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Wrapping mimicking in drug-like small molecules disruptive of protein–protein interfaces

Sebastián R. Accordino,[1] Marcela A. Morini,[1] María Belén Sierra,[1] J. Ariel Rodríguez Fris,[1] Gustavo A. Appignanesi,[1]* and Ariel Fernández[2]

[1] Sección Fisicoquímica, INQUISUR-UNS-CONICET and Departamento de Química, Universidad Nacional del Sur, Avda. Alem 1253, 8000 Bahía Blanca, Argentina

[2] Instituto Argentino de Matemática "Alberto P. Calderón", CONICET, Buenos Aires 1083, Argentina

ABSTRACT

The discovery of small-molecule drugs aimed at disrupting protein–protein associations is expected to lead to promising therapeutic strategies. The small molecule binds to the target protein thus replacing its natural protein partner. Noteworthy, structural analysis of complexes between successful disruptive small molecules and their target proteins has suggested the possibility that such ligands might somehow mimic the binding behavior of the protein they replace. In these cases, the molecules show a spatial and "chemical" (i.e., hydrophobicity) similarity with the residues of the partner protein involved in the protein–protein complex interface. However, other disruptive small molecules do not seem to show such spatial and chemical correspondence with the replaced protein. In turn, recent progress in the understanding of protein–protein interactions and binding hot spots has revealed the main role of intermolecular wrapping interactions: three-body cooperative correlations in which nonpolar groups in the partner protein promote dehydration of a two-body electrostatic interaction of the other protein. Hence, in the present work, we study some successful complexes between already discovered small disruptive drug-like molecules and their target proteins already reported in the literature and we compare them with the complexes between such proteins and their natural protein partners. Our results show that the small molecules do in fact mimic to a great extent the wrapping behavior of the protein they replace. Thus, by revealing the replacement the small molecule performs of relevant wrapping interactions, we convey precise physical meaning to the mimicking concept, a knowledge that might be exploited in future drug-design endeavors.

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Key words: protein; drug; binding; design; interface.

INTRODUCTION

A main novel avenue of progress in drug discovery is the possibility to find/design small molecules with high specificity and affinity that inhibit/block or alter protein–protein interfaces.[1–5] Several successful efforts have already been accomplished, as reviewed in a very interesting recent review.[1] An appealing observation was that some small drugs tend to perform certain mimicking of the proteins they replace.[1,6,7] One example is the case of the small molecules of the Nutlin family which disrupt the interaction between the proteins HDM2 and p53.[6] These small molecules have been shown to put hydrophobic (aromatic or aliphatic) moieties in regions of the HDM2 protein targeted by the hot spot hydrophobic residues Phe 19, Trp 23, and Leu 26 of p53.[6,1] In turn, in the complex between the protein IL2 and the small molecule SP4206, the small molecule has been shown to target virtually the same critical hot-spot residues on IL-2 that drive the binding of the partner they replace, the receptor IL-2R.[7]

Since the small drugs replace the natural partner protein, it is in fact possible that they (at least in part) establish similar interactions as the ones the latter established with the target protein. In this sense, the great difference in size between the small drug-like molecule and the partner protein it replaces might not be relevant, since protein binding has been shown to be driven by only a handful of residues or hot spots (residues which significantly contribute to the binding free energy).[8–13] However, the proposed possible mimicking performed by the drug cannot be made precise unless we understand the nature of the binding interactions involved and, up to date, it has not been feasible to completely rationalize drug–protein complexes at such first-principles level. Most notably, the molecular basis of protein–protein interactions have also remained obscure for

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*Correspondence to: INQUISUR-UNS-CONICET and Departamento de Química, Universidad Nacional del Sur, Av. Alem 1253, 8000-Bahía Blanca, Argentina. E-mail: appignan@criba.edu.ar.
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long times. In fact, the characterization of hot spots in protein binding has remained elusive, even when hot spots can usually be successfully predicted.[8–24] However, a recent advance in this field brings light onto the nature of biomolecular recognition,[13] as we show in the following, and will be useful for our comprehension of the small molecule–protein binding.

To prevail in water environments, soluble proteins protect their backbone hydrogen bonds (backbone HBs) from the disruptive effect of water attack by clustering nonpolar residues around them.[13,25–31] This exclusion of surrounding water, or wrapping effect, also enhances the electrostatic contribution by modulating the local dielectric (descreening the partial charges) and thus stabilizes the HB. Thus,[13,24–31] underwrapped interactions are adhesive, hence promoters of protein associations because their inherent stability increases upon approach of additional nonpolar residues. Hence, the integrity of the protein–protein interface in protein complexes becomes extremely reliant on intermolecular cooperativity based on three-body correlations:[13,24–31] a third nonpolar body protects an electrostatic interaction (HB) pairing the other two. Since these three-body correlations must engage the two protein molecules, the correlations must be subject to an additional constraint: One body belongs to a protein chain and the other two to its binding partner. Thus, residues in the partner protein become binding hot spots if an intramolecular hydrogen bond of the target protein relies on them in order to remain over a critical wrapping value essential on stability terms. Such a decomposition of the protein–protein interface into a web of three-body cooperative interactions enabled us to successfully predict the hot spots reported by alanine-scanning experimental studies for a set of protein–protein complexes.[13] Many different methods (knowledge-based methods and methods rooted in first-principle full detailed potentials) had shown different degree of success.[13] Nonetheless, the merit of our approach was to elucidate the physical nature of the hot spots. Hot spots were easy to predict but difficult to characterize, since the question "what makes a hot spot be a hot spot?" remained unanswered, as largely recognized in the literature.[13] This knowledge, elusive to the other previous methods, might be of great help in protein engineering and drug design. Thus, the main success of Ref. 13 was to make evident the main role of three-body wrapping interactions in characterizing hot spots. Of course, other interactions (like slat bridges among others) may play a role in protein binding as well. However, since binding sites are in fact small regions of the protein containing a bunch of hot spot residues (a few ones but usually not a single residue) it is possible that most hot spot regions do in fact contain underwrapped HBs and dehydrons. This is the case we found in our study of Ref. 13 but of course, a more extensive study would be desirable.

Within this context we shall now apply such knowledge to study wrapping three-body correlations in complexes between a set of disruptive small molecules and their target proteins and we shall compare them with the situation for the corresponding complex between the target protein and its natural protein partner. To this end, the three-body correlations between the wrapping residues/moieties and the HBs that would be underwrapped in the absence of such residues/moieties would be of great relevance. Our analysis for the different cases of study will show that the underwrapped HBs of the target protein tend to receive a similar amount of wrapping within the complex with the small molecule as that provided by the replaced partner protein. Thus, we shall demonstrate that, at the level of the relevant wrapping interactions (actually the main ingredient of protein–protein interactions, as already demonstrated[13]), the disruptive small molecules do in fact perform a substantial mimicking of the partner protein they replace.

MATERIALS AND METHODS

Given the relative abundance of hydrophilic residues on the protein surface, both the protein association process and drug binding are always confronted with the disruptive effect of polar hydration.[13,24–31] Thus, the integrity of the complex interface (protein–protein or protein–drug) becomes extremely reliant on intermolecular cooperativity.[13,24–31] We make this concept precise by invoking three-body correlations, whereupon a third nonpolar body protects an electrostatic interaction pairing the other two by contributing to the exclusion of surrounding water. Since these three-body correlations must engage the two bonded molecules, the correlations must be subject to an additional constraint: One body belongs to one molecule and the other two belong to the other. Since in this study we shall focus on the wrapping received by the target protein, we shall consider the HBs of such protein and the third nonpolar bodies will belong either to the partner protein or to the small disruptive molecule, depending on the complex under analysis.

To complete this description it is necessary to classify pairwise electrostatic interactions, HBs, in terms of an abundance distribution $P(\rho)$, where ρ is the number of three-body correlations associated with an interaction. Thus, the extent of hydrogen-bond protection can be determined directly from atomic coordinates (calculated directly from the PDB 3D structure of the complex of interest: protein–protein or protein–small molecule). This parameter indicates the number of three-body correlations engaging the HB and is also known as the wrapping of the bond[13,24–31] and denoted ρ. It is given by the number of wrapper groups: For protein–protein complexes, it is given by the number of side-chain carbonaceous nonpolar groups from the two protein molecules (CH_n, n = 0, 1, 2, 3, where the carbon atom of these groups is not bonded to an electrophilic atom or polarized group)

contained within a desolvation domain around the HB. In turn, when we consider the complex between a target protein and a small molecule, we count the number of side-chain carbonaceous groups provided by the target protein and the small molecule. In this case we also consider as wrappers the following moieties: carbon atoms bonded to halogen atoms and the halogen atoms themselves, when such halogen atoms are bonded to a benzenic ring (this is based on the fact that resonance effects substantially decrease polarity; however, such cases are few and the results we obtain do not vary qualitatively if we do not consider them and we use the same restriction for carbonaceous groups as for protein side chains). In any case, each wrapping nonpolar group represents the third body within a three-body correlation involving the HB. This domain is typically defined as the reunion of two intersecting spheres of fixed radius (~thickness of three water layers) centered at the α-carbons of the residues paired by the hydrogen bond. In structures of PDB-reported soluble proteins, backbone hydrogen bonds (BHB) are protected on average by $\rho = 26.6 \pm 7.5$ side-chain nonpolar groups for a desolvation sphere of radius 6 Å.[13,24–31] Thus, structural deficiencies lie in the tail of the ρ−distribution, i.e. their microenvironment contains 19 or fewer nonpolar groups, so their ρ-value is below the mean (=26.6) minus one standard deviation (=7.5). While the statistics on ρ-values for backbone hydrogen bonds vary with the radius, the tails of the distribution remain invariant, thus enabling a robust identification of structural deficiencies.[13,24–31] Such underprotected interactions have been called dehydrons.[13,24–31] This structural motif has been extensively discussed in the literature and identified in soluble proteins with PDB-reported structure.[13,24–31] Dehydrons are crucial in defining the binding process due to their imperative demand for additional intermolecular wrapping stabilization.[13,24–31] Thus, dehydrons are adhesive, hence promoters of protein association because their inherent stability increases upon approach of a third-body nonpolar group that enhances its dehydration, and descreens the partial charges. In this work we are dealing with protein–protein and small molecule–protein complexes, and accordingly we compute the ρ-values arising from intra and intermolecular correlations. As in Ref. 13, we only considered backbone-backbone HBs and decided to leave aside side chain—side chain hydrogen bonds from the cooperativity analysis based on the following grounds: The fluctuational nature of surface side chains imposes an entropic cost associated with HB formation which makes the latter marginally stable at best.[13] Also, the wrapping statistics for side chain HBs are essentially flat with no clear distinction of the tails of the distribution do to the conformational richness of the side chains. The algorithm to identify dehydrons, named "Dehydron Calculator", is freely accessible from the Web at the following location: http://people.cs.uchicago.edu/~arifer/.

For each case of analysis we shall first consider the protein–protein complex. This physical picture leads us to assert that relevant intermolecular cooperativity interactions in the protein–protein complex will arise in the following case: When in the protein–protein complex the partner protein contributes wrapping to an intramolecular HB of the target protein whose wrapping value ρ_A would fall below the mean value, $<\rho>$, when we solely consider the wrapping contributions coming from its own protein chain. The wrapping of such HB in the complex will be labeled as ρ_{AB} in order to indicate that the interaction is receiving wrapping both from its own chain (the target protein A) and the partner protein (protein B). Thus, ρ_A is the amount of wrapping contributed only by protein A and $\Delta\rho_{AB} = \rho_{AB} - \rho_A$ is the additional wrapping contributed by the partner protein. This situation would be most significant if ρ_A falls below the dehydron threshold ($\rho_A < 20$). A target protein may present several HBs at the complex interface which are well wrapped intramolecularly (that is, by its own chain) and thus do not require further intermolecular wrapping. If the partner protein provides additional wrapping to these HBs (whose $\rho_A > 26$), such contributions would be regarded as irrelevant, since the HBs were already intramolecularly shielded from water attack. Thus, we do not consider such instances as three-body interactions. Only in the case of a HB intramolecularly underwrapped in the target protein ($\rho_A \leq 26$) would be the additional intermolecular wrapping provided by the partner protein considered as relevant and a three-body interaction would be identified. Such analysis boils down to a decomposition of the interface into a web of three-body cooperative interactions.

In order to compare with the situation in the corresponding small molecule-target protein complex, we shall calculate the wrapping resulting in such complex for the target protein intramolecular HBs, which we shall label as ρ_{AD} (and includes wrapping coming from both its own protein chain, A, and the drug-like small molecule, D). Then, we shall compare the wrapping extent of such HBs at the small molecule–protein complex with the one at the protein–protein complex by calculating the value $\Delta\rho_{AD} = \rho_{AD} - \rho_A$ (we recall that ρ_A is always the intramolecular wrapping provided by protein A within the complex with its partner protein B).

RESULTS AND DISCUSSION

We performed our analysis of cooperative three-body interactions for the following cases of successful high affinity disruptive small molecules reviewed in the excellent work of Wells and McClendon.[1] They comprise the (protein-small molecule) complexes HDM2-Nutlin-3,[32] IL-2-SP4206,[33] BCL-XL -ABT-737[34] and ZipA-Compound 1.[35] These cases are of significant therapeutic relevance

Table I
Summary of Complexes Studied

Ligand	PDB	Affinity (μM)
HDM2		
p53-derived peptide (amino acids 15–29)	1YCR	0.6
Nutlin-3	1RV1	0.09
IL-2		
IL-2 receptor α-chain	1Z92	0.0105
SP4206	1PY2	0.06
BCL-X_L		
BAD-derived peptide (amino acids 100–126)	2BZW	0.0006
ABT-737	2YXJ	0.0006
ZipA		
FtsZ-derived peptide (amino acids 367–383)	1F47	21.6
Compound 1	1Y2F	12

We indicate the PDB entry and affinity.

and the 3D structures of the protein–small molecule and protein–protein complexes have been accurately determined and deposited in the PDB. We summarize them in Table I.

In all cases, we name the target protein as protein A, the protein–protein complex as AB, where B is the partner protein the small molecule tends to replace. In turn, the drug-like small molecule is named D and AD is the complex between the latter and the target protein A. Additionally, HB is the intramolecular HB in A (we also provide the two residues involved) wrapped by B, whose $\rho_A \leq 26$. If we follow our prior analysis of protein–protein hot spots,[13] these cases would represent theoretically predicted binding hot spots. Experimental hot spot studies (for example, by means of Alanine scanning probes) were not available for all the protein–protein complexes studied. However, we recall that our computational study of Ref. 13 showed roughly 90% accuracy when applied to 10 different proteins mutated in protein–protein complexes (for the most energetic hot spots which experimentally promoted a free energy change after mutation greater than 4 Kcal/mol).

Our results are as follows:

a) A: HDM2, B: p53, D: Nutlin-3. Please see Table II.

From Table II we can learn that the wrapping provided by the partner protein is very accurately mimicked in the Nutlin-3-HDM2 complex: From seven HBs, six of them are wrapped both by p53 and the Nutlin-3 molecule, and the 3 dehydrons (HBs with $\rho_A < 20$) are wrapped in both cases. There are additionally very few HBs wrapped by the small molecule that are not wrapped by the p53 protein, one of them with $\rho_{AD} = 18$ and with a contribution of one unit by means of the Nutlin-3. Figure 1(a) shows a 3D rendering displaying the Nutlin-3 molecule (from the complex with the HDM2 protein) together with the dehydrons of the table this molecule wraps. We also show in Figure 1(b) the Nutlin-3 molecule and the side chains of the residues of p53 which provide wrapping to such HBs in the protein–protein complex (the coordinates of such residues are obtained by alignment of the HDM2 molecules in both complexes). The latter represent hot spots of the protein–protein association process as has been found by alanine scanning[36] and also corroborated in our previous computational study of protein–protein binding hot spots.[13] We can clearly see that the hydrophobic moieties of the Nutlin-3 molecule are placed at the positions occupied by the three hydrophobic hot spots of p53 (Trp23, Phe19, and Leu26). This fact has been noted before when the binding mimicking was proposed.[6,1] However, our present study explains the reason for this mimicking: the need to provide wrapping to the otherwise underwrapped regions of HDM2.

Other conventional interactions cannot account for the binding process. For instance, there exist three intermolecular HBs and one intermolecular salt bridge between p53 andHDM2, while there are no intermolecular HBs between HDM2 and Nutlin-3. We wish to note that none of the intermolecular HBs of the protein–protein complex are backbone-backbone HBs but they involve at least one side-chain. In this regard, our previous study of protein–protein hotspots (with roughly 90% accuracy in the prediction of the most energetic hot spots for a set of protein complexes) neglected side-chain HBs grounded on the reasons already exposed in the methods section.

b) A: IL-2, B: IL-2Rα, D: SP4206. Please see Table III.

From Table III we can see that from 4 hot spot regions where the partner protein provides wrapping to IL-2, in three of them the SP4206 molecule performs similarly in which regards wrapping. An interesting situation is that of the HB between Glu68 and Lys64. Here the small molecule is not providing direct wrapping but performs what could be termed as "induced wrapping" [see Fig. 2(a)]: Within the complex with SP4206, the IL-2 protein adopts a locally different conformation that leads it to provide its own wrapping to this HB. Induced wrapping represents an interesting mechanism but a more subtle one, and *a priori* it does not seem easy to incorporate such effect in drug design strategies. However, it might be worthwhile to explore how a small molecule can produce a small conformational change in the target protein so as to make it provide its own intramolecular wrapping to an existing dehydron in its own chain (the two cases

Table II
Wrapping Study of the Complexes HDM2-p53 and HDM2-Nutlin-3

HB	ρ_A	$\Delta\rho_{AB}$	$\Delta\rho_{AD}$
L54-M50	19	3	7
F55-K51	24	1	0
M62-G58	14	12	8
V93-H73	21	8	10
I99-E95	18	1	1
Y100-H96	20	2	3
I103-I99	26	1	1

We display the values of ρ_A, $\Delta\rho_{AB}$, and $\Delta\rho_{AD}$, as indicated in the text.

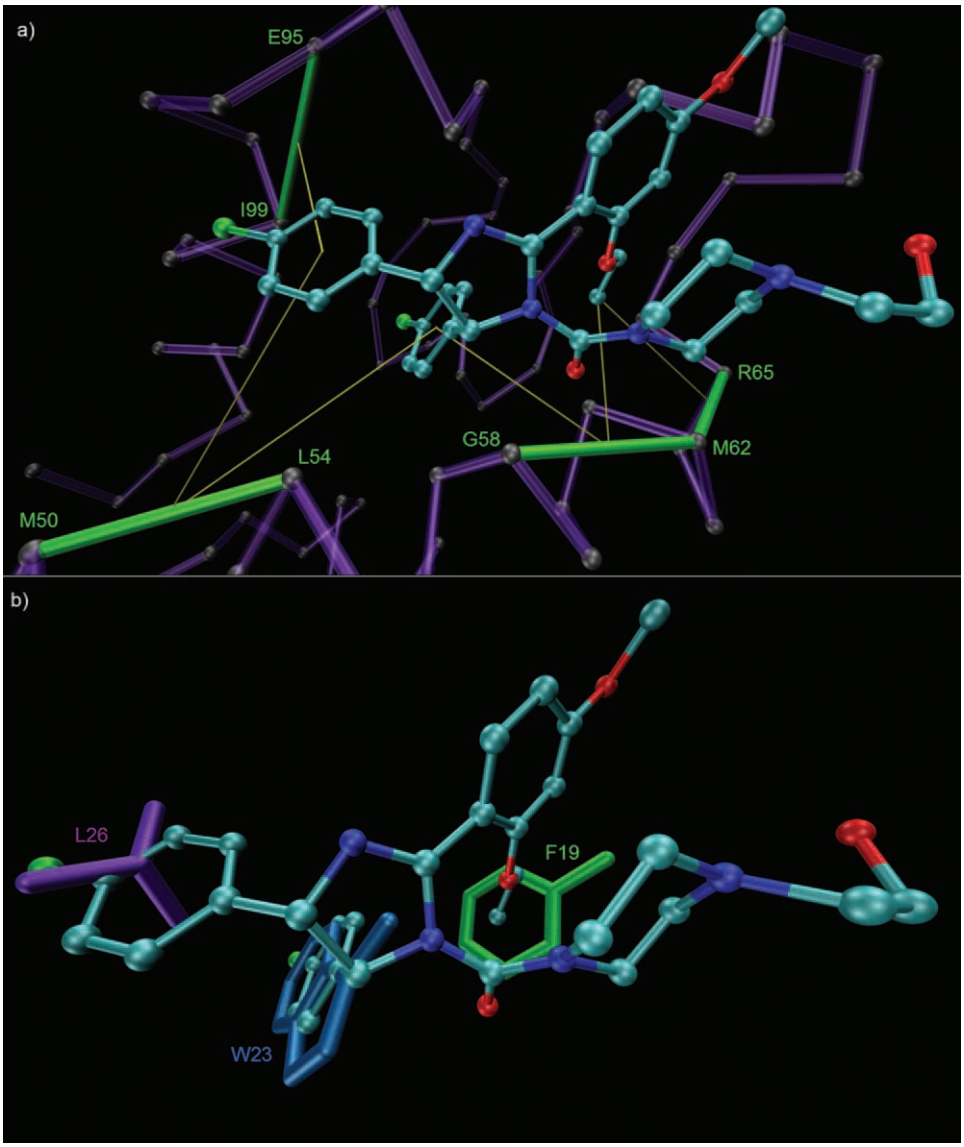

Figure 1

(**a**) The HDM2 protein in complex with Nutlin-3. We indicate dehydrons as green bars (thick, light shaded bars in the printed version) joining the two residues involved in the HB (also indicated in the figure with residue name and number) while we display wrapping three-body interactions with thin yellow lines (thin light shaded lines in the printed version). (**b**) 3D view superposition of the small molecule together with the hot spot residues of the partner protein (p53) that wrap the dehydrons of the target protein (positions are taken form both complexes after aligning the target protein positions). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

we report here involve just the flip of a side chain of a single residue but no significant change in the backbone conformation). This could be aided by molecular dynamics simulations. A possibility would be that the other conformation constituted a local minimum (higher than the global minimum represented by the PDB structure) already explored by the target protein (but seldom visited in the dynamics) and that it would be stabilized by the small molecule. We believe that it might be important that rational drug design endeavors pay attention not only to the global energy minimum of the PDB but also to the dynamics of the system. But much work would be needed to this end and, while we are indeed already exploring this possibility, this falls well beyond the scope of this present work.

Table III
Wrapping Study of the Complexes IL-2-IL-2Rα and IL-2-SP4206

HB	ρ_A	$\Delta\rho_{AB}$	$\Delta\rho_{AD}$
T41-R38	14	2	2
F44-A112	24	1	2
E61-Q57	18	2	−4
E68-K64	18	5	5

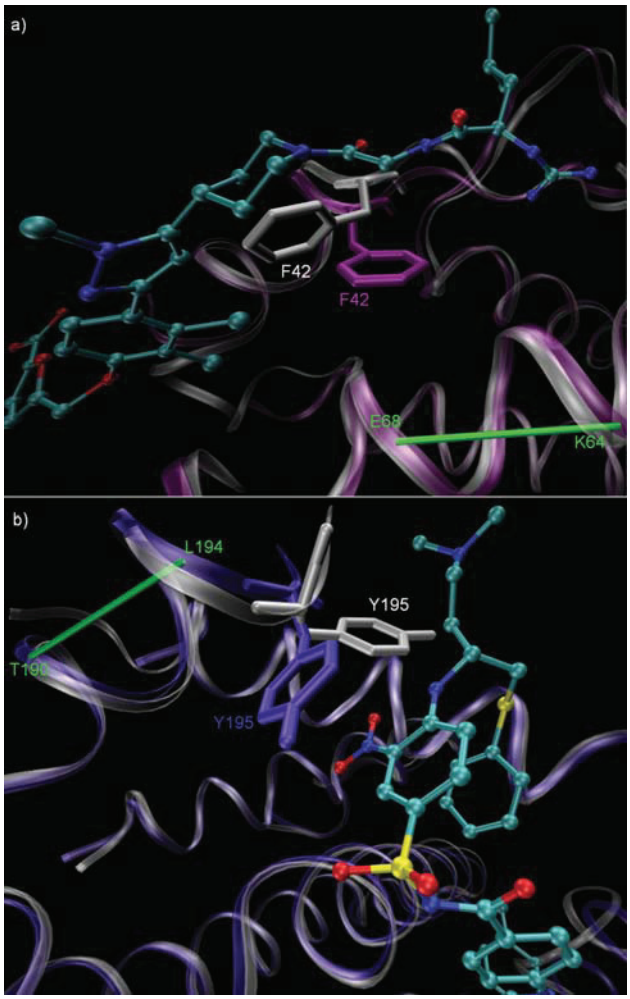

Figure 2

Examples of induced wrapping for IL-2, (a), and BCL-XL, (b), in the complex with their respective small molecules. We indicate the backbone of the target protein superimposing its position in both complexes: protein–protein (we do not display the partner protein) and protein–small molecule. We also display the residue whose side chain performs a significant displacement upon the small molecule binding in order to wrap an intramolecular dehydron in its own chain. A comparison of the conformations of the target protein in complex with the partner protein and with the small molecule shows that they share basically the same backbone conformation and only the side chain of one residue (F42 in the case of IL-2 and Y195 in BCL-XL) suffers a flip. In the case of IL-2, the small molecule does not provide direct wrapping to the HB between E68-K64 (of the IL-2 chain) as done by the partner protein. But while the partner protein contributes intermolecularly with five units of ρ to such HB, the small molecule induces the displacement of the side chain of F42 which precisely produces an increase of five units in the ρ-value of such HB. The need for such increase in the ρ-value is given by the fact that the HB between E68 and K64 is a dehydron in IL-2 ($\rho_A = 18$). A very similar situation occurs in the case of the dehydron HB Leu194-Thr190 in BCL-XL, where the induced wrapping occurs as the side chain of Y195 changes its position and thus compensates the lack of direct wrapping by the small molecule as compared with the partner protein which indeed wraps such HB with its nonpolar groups/moieties.

In summary, the small molecule SP4206 wraps three of the four underwrapped sites in IL-2 which are wrapped by the receptor protein. Additionally, there are a couple of HBs where the small molecule provides wrapping while the partner protein IL-2 Rα does not. One of them holds great relevance, since this site would constitute a dehydron in the complex if we took away the contribution of SP4206. This site is the HB between Leu72 and Glu68 which receives a wrapping of 7 units by SP4206 (to reach a total of $\rho_{AD} = 24$ in the complex). This HB is not even formed in the complex between IL-2 and the receptor protein, probably since its large underwrapping makes it utterly unstable.

These results of the substantial wrapping mimicking of SP4206 can be appreciated in Figure 3(a), where we plot the small molecule superimposed with the underwrapped HBs of IL-2 and in Figure 3(b) where we show the hot spot residues of the IL-2 Rα superimposed with SP4206. Here, unlike the case of Nutlin-3, the hotspots and the wrappers of the protein are not similar (thus the physical or spatial mimicking is not achieved) but nevertheless the wrapping provided by the protein is very well mimicked by the small molecule.

We also note that there are seven intermolecular HBs in the complex IL2-IL2Rα but none of them are expected to be very relevant since none of them are backbone-backbone HBs. In turn, the complex IL2- SP4206 has three intermolecular HBs, only two of them replacing intermolecular HBs in IL2-IL2Rα. Additionally, there are five intermolecular salt bridges in the complex IL2-IL2Rα and only two of them are established in the complex IL2- SP4206.

c) A: BCL-XL, B: BAD, D: ABT-737. Please see Table IV.

Here again, Table IV tells us that the small molecule also performs a very good mimicking of the wrapping behavior of the partner protein. In this case, the small molecule ABT-737 wraps seven underwrapped HBs of BCL-XL of the eleven ones wrapped by BAD. The HB Leu194-Thr190 represents a case of induced wrapping [see Fig. 2(b)]. In turn, ABT-737 wraps some underwrapped residues of BCL-XL not wrapped by BAD. In particular, a couple of dehydrons: Leu108-Ala104 (which is not found as a HB in the protein–protein complex presumably given its large underwrapping) and Arg132-Glu129, which is a highly underwrapped HB in the protein–protein complex.

In Figure 4(a) we can see the 3D plot of the small molecule superimposed with the HBs it wraps in the target protein and in Figure 3(b) the wrapper residues of the partner protein superimposed with ABT-737.

In this case there are three intermolecular HBs in BCL-XL-BAD but none of them are backbone-backbone HBs. In turn, there are two intermolecular HBs in BCL-XL-ABT-737, but none of them involving residues of BCL-XL which form intermolecular HBs with BAD. Additionally, there are two intermolecular salt bridges in BCL-XL-BAD, but none in the complex BCL-XL- ABT-737.

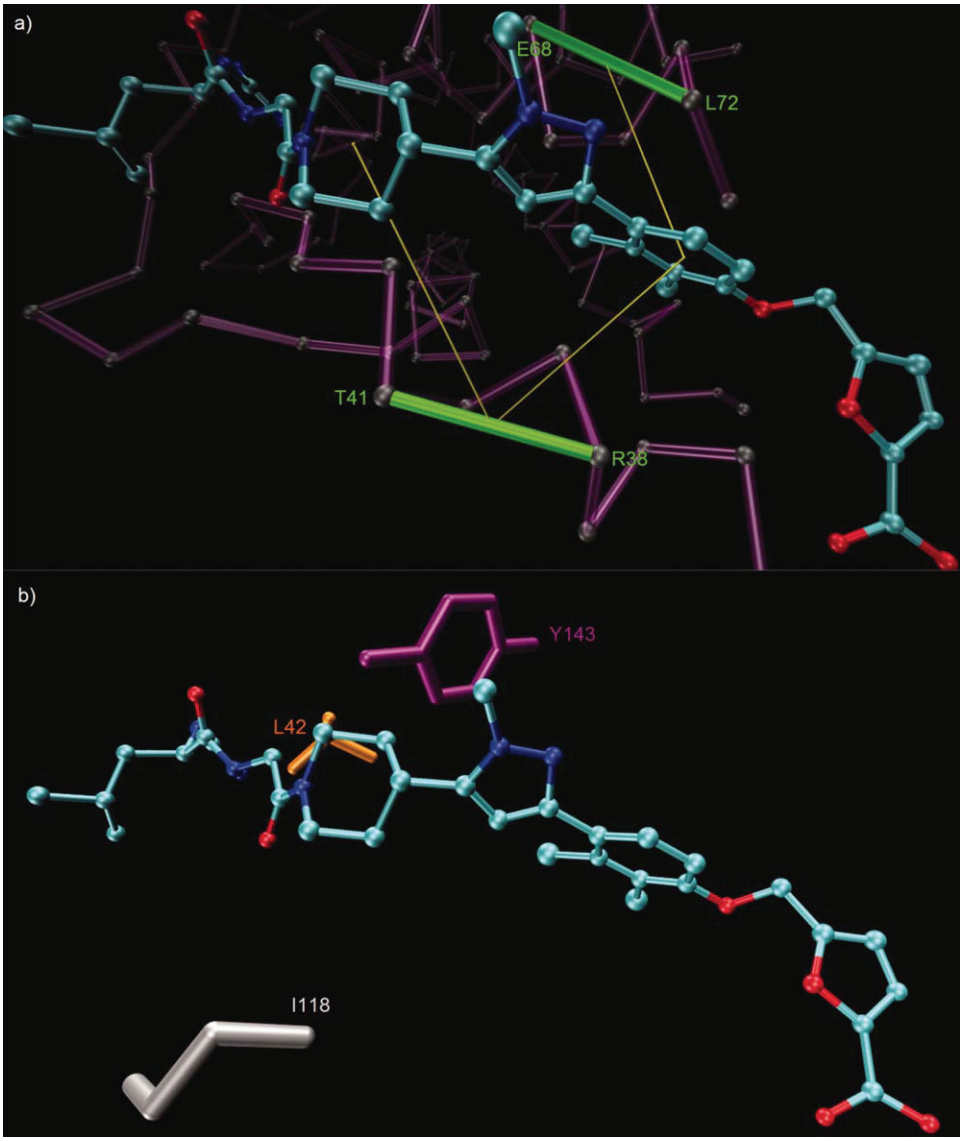

Figure 3

Idem to Figure 1, but for the complexes IL-2-SP4206 and IL-2 - IL-2Rα. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table IV
Wrapping Study of the Complexes BCL-XL-BAD and BCL-XL-ABT-737

HB	ρ_A	$\Delta\rho_{AB}$	$\Delta\rho_{AD}$
A93-A89	25	2	−2
E96-E92	18	2	−2
R100-E96	17	2	3
S122-T118	13	3	−1
Q125-Q121	17	5	−2
V126-S122	21	6	2
E129-Q125	12	3	2
L130-V126	23	9	11
A142-G138	24	13	14
L150-F146	23	1	3
L194-T190	12	5	5

In Figure 4(b) we explicitly show one of the dehydrons wrapped by both BAD and ABT-737 since in this case the wrapping side chains of BAD and the small molecule are far apart from each other but nonetheless both of them provide wrapping to such HB of BCL-XL.

d) A: ZipA, B: FtsZ, D: Compound 1. Please see Table V.

The affinity of this compound (12 μM) is much lower than the other three cases which were below micromolar. As we can see in Table V, here the performance of the small molecule is clearly suboptimal, wrapping only one of the three underwrapped ($\rho < 26$) HBs of the ZipA protein which were wrapped by the partner protein (but it wraps one of the two dehydrons, $\rho_A < 20$). In turn, the small molecule wraps some HBs ($\rho_A \leq 26$) of ZipA which are

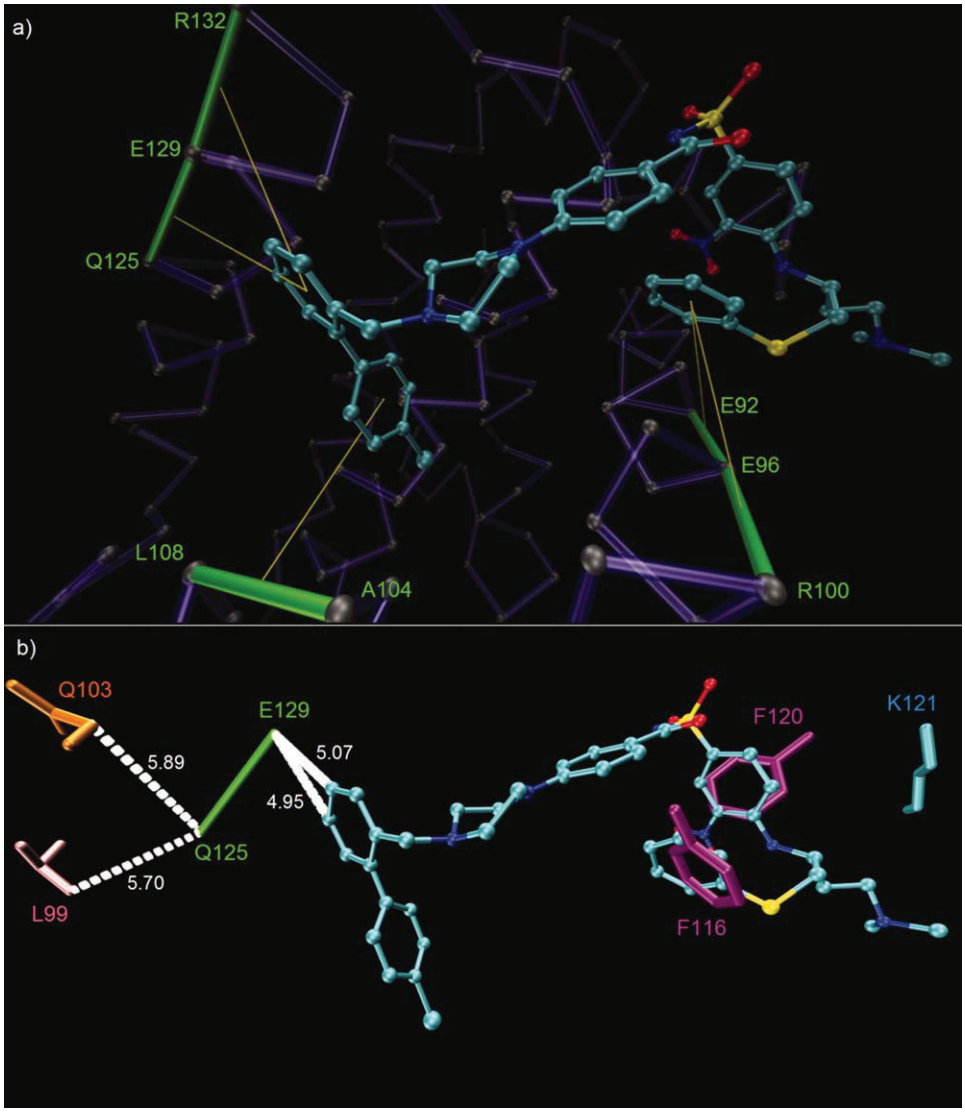

Figure 4

Idem to Figure 1, but for the complexes BCL-XL – BAD and BCL-XL – ABT-737.

not wrapped by FtsZ, but none of them falls beyond the critical dehydron value ($\rho_A < 20$). We also show the corresponding 3D representation [see Fig. 5(a,b)]. Additionally, the complex ZipA-FtsZ presents two intermolecular HBs (both backbone–backbone HBs) but there are no intermolecular HBs in the complex ZipA- Compound 1. None of the two complexes presents intermolecular salt bridges. We note that not only Compound 1 shows a low affinity for ZipA. The partner protein, FTsZ, also exhibits a low affinity. And thus, since the binding of the partner protein is not strong, it cannot be expected to be a good behavior to mimic by the small molecule. It is also important to consider that this low affinity can also be rationalized by our ideas since in Table 5 there are only three underwrapped interactions which receive wrapping from the partner protein. In fact, one of them has a ρ_A-value relatively high ($\rho_A = 23$) and only receives an additional wrapping of one unity from FTsZ. Thus, this interaction would not be relevant to the binding process which should thus be accounted for by only two three-body interactions (the two intramolecular dehydrons, $\rho_A \leq 19$). In turn, the small molecule provides wrapping to one of these two dehydrons

Table V

Wrapping Study of the Complexes ZipA-FtsZ and ZipA-Compound 1

HB	ρ_A	$\Delta\rho_{AB}$	$\Delta\rho_{AD}$
I44-D41	23	1	−1
N63-G68	18	7	3
M64-G81	19	5	−2

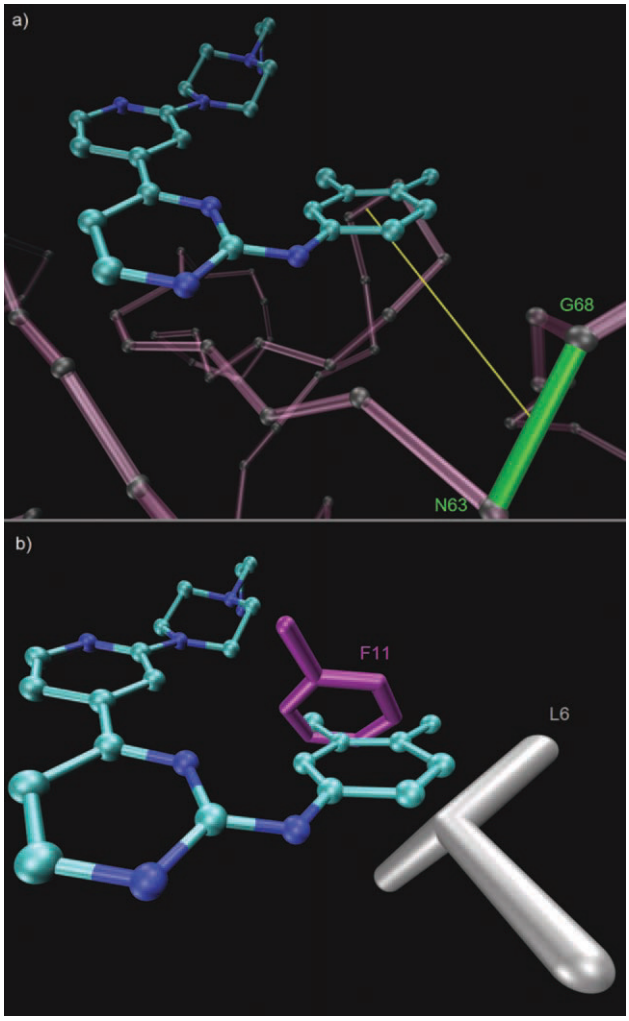

Figure 5

Idem to Figure 1, but for the complexes ZipA – FtsZ and ZipA-Compound 1.

while there is no other dehydron wrapped by this molecule. Thus, while the affinity is also expected to be low, certain mimicking, albeit suboptimal, is also achieved.

For comparison, we also analyzed other alternative cases for the complexes of the proteins IL-2 and BCL-XL. We must note, however, that our method is only qualitative and thus, while it can evidence a tendency, it certainly would not provide quantitative results.

In the case of IL-2 where the small molecule studied above was SP4206 (affinity = 0.06 μM), we also studied the molecule called Compound 2 in Ref. 37, which is one of the lead compounds considered on the way to SP4206. Compound 2 presents an affinity 100 times lower than SP4206 (namely, K_d = 6 μM). Our above-expounded study of SP4206 (cf. Table III) showed that the small molecule provided wrapping to two of the four underwrapped HBs ($\rho_A \leq 26$) of IL-2 which were targeted (wrapped) by the partner protein. Both cases constituted dehydrons ($\rho_A \leq 19$). Additionally SP4206 also wrapped two underwrapped HBs of the protein which were not wrapped by the partner protein, one of them being a dehydron (Leu72-Glu68). Thus, in summary, SP4206 wrapped a total amount of three dehydrons. In turn, Compound 2 (pdb code of the complex: 1PW6) also wraps two underwrapped HBs of IL-2 which were wrapped by the partner protein, but only one of them was a dehydron. Additionally, Compound 2 also wrapped the dehydron Leu72-Glu68. Thus, Compound 2 wraps a total number of two dehydrons of IL-2, one dehydron less than the high affinity small molecule SP4206.

In the case of BCL-XL (where the small molecule above-studied was ABT-737, with a K_d = 0.0006 μM) we also studied the small molecule called Compound 31 (pdb of the complex: 1YSI) from Ref. 38. This molecule exhibits an affinity 60 times lower (K_d = 0.036 μM).[34] From our results previously shown (cf. Table IV), we learn that ABT-737 wraps 7 underwrapped ($\rho_A \leq 26$) HBs of BCL-XL which are wrapped by the partner protein it should replace, three of them being dehydrons ($\rho_A \leq 19$). It also wraps a few other underwrapped HBs not targeted by the partner protein, two of them representing dehydrons. Thus, it wraps a total of 5 dehydrons. In turn, Compound 31 wraps only 4 underwrapped interactions of BCL-XL targeted by the partner protein, but none of them represents a dehydron. It also wraps a few underwrapped HBs not wrapped by the partner protein, one of them being a dehydron. Thus, Compound 31 wraps a few underwrapped HBs of BCL-XL but only one of them is a dehydron. This behavior looks much suboptimal as compared to ABT-737 which wraps a total amount of 5 dehydrons. However, its affinity, while being 60 times lower than ABT-737, is not low. At this point we must consider that other kind of interactions not taken into account by our method can also be at play. In fact, a possible explanation for the relatively high affinity of Compound 31 has been provided[38] by considering the formation of an extensive π-stacking arrangement.

We also studied a couple of compounds which bind to BCL-XL with low affinity. These molecules constituted lead compounds studied in the work that led to the discovery of Compound 31.[38] The combined results of the binding of these two compounds, named Compound 1 and Compound 20, showed that they wrapped three dehydrons of BCL-XL when they both formed the complex with BCL-XL, two dehydrons less than ABT-737. The pdb of the complex of the two small molecules with BCL-XL was 1YSG (here we should consider that we used a different PDB entry for the complex between BCL-XL and BAD,[38] since the protein presented slight differences in the primary sequence: 1G5J instead of 2BZW). Again, we recall that our method can only provide qualitative results. However, the trends observed for all the cases studied are basically in accord with the experimentally found affinities.

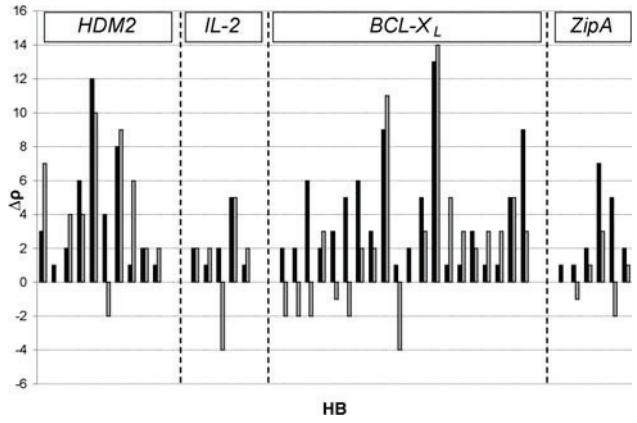

Figure 6

Comparison of the wrapping provided by the partner protein and the small molecule in the four cases studied. We plot $\Delta\rho_{AB}$ (black bars) and $\Delta\rho_{AD}$ (light gray bars). The HBs examined (abscissa) are only indicated in a generic form. This study includes both direct and induced wrapping. However, we must recall that there are very few instances of the latter (in all the four protein–small molecule complexes studied, only two of the HBs with ρ_A within the dehydron range are wrapped by such mechanism).

Finally, in order to get a global idea of the extent at which the different small molecules (cases a) to d) above considered) mimic the wrapping behavior of the proteins they replace, we calculated $\Delta\rho_{AB}$ for all HBs which are wrapped by the partner proteins in the four protein–protein complexes ($\Delta\rho_{AB} > 0$) for all ρ_A values (that is, without the restriction of $\rho_A \leq 26$) and we also calculated the corresponding $\Delta\rho_{AD}$ values, the wrapping acquired upon binding with the small molecule. Figure 6 shows the results for the four cases (the abscissa indicates generically the different HBs in the corresponding target protein). We can see a good overall tendency for the small molecules to follow the wrapping behavior of the protein they tend to replace.

To better quantify this fact we calculated the cross-correlation function between both functions as follows:

$$R_i = \frac{(x_i - \bar{x})(y_i - \bar{y})}{\left(\sum_i (x_i - \bar{x})^2\right)^{1/2}\left(\sum_i (y_i - \bar{y})^2\right)^{1/2}}$$

Where in this case $x_i = \Delta\rho_{AB}$ and $y_i = \Delta\rho_{AD}$. We get a value of $R = \Sigma_i R_i = 0.6$ which speaks of the good overall correlation between both functions (we recall that a value of $R = -1$ implies a complete anticorrelation, $R = 0$ represents no correlation and $R = 1$ is a perfect correlation).

CONCLUSIONS

From our analysis we can learn that the different drug-like small molecules studied tend to engage in a behavior similar to the protein they are meant to replace, especially in regards to intermolecular wrapping. Only in the case of Compound 1, which binds to ZipA, the behavior is clearly suboptimal, but we must bear in mind that this complex exhibits a low affinity value. While only the Nutlin-3 molecule performs a clear three dimensional or spatial mimicking, even replacing residues of the partner protein by similar hydrophobic moieties, all small molecules studied mimic to a great extent the wrapping the partner protein provides to underwrapped HBs of the target protein. This is not surprising since such three-body correlations have been shown to represent a major driving force for binding and have been able to explain (when considered as the only ingredient) most of the binding hot spots in several protein–protein complexes.[13] In turn, classical interactions like intermolecular HBs and salt bridges are not generally significant in number and do not show a good match between both complexes (protein–protein and protein–small molecule). In turn, we can envision that in the cases when the wrapping performance of the small molecule is suboptimal it might be possible to add certain functional groups/moieties in order to enhance its mimicking of the partner protein. Thus, this knowledge can be exploited in rational drug design since the regions of a protein singled out by this wrapping targeting may serve as blueprints to engineer small-molecule disruptive drugs or to reengineer already existing lead compounds.

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