Corrections and Retraction

CORRECTIONS

BIOPHYSICS. For the article “An allosteric model for heterogeneous receptor complexes: Understanding bacterial chemotaxis responses to multiple stimuli,” by Bernardo A. Mello and Yuhai Tu, which appeared in issue 48, November 29, 2005, of Proc. Natl. Acad. Sci. USA (102, 17354–17359; first published November 17, 2005; 10.1073/pnas.0506961102), the authors note that the citation given for ref. 24 [Ames, P. & Parkinson, J. S. (2004) Proc. Natl. Acad. Sci. USA 101, 2117–2122] was incorrect. The citation should have read as follows:


www.pnas.org/cgi/doi/10.1073/pnas.0600530103

GENETICS. For the article “Mutagenic specificity of endogenously generated abasic sites in Saccharomyces cerevisiae chromosomal DNA,” by Paul Auerbach, Richard A. O. Bennett, Elisabeth A. Bailey, Hans E. Krokan, and Bruce Demple, which appeared in issue 49, December 6, 2005, of Proc. Natl. Acad. Sci. USA (102, 17711–17716; first published November 28, 2005; 10.1073/pnas.0504643102), the authors note that ODG was erroneously listed as an abbreviation for “oxanine-DNA glycosylase.” In fact, ODG represents the empty vector for parallel experiments to the CDG and TDG vectors. In some but not all places, ODG was corrected to read “pODG”; this correction should have been made throughout the paper. These errors do not affect the conclusions of the article.

www.pnas.org/cgi/doi/10.1073/pnas.0600853103

IMMUNOLOGY. For the article “A pilot clinical trial of a recombinant ricin vaccine in normal humans,” by Ellen S. Vitetta, Joan E. Smallshaw, Elaine Coleman, Hasan Jafri, Callie Foster, Robert Munford, and John Schindler, which appeared in issue 7, February 14, 2006, of Proc. Natl. Acad. Sci. USA (103, 2268–2273; first published February 3, 2006; 10.1073/pnas.0510893103), the authors note the following. “E.S.V. and J.E.S. are patent holders on RiVax, a recombinant ricin vaccine, and would be eligible to accept royalties if the vaccine is commercialized. In the case of E.S.V., royalties would be signed over to the University of Texas Southwestern Medical Center.”

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PHYSIOLOGY. For the article “Regulation of sodium-proton exchanger isoform 3 (NHE3) by PKA and exchange protein directly activated by cAMP (EPAC),” by Katharina J. Honegger, Paola Capuano, Christian Winter, Desa Bacic, Gerti Stange, Carsten A. Wagner, Jürg Biber, Heini Murer, and Nati Hernando, which appeared in issue 3, January 17, 2006, of Proc. Natl. Acad. Sci. USA (103, 803–808; first published January 9, 2006; 10.1073/pnas.0503562103), the authors note that the concentrations for EPAC1 (40) and PKA (1) affinities were published incorrectly due to a printer’s error. On page 803, in lines 12–14 of the first full paragraph, right column, “EPAC1 and the B domain of EPAC2 bind cAMP in vitro with a K_m of ≈40 mM, whereas the K_m of PKA is ≈1 mM (17) should read: “EPAC1 and the B domain of EPAC2 bind cAMP in vitro with a K_m of ≈40 µM, whereas the K_m of PKA is ≈1 µM (17).” These errors do not affect the conclusions of the article.

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RETRACTION

BIOPHYSICS. For the article “Packing defects as selectivity switches for drug-based protein inhibitors,” by Ariel Fernández, Ridgway Scott, and R. Stephen Berry, which appeared in issue 2, January 10, 2006, of Proc. Natl. Acad. Sci. USA (103, 323–328; first published December 30, 2005; 10.1073/pnas.0509351102), the editors note that there is substantial overlap in the figures and text of this PNAS article with the article by A. Fernández that appeared in the December 2005 issue of Structure (13, 1829–1836) titled, “Incomplete protein packing as a selectivity filter in drug design.” The latter article, which is copyrighted by Structure, is not cited in the PNAS article, and all panels of the figures appearing in the Structure article are reproduced in the PNAS article without reference to the Structure article. PNAS policy states that articles must not be previously published and that permissions must be obtained for any previously published portions of the work prior to publication.

Because this article does not meet these requirements, PNAS is withdrawing it.

Nicholas R. Cozzarelli, Editor-in-Chief

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Packing defects as selectivity switches for drug-based protein inhibitors

Ariel Fernández*†§, Ridgway Scott‡¶, and R. Stephen Berry§**

*Department of Bioengineering, Rice University, Houston, TX 77005; †Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202; and Departments of ‡Computer Science, §Mathematics, and **Chemistry and Institute for Biophysical Dynamics, University of Chicago, Chicago, IL 60637

Contributed by R. Stephen Berry, October 26, 2005

The conservation of structure across homolog proteins often diffuses the impact of drug-based inhibition by promoting alternative protein–ligand associations that may lead to toxic side effects. However, sticky packing defects are typically not conserved across homologs, making them valuable a priori targets to enhance specificity. By introducing a homology to quantify packing differences among proteins, we enable a previously underscribed strategy for the design of highly selective drug inhibitors involving ligands that wrap nonconserved packing defects. The selectivity of these ligands is validated by performing affinity assays on a cancer-related pharmacokinome. Minor reengineering of a powerful inhibitor guided by wrapping differences across its target kinome can selectively direct its impact toward a specific kinase. Thus, nonconserved packing defects may be used as selectivity switches across homolog targets, using spatial displacements of packing defects across aligned protein structures.

Contributed by R. Stephen Berry, October 26, 2005

Conflict of interest statement: No conflicts declared.

Abbreviations: Lck, lymphocyte kinase; PDB, Protein Data Bank; PST, packing-similarity tree.

*To whom correspondence may be addressed. E-mail: arifer@rice.edu or berry@uchicago.edu.

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strating experimentally that targeting dehydrons that are not conserved across paralogs constitutes a useful strategy to enhance the selectivity of the inhibitory impact. Thus, the notion of packing similarity will be rigorously introduced and used selectively to modify a multiple-target inhibitor to achieve a higher specificity toward a particular target and subsequently to redirect its impact toward an alternative target.

Methods

Structure-Based Dehydron Identification. Packing defects in the form of dehydrons or underwrapped backbone H-bonds may be identified from the atomic coordinates of a protein structure in a single or multidomain chain or a protein complex in a PDB entry, according to these simple tenets (2–6). (i) The extent of intramolecular H-bond desolvation, \( \rho \), in a monomeric structure may be quantified as the number of nonpolar groups (carbonaceous, not covalently bonded to an electrophilic atom) contained within a desolvation domain. (ii) The desolvation domain is defined as two intersecting balls of fixed radius centered at the \( \alpha \)-carbons of the residues paired by the backbone amide-carbonyl H-bond. (iii) Determining the extent of desolvation of an intramolecular H-bond within a protein–ligand or protein–protein complex requires that the count include nonpolar groups from the monomer itself as well as those from its binding partner(s). The statistics of H-bond wrapping depend on the choice of desolvation radius, but the tails of the distribution invariably single out the same dehydrons in a given structure over a 6.2–7 Å range in the adopted radius. In this work the value 6.4 Å was used.

In most (\sim 92% of PDB entries) stable protein folds, at least two-thirds of the backbone H-bonds are wrapped on average by \( \rho = 26.6 \pm 7.5 \) nonpolar groups [or \( 14.0 \pm 3.7 \), counting only side-chain groups and excluding those from the H-bonded residue pair (8)]. Dehydrons are here defined as H-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 \( \rho \)-value is one Gaussian dispersion below the mean. Dehydrons are dominant factors driving association in 38% of the PDB complexes (the number of dehydrons per 1,000 Å² at protein–protein interfaces is \( \sim 3/2 \) the average density on individual monomers). Furthermore, dehydrons constitute significant factors (interface dehydron density larger than average) in 92.9% of all PDB complexes (8).

Given the inherent stickiness of packing defects in soluble proteins (8, 18) and the fact that interfacial water removal from a concave or flat region of the protein surface entails far less thermodynamic work than removal from a convex water-clathrated region (26), we infer that dehydrons in cavities are suitable targets for ligand design. These structural features become of paramount importance when the hydrophobicity of the cavity (25) is not significantly higher than the average for a soluble protein surface (8).

Packing Homology. Assessing packing similarity among protein homologs requires comparing the microenvironments of backbone H-bonds (8) that can be effectively carried out following structural alignment (ref. 30; see also www.ncbi.nlm.nih.gov/structure/CN3D/cn3d.shtml). For a protein chain of length \( N \), a matrix of dehydrons or underwrapped H-bonds \( D_{ij} \), \( i, j = 1, 2, \ldots, N \) is constructed by choosing \( D_{ij} = 1 \) if residues \( i \) and \( j \) are paired by a dehydron and \( D_{ij} = 0 \) otherwise. When the dehydron matrices for two proteins \( m \) and \( n \) are aligned, they are trimmed by restricting them to those residues that structurally align in a one-to-one correspondence. Then a Hamming distance \( M_{ij}(m, n) = \sum_{i,j}[D_{ij}(m) - D_{ij}(n)] \) is computed to serve as indicator of the packing similarity between proteins \( m, n \). Given a collection of paralogs \( m, n = 1, \ldots, F \), and its corresponding packing-similarity matrix \( M_{ij} = [M_{ij}(m, n)] \), a packing-similarity tree (PST) may be easily constructed from the premise that the minimal pathway between protein-nodes \( m \) and \( n \) crosses a number of nodes equal to \( M_{ij}(m, n) \).

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 (31) in which the adenosine diphosphate production is coupled to the NADH oxidation and determined by absorbance reduction at 340 nm. Reactions were carried out at 35°C in 500 µl of buffer (100 mM Tris-HCl/10 mM MgCl₂/0.75 mM ATP/1 mM phosphoenol pyruvate/0.33 mM NADH/95 units/ml pyruvate kinase, pH 7.5). The adopted peptide substrates (Invitrogen/Biaffin) for kinase phosphorylation are AEEEYGG-EFEEAKKKKG for unphosphorylated Brc-AbI (31), KVVEE-INGNNYYYIDPTOLPY for C-ktI (32), GLARLIEDNEYTAR-EGAKFPI for LCK (33), GCSPLALKRSHSDLHDFIQL for ChkI (34), and EGLGPGDTSTTSCGPTYAP for PdkI (35).

Results

Protein–Inhibitor Complexes and Wrapping of Packing Defects. The interfaces of the 814 protein–inhibitor PDB complexes were reexamined to determine whether inhibitors were “dehydron wrappers,” that is, whether nonpolar groups of inhibitors penetrated the desolvation domain of dehydrons. This feature was found in 631 complexes and invariably found in the 488 complexes whose binding cavities presented average or no surface hydrophobicity (25). Figs. 1 and 2 show such wrapping for the HIV-1 protease (21, 36) and urokinase-type plasminogen activator (23), respectively. The inhibitor contribution to improve the protein packing is not fortuitous because the substrate must be anchored and water must be expelled from the enzymatic site. Thus, wrapping of dehydrons is an effective, if unintended, strategy for drug design (6).

The Merck inhibitor Indinavir (Crixivan) bound to the functionally dimeric HIV-1 protease (PDB ID code 2BPX) is shown in Fig. 1 (21, 36). The dehydrons are marked in green. On each monomer, these dehydrons are backbone H-bonds between these residue pairs: Ala-28–Arg-87, Asp-29–Asn-88, Gly-49–Gly-52, and Gly-16–Gln-18. The cavity associated with substrate binding contains the first three dehydrons, with dehydron 49–52 located in the flap and dehydrons 28–87 and 29–88 adjacent to the catalytic site (Asp-25) to anchor the substrate. This “sticky track” determined by dehydrons 28–87 and 29–88 is required to align the substrate peptide across the cavity as needed for nucleophilic attack by the Asp-25. The flap, conversely, needs an exposed, and hence labile, H-bond to confer the flexibility associated with the gating mechanism. The lack of protection on the flap (49–52) H-bond becomes reason for its stickiness, because the bond can be strengthened by the exogenous removal of surrounding water (Fig. 1C). The positioning of all three dehydrons in the cavity (six in the dimer) promotes inhibitor association (Fig. 1A and B).

Indinavir is an wrapper of packing defects in the enzymatic cavity; it contributes 12 desolvating groups to the 49–52 H-bond (Fig. 1C), to the 28–87 H-bond, and to the 29–88 H-bond. All functionally relevant residues are either polar or expose the polarity of the peptide backbone (Asp-25, Thr-26, Gly-27, Ala-28, Asp-29, Arg-87, Asn-88, Gly-49, and Gly-52) and thus are not themselves promotoers of association to hydrophobic groups of the ligand. The strategic position of dehydrons involving these residues in their microenvironments becomes a decisive factor to drive water removal or charge descreening required in facilitating the enzymatic nucleophilic attack.

Fig. 2 shows an inhibitor acting as wrapper of packing defects in its complexation with the urokinase-type plasminogen activator (PDB ID code 1CSW), a protease associated with tumor metastasis and invasion (23). Fig. 2A and B reveals dehydrons Cys-191–Asp-194, Asp-194–Gly-197, and Gln-192–Lys-143 in the protein cavity.
Strikingly, none of the hydrophobic residues in the cavity contributes to the inhibitor binding (Fig. 2C).

Enhancing Specificity by Targeting Packing Defects. Central to drug design is the minimization of toxic side effects. Because paralog proteins are likely to share common domain structures (37), the possibility of multiple binding partners for a given protein inhibitor arises, unless it specifically targets nonconserved features. This problem may be circumvented by targeting dehydrons because, in contrast to the fold, the wrapping is generally not conserved (3), making dehydron wrapping a selectivity filter in drug design.

To determine whether dehydron targeting is likely to reduce side effects, we first investigated the extent of conservation of dehydrons across human paralogs in PDB. The paralogs for every crystallized protein–inhibitor complex were identified, and dehydron patterns at binding cavities were compared. A 30% minimal sequence alignment was required for paralog identification. Packing defects were found to be a differentiating marker in paralogs of 527 of the investigated 631 proteins crystallized in complex with inhibitors.
Ser-195–Gly-197 dehydron in pattern of the cavity (compare Figs. 2 if the inhibitor had been tailored to target the unique dehydron of the plasminogen activator.

Although the inhibitor complexed with α-thrombin, revealing its role as wrapper of packing defects in the cavity. Notice the difference in the dehydron pattern of the cavity, distinguishing the α-thrombin from its paralog shown in Fig. 2.

Protein chains are often reported in complexes with different inhibitors.

The PDB contains 440 redundancy-free pairs of human paralogs. Of these, 305 involve some of the 527 proteins with binding dehydrons. In 269 pairs, the intramolecular wrapping at the binding cavity differs in the location or presence of at least one dehydron, and in 203 pairs, the difference extends to two or more dehydrons. Thus, the probability of avoiding side effects by selectively wrapping packing defects is estimated at 88% (269 of 308).

For instance, α-thrombin (PDB ID code 1A3E) (38), a paralog of the plasminogen activator (PDB ID code 1C5W) sharing a common domain structure but different wrapping. (B) Location of inhibitor complexed with α-thrombin, revealing its role as wrapper of packing defects in the cavity. Notice the difference in the dehydron pattern of the cavity, distinguishing the α-thrombin from its paralog shown in Fig. 2.

Poor Dehydron Wrappers Make Poor Drugs. The natural product staurosporine has been singled out as the ligand inhibitor most promiscuously interactive with the human kinome (39). Its complexes, extensively reported in the PDB, reveal inhibitory impact over the following kinases: HCK, Csk, Phosphoinositide 3k, Cdk2, Pck1, Pck2, Src, Chk, Map Kap k2, Gsk-3β, lymphocyte kinase (Lck), Itk, Zap70 k, cAMP-dependent protein kinase, Syk tyrosine k, Jak3 k, Pim1 k, and Ser–Thr protein kinase 16. We have examined all 96 staurosporine–protein PDB complexes and found no instance of the inhibitor acting as dehydron wrapper. All interactions were hydrophobic, involving nonpolar groups on the protein surface. An illustration of such binding is given in Fig. 4, where the Syk tyrosine kinase–staurosporine complex (PDB ID code 1XBC) is shown to hinge on three pivotal hydrophobic interactions engaging Phe-382, Val-385, and Leu-377. Note that staurosporine does not wrap the single dehydron closest to the binding site, Gly-380–Gly-383. Thus, because surface hydrophobic residues tend to be conserved as markers for interactivity and staurosporine does not discriminate among packing differences in the kinome, its promiscuity becomes entirely expected, and so is its uselessness as a therapeutic agent.

Wrapping as a Selectivity Filter: Proof of Principle. We now provide a proof of principle of the enhanced selectivity achieved with the proposed wrapping technology. Because of the evolutionary promiscuity of kinases, side effects arising from off-target ligand binding often arise with kinase inhibitors, especially in cancer therapy (39). Thus, a need arises to sharpen the binding affinity within the pharmacokinome associated with a specific drug. For example, selective inhibition of the Brc-Abl (Abelson tyrosine kinase), the fusion product of a chromosomal translocation, is crucial to treat chronic myeloid leukemia (31). Brc-Abl has been proven to be a target for the potent inhibitor Gleevec (31) but is not its only target (39). Of the alternative targets with reported structure, the C-kit tyrosine kinase can be a binding partner, making Gleevec a therapeutic agent for colorectal cancer (40, 41). In addition, Gleevec binds tightly to the Lck (33). Thus, we sought to modify Gleevec to improve its selectivity for Brc-Abl by targeting dehydrons not conserved across paralogs.

The protein–inhibitor complex with PDB ID code 1FPU (Fig. 5A) reveals three electrostatic interactions in Brc-Abl, the dehydrions Gly-249–Gln-252 and Gln-300–Glu-316 and the salt bridge Lys-271–Glu-286, that can be better wrapped by methylating residues I, II, and III would presumably improve the wrapping of dehydrions 249–252 and 300–316 and salt bridge 271–286, respectively. A structural alignment of the paralogs of Brc-Abl was performed using the program CN3D (30) to investigate the microenvironment conservation for these intramolecular interactions. The six kinases reported in PDB that aligned with Brc-Abl are C-kit (PDB ID code

Fig. 3. The nonconserved wrapping across paralogs sharing common folds with drug-targeted proteins. (A) Dehydron pattern in α-thrombin (PDB ID code 1A3E), a paralog of the plasminogen activator (PDB ID code 1C5W) sharing a common domain structure but different wrapping. (B) Location of inhibitor complexed with α-thrombin, revealing its role as wrapper of packing defects in the cavity. Notice the difference in the dehydron pattern of the cavity, distinguishing the α-thrombin from its paralog shown in Fig. 2.

Fig. 4. Staurosporine does not wrap dehydrons, and, thus, it does not discriminate proteins with different packing, which makes it highly promiscuous within the kinome. The staurosporine–Syk Tyr kinase complex (PDB ID code 1XBC) reveals a binding mode determined exclusively by hydrophobic interactions engaging residues Leu-377, Phe-382, and Val-385. The closest Gly-380–Gly-383 dehydron is not wrapped by staurosporine.
The alignment is shown in Fig. 5B. The dehydron 249–252 (crankshaft-like kink marked in yellow on Fig. 5B) is not conserved in any of the six paralogs of Brc-Abl, whereas the dehydron 300–316 becomes well wrapped in the paralogs. Conversely, the microenvironment of the salt bridge 271–286 is conserved.

The packing similarities among all seven paralogs are quantified by introducing a "packing homology." Thus, a distance between two aligned protein structures is established by first determining their respective dehydron matrices and then computing the Hamming distance between the aligned matrices. The $ij$-entry is 1 if residues $i$ and $j$ are paired by a dehydron; otherwise, the entry is 0. A PST then may be built so that the minimum number of nodes along a walk from one protein node to another is proportional to their Hamming distance. The resulting PST (Fig. 5C) is identical to the phylogenetic tree limited to the seven proteins (42), implying that dehydron structure alone encodes functional differences in proteins. We also have restricted the PST computation to the H-bonds/dehydrons in Brc-Abl, which become wrapped by Gleevec upon association (Fig. 5C). Adopting this restricted measure, the closest proteins to Brc-Abl become Lck and C-kit, precisely the alternative Gleevec targets with reported structure.

To enhance affinity and selectivity for Brc-Abl, we modified the inhibitor methylaing at positions I and II (Fig. 5D) (43). To test whether the specificity and affinity for Brc-Abl improved, we conducted a spectrophotometric assay to measure the phosphorylation rate of peptide substrates (31–35) in the presence of the kinase inhibitor at different concentrations. As indicated in Fig. 5E, the inhibition of unphosphorylated Brc-Abl by the wrapper of the 249–252 and 300–316 dehydrons (the I,II methylation product) improved over Gleevec levels. Furthermore, the inhibitory impact of the dehydron wrapper became selective for Brc-Abl vis-à-vis C-kit and Lck. Dehydrons 249–252 and 300–316 are absent in the latter kinases, and consistently, the drug designed to better wrap them has very low inhibitory impact against C-kit and Lck. Finally, neither Gleevec nor its modified version showed detectable inhibitory impact on the remaining paralogs, Chk1, Pdk1, for which substrate peptides have been reported.

Selectivity Switch Based on Packing Differences. In accord with the fact that packing defects may serve as selectivity filters, we may take

1T45), Lck (3LCK), Pdk1 kinase (1UVR), Cdc42-associated Tyr kinase Ack1 (1U54), EGF receptor kinase (1M17), and the checkpoint kinase Chk1 (1IA8).
selected at positions II and IV. Notice the selective and enhanced inhibition of C-kit.

methylating Gleevec at the 5 positions II and IV. Notation indicative of the functional fine tuning that needs to be discriminated in drug therapy. As this work reveals, such packing differences offer a previously undescribed control in constructing the selectivity filters and selectivity switches that may endow the wrapping technology with a high therapeutic value.

Discussion

This work substantiates a previously undescribed concept in inhibitor design: packing defects (dehydrogens) in proteins may be used as targets for ligands designed to wrap them. Because dehydrogens are generally not conserved across homolog proteins, the inhibitory impact of this wrapping technology is likely to be highly selective.

Functional differences across homolog proteins sharing a common fold are very frequent in biology. Previous studies revealed that the wrapping constitutes a molecular dimension explored when evolution is constrained to preserve the fold for functional reasons. Thus, it is likely that packing differences across paralogs are indicative of the functional fine tuning that needs to be discriminated in drug therapy. As this work reveals, such packing differences offer a previously undescribed control in constructing the selectivity filters and selectivity switches that may endow the wrapping technology with a high therapeutic value.

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