Trends in Biotechnology

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Opinion

Advanced Modeling Reconciles Counterintuitive Decisions in Lead Optimization

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Lead optimization (LO) is essential to fulfill the efficacy and safety requirements of drug-based targeted therapy. The ease with which water may be locally removed from around the target protein crucially influences LO decisions. However, inferred binding sites often defy intuition and the resulting LO decisions are often counterintuitive, with nonpolar groups in the drug placed next to polar groups in the target. We first introduce biophysical advances to reconcile these apparent mismatches. We incorporate three-body energy terms that account for the net stabilization of preformed target structures upon removal of interfacial water concurrent with drug binding. These unexplored drug-induced environmental changes enhancing the target electrostatics are validated against drug–target affinity data, yielding superior computational accuracy required to improve drug design.

Counterintuitive Drug Design

We are concerned with molecular targeted therapy, specifically with designing small molecules that bind dysfunctional proteins that need to be blocked for therapeutic purposes [1–4]. Once a target has been validated, drug discovery commences with the identification of a lead. The lead is a compound with nanomolar or submicromolar target affinity and is typically found via **high-throughput screening** (see Glossary) against a proprietary compound library usually covering vast chemical combinatorial possibilities [1,2,4]. Once the lead has been identified, considerable optimization is required to obtain a molecule that may become an effective therapeutic agent that fulfills the stringent requirements for safety, efficacy, selectivity, and deliverability [2–8]. This optimization represents a major bottleneck in the drug discovery pipeline because the underlying physical principles governing drug–target affinity and selectivity are not fully understood [4–6]. For this reason, chemical combinatorial variations of the lead scaffold are often screened to maximize affinity without introducing human biases. The resulting LO decisions end up being more serendipitous than rational and often entail what *a priori* seem to be counterintuitive steps [5–10], where a nonpolar group in the ligand is introduced next to a polar group in the target. Here we describe new optimization technology rooted in recent advances in biophysics to reconcile this conundrum and improve drug design beyond engineering matched pairs across the drug–target interface.

During the past decade, rational decisions regarding LO have been increasingly influenced by the identification of labile water molecules at the interface with the target protein [5–10]. These molecules are expected to be displaced upon drug binding, an operational premise widely adopted [5–7] and introduced in 2007 [5,9] in what may be regarded as a precursor to the

Trends

As first described in maps of local dewetting propensities, the ease with which water is locally removed from around the target influences lead optimization (LO) decisions in drug design.

Common hotspots for water displacement often defy intuition, resulting in 'counterintuitive"' LO decisions.

We introduce biophysical advances on dielectric modulation down to single-water-molecule contributions to reconcile mismatches across drug–target interfaces resulting from counterintuitive LO decisions.

We incorporate three-body energy terms that account for the net stabilization of target structure on removal of interfacial water concurrent with drug–target association.

Unexplored drug-induced environmental changes affecting the target electrostatic interactions are validated against affinity data, yielding the computational accuracy required to improve drug design.

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Box 1. Dehydron Patterns and WaterMap® Blueprints for Drug Targeting

The engineering of drug–target associations with controlled affinity and specificity is at the core of the drug discovery process. As noted in 2007 [5,9], labile hydration patterns in the target protein provide suitable 'epistructural' (around the structure) blueprints for the type of molecular engineering usually adopted by the pharmacological industry. Thus, it has been shown that water becomes easily removable when found in the vicinity of certain packing defects in proteins known as dehydrons or water-exposed backbone hydrogen bonds, while drug leads may be optimized to expel dehydron-neighboring water upon binding. Because dehydron patterns are not conserved across proteins of common ancestry (homologs) [26], dehydrons have become targetable features for the control of drug specificity and enhancement of affinity. After these initial solvent-centric approaches to drug design, the reversible work (i.e., the free-energy change) needed to transfer water molecules from the protein interface to the bulk solvent became computationally accessible through the WaterMap® software [6–8]. WaterMap performs molecular dynamics (MD) simulations with OPLS force fields adopting a protocol of successive minimization and heating steps using initial protein structures obtained from the PDB eventually subject to positional restraints and solvated within a TIP4P water box ([7], see also references therein). Thus, the identification of labile water molecules at the interface, also known as 'dewetting patterns' [4,5,9], ultimately gave rise to an alternative computational strategy whereby 'hot' water molecules were identified as those with a higher free-energy content than those in bulk solvent. Naturally, WaterMap-based drug designs were guided by the overarching principle that hot interfacial water molecules could be displaced without the need to perform any reversible work upon drug–target association; thus, their removal would in fact enhance drug affinity. This premise is reasonable and clearly inspired by the dewetting patterns of 2007 [4]. Nevertheless, the WaterMap concept of the free-energy content of a single water molecule appears to be somewhat difficult to grasp, as no obvious thermodynamic ensemble may be associated with a single water molecule within a solvated protein system. Absent an appropriate statistical thermodynamics framework, it is hard to visualize the true meaning of the free-energy content of a single water molecule located at the protein interface.

WaterMap® software (Schrödinger, Inc.; Box 1) [6,7]. Thus, the maps of 'local dewetting propensities' scanning the protein interface [5] may be regarded as epistructural blueprints for drug discovery and in fact constitute early models that complement WaterMap computations. Such computations estimate the **reversible work** (i.e., the free-energy cost) required to transfer water molecules from the protein–water interface to bulk solvent [7]. Thus, labile water molecules that entail minimal work to remove become 'hotspots' guiding drug design, while 'colder' water molecules are sometimes purposely retained upon drug binding [10].

A rather counterintuitive yet ubiquitous and highly effective way of optimizing a lead scaffold arises as the ligand is modified through the incorporation of a nonpolar moiety, usually a methyl group, to displace water from the vicinity of a backbone amide in a structured region of the protein target [6–8]. By vicinal, we mean a water molecule whose oxygen atom is within 4 Å of a protein-heavy (i.e., non-hydrogen) atom. The water vicinal to the backbone amide is known to be the 'hottest' in terms of its free-energy content relative to bulk water (on average +1.95 kcal/mol) ([7], see Table I). Thus, such solvated backbone amides are effective structural targets for the improvement of ligand affinity, but these targets are counterintuitive given the polarity of the amide group and the nonpolarity of the water-displacing group added to the ligand [4–8].

This analysis prompts us to revisit the physical insights that guide the decisions that ultimately lead to the creation of pairwise interactions across the drug–target interface. These interactions are inherent to the force fields used in free-energy computations and thermodynamic calculations of water displacement. Such thermodynamic calculations incorporate solute–solvent and solvent–solvent terms [6,7]. However, as shown in the next section, there is a term missing in such an approach that not only reconciles the seeming counterintuition but also justifies the ubiquity of the exposed backbone amide as a targetable feature.

Reconciling Counterintuitive Drug Design by Incorporating Three-Body Energy Terms

A targetable backbone amide is known to occur typically in structured regions [7], where it is paired with a backbone carbonyl forming a hydrogen bond. Such water-exposed backbone hydrogen bonds are called **dehydrons** [4,11–13]. Due to confinement at subnanometer scales, water vicinal to such dehydrons is **frustrated** in its hydrogen bonding possibilities as it binds to the backbone carbonyl (Figure 1). This is consistent with the 'high free-energy content'

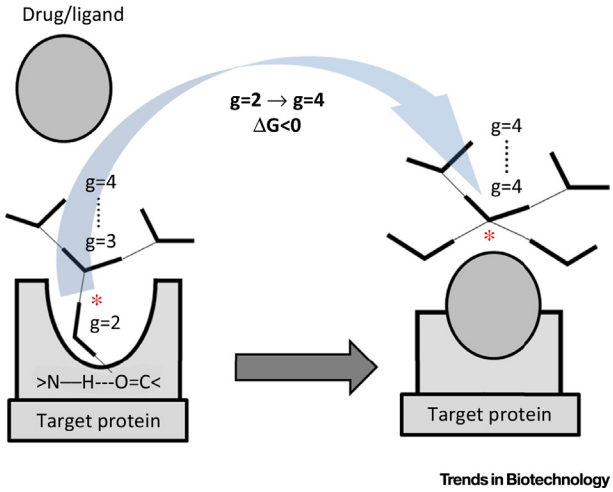

Figure 1. Favorable Displacement of Confined Interfacial Water in the Vicinity of a Backbone Amide within a Structured Region of the Target Protein. The structured region involves a solvent-exposed backbone hydrogen bond (dehydron). Here, *g* represents the degree of hydrogen bond coordination. The preferential hydration of the dehydron utilizes the available electron pair on the carbonyl oxygen and forces the interfacial water molecule (thick angular segment) into a frustrated conformation whereby it loses hydrogen-bonding opportunities ($g = 2$), when compared with bulk solvent ($g = 4$) [4]. The 'hot' water molecule is marked by an asterisk. As noted in WaterMap® and in previous computations, the transference of hot water molecules to bulk solvent ($g = 2 \rightarrow g = 4$) occurring on protein–ligand association is thermodynamically favorable.

(low entropy) of water around backbone amides as computed by WaterMap [6,7]. However, as we examine the situation from a wider perspective (Figure 2, Key Figure), we notice that removing water from the vicinity of the dehydron stabilizes and strengthens the underlying hydrogen bond [11–13]. This is a favorable three-body effect (Figure 2) not included in the WaterMap computation [7] that nevertheless contributes to lowering the reversible work required to displace the water molecule. In this case, the three bodies are the amide, the carbonyl, and the introduced nonpolar moiety in the ligand (Figure 2A). The water-displacing nonpolar group from the ligand can interact favorably with the two polar entities when the latter are paired by a hydrogen bond [11–13]. This observation reconciles the counterintuitive pairwise mismatch dictated by the WaterMap computation. The analysis from a broader context shows that the mismatch is actually a favorable three-body interaction and the vicinal water molecule is more easily displaced than originally thought.

More precisely, as the water molecule is removed from the vicinity of the polar pair (Figure 2B), the dielectric environment is modified to enhance the electrostatic interaction between the amide and the carbonyl [4,11,13]. To capture such an effect with conventional pairwise potentials, the partial charges of the amide and carbonyl would need to depend on the environment so that the removal of the water molecule enhances the preexisting intramolecular dehydron interaction due to a nanoscale modulation of the dielectric. This three-body effect is not captured in either the WaterMap computation [7] or free-energy computations of protein–ligand affinity [8]. Furthermore, it is not included in the solute–solvent or solvent–solvent terms used to develop such calculations [6,7]. Thus conventional computations may fail when put to a quantitative test in particular contexts for drug design, as we show below.

Standard free-energy computations, including WaterMap analysis, accurately estimate the low entropy content of water vicinal to a structured backbone amide. A water molecule that hydrates a dehydron must lose binding partners and become frustrated, as shown in Figure 1, due to partial confinement at the solvent-exposed backbone cavity. However, standard computations do not account for the fact that the removal of the frustrated water molecule from such an

Glossary

Breakpoint cluster region (BCR)–Abelson murine leukemia (ABL): results from a chromosomal translocation whereby the ABL viral oncogene homolog 1 (ABL1) gene from human chromosome 9 is juxtaposed onto the BCR gene from chromosome 22, encoding a hybrid constitutively active signaling protein, the BCR–ABL kinase, that causes cells to divide uncontrollably.
Chimera: hybridization and fusion of two or more gene products into a single molecule.
Chromosomal translocation: genetic abnormality caused by rearrangement of parts between two or more nonhomologous chromosomes.
c-KIT: human gene encoding the mast/stem cell growth factor receptor (SCFR) tyrosine kinase CD117.
Dehydron: water-exposed backbone hydrogen bond in a protein chain.
Frustration: reduction in the expected number of simultaneous hydrogen bonds involving a single water molecule at a spatial location.
High-throughput screening: robotics-based assessment of target affinity against a library of chemical compounds tested for lead candidacy.
Kinase: regulated signaling protein that transfers a phosphate group from the high-free-energy phosphate-donating molecule ATP to a specific substrate.
LCK: lymphocyte-specific protein tyrosine kinase.
Reversible work: work performed along a path of well-defined quasi-equilibrium states visited through infinitesimal displacement.

Key Figure

Three-Body Effects Reconcile Pairwise Mismatches across the Drug–Target Interface

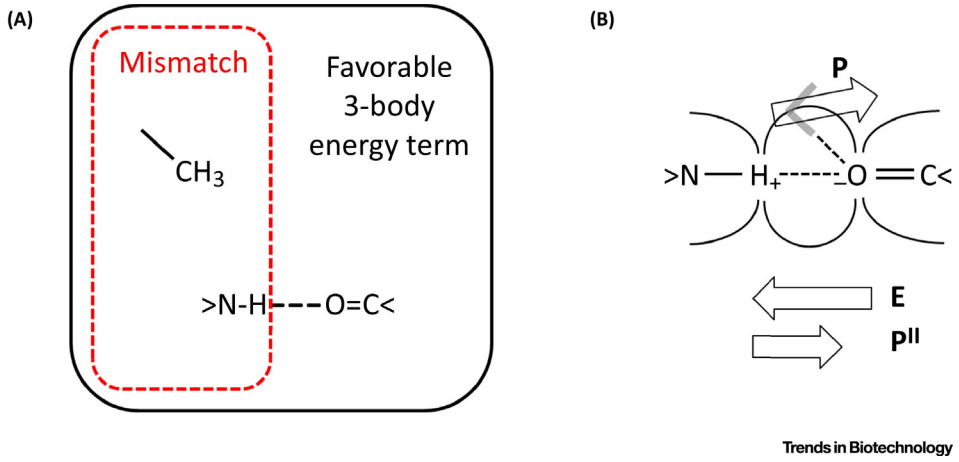

Figure 2. For a Figure360 author presentation of Figure 2, see http://dx.doi.org/10.1016/j.tibtech.2016.12.003#mmc1. (A) The proximity of a methyl group from the drug to a backbone amide from the target protein may be regarded as a hydrophobic–polar mismatch, but a broader perspective including three-body effects shows that the approach is favorable provided the amide is hydrogen bonded to a carbonyl group. (B) The interaction of interfacial water with the dehydron weakens it. An induced polarization **P** results from the positioning of the water dipole along the field lines of the electrostatic field **E** created by the backbone hydrogen bond while hydrogen bonding with the backbone carbonyl (Figure 1). The projection $\mathbf{P}^{II}$ of water polarization along the direction of **E** opposes the field **E**, describing the dielectric shielding at the single-molecule level. As the drug with its nonpolar group displaces the water molecule at the dehydron interface, the **E**-opposing field $\mathbf{P}^{II}$ is removed. Thus, the expulsion of water strengthens the preformed hydrogen bond by enhancing the electrostatic interaction. This three-body effect represents a dielectric modulation (i.e., a decrease in water-polarization effects) and involves the ligand nonpolar moiety and the two polar groups paired by the backbone hydrogen bond.

environment strengthens the backbone hydrogen bond (Figure 2B) and enhances its stability by destabilizing the unbound state [4,11–13].

By hampering the hydration of an unpaired amide and carbonyl, the nearby nonpolar moiety from the ligand destabilizes the unbound state, which is exactly equivalent to saying that it stabilizes the bound state (i.e., the backbone hydrogen bond). In other words, the nonpolar moiety from the ligand is not unfavorably mismatched against a polar group in the protein target if that polar group is in turn matched to another polar group (Figure 2A). This largely overlooked three-body effect is expected to have paramount consequences for rational drug design, while it reconciles the unintuitive creation of drug–target mismatches.

Pairwise potentials fail to represent the context dependence of the electrostatics of hydrogen bonds. It has been known for some time that hydrogen bonds cannot be faithfully represented by fixed partial charges even in a fixed context [14,15]. When the context changes due to water removal, the situation is even more difficult. It may be possible in the future to augment force fields to capture such context-dependent behavior [15–18]. However, for now we can utilize the three-body model (Figure 2A), together with experimental data for such a system, to improve the accuracy of current models as demanded by rational drug design.

Drug Design Guided by Three-Body Energy Contributions: Reworking Imatinib

To illustrate the overlooked effects of three-body contributions, we need to focus on a LO case that involves exclusively the incorporation of a nonpolar group to the lead scaffold with the express intent of removing water from the vicinity of a specific dehydron in the target protein. Such an example exists: the modification of the cancer drug imatinib by the incorporation of an extra methyl group to enhance the drug affinity and specificity, an optimization decision that resulted in the compound WBZ_4 [5].

Imatinib was originally intended as a therapeutic agent against chronic myeloid leukemia (CML), the outcome of a **chromosomal translocation** that produces the constitutively active **chimera breakpoint cluster region (BCR)–Abelson murine leukemia (ABL) kinase** [19]. Besides binding to the ABL viral oncogene **kinase** in the inactive form, imatinib also binds to other targets, like the **c-KIT** kinase, a target for the treatment of gastrointestinal stromal tumors (GISTs) [5]. However, some crossreactivities of imatinib are undesirable [5,20], such as its affinity for **LCK**, which causes immunosuppression [21]. Even the activity of imatinib against its primary target ABL has some health-threatening consequences resulting from cardiotoxicity [20]. Therefore, imatinib was redesigned with three objectives: (i) retain or improve its therapeutic potency against GISTs by enhancing its affinity for c-KIT; (ii) remove its cardiotoxicity by reducing its affinity for the ABL kinase; and (iii) remove its drug-induced immunosuppression by reducing its affinity for LCK. To achieve these goals, a methyl group was added to the imatinib scaffold resulting in the compound WBZ_4 [5].

To rationalize this optimization decision, we examine the hydration patterns for the three targeted kinases. WaterMap points to a hot water molecule in the vicinity of dehydron C673–G676 in c-KIT (PDB.1T46) as shown in Figure 3 [6]. This hot water molecule aligns with a colder (i.e., more difficult to displace) water molecule interfacing with the anhydrous backbone hydrogen bond M318–G321 in the inactive form of ABL (PDB.2HYY) [5]. Furthermore, the hot water molecule vicinal to dehydron C673–G676 in c-KIT also aligns with a cold water molecule in the vicinity of the anhydrous hydrogen bond M319–G322 in LCK (PDB.3LCK). As computed by WaterMap, the difference in free-energy content at the c-KIT hydration site versus the corresponding ABL or LCK hydration site is 0.9 kcal/mol ([6], see Figure 5). In contrast with imatinib, due to the extra methyl group WBZ_4 displaces the hot interfacial water molecule in c-KIT upon binding as well as the colder (less labile) water molecules in ABL and LCK. Imatinib does not displace any of the three molecules (Figure 3).

This analysis shows that the extra methyl group in WBZ_4 is responsible for its enhanced selectivity towards c-KIT compared with imatinib. Upon drug association with the target, the extra methyl group will displace an easily removable water molecule from c-KIT that becomes much harder to displace from ABL or LCK, lowering the affinity for these kinases. The affinity of WBZ_4 is predicted to be slightly greater than the affinity of imatinib for c-KIT, while the affinity of WBZ_4 is expected to be lower than that of imatinib for ABL and LCK.

This is indeed what the results show [5], and what WaterMap predicts [6], but when tested quantitatively the WaterMap computation needs to be supplemented with the three-body terms previously introduced [5], as shown subsequently.

The standard thermodynamic definition of dissociation constant (K_d) [4,22] holds that

$$\rho(c{-}KIT) = \frac{K_d(WBZ\_4, c{-}KIT)}{K_d(imatinib, c{-}KIT)} = e^{\Delta G_x(c-KIT)/RT} \quad (1)$$

where R is the universal gas constant, T is the absolute temperature, and $\Delta G_x(c - KIT)/RT$ denotes the change in free energy due to the removal of the specific water molecule that is not

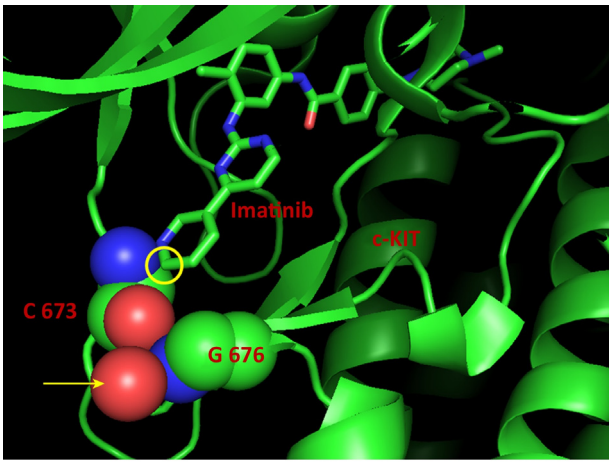

Figure 3. Redesign of Anticancer Drug Imatinib into WBZ_4, an Optimization Product Designed to Enhance Affinity and Selectivity for the c-KIT Kinase (PDB.1T46). Methylation at the position circled is intended to expel the interfacial water molecule (arrow) identified as 'hot' by WaterMap®. This molecule surrounds dehydron C673–G676, which becomes strengthened and further stabilized upon approach by the nonpolar moiety in the ligand. This three-body contribution reinforces the effect estimated by WaterMap and reconciles the 'counterintuitive' nature of the imatinib redesign.

displaced upon imatinib/c-KIT association but is displaced when WBZ_4 binds to c-KIT (Figure 3). In effect, this free-energy increment corresponds to the displacement of the water molecule by the methyl from WBZ_4 upon ligand–protein association. Similarly,

$$\rho(ABL) = \frac{K_d(WBZ\_4, ABL)}{K_d(imatinib, ABL)} = e^{\Delta G_x(ABL)/RT} \quad (2)$$

where $\Delta G_x(ABL)$ denotes the change in free energy due to the exchange of a water molecule with the extra methyl group in WBZ_4 when the ligand associates with ABL. An analogous relation holds for LCK. The free-energy difference

$$\Delta G_x(c{-}KIT) - \Delta G_x(ABL) \approx -0.9 \frac{kcal}{mol} \quad (3)$$

is reported in ([6], see Figure 5), estimated by comparing the difference between the reversible work needed to displace the respective water molecules. These WaterMap estimations may be contrasted with experimental measurements as shown below. Thus, the experimental data on WBZ_4/imatinib affinity ratios for c-KIT, ABL, and LCK yield [5]

$$\frac{K_d(WBZ\_4, c{-}KIT)}{K_d(imatinib, c{-}KIT)} \approx 0.8; \quad \frac{K_d(WBZ\_4, ABL)}{K_d(imatinib, ABL)} \approx 20; \quad \frac{K_d(WBZ\_4, LCK)}{K_d(imatinib, LCK)} \approx 20. \quad (4)$$

In turn, these experimental values yield

$$\frac{\rho(c{-}KIT)}{\rho(ABL)} \approx \frac{\rho(c{-}KIT)}{\rho(LCK)} \approx \frac{0.8}{20} = 0.04 \approx e^{-3.2}, \quad (5)$$

which differs by an order of magnitude from the value yielded by WaterMap at $T = 303$ K [6]:

$$\frac{\rho(c{-}KIT)}{\rho(ABL)} = \frac{\rho(c{-}KIT)}{\rho(LCK)} = \exp\{[\Delta G_x(c{-}KIT) - \Delta G_x(ABL)]/RT\} = e^{-0.9/0.6} \approx 0.22. \quad (6)$$

Thus, the WaterMap estimate appears insufficient to account for the enhanced selectivity of WBZ_4 towards the c-KIT kinase relative to imatinib compared with LCK or ABL.

The corrective factor of two in the exponent (equation 6) is accounted for by the additional three-body contribution to the binding free energy that arises due to net stabilization of the C673–G676 preformed dehydron in c-KIT (PDB.1T46) due the proximity of the methyl group added in WBZ_4, as shown in Figure 3. This contribution is absent in LCK and ABL since the aligned backbone hydrogen bonds are already anhydrous in the *apo* forms of those proteins [5]. It has been experimentally determined that water removal from a preformed dehydron lowers free

energy by 0.9 kcal/mol [22], a value comparable in magnitude with the thermodynamic effect captured by WaterMap, albeit complementary to it. Thus, correcting the WaterMap estimation to include the three-body effect yields

$$\frac{\rho(c{-}KIT)}{\rho(ABL)} \approx \frac{\rho(c{-}KIT)}{\rho(LCK)} \approx e^{-\frac{0.9+0.9}{0.6}} = e^{-3} \approx 0.05, \quad (7)$$

which is in satisfactory agreement with the experimental value in Equation 5.

As this detailed and specialized example shows, the quantitative assessment of the binding free energy can be improved by incorporating a model for the strengthening and stabilization of the preformed target structure due to the removal of interfacial water upon drug binding. The incorporation of this three-body effect appears to yield the level of accuracy required for rational drug design.

Concluding Remarks and Future Perspectives

The end product of the drug discovery process is often far from what one would expect in terms of pairwise matching across the drug–target interface [4–9]. This tells us that LO does not follow a rational path, itself a symptom that the underlying physical principles governing drug–target affinity are not fully understood. LO is mainly guided by screening chemical combinatorial possibilities for lead modification and the net result is seldom intuitively appealing. This black-box approach makes the overall process inefficient and cost-ineffective.

This Opinion article seeks to transform this reality by introducing novel physical insights to rationalize design steps and improve the overall efficiency of the discovery pipeline. Here we argue that three-body energy terms play a significant role in guiding LO by squarely addressing the question of why there are pairwise mismatches across the drug–target interface (see Outstanding Questions). To answer this question, we revisited the structure-based modeling that typically serves as guidance for the optimization decisions. As first noted in [5,9] and developed subsequently [23,24], the exclusion of labile water molecules located at the interface with the target protein provides important cues for drug design. Thus, nonpolar groups are strategically placed in the ligand to expel (upon binding) interfacial water molecules known to be labile; that is, requiring minimal work to be transferred to bulk solvent [25]. However, the most common structural motif yielding movable water at the protein interface has been identified as the water-exposed backbone amide group [7,8], which is obviously polar. Thus, the water-displacing ligand designed according to the WaterMap blueprint [23,24] is likely to generate a nonpolar–polar mismatch upon binding to the target, which is *prima facie* a counterintuitive result.

This conundrum is resolved in this Opinion article as the complementary approach introduced in [4,5,9,13,22] is brought into the picture. The latter approach includes the energetic benefit of strengthening a preformed intramolecular hydrogen bond upon removal of surrounding water. As it turns out, the solvent-exposed backbone amides that generate hot water according to WaterMap occur predominantly in structured regions [7]. This fact implies that the amide is actually paired to a backbone carbonyl, forming what is known as dehydron; that is, a solvent-exposed backbone hydrogen bond. Thus, the displacement of the nearby water molecule to the bulk becomes favorable not only because of the gain in entropy and the restoration of full hydrogen-bond coordination ($g < 4 \rightarrow g = 4$; Figure 1), as previously asserted [6,7] but also because the target dehydron becomes shielded upon ligand binding, thereby becoming strengthened and more stable (Figure 2B). The latter is a three-body effect involving the nonpolar water-expelling group from the ligand and the backbone polar amide and carbonyl groups from the target protein (Figure 2A). These dielectric-modulation effects are not usually included in the standard analysis of water-exclusion propensities. As shown in this Opinion article, they need to be incorporated to predict drug–target affinity with the accuracy required

Outstanding Questions

Are all polar–nonpolar mismatches across the drug–target interface accounted for by introducing higher-order energy terms in the electrostatic potential?

What structure-based indicators may lead the rational designer to purposely engineer such pairwise mismatches?

How can we implement novel drug designs that stabilize the structure of the protein target, thereby increasing affinity by increasing the stability of the drug–target complex?

How can we realize the next generation of drugs serving as dielectric modulators of preformed electrostatic interactions in the target?

How can we operationally supplement extant epistructural modeling, such as WaterMap® (Schrodinger, Inc.), to include the three-body energy terms that may steer the engineering of pairwise mismatches in the drug–target complex?

How do we parse chemical space to generate novel designs steered by desired environmental effects that lead to dielectric modulation of preexisting electrostatics?

Can molecular designs guided by the three-body energy terms described ('electrostatic wrapping') result in a significant increase in efficacy to better fulfill the demands of drug therapy?

How can drug designs based on electrostatic wrapping show an advantage in terms of efficacy relative to standard structure-based design?

for drug design. It is therefore expected that the incorporation of the three-body energy terms will sharpen our design intuition as we reconcile 'counterintuitive' LO steps.

The example worked out in detail suggests that the missing three-body effects driving drug–target association may be in thermodynamic terms of the same magnitude as the conventional terms adopted to identify binding hotspots. Creating the correct nonpolar environment around target polar pairs is as important for drug design as engineering pairwise matches across the drug–target interface. These observations argue for the need to include advanced modeling to improve LO and reconcile what *prima facie* appear to be counterintuitive designs.

Extensive research on structure-based molecular evolution has shown that, despite the high degree of structural conservation, labile hydration patterns are not conserved across proteins of common ancestry [5,26]. This implies that the three-body energy terms are unique to specific drug–target pairs, a result with profound implications for the control of drug specificity. Thus, we envision ligands that behave as dielectric modulators of the target electrostatics, heralding the next generation of safer drugs with controlled specificity towards clinically relevant targets.

Molecular evolution has not played a visible role in drug discovery so far, but this opinion article argues that it should, particularly since dehydrons constitute evolutionary markers that may be targeted by purposely designed drugs.

Supplemental Information

Supplemental information associated with this article can be found online at http://dx.doi.org/10.1016/j.tibtech.2016.12.003.

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