2014

Intuitive, But Not Simple: Including Explicit Water Molecules in Protein-Protein Docking Simulations Improves Model Quality

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Intuitive, But Not Simple: Including Explicit Water Molecules in Protein-Protein Docking Simulations Improves Model Quality

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Abstract: Characterizing the nature of interaction between proteins that have not been experimentally co-crystallized requires a computational docking approach that can successfully predict the spatial conformation adopted in the complex. In this work, the Hydropathic INTeractions (HINT) force field model was used for scoring docked models in a data set of 30 high-resolution crystallographically characterized "dry" protein-protein complexes, and was shown to reliably identify native-like models. However, most current protein-protein docking algorithms fail to explicitly account for water molecules involved in bridging interactions that mediate and stabilize the association of the protein partners, so we used HINT to illuminate the physical and chemical properties of bridging waters and account for their energetic stabilizing contributions. The HINT water Relevance metric identified the 'truly' bridging waters at the 30 protein-protein interfaces and we utilized them in "solvated" docking by manually inserting them into the input files for the rigid body ZDOCK program. By accounting for these interfacial waters, a statistically significant improvement of ~24% in the average *hit-count* within the top-10 predictions the protein-protein dataset was seen, compared to standard "dry" docking. The results also show scoring improvement, with medium and high accuracy models ranking much better than incorrect ones. These improvements can be attributed to the physical presence of water molecules that alter surface properties and better represent native shape and hydropathic complementarity between interacting partners, with concomitantly more accurate native-like structure predictions.

Keywords: HINT, protein-protein docking, HINT Water-Relevance metric, solvated docking

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This is the peer reviewed version of the following article: Parikh, H. I. and Kellogg, G. E. (2014), Intuitive, but not simple: Including explicit water molecules in protein–protein docking simulations improves model quality. Proteins, 82: 916–932, which has been published in final form at doi:10.1002/prot.24466. This article may be used for non-commercial purposes in accordance With Wiley Terms and Conditions for self-archiving.

INTRODUCTION

Protein-protein interactions play a fundamental role in most biological events and many pathological processes. Virtually every molecular process in a cell is carried out via interactions between two macromolecules: DNA synthesis, gene expression, post-translational modifications, transport, signal transduction, etc. Various genetic, biochemical, or bioinformatics studies have identified tens of thousands of proteins interacting with each other forming millions of putative complexes. A detailed atomic understanding of the nature of these often-transient interactions is a key step to exploiting/inhibiting these biomolecular associations as potential new routes to disease therapeutics.

A large number of datasets exist that contain experimentally verified protein-protein interactions, like HPRD (Human Protein Reference Database),[1] BIND (Biomolecular Interaction Network Database),[2] MINT (Molecular Interactions Database),[3] etc. These databases contain, as of September 2013, more than 240,000 binary protein-protein interactions, and thereby provide a wealth of information pertaining to the human proteome. These data are related to thousands of protein-protein interactions, post-translational modifications, enzyme/substrate relationships, disease associations and more.

In contrast, the RCSB Protein Data Bank (http://www.rcsb.org/pdb/) only contains a few hundred protein-protein complex structures. One of the reasons for this lack of structural information is that experimental structural determinations using techniques like X-ray crystallography, Nuclear Magnetic Resonance (NMR) and Electron Microscopy (EM) are very demanding.[4]

Consequently, there has been a rapid emergence of computational algorithms to predict, model and understand these interactions – by producing a class of tools generically known as protein-protein docking methods. The first such predictive algorithm, which generated possible orientations of one protein relative to another, was developed Wodak and Janin in the late 1970s.[5] Since experimental structural determination techniques, although powerful, have low throughput, predictive methods, even of dubious quality, have been routinely used since then. Computational predictions that are accurate and reliable could obviously prove to be even more useful for the generation of testable hypotheses such as inferring how two proteins bind, giving valuable functional information about the interacting proteins and also helping guide new genetic and biochemical experiments – *if* the predictions can be validated. Notably, as docking algorithms and scoring functions have proliferated, the CAPRI (Critical Assessment of PRedicted Interactions) communitywide experiment has regulated the quality and utility of these tools through blind prediction competitions.[6]

Protein-protein docking

As the field developed, docking algorithms have become more sophisticated, partly due to the rapid progress in computer hardware with an ever-increasing availability of cost-effective computational resources, but also due to our improving understanding of biomacromolecular structure. Docking protocols have evolved from simple *rigid-body* docking (where both interacting partners are treated as rigid),[7, 8] to *soft body* docking (where side-chain and backbone flexibility is allowed in either or both molecules),[9-12] to incorporation of short molecular dynamics (MD) simulations (i.e., induced fit),[13-15] to the inclusion of implicit solvent models and explicit solvent

molecules.[16-18] The process of docking macromolecules is multi-step and usually computationally demanding.[19]

In most current docking approaches, the protein surface is represented atomically at its solvent-exposed residues with mathematical models, e.g., geometric shape descriptors like Connolly surfaces.[20] This description of the shape function[21, 22] can be combined with affinity grids encoding force field potentials.[23] Search of conformational space can be referenced to matches of surface complementarity at the protein-protein interface,[24] the combination of geometric complementarity with pairwise amino acid affinities,[9] or interface contacts analyzed by Fourier correlation, e.g., Katchalski-Katzir *et al.*[25] geometric hashing,[21, 26-28] genetic algorithms,[29] Brownian dynamics simulations,[30] and Brownian simulations combined with energy minimization[31] have all been utilized to generate energetically viable poses prior to scoring. A number of issues are related to the conformational changes that may occur upon binding, i.e., flexibility, presaged by Koshland's suggestion of induced fit in 1958.[32] A recent review by Andrusier *et al*.[33] described the treatment of protein flexibility during different stages of the docking process; most methods focus on the easier to simulate interfacial sidechains,[34,35] because implementing full backbone flexibility is far more challenging.

All docking protocols generate a huge number of potential solutions, from which the one or few corresponding to the lowest free energies of binding must be identified with a scoring function. Ideally, scoring functions should be able to distinguish between native-like models and false models. During protein-protein complex prediction, metrics such as shape complementarity between interacting protein surfaces are used as filters to eliminate incorrect predictions, but are insufficient to evaluate the complete

energetics of protein-protein associations. Thus, filtering is usually followed by applying scoring functions that rank the solutions by quantitating geometric complementarity, electrostatic interactions, hydrogen bonding and/or desolvation energy.[8,36,37] Most scoring functions are designed to predict the free energy of binding, $\Delta G_{binding}$, which is neither trivial nor a solved problem because the associated algorithms are imperfectly able to completely characterize processes of biomacromolecular association.[23] Scoring remains perhaps the most significant challenge in modeling the protein-protein (or even protein-small molecule) docking process.

Since 2003, the CAPRI (Critical Assessment of PRedicted Interactions)[6] experiment has enabled evaluation of protein-protein docking algorithms through blind predictions of target protein-protein or protein-DNA complexes for which X-ray or NMR structures have been strategically withheld from publication. First, a target complex is made available by experimentalists to CAPRI management. From the coordinates of the interacting partners, the participants generate and submit models of the complex that are then compared to the experimental structure based on standard criteria.[6, 38-40] A recent review[23] that analyzed the quality of submitted models showed that ICM-DISCO,[41] ZDOCK,[37] HADDOCK[42] and RosettaDock[8] have been the best predictors over the past decade.[23] Literature citations suggest that HADDOCK, RosettaDock and ClusPro[43] followed by PatchDock[10] and ZDOCK are the most popular.[23]

The hydropathic roles of water in biological associations

Water is a vital component of all living organisms and plays a crucial role in all biological processes. Particularly with proteins, the dynamics of water interactions govern many molecular phenomena like protein folding and molecular recognition,[44] as

well as maintenance of structural integrity.[45] The strongly bound or "conserved" waters are, at a minimum, able to modify protein surface properties like shape and charge. Bogan and Thorn claimed, in their O-ring hypothesis for interfaces, that occlusion of solvent by "hot spot" residues is necessary for energetically favorable interactions,[46] but the abundant presence of water at protein-protein and protein-DNA interfaces belies this model and instead highlights the vital role played by water in the polar interactions that stabilize such complexes. Janin's structure-based examination of protein-protein and protein-DNA recognition sites revealed that these interfaces contain at least as many water-mediated interactions as direct hydrogen bonds or salt bridges.[47] The displacement of waters upon association is, however, a recipe for increased free energy of binding through the entropy those waters gain.

Likewise, we have long been interested in understanding the functional and energetic contribution of water molecules in various biological environments. The empirical HINT forcefield (*vide infra*) models both hydrophobic and polar non-covalent interactions between molecules[48] and is the basis for our analyses. Previously, we analyzed the structures of mutant hemoglobins and calculated the contribution of crystallographically important water molecules in dimer-tetramer assembly,[49] mapped the energetics of water-protein and water-ligand interactions at protein-ligand interfaces in HIV-1 protease-ligand complexes with a significant improvement in binding energy prediction when bridging water molecules were scored,[50] evaluated protein-water and water-ligand interactions in paired sets of uncomplexed and ligand-complexed proteins with scores calculated by HINT[51] and the geometric Rank algorithm,[52] and quantified the key energetic role of bridging interfacial waters in protein-DNA associations.[53]

HINT free energy scoring and Rank were combined in the statistically validated water Relevance metric,[54] which classifies water molecules in protein active sites in terms of a continuum from easily displaced (Relevance ~ 0) to generally conserved (Relevance > 0.5). High Relevance waters are not likely to be casually displaced by a ligand and should be explicitly considered when building geometrically and functionally correct models of the binding site. Ligands designed to specifically target such waters can be comparatively more potent if they have polar functional groups capable of mimicking the waters' hydrogen bonds.

Recently, we performed a comprehensive study on the multiple roles of bound water at protein-protein interfaces.[55] Analysis of 4741 water molecules at the interfaces of 179 high-resolution (< 2.3 Å) heterodimeric protein-protein complex structures showed that 21% of waters are involved in bridging interactions with both proteins, while 53% and 26% are involved with one or neither of the proteins, respectively. The total energetic contribution of bridging water is not insignificant as it ranges up to -11.35 kcal mol^{-1} per protein pair. This emphasized the importance of characterizing the behavior of biological waters at biomacromolecular interfaces, as they clearly influence complex assembly. Also, these data have contributed, in our view, towards the establishment of a rational basis for including the effects of individual waters in macromolecular docking.

Solvated docking

Substantive effort has been applied towards incorporation, both implicitly and explicitly, of water molecules in protein-ligand docking protocols. Several of the methods available for identifying/predicting protein-ligand interfacial waters have shown promise; e.g., GRID,[56] AQUARIUS,[57] CS-Map[58] and Fold-X.[59] A more recently

available tool, WaterMap,[60,61] predicts active site bound water molecules by solvating the site and calculating its thermodynamic properties. Some protein-ligand docking programs like Flex-X,[62] Autodock,[63] GOLD,[64] and GLIDE[65, 66] have shown significant improvements in docking performance[67] by developing algorithms to include contributions from interfacial waters.

Although the challenges remain significant, the majority of tools for protein-protein docking *have* been reasonably successful at modeling these associations, as seen from the recent CAPRI experiment.[40] Overall, 67% of the participating research teams produced acceptable models for at least one target. However, no evident correlation has been seen between the ranking of models and their accuracy,[68] underscoring the weakness of current scoring function methodology. Importantly, however, water has been *neglected* in almost all protein-protein docking algorithms. Instead, developments in solvated protein-protein docking have been focused on implicit treatment of solvent molecules, which has reduced computational cost compared with explicit treatment. Chen *et al.*[69] recently reviewed the progress from *in vacuo* to *in solutio* docking, using implicit solvent-based methods. While this approach has shown some promise, a more detailed understanding of protein-protein interfaces will likely be achieved with explicit treatment of waters molecules.

HADDOCK is one of the very few docking programs designed to explicitly treat water molecules in macromolecule docking.[17,70,71] The most recent[70] implementation of the protein-protein docking algorithm: 1) hydrates the individual protein molecules, 2) performs a rigid-body docking that results in a water layer between the two proteins, 3) removes all non-interfacial water molecules and 4) subjects the remainder to a biased

Monte Carlo procedure that disqualifies waters with respect to water-mediated contact probabilities derived from the Kyte-Doolittle[72] hydrophobicity scale. Only waters having favorable interaction energies with the surrounding protein(s) are then retained in the final predictions. This methodology resulted in improvements, both in quality and scoring, over "unsolvated" HADDOCK docking.[70] It should be noted, however, that Kyte-Doolittle contact propensities would fail to detect bridged interactions that involve backbone atoms, which we previously reported account for 21.5% of all water-mediated protein-protein interactions.[55] Also, as used, the K-D scale does not take into account hydrogen bond directionality and, thus, their quality.

Our interest in this problem, and our previous studies showing the utility of the HINT scoring function in various biological environments,[50,73-77] have led us to a long-range goal of incorporating the HINT force field, including its ancillary tools[54,75,78] into a new protein-protein docking algorithm. In this work, we are testing HINT scoring and water Relevance in second stage refinement and model scoring. While we believe that protein-protein docking algorithms should account for physical effects such as shape complementarity and residue flexibility, as well as chemical effects like hydropathic complementarity, residue ionization states and explicit consideration of interfacial water molecules, will yield more realistic models, many of these effects will need to be incorporated during the search stage. Nevertheless, much can be learned about the importance of water in protein-protein associations through careful model preparation and during the scoring stage. Thus, we identified interfacial waters relevant to both interacting partners using the HINT Relevance metric and we used ZDOCK, a rigid-body docking program, for the docking search stage. We forced ZDOCK to include the

Relevant waters as atoms in one of the two interacting proteins, and show that more accurate results are obtained when water is ***not*** ignored.

Methods

Data set preparation

A non-redundant benchmark for protein-protein docking algorithms, which contains test cases where 3D structures of the complex and both unbound components are available, was designed by Weng and colleagues.[79] We filtered this data set for structures where the resolution of the bound complex is ≤ 2.0 Å to ensure that the interfacial water molecules were well-structured and thus more reliable. Coordinates for all complexes in the data set were obtained from the RCSB Protein Data Bank (http://www.pdb.org/).[80] Ligands and/or cofactors were deleted from each complex structure. For cases of multimer assembly, only one chain of each component forming the complex was retained. Hydrogen atoms were added with Sybyl 8.1, and minimized with the Tripos force field (1000 iterations, 0.01 kcal mol^{-1} $Å^{-1}$ gradient, Gasteiger-Hückel charges), while the coordinates of all heavy atoms were fixed. Interfacial waters, defined as those within 4 Å of atoms on both interacting proteins, were retained with each protein-protein complex. The larger of the two proteins was designated as the "receptor" protein, to be held static during the docking process, while the smaller one as the "ligand" protein.

Hydropathic scoring of protein-protein interfaces

Intermolecular interaction scores were calculated between each receptor-ligand pair using the HINT scoring function, which has been described previously.[48] First,

direct HINT interaction scores were calculated for every complex, without accounting for the contributions made by interfacial waters, using HINT parameters and controls similar to those in previous studies;[51,53,76] protein molecules were partitioned with the *dictionary*, with *essential* hydrogen treatment (explicit polar hydrogens and implicit non-polar hydrogens), and with the usual 30 Å^2 correction for the solvent accessible surface areas of backbone amide nitrogens.

Identification of "bridging" interfacial waters

The orientation of every interfacial water molecule was optimized using an algorithm that performs an exhaustive rotational search to assign H-atom positions.[78] This algorithm individually treats each water molecule as a small "ligand" and the surrounding atoms within 8 Å from both proteins as its "binding site". HINT scores are calculated and maximized between this "ligand" and "binding site" through rotation of the water around its three axes and allowing for limited (< 0.5 Å) translation of its O centroid.

The water Relevance[54] was calculated for each optimized water molecule. Relevance is a metric combining the water's HINT score (as above) with its Rank.[78] Rank is a geometric evaluation of the water's potential for hydrogen bond formation in its site, calculated as:

$$\boldsymbol{Rank} = \boldsymbol{\Sigma_n}\left\{\left(\boldsymbol{2.80}\ \text{Å}/\boldsymbol{r_n}\right) + [\boldsymbol{\Sigma_m}\ \boldsymbol{cos}(\boldsymbol{\theta_{Td}} - \boldsymbol{\theta_{nm}})]/\boldsymbol{6}\right\} \quad (1)$$

where r_n is the distance between the water's O and target heavy atom n (n = 1 to number of targets), θ_{Td} is the ideal tetrahedral angle (109.5°) and θ_{nm} is the angle between targets n and m (n = m to number of valid targets). Rank values range from 0 for waters that do not form any hydrogen bonds to about 6 for waters forming four

hydrogen bonds (two as donor, two as acceptor) with excellent bond lengths and bond angle geometries. The Relevance of a water molecule is calculated using the weighted probability equation:

$$\boldsymbol{P_A} = \frac{\boldsymbol{P_R}(|\boldsymbol{W_R}| + \mathbf{1})^{\mathbf{2}} + \boldsymbol{P_H}(|\boldsymbol{W_H}| + \mathbf{1})^{\mathbf{2}}}{(|\boldsymbol{W_R}| + \mathbf{1})^{\mathbf{2}} + (|\boldsymbol{W_H}| + \mathbf{1})^{\mathbf{2}}} \quad (2)$$

where P_A is the overall probability or Relevance for a water molecule, P_R and P_H are the probabilities for water conservation based on Rank and HINT score, and W_R and W_H are the weights for these probabilities, respectively. Water Relevance was trained such that a water molecule in an unliganded protein with $P_A \geq 0.5$ is "conserved", meaning it would be present in the ligand-bound complex.[54] Relevance has been extended to protein-protein complexes[55] to identify waters contributing bridging interactions.

The Relevance for each interfacial water molecule was calculated as above. An interfacial water molecule that is involved in bridging interactions should be Relevant with respect to both proteins; as before,[55] our criterion for "bridging" was that such waters have Relevance scores of ≥ 0.25 with respect to both proteins (thus, a total value of ≥ 0.5). Water molecules meeting this condition were carried through to the next step.

Solvated docking using ZDOCK

ZDOCK v3.0.2,[81] which incorporates a 3D convolution library to improve its efficiency, was obtained from http://zdock.umassmed.edu/software/. 100 solutions were generated for each receptor-ligand (protein-protein) pair in the data set. Since bound-bound docking was performed, a seed integer was specified for randomization of the ligand's starting coordinates. Rotational sampling was set as *dense*, i.e., the rotational search was performed in 6° steps. The receptor protein's coordinates were fixed,

preventing its rotation or switching with ligand during execution. Using these parameters, we designed and evaluated two different docking protocols for each member of our protein-protein complex data set: 1) *unsolvated docking*, with standard rigid-body methodology and the absence of interfacial water molecules; and 2) *solvated docking*, again with rigid-body methodology, but with explicit inclusion of the bridging water molecules identified as described above. In order to assess the influence of incorporating waters that are *not* Relevant to either interacting partner, we performed a third, "negative control", protocol with incorrect waters, i.e., with Relevance < 0.25, with the same rigid-body methodology on five randomly selected cases from our dataset.

For solvated docking, the bridging water molecules were added to the receptor file and considered as a part of that protein. The ZDOCK program is not parameterized to include cofactors such as explicit waters in its algorithm; thus, we simulated their effect by manually inserting: the atomic contact energy (ACE) type (0), atomic radius (1.38 Å) and atomic charge, (-0.25 for those acting as H-bond donors and -0.55 for those acting as H-bond acceptors with respect to the ligand protein) for each water's O-atom into the ZDOCK input files.

ZDOCK's output results are the rotation and translation matrices for the ligand with respect to its initial positioning. From this, the model for the protein-protein complex was generated for each prediction; hydrogens stripped by ZDOCK were re-added and subjected to minimization under the Tripos force field (1000 iterations, 0.01 kcal mol^{-1} $Å^{-1}$ gradient, Gasteiger-Hückel charges). Next, for unsolvated docking, HINT interaction scores were calculated between the receptor protein and ligand protein for each prediction. In the case of solvated docking, the water molecules at the interface

were first optimized using the water optimization algorithm, followed by calculation of the HINT interaction score as $H_{TOTAL} = H_{prot1\text{-}prot2} + H_{prot1\text{-}water} + H_{prot2\text{-}water}$, where the terms represent scores between the two proteins and between each protein and the water set. For the purpose of comparisons between proteins in the data set, HINT scores were normalized with respect to the top HINT score for each case. Predictions were then ranked based on these scaled HINT scores.

The CAPRI assessment protocol

The standard CAPRI assessment criteria was used to evaluate our predictions against the target crystallographic structures.[38] Three characteristics of predicted complexes were evaluated: root mean square deviations (interfacial and ligand) and the identification of correct residue-residue contact pairs (see Supporting Information, Figure S1). The ligand root mean square deviation (*l-RMSD*) and interface root mean square deviation (*i-RMSD*) are parameters that evaluate the 3D fit between the predicted complexes and target structures. The *l-RMSD* represents the global geometric fit, defined as the RMSD between the ligand backbone atoms in the predicted complexes versus in the target structure, after superimposition of the receptor. The *i-RMSD* is calculated between predicted and target structures for the backbone atoms of all interfacial residues, i.e., those within 10 Å of their partner molecule. Calculations of l-RMSD and i-RMSD were performed using the McLachlan algorithm[82] as implemented in ProFit (Martin and Porter, http://www.bioinf.org.uk/software/profit/). Residues on either protein at the interface were considered to be in contact if any of their atoms were within 5 Å of atoms of each other. The total number of residue-residue contact pairs was

calculated for each target (crystallographic) and for each predicted structure. *fnat* is defined as the fraction of predicted contact pairs relative to experimental.

The predicted structures were classified into one of four categories – incorrect models, acceptable models (*), medium accuracy models (**) and high accuracy models (***) based on the criteria listed in Table I. Predictions of medium accuracy or better (** or ***) were considered to be "hits". Hit counts, C_N, and average hit counts, C_N^{ave}, were calculated for the top N predictions for both the unsolvated and solvated docking protocols. To more quantitatively measure the success of the two docking protocols, the weighted quality, Q_N, and average weighted quality, Q_N^{ave}, were calculated by giving a value of 0, 1, 2, or 3 to the incorrect, acceptable, medium and high accuracy predictions, respectively, for the top ranked N models. All statistical analyses were performed at significance level $\alpha = 0.05$ using JMP v.10.[83]

Results and Discussion

Organization, preparation and docking of data set

Weng's protein-protein docking benchmark,[79] is a set of 176 cases classified into three classes based on the extent of conformational change at the interface upon complex formation: rigid body cases (123), medium difficulty cases (29) and difficult cases (24). The high-resolution (< 2.0 Å) subset of 42 complexes contains cases from all three classes defined by Weng and also samples well the protein interface sizes,[84] with changes in accessible surface areas (ΔASA) on complex formation ranging from 808 to 3347 Å^2.[79] Within this subset, complete hydropathic analyses of the protein-protein interfaces were performed using HINT. Only those water molecules that were

Relevant to *both* proteins were retained within their protein-protein complexes, while other waters, even at the interface, were deleted. Twelve of the forty-two had no bridging waters and were removed from the data set. Table II lists the 30 protein-protein complexes used for this study with their crystallographic data, chain IDs within the complex for the proteins designated as receptor (larger of the two) and ligand (smaller of the two), the total numbers of interfacial and bridging waters, and their HINT interaction scores calculated from the crystal structures, with and without accounting for the bridging waters.

It is clear from Table II that including the effects of water in the HINT score calculations is beneficial to the overall structure. Only one of the thirty is not stabilized by water, and that structure, 1fle, has only one bridging water molecule. On average, the thirty structures are stabilized by 1123 HINT score units, which based on our previous studies,[84,85] represents a $\Delta\Delta G$ of -2.18 kcal mol^{-1}. Each of the bridging waters contributes 232 score units (-0.45 kcal mol^{-1}), which is very similar to values reported in other studies from our group,[51,55,87] and in the range of other techniques.[67,88,89]

Re-docking of protein-protein complexes

ZDOCK, which applies a Fourier Transform (FFT) algorithm[90] to model the 3D structure of a protein complex starting from the structures of individual components, was used to recreate by docking the protein-protein complexes. It proceeds by optimizing three parameters: shape complementarity, electrostatics and desolvation free energy.[81] Each individual protein is analyzed with the *mark_sur* algorithm that calculates the accessible surface area (ASA) for each atom (using a water probe) and marks its atom type based on atomic contact energy (ACE).[91] This is followed by a search in the 3D

translational space with the ligand protein rotated in 6° steps, resulting in tens of thousands of angle sets, of which top 2000 translations with the high internal ZDOCK scores are retained. ZDOCK scores physical and biochemical properties: 1) pair-wise shape complementarity (PSC) combining a favorable term measuring the number of atom pairs between the receptor and ligand proteins within a cutoff distance and a penalty term for the number of overlapping grid points; 2) an electrostatic energy term that correlates the electric potential generated by the receptor with the charges of the ligand; and 3) a desolvation free energy term calculated from atomic contact energies.[92]

HINT scores predict correct re-docked geometry

HINT scores have been previously shown to correlate with the free energy of binding in protein-ligand systems,[86,93-95] and in a few cases of protein self assembly.[49] The HINT scoring function is designed and calibrated around these predictions, rather than, like most scoring functions for docking, recreation of crystal structure geometries.[93] First, we needed to demonstrate that, regardless of its training, the HINT scoring function can accurately predict geometry in protein-protein experiments. To test this, we performed a rigid-body docking with ZDOCK on the data set described above. Using the unsolvated protocol, we obtained 100 predictions for each of the 30 protein-protein complexes. The intermolecular interaction score for each prediction was calculated using HINT, and they were ranked based on their scaled HINT scores. The accuracy of each prediction was evaluated using the CAPRI criteria, *fnat*, *l-RMSD and i-RMSD*, as described above. Then, as defined in Table I, predictions were classified as incorrect, acceptable accuracy, medium accuracy and high accuracy models.

A prediction with high *fnat* (approaching 1) indicates correct identification of the interface. Figure 1A shows a plot of *fnat* vs. scaled HINT score for all predictions (n = 30 × 100 = 3000) obtained from the unsolvated docking of fifteen protein-protein complexes. A significant positive linear correlation is observed between the scaled HINT scores and *fnat* ($r = 0.252$, $p < 0.0001$), i.e., predictions with high HINT scores have *fnat* values close to 1, and those with lower HINT scores have *fnat* values close to 0. The *fnat* values of the top-10 and lowest-10 HINT-ranked predictions for each test case were also examined (see Supporting Information, Figure S2). For the top-10, 160 of the 300 predictions (53%) have *fnat* values of ≥ 0.3 (one of the criteria for medium or better accuracy); with 17 of the 30 (57%) top-1 ranked predictions with *fnat* > 0.5 (high accuracy criterion). On the other extreme, 226 of 300 (75%) of the lowest-10 ranked predictions have *fnat* < 0.3 and 21 of the 30 (70%) lowest-1 ranked predictions have *fnat* < 0.1 (incorrect prediction). Lastly, 1053 out of 1263 predictions (83%) with *fnat* = 0, i.e., predictions that did not identify a single native residue-residue contact, have scaled HINT scores < 0.5. Clearly, high HINT-ranked predictions have *fnat* values close to 1, and the HINT score is an effective filter for pose selection.

Unsolvated docking vs. solvated docking

The protein-protein docking problem remains difficult due to the inherent complexity of these biological systems. There are many more degrees of freedom involved in bringing two proteins together as opposed to docking a small molecule in a pocket, and much algorithm development has been devoted to the problem. Yet, one of the most critical factors influencing the assembly of proteins – water – is almost always ignored. The purpose of this work is to test whether simply *including* “bridging” waters,

or as we have defined it, Relevant interfacial waters, in a docking protocol would improve its accuracy and reliability. After determining the Relevance of all interfacial waters in our dataset, and deleting those not Relevant (< 0.25) with respect to either protein, we forced ZDOCK to include the remaining waters as atoms of the receptor protein and generated 100 solutions for each of the 30 complexes in our data set. Next, those waters were extracted and individually optimized for orientation using the HINT-based algorithm. HINT interaction scores for the solvated complex were calculated: $H_{TOTAL} = H_{prot1\text{-}prot2} + H_{prot1\text{-}water} + H_{prot2\text{-}water}$, and the predictions were ranked, as before, based on their scaled HINT scores, evaluated with CAPRI criteria, and classified as described in Table I.

The overall performance of unsolvated vs. solvated docking was compared by calculating the hit count (C_N) for each complex, total hit counts (C_N^{TOT}) for all complexes, average hit counts (C_N^{ave}), weighted quality (Q_N) for each complex, total weighted quality (Q_N^{TOT}) for all complexes, and average weighted quality (Q_N^{ave}) (see Methods). Table III lists C_N for the top N (1, 10, 25, 50 and all) predictions for both unsolvated and solvated docking of each complex. As can be seen, solvated docking performs better overall than unsolvated docking in terms of hit counts, especially in the important top 10% regime, i.e., C_{10}. Note that C_{10} = 5 means that five of the top-10 predictions were of medium (**) or high accuracy (***). C_{10}^{TOT}, the total of all C_{10} values, increases from 156 in unsolvated docking to 194 in solvated docking. This is a significant (paired t-test, $p < 0.05$) improvement of 24.42%. Similarly, the quality metric for predictions, Q_N, which rewards high, medium, acceptable and incorrect predictions differentially (see Methods), tells the same story. The solvated docking protocol performs better than the

standard protocol, as measured by Q_N (see Table S1, Supporting Information). Table IV summarizes the overall result well: the counts of high accuracy (***) predictions are drastically better for solvated docking with 8/30 (27%) top-1 scoring high accuracy models compared to only 3/30 (10%) using the unsolvated protocol. Thus, more accurate and reliable results are obtained when bridging interfacial waters are ***not*** ignored.

Figure 1B shows the plot of *fnat* vs. scaled HINT scores for all predictions from the solvated docking protocol, colored by quality. A total of n = 194 out of 300 (65%) top-10 predictions were of medium accuracy or better (Table III), i.e., improving not only the number of hits, but also their scores, as more high/medium accuracy models are found in the upper right region of the Figure 1B plot. In contrast, the "negative control" experiment, where crystallographic, but non-Relevant, water molecules were included in the models for five complexes, the performance was poor compared to solvated docking, as expected, but even compared to unsolvated docking: only n = 27 out of 50 (54%) top-10 predictions were of medium accuracy or better (see Table S3).

Illustrative case 1: HyHEL-63 antibody complexed with HEL (1dqj)

With this example, the anti-lysozyme antibody HyHEL-63 complexed with hen egg white lysozyme HEL (PDB ID: 1dqj), we will illustrate how docking results and interpretability can improve when a solvated docking approach is applied. The crystal structure of the complex is relatively high-resolution (2.0 Å) and 17 interfacial waters (within 4.0 Å of both proteins) are reported.[96] After optimization of each water molecule's orientation, the Relevance of each was calculated. Seven water molecules were found to be Relevant (≥ 0.25) with respect to both proteins, thus forming bridging

interactions with interfacial residues (see Table V and Figure 2A and B). For solvated docking, those bridging waters were considered as a part of the receptor protein.

For unsolvated docking, clustering of the top-10 predictions (Table VI) revealed three distinct poses for the ligand proteins: cluster a1, consisting of five predictions (1, 2, 3, 4 and 8), was clearly an incorrect pose (8.55 ≤ *i-RMSD* ≤ 9.86, 11.00 ≤ *I-RMSD* ≤ 12.33); cluster a2, consisting of four predictions (5, 6, 7 and 9) was similarly poor (7.95 ≤ *i-RMSD* ≤ 8.39, 12.73 ≤ *I-RMSD* ≤ 13.44); and cluster a3, consisting of one prediction (10), was a medium accuracy pose (i-RMSD = 1.72, I-RMSD = 2.32). The top-10 predictions from solvated docking were, in contrast, notably more accurate. Clustering these (Table VII) revealed two distinct poses: cluster b1, consisting of two predictions (1 and 3), was an incorrect pose (*i-RMSD* = 6.44 & 7.19, *I-RMSD* = 7.04 & 7.47); while cluster b2, consisting of the remaining eight predictions was the native-like pose, with six predictions of medium accuracy (1.05 ≤ *i-RMSD* ≤ 2.48, 1.21 ≤ *I-RMSD* ≤ 3.06) and two predictions (7 and 8) of high-accuracy (*i-RMSD* = 0.90 & 0.95, *I-RMSD* = 0.64 & 1.72).

It is clear that the presence of water molecules, which change the physical and chemical properties of the receptor protein interface, have brokered this improvement in docking performance. To zero in on a single interaction: water HOH 143 is seen in the crystal structure (Figure 2C) to be involved in bridging interactions between B/Tyr58 of HyHEL–63 (receptor) and C/Val99 and C/Asp101 of HEL (ligand). The interaction with B/Tyr58 is incorrectly construed in two of the solution clusters from unsolvated docking (see Figure 3) to be direct interactions between Tyr's OH and C/Gly22 in cluster a1 or C/Gln57 in cluster a2. The third solution cluster, a3, while of overall medium quality,

does not show the interaction between B/Tyr58 and C/Val99. However, applying the solvated docking protocol (see Figure 4) results in better shape and hydropathic complementarity of the receptor with the ligand surface and leads to more accurate and native-like predictions: cluster b2 correctly predicts the water-mediated interaction between B/Tyr58 and both C/Val99 and C/Asp101 that was seen in the crystal structure.

Illustrative case 2: Potassium channel KcsA complexed with Fab (1k4c)

For the KcsA potassium channel complexed with Fab (PDB ID: 1k4c),[97] a noteworthy improvement was seen in not only the number and quality of hits obtained, but also in their rankings (Table III, S1). A total of n = 6 hits were obtained for the unsolvated docking protocol, with no hits identified in the top-10 predictions. The solvated docking protocol (incorporating three bridging water molecules, Table II), however, performed significantly better, with a total of n = 15 hits identified, with all of the top-10 predictions being hits.

We focus on a residue-residue contact pair observed in the crystal structure: A/Glu62 of Fab (receptor protein) is within 5 Å of C/Gly53 of KcsA (ligand) as illustrated in Figure 5. HINT analysis of the dry interface indicates this interaction to be energetically unfavorable (+0.22 kcal mol^{-1}). However, a water molecule, HOH 2016, bridges between the side-chain carboxylic group of A/Glu62 and the backbone carbonyl of C/Gly53, transforming this to an overall favorable interaction (-0.62 kcal mol^{-1}). Table VIII tabulates the interaction energetics of this particular interaction for all the hits obtained from both unsolvated and solvated docking. For all but one of the unsolvated docking hits, direct interaction between the two residues not detected (i.e., they were at least 5 Å from each other). However, this interaction was conserved in all fifteen

solvated docking hits, with seven of them revealing the water molecule having favorable interaction energies with both residues. For these hits, the average water-mediated interaction energy was -0.61 kcal mol^{-1}. Also of note was that the top-10 HINT-ranked models were hits while the best ZDOCK-ranked model was 11^{th} overall. Clearly, explicitly accounting for water-mediated interactions affords better scores for identifying native-like predictions, resulting in better ranking.

These two cases were obviously chosen for their dramatic results, but there are *many* water-mediated interactions at protein-protein interfaces, some of which will certainly be even more important. Dismissing water and water-mediated interactions as irrelevant in protein-protein docking reduces our ability to truly understand biological associations at an atomic level.

Limitations and alternate protocols

This study focused on understanding the **direct** influence of interfacial water on the quality of structure prediction for protein-protein complexes. We performed a bound-bound docking, which means that the starting structures of the two proteins were obtained from the crystal structure of the bound complex. This eliminates two major issues that might result in incorrect predictions: 1) protein flexibility and conformational adjustments that could be seen with unbound/unconstrained docking and 2) finding positions for important water molecules. Ideally, we would like to like to start with native, unbound structures for the interacting partners, identify *ab initio* the locations of important waters, and then predict the bound complex, but this would add many more degrees of freedom to an already spectacularly underdetermined problem. Just as an aside, we applied a few unbiased protocols to this problem: 1) without assessing the

Relevance of crystallographically-determined waters at an interface, we toggled each one, and all combinations of them, to see if we could determine which water(s) were most influential (and presumably Relevant) for protein-protein docking. Unfortunately, this problem scales as 2^n, where n is the number of waters; 2) we *de novo* solvated, with both HINT[98] and GRID[56] tools, the "receptor" protein surface with high Relevance (-8 kcal mol^{-1} binding energy in GRID) waters and repeated the docking protocol. The resulting models were poorer, even, than those from unsolvated docking. In simple terms, waters with high Relevance with respect to one protein are not likely to be those that are bridging in the final complex and low Relevance waters are both numerous and hard to characterize; and 3) we solvated[98] the protein-protein interface of all unsolvated predictions from a single complex, and scored and re-ranked the resulting models. Only a statistically insignificant improvement in ranking was found, but this approach would appear to hold more promise and/or accessibility than the others.

Conclusions

Successfully *predicting* the conformation adopted by two proteins within a complex requires enhanced understanding of interfacial interactions and integrating this knowledge to the docking problem. Most current docking programs only take into account the underlying physics of protein-protein interactions, ignoring, in a sense, chemistry – like the roles of water molecules. Interfacial waters have been shown to contribute immensely to the kinetics and thermodynamics underlying protein-protein interactions. Also, these water molecules are not just randomly trapped in the protein–protein interface, but are part of the recognition code facilitating interactions that are less favorable in their absence.[99] It may even be possible to say that water-mediated

residue-residue interactions have a structural advantage over their dry counterparts, as they are less susceptible to disruptions by changes in pH.[55]

The conformational search step of conventional “dry” docking is generally performed in vacuum and, thus, does not account for the presence of any water molecules. Some docking algorithms attempt to mitigate this problem by incorporating a desolvation term in their scoring functions, implicitly accounting for water, which does improve the ranking of docked predictions and subsequent identification of correct configuration.[69] However, implicitly treating water introduces various concomitant approximations and thus results in a coarse description of energetics.

Our solvated docking protocol, which utilizes HINT-based tools for identifying and optimizing bridging water molecules and rationally scoring the final solvated models, can improve protein-protein docking results. It is also likely that other paradigms that accomplish the same task would also be profitably applied to this problem. This is intuitive in concept but **not** simple in execution. It should be noted that this approach as we performed it is woefully crude because water is represented as only a single immobile atom that is part of the receptor during the docking search stage and thus does not really reproduce water’s true chemical properties, i.e., as a potential hydrogen bond donor to two partners *and* as an acceptor for two partners. It is only during the scoring stage, when protons are re-added and the water molecules are optimized, that their complete set of properties is incorporated.

An overarching goal of our studies in protein-protein interactions is to lay the groundwork for a docking tool that accomplishes the above result. We have shown here that the HINT forcefield and water Relevance metric add context to evaluating the

roles of water at docked protein-protein interfaces. Analyses of interfacial water at static structures,[55, 87] are also ongoing and informing the direction of research. Other tools, like extension of the HINT-based computational titration algorithm, which would provide a rational approach to optimizing the ionization states of interfacial residues, and Relevance-driven *ab initio* positioning of water molecules, are under development. Basically, if we can introduce such HINT functionality in the first stage search of conformational space to ascertain the viability of a particular pose, we will have more accurate predictions for the subsequent refinement and scoring stages, and improve the success rate of docking. Even in this current work, where we explicitly accounted for interfacial waters by "tricking" ZDOCK, we showed that using hydropathic complementarity and *not* ignoring Relevant waters in modeling protein complexes does show a statistically significant improvement in the quality of docking predictions.

Acknowledgments

We gratefully acknowledge the technical and philosophical assistance and advice of our colleagues Drs. Philip D. Mosier, J. Neel Scarsdale and H. Tonie Wright. Mostafa H. Ahmed and Claudio Catalano contributed to the study design and preparation of this manuscript. We also thank Dr. Francesca Spyrakis at the University of Modena and Reggio Emilia for assistance with GRID.

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TABLES:

Table I. Predicted model quality classification criteria from CAPRI experiments.[38]

Model Quality	**Criteria**
Incorrect	(*fnat* < 0.1) OR [(*l-RMSD* > 10.0 Å) AND (*i-RMSD* > 4.0 Å)]
Acceptable (*)	[(*fnat* ≥ 0.1) AND (*fnat* < 0.3)] AND [(*l-RMSD* ≤ 10.0 Å) OR (*i-RMSD* ≤ 4.0 Å)] OR [(*fnat* ≥ 0.3) AND (*l-RMSD* > 5.0 Å) AND (*i-RMSD* > 2.0 Å)]
Medium (**)	[(*fnat* ≥ 0.3) AND (*fnat* < 0.5)] AND [(*l-RMSD* ≤ 5.0 Å) OR (*i-RMSD* ≤ 2.0 Å)] OR [(*fnat* ≥ 0.5) AND (*l-RMSD* > 1.0 Å) AND (*i-RMSD* > 1.0 Å)]
High (***)	(*fnat* ≥ 0.5) AND [(*l-RMSD* ≤ 1.0 Å) OR (*i-RMSD* ≤ 1.0 Å)]

Table II. Solvated protein-protein docking data set with crystal structure data.

	PDB ID	Resolution (Å)	Chain ID Recep / Lig	Water count		HINT Score of X-ray structure	
				Interfacial	Bridging	Without HOH	With HOH
1	1acb	2.0	E / I	7	1	-17	79
2	1avx	1.9	A / B	8	2	2122	2365
3	1clv	2.0	A / I	30	8	-167	2319
4	1dqj	2.0	AB / C	17	7	1787	2864
5	1eer	1.9	BC / A	22	8	4864	6128
6	1fle	1.9	E / I	12	1	1063	1035
7	1gcq	1.7	C / B	6	1	80	332
8	1i2m	1.8	B / A	28	11	5492	9079
9	1iqd	2.0	AB / C	25	6	2012	2589
10	1j2j	1.6	A / B	16	6	1035	2327
11	1jiw	1.7	P / I	29	3	-2856	-1540
12	1jps	1.9	HL / T	8	3	1172	1458
13	1k4c	2.0	AB / C	15	3	2586	3150
14	1klu	1.9	AB / D	8	3	1250	1586
15	1ppe	2.0	E / I	19	2	536	820
16	1pxv	1.8	A / C	24	5	451	2509
17	1r0r	1.1	E / I	27	6	-1361	-497
18	1r8s	1.5	E / A	24	5	118	1975
19	1vfb	1.8	AB / C	28	7	1047	3185
20	1wej	1.8	HL / F	10	6	1848	2735
21	1z0k	1.9	A / B	26	5	1567	2657
22	1z5y	1.9	E / D	17	2	-809	-591
23	1zhh	1.9	A / B	32	10	-411	2223
24	2a5t	2.0	A / B	25	4	-1477	-822
25	2hqs	1.5	A / H	46	7	-421	1524
26	2i25	1.8	L / N	13	6	3081	4763
27	2nz8	2.0	B / A	42	10	-722	1996
28	2sic	1.8	E / I	17	1	-1	159
29	2z0e	1.9	A / B	23	4	-4889	-3891
30	3sgq	1.8	E / I	11	2	-617	-470

Table III. Hit counts for top *N* predictions for unsolvated and solvated docking protocols

PDB ID	Unsolvated Docking					Solvated Docking					Diff. (Solv. – Unsolv.)				
	C_1	C_{10}	C_{25}	C_{50}	C_{all}	C_1	C_{10}	C_{25}	C_{50}	C_{all}	ΔC_1	ΔC_{10}	ΔC_{25}	ΔC_{50}	ΔC_{all}
1acb	0	0	0	6	17	0	0	0	4	16	0	0	0	-2	-1
1avx	1	8	20	36	54	0	9	22	41	63	-1	1	2	5	9
1clv	1	10	25	50	95	1	10	25	50	99	0	0	0	0	4
1dqj	0	1	10	16	16	0	8	19	31	32	0	7	9	3	16
1eer	1	10	24	48	91	1	10	23	48	93	0	0	-1	0	2
1fle	1	5	5	5	5	1	5	8	9	9	0	0	3	4	4
1gcq	0	0	0	2	5	0	1	3	3	10	0	1	3	1	5
1i2m	1	10	24	47	71	1	9	21	45	72	0	-1	-3	-2	1
1iqd	1	10	25	50	76	1	10	25	49	85	0	0	0	-1	9
1j2j	1	10	23	25	25	1	9	17	32	39	0	-1	-6	7	14
1jiw	0	4	15	25	32	1	7	17	27	33	1	3	2	2	1
1jps	0	0	1	1	1	0	2	2	2	2	0	2	1	1	1
1k4c	0	0	1	3	6	1	10	14	15	15	1	10	13	12	9
1klu	1	2	2	2	2	1	4	5	5	5	0	2	3	3	3
1ppe	1	10	25	50	99	1	10	25	50	100	0	0	0	0	1
1pxv	1	10	25	49	59	1	10	24	43	62	0	0	-1	-6	3
1r0r	0	0	0	0	13	0	0	0	0	25	0	0	0	0	12
1r8s	1	10	24	48	96	1	10	25	50	93	0	0	1	2	-3
1vfb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1wej	0	4	4	4	4	1	10	11	11	11	1	6	7	7	7
1z0k	1	10	20	35	58	1	10	18	35	54	0	0	-2	0	-4
1z5y	0	0	3	18	61	0	0	2	20	68	0	0	-1	2	7
1zhh	1	6	8	9	9	1	7	16	20	20	0	1	8	11	11
2a5t	0	0	0	0	1	0	0	1	2	3	0	0	1	2	2
2hqs	1	9	16	19	19	1	9	16	18	18	0	0	0	-1	-1
2i25	1	10	25	50	89	1	10	25	48	87	0	0	0	-2	-2
2nz8	1	6	14	26	32	1	9	16	26	33	0	3	2	0	1
2sic	1	10	24	47	74	1	10	22	46	72	0	0	-2	-1	-2
2z0e	0	1	4	13	54	0	5	10	27	67	0	4	6	14	13
3sgq	0	0	0	0	1	0	0	0	0	0	0	0	0	0	-1
C_N^{TOT}	17	156	367	684	1165	19	194	412	757	1286	2	38	45	61	121
C_N^{Avg}	0.57	5.20	12.23	22.80	38.83	0.63	6.47	13.73	25.23	42.87	0.07	1.27	1.50	2.03	4.03

Table IV. Count of high accuracy (***) models in top *N* predictions

PDB ID	Unsolvated Docking					Solvated Docking				
	C_1	C_{10}	C_{25}	C_{50}	C_{all}	C_1	C_{10}	C_{25}	C_{50}	C_{all}
1acb	0	0	0	3	4	0	0	0	3	7
1avx	0	1	3	7	8	0	0	5	6	7
1clv	1	10	25	49	94	1	10	25	50	98
1dqj	0	0	4	4	4	0	2	5	7	7
1eer	0	3	3	5	5	1	4	4	5	5
1fle	0	4	4	4	4	1	5	8	9	9
1gcq	0	0	0	2	5	0	0	0	0	2
1i2m	0	4	4	4	4	1	5	6	7	7
1iqd	0	3	9	12	16	0	1	6	10	14
1j2j	0	6	7	8	8	0	4	10	14	14
1jiw	0	0	4	9	9	0	2	5	11	11
1jps	0	0	1	1	1	0	0	0	0	0
1k4c	0	0	0	0	0	0	1	4	4	4
1klu	1	2	2	2	2	1	4	5	5	5
1ppe	0	0	0	7	35	0	0	4	11	42
1pxv	0	1	1	3	3	0	1	1	5	6
1r0r	0	0	0	0	11	0	0	0	0	21
1r8s	0	0	0	0	3	0	0	1	2	2
1vfb	0	0	0	0	0	0	0	0	0	0
1wej	0	2	2	2	2	1	6	6	6	6
1z0k	1	4	8	10	12	0	3	5	9	9
1z5y	0	0	0	2	12	0	0	0	5	14
1zhh	0	2	2	2	2	0	0	1	1	1
2a5t	0	0	0	0	1	0	0	0	0	1
2hqs	0	5	8	11	11	1	5	8	9	9
2i25	0	5	13	18	21	0	4	13	19	25
2nz8	0	1	2	4	5	0	0	3	6	6
2sic	0	6	12	18	19	1	8	15	20	21
2z0e	0	0	0	1	10	0	0	0	0	7
3sgq	0	0	0	0	0	0	0	0	0	0
C_N^{TOT}	3	59	114	188	311	8	67	140	224	360
C_N^{Avg}	0.10	1.97	3.80	6.27	10.37	0.27	2.23	4.67	7.47	12.00

Table V. HINT water Relevance report for interfacial waters[a] in anti-lyzozyme antibody HyHEL-63 / lyzozyme HEL complex crystal structure (1dqj).

Water #	Monomer Name	Target One - Anti-Lyzozyme Antibody HyHEL-63 (Chain A+B)			Target Two -Lyzozyme HEL (Chain C)		
		Rank	HINT Score	Relevance	Rank	HINT Score	Relevance
1	**HOH 130**	**4.1**	**68.60**	**0.65**	**1.5**	**72.20**	**0.37**
2	HOH 131	1.2	122.40	0.39	2.3	-314.10	-0.47
3	**HOH 133**	**2.7**	**124.60**	**0.56**	**2.7**	**-36.50**	**0.39**
4	HOH 134	1.0	-5.70	0.25	2.5	-218.80	-0.24
5	HOH 138	1.4	-58.90	0.21	3.5	-195.60	-0.19
6	HOH 140	0.0	-64.00	-0.04	2.2	196.70	0.57
7	**HOH 141**	**2.5**	**48.70**	**0.45**	**1.3**	**16.80**	**0.30**
8	**HOH 143**	**1.4**	**117.60**	**0.41**	**3.9**	**48.20**	**0.62**
9	HOH 146	0.9	-42.40	0.20	1.2	272.00	0.44
10	HOH 152	1.0	-43.40	0.22	1.1	121.50	0.36
11	**HOH 182**	**2.4**	**123.30**	**0.52**	**1.3**	**29.50**	**0.31**
12	**HOH 222**	**3.0**	**270.70**	**0.71**	**2.8**	**113.00**	**0.55**
13	HOH 243	1.0	-78.60	0.19	1.0	116.60	0.35
14	HOH 263	1.1	60.30	0.32	0.0	-153.80	-0.07
15	HOH 327	0.9	7.00	0.24	0.9	42.50	0.27
16	HOH 335	1.0	166.90	0.34	0.0	-79.10	-0.04
17	**HOH 388**	**1.1**	**43.70**	**0.30**	**0.9**	**36.50**	**0.26**

[a]Waters within 4.0 Å of atoms on both proteins.
Relevant/bridging waters (having relevance ≥ 0.25 with respect to both proteins) are shown in bold.

Table VI. Unsolvated docking results for HyHEL-63 / HEL complex.[a]

Prediction[b]	HINT Score	Scaled HINT Score	*i-RMSD*	*l-RMSD*	*fnat*	Cluster / Pose[c]	CAPRI Quality[d]
model 1	4806	1.000	8.55	11.00	0.127	a1	-
model 2	4355	0.906	9.86	12.26	0.113	a1	-
model 3	4237	0.882	9.81	12.17	0.127	a1	-
model 4	4124	0.858	8.84	11.37	0.127	a1	-
model 5	4116	0.856	7.95	12.73	0.028	a2	-
model 6	4105	0.854	8.26	13.21	0.028	a2	-
model 7	4037	0.840	8.39	13.44	0.028	a2	-
model 8	3881	0.807	9.57	12.33	0.141	a1	-
model 9	3836	0.798	8.24	13.06	0.014	a2	-
model 10	**3796**	**0.790**	**1.72**	**2.32**	**0.915**	**a3**	****

[a]Top-10 predictions, ranked/named based on their HINT scores.
i-RMSD, l-RMSD and *fnat* values calculated with respect to the complex crystal structure.
[b]Predictions in bold are hits, i.e., medium or high accuracy.
[c]Clustering the ten solutions revealed three pose clusters for the ligand protein (RMSD within 2.0 Å).
[d]CAPRI quality criteria: high accuracy (***), medium accuracy (**), acceptable (*) and incorrect (-).

Table VII. Solvated docking results for HyHEL-63 / HEL complex.[a]

Prediction[b]	HINT Score	Scaled HINT Score	*i-RMSD*	*l-RMSD*	*fnat*	Cluster / Pose[c]	CAPRI Quality[d]
model 1	6099	1.000	6.44	7.04	0.00	b1	-
model 2	**5328**	**0.874**	**1.62**	**2.22**	**0.94**	**b2**	**
model 3	5046	0.827	7.19	7.47	0.00	b1	-
model 4	**4983**	**0.817**	**1.50**	**1.21**	**0.99**	**b2**	**
model 5	**4855**	**0.796**	**1.08**	**1.31**	**0.96**	**b2**	**
model 6	**4852**	**0.796**	**1.05**	**2.93**	**0.76**	**b2**	**
model 7	**4774**	**0.783**	**0.95**	**1.72**	**0.90**	**b2**	***
model 8	**4715**	**0.773**	**0.90**	**0.64**	**0.96**	**b2**	***
model 9	**4638**	**0.761**	**2.48**	**2.46**	**0.87**	**b2**	**
model 10	**4634**	**0.760**	**1.41**	**3.06**	**0.78**	**b2**	**

[a]Top-10 predictions, ranked/named based on their HINT scores.
i-RMSD, l-RMSD and *fnat* values calculated with respect to the complex crystal structure.
[b]Predictions in bold are hits, i.e., medium or high accuracy.
[c]Clustering the ten solutions revealed two pose clusters for the ligand protein (RMSD within 2.0 Å).
[d]CAPRI quality criteria: high accuracy (***), medium accuracy (**), acceptable (*) and incorrect (-).

Table VIII. HINT-based interaction energy between A/Glu62 (receptor protein) and C/Gly53 (ligand protein) of KcsA/Fab complex, bridged via HOH 2016.*

	Predictions[a]	ZDOCK rank	Model Quality[b]	Interaction Energy (kcal mol^{-1})[c]			
				A/Glu62 - C/Gly53	A/Glu62 – HOH 2016	HOH 2016 - C/Gly53	Total residue-residue interaction
Crystal Structure 1k4c				0.22	-0.67	-0.17	-0.62
Unsolvated Docking	model 12	72	**	0.27			0.27
	model 27	63	**	-			-
	model 46	31	**	-			-
	model 58	25	**	-			-
	model 66	39	**	-			-
	model 82	67	**	-			-
Solvated Docking	model 1	97	**	0.19	-0.93	0.09	-0.65
	model 2	**54**	****	**0.24**	**-0.87**	**-0.06**	**-0.69**
	model 3	**63**	****	**0.34**	**-0.87**	**-0.10**	**-0.62**
	model 4	**83**	****	**0.26**	**-0.88**	**-0.09**	**-0.71**
	model 5	**31**	****	**0.23**	**-0.89**	**-0.01**	**-0.67**
	model 6	65	**	0.19	-0.90	0.04	-0.66
	model 7	60	**	0.21	-0.85	0.10	-0.53
	model 8	11	**	0.22	-0.89	0.07	-0.60
	model 9	18	**	0.22	-0.86	0.12	-0.52
	model 10	**59**	*****	**0.50**	**-0.88**	**-0.19**	**-0.57**
	model 11	**72**	*****	**0.28**	**-0.92**	**-0.03**	**-0.67**
	model 12	56	***	0.23	-0.83	0.09	-0.51
	model 13	**21**	****	**0.24**	**-0.84**	**-0.01**	**-0.61**
	model 14	44	***	0.14	-0.89	0.11	-0.64
	model 35	70	**	0.28	-0.85	0.06	-0.52

[a]Only predictions of medium accuracy or better are shown, ranked/named based on their HINT scores.
[b]CAPRI quality criteria: high accuracy (***), medium accuracy (**) and acceptable (*).
[c]Interaction energy calculated based on HINT scores (previous studies show ~515 HINT units = 1 kcal mol^{-1}).[86]
*Solvated docking hits with HOH 2016 showing favorable interaction energies with both residues are shown in bold. "-" indicates interaction not present in model.

FIGURES and CAPTIONS

Figure 1. Scatterplots of *fnat* vs scaled HINT scores for all predictions (n = 3000 for 100 poses in each complex) grouped based on their quality: blue (incorrect), green (acceptable, *), yellow (medium, **), red (high, ***). (A) Results from unsolvated docking. There is a positive linear correlation (not shown) between scaled HINT scores and *fnat* values ($r = 0.252$, $p < 0.0001$). (B) Results from solvated docking. On average, predictions of high/medium accuracy rank much better than the incorrect ones. Predictions with higher HINT scores have *fnat* values closer to 1, indicating the ability of HINT to identify correct poses. For clarity, points with scaled HINT scores < -2.0 are not shown.

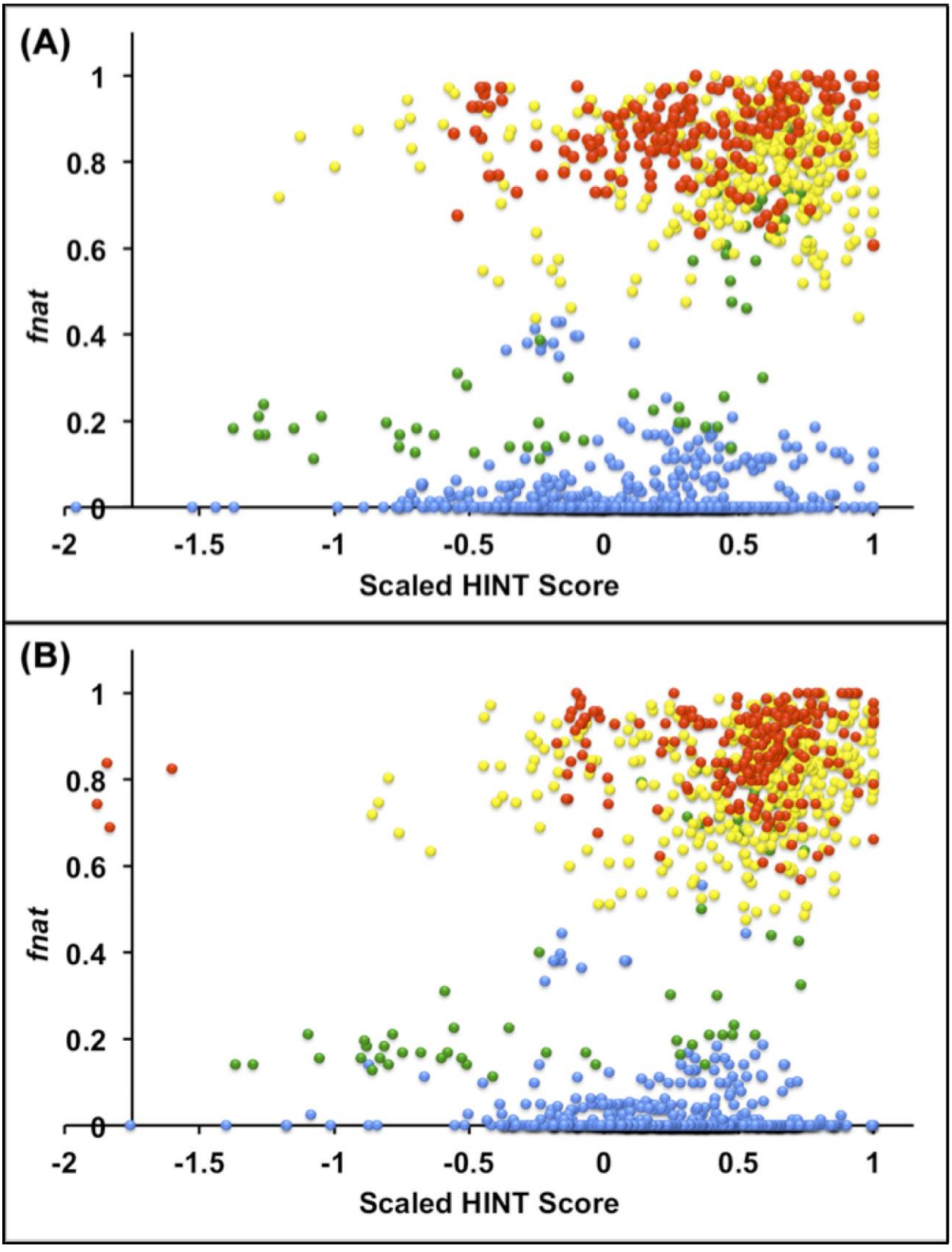

(A)
1
0.8
0.6
0.4
0.2
0
fnat
-2
-1.5
-1
-0.5
0
0.5
1
Scaled HINT Score
(B)
1
0.8
0.6
0.4
0.2
0
fnat
-2
-1.5
-1
-0.5
0
0.5
1
Scaled HINT Score

Figure 2. The anti-lysozyme antibody HyHEL-63 / lysozyme HEL complex (PDB 1dqj). (A) Crystal structure of HyHEL-63 (green) / HEL (cyan) in complex. The image shows the presence of Relevant interfacial waters (red spheres). (B) Detailed view of the interface showing bridging interactions of Relevant waters with residues on both proteins. (C) Bridging interactions formed by Relevant interfacial water HOH 143 with Tyr58 of HyHEL-63 and Val99 and Asp101 of HEL. Image prepared using PyMOL.[100]

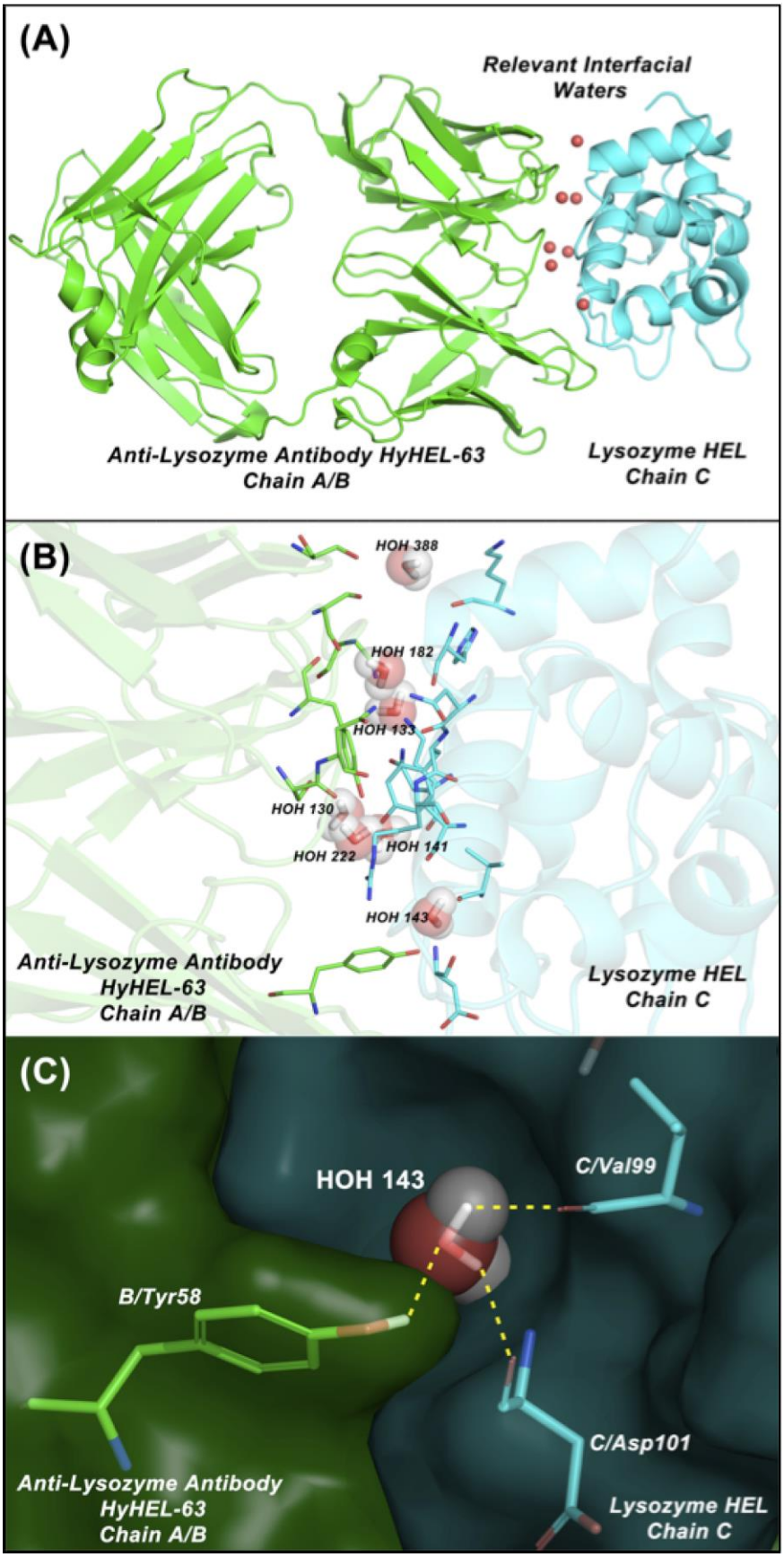

(A)
Relevant Interfacial Waters
Anti-Lysozyme Antibody HyHEL-63 Chain A/B
Lysozyme HEL Chain C
(B)
HOH 388
HOH 182
HOH 133
HOH 130
HOH 222
HOH 141
HOH 143
Anti-Lysozyme Antibody HyHEL-63 Chain A/B
Lysozyme HEL Chain C
(C)
HOH 143
C/Val99
B/Tyr58
C/Asp101
Anti-Lysozyme Antibody HyHEL-63 Chain A/B
Lysozyme HEL Chain C

Figure 3. Unsolvated docking results for HyHEL-63 / HEL complex. The left panels overlay the predicted ligand poses (cyan) with the crystal structure (red); the right panels illustrate the interactions of B/Tyr58 with ligand residues. (A) model representative of cluster a1 (n=5); (B) model representative of cluster a2 (n=4); (C) model representative of cluster a3 (n=1). Neither cluster a1 nor cluster a2 show native residue-residue contacts, and are thus incorrect predictions, but cluster a3 is of medium accuracy. Image prepared using PyMOL.

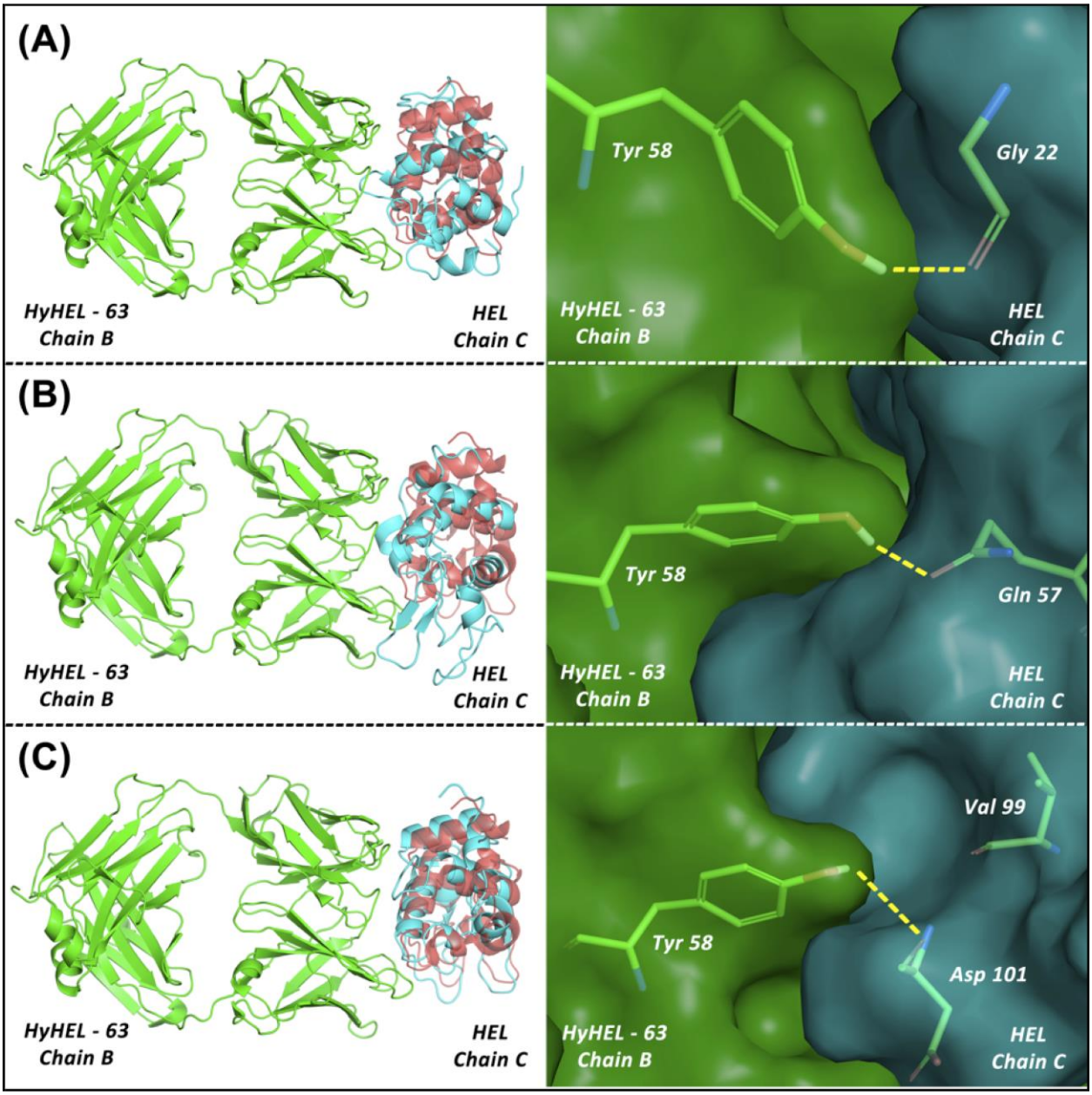


Figure 4. Solvated docking results for HyHEL-63 / HEL complex. Unsolvated docking results for HyHEL-63 / HEL complex. The left panels overlay the predicted ligand poses (cyan) with the crystal structure (red); the right panels illustrate the interactions of B/Tyr58 with ligand residues. (A) model representative of cluster b1 (n=2); (B) model representative of cluster b2 (n=8). Cluster b1 are non-native-like predictions showing water-mediated interaction between B/Tyr58 and C/Lys116. In cluster b2 native water-mediated residue-residue contacts are retained, with B/Tyr58 showing a water-mediated hydrogen-bonding network with C/Val99 and C/Asp101. Image prepared using PyMOL.

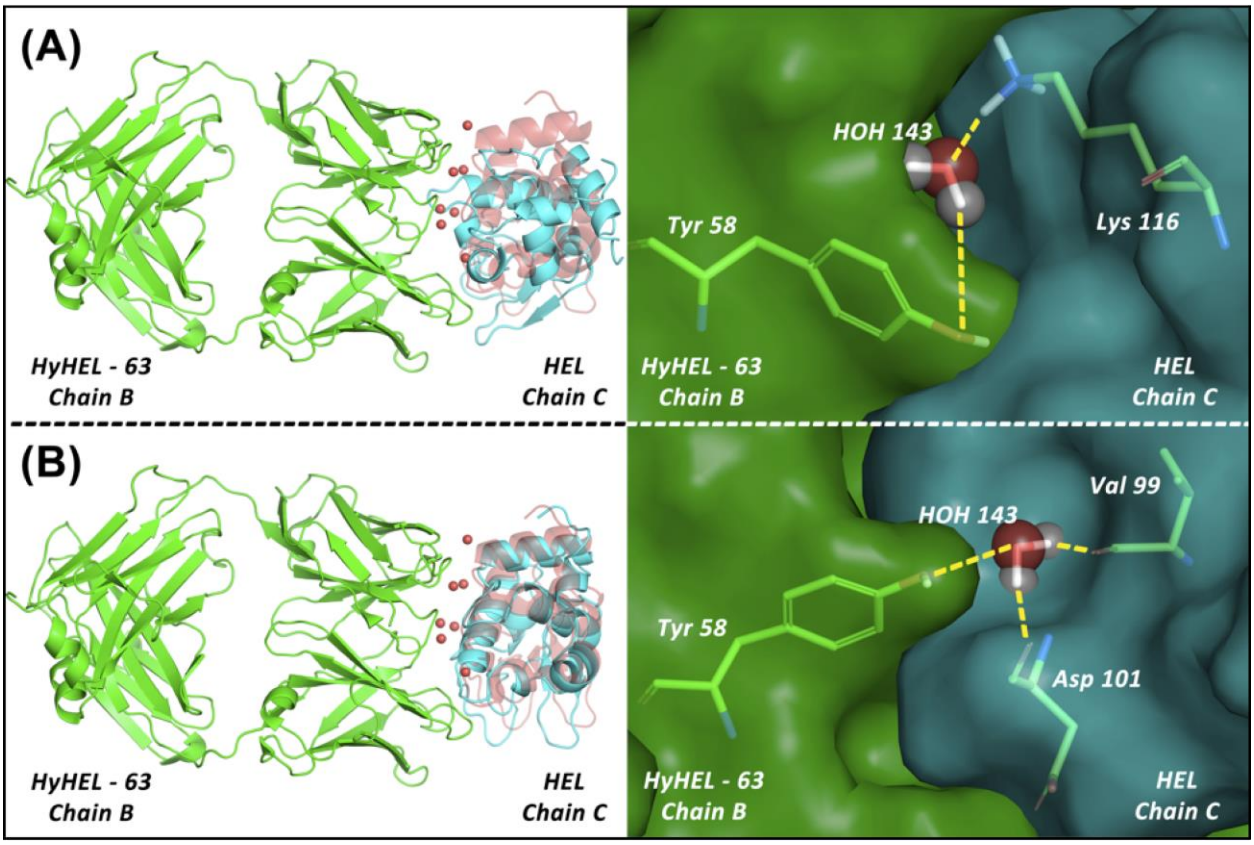
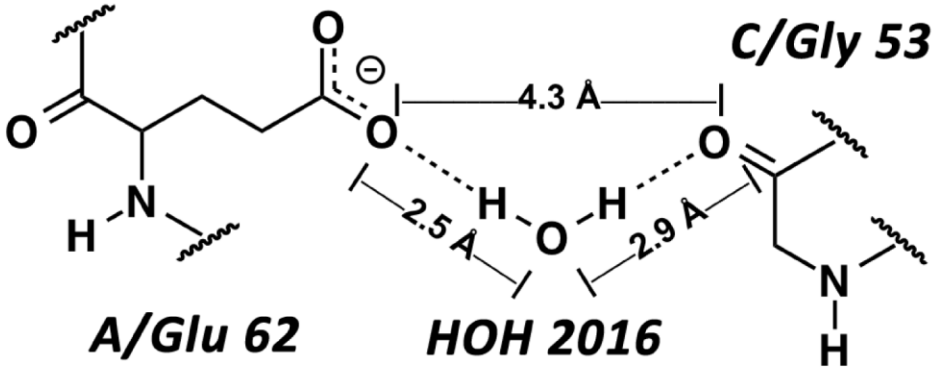
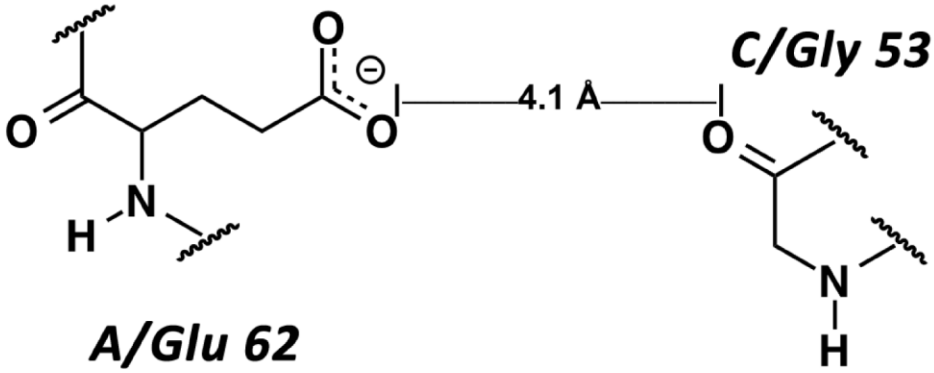


Figure 5. Interfacial residue-residue contacts between A/Glu62 of Fab and C/Gly53 of KcsA: (A) energetically stabilized by a water molecule, HOH 2016, as observed in the crystal structure of the complex (PDB ID: 1k4c), distances shown in Å; (B) A/Glu62–C/Gly53 interaction as observed in top HINT-ranked "hit" (model 12) for unsolvated docking (the interaction is scored unfavorably, Table VIII); (C) A/Glu62–HOH2016–C/Gly53 interaction as observed in second HINT-ranked "hit" (model 2) for solvated docking. Model 2 was selected because HOH 2016 shows favorable interactions with respect to both proteins. Now, the overall interaction is favorable and energetically similar to the native (Table VIII).

(A) Crystal Structure - 1k4c



(B) Unsolvated Docking Prediction - Model 12



(C) Solvated Docking Prediction - Model 2