurosporine (18, 19) entails replacing the imide hydrogen in the indole ring with a methyl group, a substitution known to severely impair the capacity of the ligand to become engaged as donor in an intermolecular hydrogen bond with the ATP pocket. Methylation at indole N6 may be achieved by two routes: a) Recapitulating the staurosporine synthesis using methyl substitution on the indole N6 as protective group, and retaining the substitution throughout the synthesis; b) using staurosporine as starting point and methylating with NaH/DMF (sodium hydride/dimethyl formamide) with prior protection of alternative N-methylation sites (19). The latter route was selected for simplicity.

To test whether the specificity and affinity for Src improved as the staurosporine derivative is compared with the parental compound, we conducted kinetic spectrophotometric assays as indicated above. These assays were geared at measuring the phosphorylation rate of peptide substrates in the presence of the kinase inhibitor at different concentrations (Figure 9). As indicated in Figure 9, the inhibition of the Src by the drug-wrapper of dehydron Gln250-Glu267 *improved* when compared with

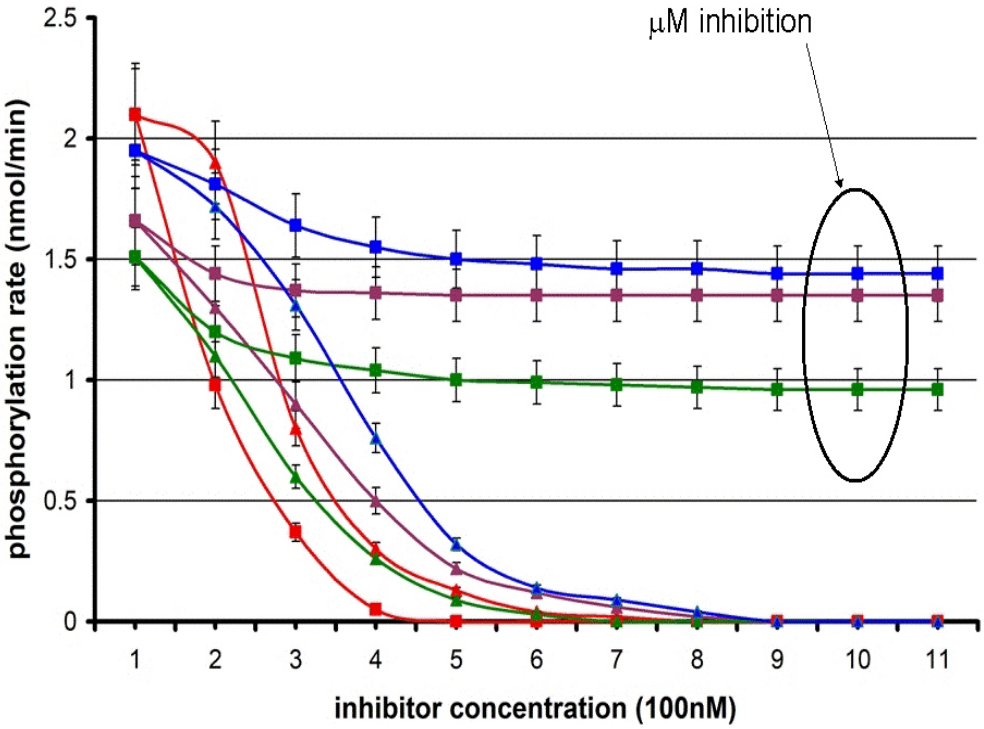

Figure 9. Phosphorylation rates of Src (red), CDK2 (blue), Chk1 (purple) and Pdk1 (green) in the presence of staurosporine (triangles) and in the presence of the staurosporine methylated at the imide N6 of the indole ring (squares). The latter compound was designed to better wrap the nonconserved dehydron Gln250-Glu267 in Src kinase. Error bars represent dispersion over 10 runs for each kinetic assay. Within the means of detection, the kinase phosphorylation rates do not vary appreciably in the range 0-100nM inhibitor concentration. **Spectrophotometric kinetic assay.** To determine the level of selectivity of drug inhibitors designed by adopting the wrapping technology, kinetic assays of the inhibition of multiple kinases have been conducted. To measure the rate of phosphorylation due to kinase activity in the presence of inhibitors, a standard spectrophotometric assay has been adopted in which the adenosine diphosphate production is coupled to the NADH oxidation and determined by absorbance reduction at 340nm. Reactions were carried out at 35ºC in 500μl of buffer (100mM Tris-HCl, 10mM $MgCl_2$, 0.75mM ATP, 1mM phosphoenol pyruvate, 0.33mM NADH, 95 units/ml pyruvate kinase). The following peptide substrates (Invitrogen/Biaffin) for kinase phosphorylation were chosen for their high specificity: KVEKIGEGTYGVVYK for SRC; HHASPRK for CDK2; GCSPALKRSHSDSLDHDIFQL for Chk1; and EGLGPGDTTSTFCGTPNYIAP for Pdk1.

staurosporine levels. Furthermore, the inhibitory impact of the ligand-wrapper in the form of a methylated staurosporine derivative became selective for Src vis-à-vis CDK2, Chk1 and PDK1. Dehydron Gln250-Glu267 is either absent or well-wrapped in the latter PDB-reported kinases, and consistently, the drug designed to better wrap this dehydron has very low inhibitory impact against the other PDB-reported paralog kinases. Thus, we have demonstrated that packing differences across protein paralogs may guide molecular design to significantly enhance specificity. The illustration described in this section is critical in this regard, since it entails the redesign of the most promiscuous kinase inhibitor known to date.

9. PERSPECTIVE: WRAPPING DRUGS TO TREAT CANCER PROGRESSION

Due to its commanding role in transducing extracellular signals to modulate cell adhesion and motility, the focal adhesion kinase (FAK) has been recently identified as a major potential target to treat cancer metastasis (20, 21). The therapeutic interest in the kinase role of FAK and of its signaling partner, the Src kinase, arises from the fact that the FAK/SRC signaling axis has been identified as playing a central role in promoting invadopodia formation and carcinoma cell invasion, in part through the induction of Rac activity (22). Thus, the FAK/Src signaling axis has been unambiguously implicated in the metastatic cell phenotype (23).

What follows is an "*in-silico* design exercise", aimed at delineating the possibilities of the wrapping technology in guiding the molecular engineering of highly selective inhibitors to target the FAK/Src axis. This exercise is aimed at chartering the preliminary work that will be required to ultimately develop therapeutic molecular agents to hamper cancer metastasis.

9.1. *In silico* design of a FAK wrapping inhibitor

From a structural biology perspective, there are major challenges associated with a rational design of highly selective FAK inhibitors. A promising target results from the existence of a nonconserved dehydron depicted in Figures 10, 11.

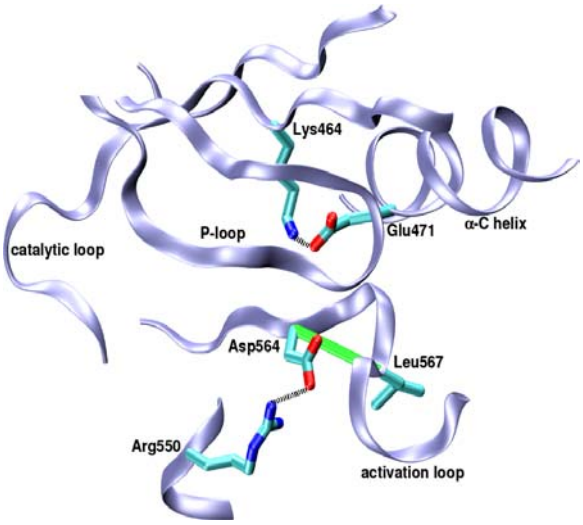

Figure 10. The nonconserved dehydron Asp564-Leu567 (green segment joining the α-carbons) in FAK.

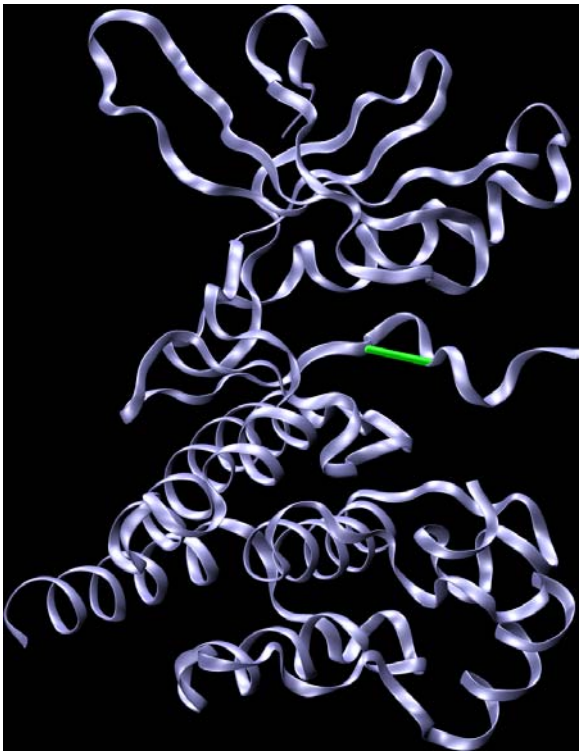

Figure 11. Position of the nonconserved FAK dehydron in the overall conformation.

From the perspective of the wrapping technology, the central problem we ultimately need to address is: How do we redesign an existing compound with affinity for FAK and turn it into a selective FAK inhibitor? This problem may be tackled since FAK possesses a nonconserved dehydron (Asp564-Leu567) involving the highly conserved Asp564 from the DFG catalytic triad located in the activation loop (Figures 10, 11) and thus can be turned into a targettable feature to promote specificity. Taking into account these considerations, we may eventually design an inhibitor inspired in a basic chemical lead but with the wrapping components that would make it specific for FAK.

9.2. *In silico* design of an Src kinase wrapping inhibitor

The full enzymatic activity of FAK is known to be contingent on the phosphorylation of FAK by the Src kinase on positions Tyr576 and Tyr577 (other phosphorylation sites have been identified) (20). Thus, the FAK/Src signaling complex triggers the cascade that leads to the onset of the motile and ultimately invasive phenotype. These considerations prompt us to design also an Src inhibitor as a means to potentially hamper the full activation of FAK.

The likelihood of success of the wrapping technology in achieving specificity on an Src inhibitor appears to be also high: this kinase possesses a highly nonconserved packing defect in the form of an under-wrapped salt bridge (named *desolvon* (13)) involving the polar pair Lys295-Asp404. This salt bridge involving the Asp404 within the catalytic triad in the activation loop is most unusual and not found in any other kinase: A structural alignment with any other of the 86 human kinases reported in PDB would lead us to infer that the highly conserved Lys295 would interact with the also highly conserved helical Glu310. This is not the case, however, for the Src kinase, and we may take advantage of this singularity to develop a highly selective wrapping inhibitor for the Src kinase (Figure 12).

The two *in silico* design "exercises" presented above explore unchartered territory in the design of wrapping ligands: Their focus is the development of molecular prototypes that should be ultimately translated into therapeutic tools to treat cancer progression and metastasis, rather than cancer development or the onset of malignancy. Thus, the manifold interrelated components of wrapping technology will need to be deployed if such drawing-board products are to be ultimately turned into inhibitors of therapeutic value.

To conclude, we believe that the foundational steps have been undertaken to develop a novel technology in drug discovery based on singularities in the structure of soluble proteins that have not been previously described. In contrast with standard drug-design approaches, the wrapping of a protein, or more precisely, its pattern of packing defects, may be used as a selectivity filter and a selectivity switch to design small-molecule inhibitors. Thus, a novel form of rational design is now available to tackle the long-standing problem of avoiding side effects in drug therapy and will contribute to sharpen the inhibitory impact of drugs on the oncokinome.

Using chemical leads emerging from screening and other medicinal chemistry studies, we should be able to introduce a variety of wrapping modifications, test them in cell lines for enhanced affinity and specificity, and in xenografts and ultimately, in humans.

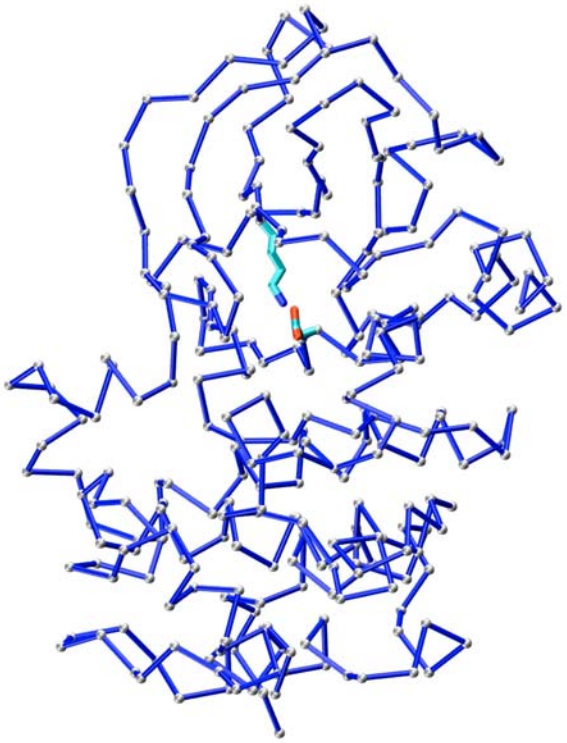

Figure 12. The nonconserved Lys295-Asp404 desolvon in Src Kinase.

Future developments are envisioned as we couple the wrapping technology with novel high-throughput screening assays based on libraries of bacteriophage-expressed kinases. These techniques will no doubt enable an unprecedented level of drug-affinity profiling against hundreds of kinases. Our wrapping compounds are currently subject to this level of screening in order to fine tune our engineering of controlled inhibitory impact. We are already embarked on a significant effort to combine our modeling design with the novel screening tool.

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11. REFERENCES

1. A. Levitski and A. Gazit: Tyrosine kinase inhibition: an approach to drug development. *Science* 267, 1782-1788 (1995)

2. N. J. Donato, and M. Talpaz: Clinical use of tyrosine kinase inhibitors: Therapy for chronic myelogenous leukemia and other cancers. *Clin. Cancer Res.* 6, 2965-66 (2000)

3. B. J. Druker: Molecularly targeted therapy: have the floodgates opened? *Oncologist* 9, 357-360 (2004)

4. Z. A. Knight and K. M. Shokat: Features of selective kinase inhibitors. *Chemistry & Biology* 12, 621–637 (2005)

5. M. A. Fabian, W. A. Biggs, D. K. Treiber, C. E. Atteridge, M. D. Azimioara, M. G. Benedetti, T. Carter, P. Ciceri, P. T. Edeen, M. Floyd, J. M. Ford, M. Galvin, J. L. Gerlach, R. M. Grotzfeld, S. Herrgard, D. E. Insko, M. A. Insko, A. Lai, J.-M. Lélias, S. Mehta, Z. V. Milanov, A. M. Velasco, L. M., Wodicka, H. K. Patel, P. P. Zarrinkar and D. A. Lockhart: A small molecule-kinase interaction map for clinical kinase inhibitors. *Nature Biotechnology* 23, 329-336 (2005)

6. J. Bain, H. McLauchlan, M. Eliott and P. Cohen: The specificities of protein kinase inhibitors: an update. *Biochem. J.* 371, 199-204 (2000)

7. A. Fernández, K. Rogale, L. R. Scott and H. A. Scheraga: Inhibitor design by wrapping packing defects in HIV-1 proteins. *Proc. Natl. Acad. Sci. USA* 101, 11640-11645 (2004)

8. A. Fernández: Incomplete protein packing as a selectivity filter in drug design. *Structure* 13, 1829-1836 (2005)

9. A. Fernández and S. Maddipati: The *a-priori* inference of cross reactivity for drug-targeted kinases. *Journal of Medicinal Chemistry* 49, 3092-3100 (2006)

10. J. Chen, X. Zhang and A. Fernández: "Molecular basis for specificity in the druggable kinome: sequence-based analysis". Bioinformatics, published in Advanced Access: doi:10.1093/bioinformatics/btl666, in press (2007)

11. A. Fernández and H. A. Scheraga: Insufficiently dehydrated hydrogen bonds as determinants of protein interactions. *Proc. Natl. Acad. Sci. USA* 100, 113-118 (2003)

12. A. Fernández and R. S. Berry: Molecular dimension explored in evolution to promote proteomic complexity. *Proc. Natl. Acad. Sci. USA* 101, 13460-13465 (2004)

13. A. Fernández: Keeping dry and crossing membranes. *Nature Biotechnology* 22, 1081-1084 (2004)

14. C. Deremble and R. Lavery: Macromolecular Recognition. *Curr. Opin. Struc. Biol.* 15, 171-175 (2005)

15. A. Fernández and L. R. Scott: Dehydron: A structurally encoded signal for protein interaction. *Biophys. J.* 85, 1914-1928 (2003)

16. S. Faderl, M. Talpaz, Z. Estrov, S. O'Brien, R. Kurzrock and H. M. Kantarjian: The biology of chronic myeloid leukemia. *N. Engl. J. Med.* 341, 164-172 (1999)

17. A. L. Hopkins, J. S. Mason and J. P. Overington: Can we rationally design promiscuous drugs? *Curr. Opin. Struct. Biol.* 16, 127-136 (2006)

18. J. T. Link, S. Raghavan and S. J. Danishefsky: First total synthesis of staurosporine and ent-staurosporine. *J. Am. Chem. Soc.* 117, 552-553 (1995)

19. H. J. Knölker and K. R. Reddy: Isolation and synthesis of biologically active carbazole alkaloids. *Chem. Rev.* 102, 4303-4427 (2002)

20. G. W. McLean, N. L. Carragher, E. Avizienyte, J. Evans, V. G. Brunton and M. C. Frame: The role of focal adhesion kinase in cancer: A new therapeutic opportunity. *Nature Reviews Cancer* 5, 505-515 (2005)

21. S. K. Mitra, D. A. Hanson and D. D. Schlaepfer: Focal adhesion kinase: In command and control of cell motility. *Nature Reviews Mol. Cell. Biol.* 6, 56-68 (2005)

22. D. A. Hsia, S. K. Mitra, C. R. Hauck, D. N. Streblow, J. A. Nelson, D. Ilic, S. Huang, E. Li, G. R. Nemerow, J. Leng, K. S.R. Spencer, D. A. Cheresh and D. D. Schlaepfer: Differential regulation of cell motility by FAK. *J. Cell. Biol.* 160, 753-767 (2003)

23. C. R. Hauck, D. A. Hsia, X. S. Puente, D. A. Cheresh and D. D. Schlaepfer: FRNK blocks v-Src stimulated invasion and experimental metastasis without effects on cell motility or growth. *EMBO J.* 21, 6289-6302 (2002)

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Send correspondence to: Dr. Ariel Fernandez, Department of Bioengineering, Rice University, Houston, TX77005, Tel: 713-348-3681, Fax: 713-348-3699, E-mail: arifer@rice.edu

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