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ORIGINAL RESEARCH

Refinement of rigid-body protein–protein docking using backbone and side-chain minimization with a coarse-grained model

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Abstract: Understanding protein–protein recognition is one of the main goals in structural biology. Most of the key biological processes involve the formation of specific protein complexes, for which a detailed structural knowledge is essential to understand the mechanism of protein association and their functional implications. Computational docking methods are currently able to predict the structure of a protein–protein complex with a high degree of accuracy in some cases. However, in the majority of cases, with conformational movements upon binding, we have to go beyond the current rigid-body approach and introduce flexibility. Given the difficulties of using full-atom descriptions during flexible docking, we need to focus our efforts in coarse-grain models. Here, we have implemented and tested a version of the united residue (UNRES) forcefield for protein–protein docking refinement. The results indicate improvement in the geometry of the docking solutions, and better docking energy landscapes, although in general, the scoring did not improve with respect to rigid-body pyDock function. However, as opposed to other scoring algorithms, the UNRES scoring does not seem to be biased towards cases that are over-represented in the structural databases (typically enzyme-inhibitor and antibody-antigen cases). This consistency among all types of complexes suggests its use as a solid basis for developing better unbiased scoring methods.

Keywords: molecular recognition, structural prediction, protein–protein association, global energy

Introduction

The behavior of the cell, both internally and with respect to the external environment, relies on a vast number of different molecular interactions. Among all these interactions, protein–protein interactions are responsible for the most important biological processes, including signal transduction, transportation, enzymatic activity and immune activities. It is thus essential to understand the structural and physicochemical basis of protein–protein recognition and to be able to predict the structure of the specific complexes formed between interacting proteins. The low number of protein–protein complexes experimentally assessed stresses even more the importance of this endeavor.

In recent years, computational approaches have boosted the structural biology field, and with increasing frequency more studies are focusing on protein–protein associations. Protein–protein docking addresses the problem of predicting the three-dimensional structure of a protein–protein complex given the structure of the subunits. Since a full *ab initio* physical calculation is computationally prohibitive, due to the huge number of degrees of freedom that appear to be in a standard system, many algorithms have been developed that try to combine different disciplines, from

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mathematics to biology.[1] In most cases, docking algorithms generally include a rigid body orientational sampling part, followed by a scoring and post-analysis selection of small numbers of conformations for a final refinement. Despite improvements in these methods during the last few years, as can be seen from the results of the CAPRI experiment[2–4] (see http://www.ebi.ac.uk/msd-srv/capri), most methods usually fail when there are conformational changes upon complexation. Thus, a proper inclusion of conformational flexibility in the docking algorithms is essential to make progress in this field.

Coarse-grained models for proteins have experienced significant advances during the last few years, which have aroused a renewed interest in this field.[5] One can simplify the geometry, describing each residue with a small number of beads, or use simplified forcefields, elastic network models, or even Gō-like models.[6] In general, the coarser the description, the larger the system can be treated. However, with coarser models it also becomes more difficult to parameterize forcefields for being both accurate and transferable. In this framework the united residue (UNRES) forcefield,[7–17] with two beads per residue, achieves an acceptable compromise. Since it has been derived from first principles, averaging the degrees of freedom that the model neglects, its applicability becomes largest. Thus, the UNRES forcefield has proven to be quite adequate for several molecular mechanics problems such as in protein folding – as shown in recent CASP experiments[18] – in multichain protein folding,[19] or in free energy calculations with multicanonical algorithms.[20] However, it has not yet been applied to the broad problem of protein–protein association or benchmarked as part of a general protein–protein docking and refinement protocol.

In this work, we will apply the UNRES model methodology to the refinement of protein–protein rigid-body docking poses. We will evaluate different UNRES forcefield parameters and will analyze the improvement of the docking landscapes.

Methods

UNRES forcefield

The UNRES model for protein modeling[7–17] offers a coarse grained description of the molecule together with a physical based forcefield. Two beads per residue are used as interaction sites, one for the main chain (p) and the other for the side chain (SC). The SC beads are different-sized ellipsoids according to the specific size and shape of the amino acid side chain.[7] The C^{α}s are used only to construct the chain, and to place the peptide group p halfway between them (see Figure 1), although they are not used to compute the energy. The distance between consecutive C^{α}s is fixed, just as the distance between the side chain centers and the corresponding C^{α}. Thus, each amino acid is described by four degrees of freedom, they are the virtual bond bending θ, the virtual bond dihedral angle γ and the angles α and β, for the orientation of the side chain with respect to the backbone.

The force field has been derived as a potential of mean force (PMF), or restricted free energy (RFE) by averaging out the degrees of freedom of the chain not present in the two-bead model, as the ones belonging to the solvent molecules (the reader is referred to Alberts[21] for an introduction to PMF, and to Liwo et al[10] for a theoretical basis of the UNRES forcefield). Performing a factorization of the RFE, into one-, two- and multibody-terms, according to the cluster expansion of Kubo,[22] analytical expressions for the description of the energy are obtained. The expression for the calculation of the intrachain potential energy is then given by equation 1 below:

$$\begin{aligned} U_{intrachain} = {} & w_{sc}\sum_{j}\sum_{i<j}U_{SC_iSC_j} + w_{SCp}\sum_{i\neq j}U_{SC_ip_j} \\ & + w_{el}\sum_{i<j-1}U_{p_ip_j} + w_{tor}\sum_{i}U_{tor}(\gamma_i) \\ & + w_{tord}\sum_{i}U_{tord}(\gamma_i,\gamma_{i+1}) + w_b\sum_{i}U_b(\theta_i) \\ & + w_{rot}\sum_{i}U_{rot}(\alpha_{SC_i},\beta_{SC_i}) \\ & + \sum_{i=3}^{4}w_{corr}^{(i)}U_{corr}^{(i)} + \sum_{i=3}^{4}w_{turn}^{(i)}U_{turn}^{(i)} \end{aligned} \quad (1)$$

where $U_{SC_iSC_j}$, $U_{SC_ip_j}$, and $U_{p_ip_j}$ stand for the potential energy accounting for the interactions in water between different side chains, between side chains and peptide groups, and between different peptide groups, respectively (the term "peptide group" refers to the backbone portion of each residue).The term U_b describes the bending of the virtual bond angles, U_{tor} and U_{tord} the virtual bond torsional angle and coupled (double) torsional angle, and U_{rot} the energetics of the rotameric state of the side chains. Finally the terms $U_{corr}^{(i)}$ refer to three- and four-body interactions between non-contiguous parts of the chain, while $U_{turn}^{(i)}$ accounts for the same interactions between contiguous parts of the chain. These terms come from the truncated expansion of the free energy into independent clusters (together with U_{tor} and U_{tord}), and involve mixed interactions between local energies (averaged all atom energy of the

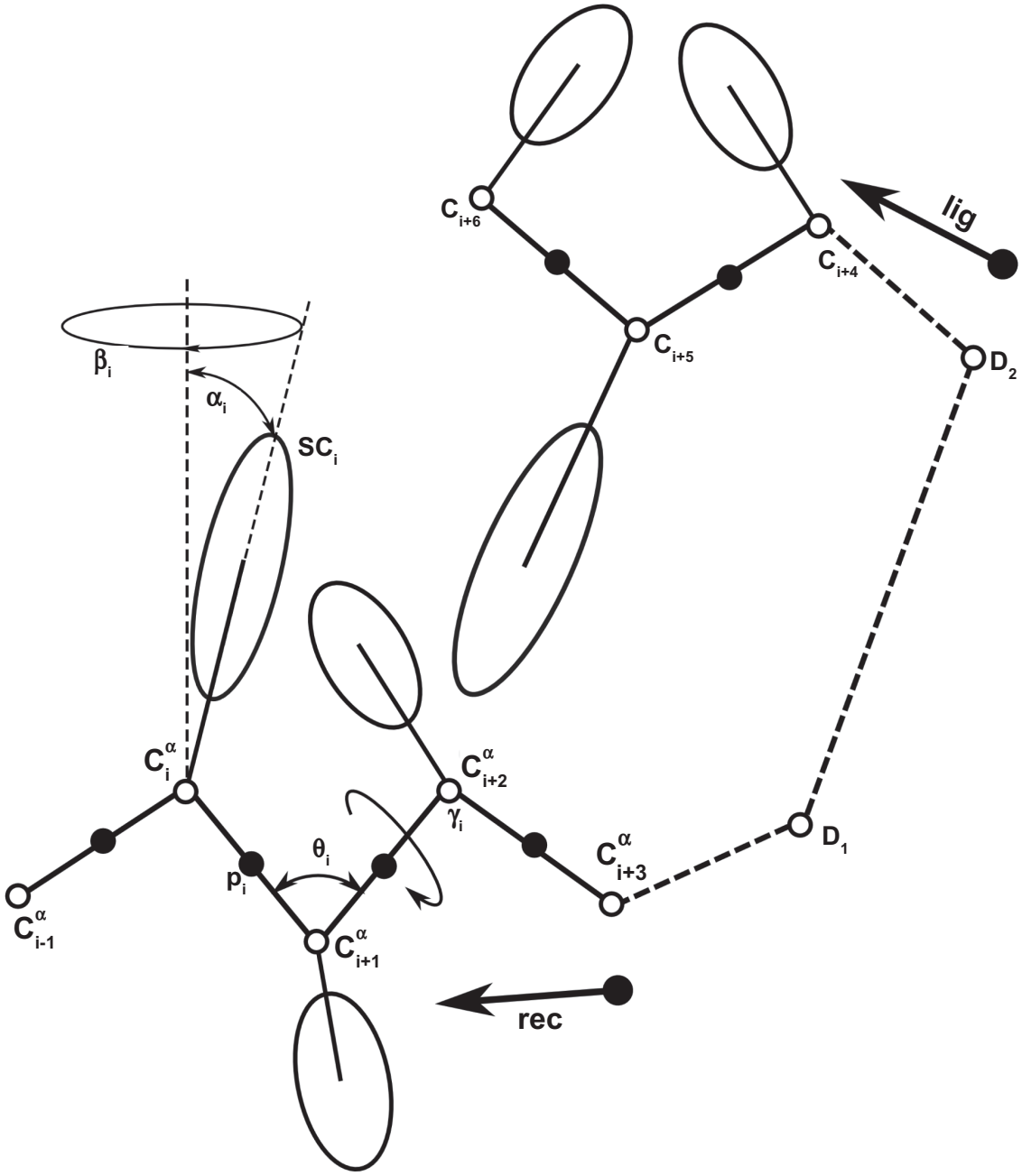

Figure 1 The UNRES model uses a 2-bead coarse-grained description for each amino acid residue.
Notes: Four angle values describe the geometry of each peptide, θ and φ for the backbone and α and β for the side chain (SC). The interacting sites are different-sized ellipsoid beads corresponding to the SC, and peptide groups *p* represented as black circles. The bond lengths are kept fixed and two extra dumb residues are placed between receptor and ligand to allow extra mobility.

backbone for each peptide) and the electrostatic interaction between peptide groups.

The use of this force field for protein–protein interactions requires a term for the interaction between proteins, as in a previous work about multichain protein folding.[19] Following that study, we have used the same nonbonded terms as in the single chain forcefield, with the same derived weights. Hence, the interacting energy between two different chains *k* and *l* is described by equation 2 below:

$$U^{k,l}_{interchain} = w_{sc}\sum_i\sum_j U_{SC_i^k SC_j^l} + w_{SCp}\sum_i\sum_j U_{SC_i^k p_j^l} + w_{SCp}\sum_i\sum_j U_{p_i^k SC_j^l} + w_{el}\sum_i\sum_j U_{p_i^k p_j^l} + \sum_{i=3}^{4} w_{corr}^{(i)} U_{corr}^{(i)} \quad (2)$$

Depending on the energy terms, the parameterization comes from fitting to distributions derived from the Protein Data Bank[23] (PDB) ($U_{SC_iSC_j}$, $U_{SC_ip_j}$, U_b, and U_{rot}) or from fitting to averaged free energy surfaces (the rest of terms). The set of weighting factors for the different energy terms

can define the behavior of the proteins. In this work we have compared the results produced from three different sets of weights, previously described. The first set of weighting factors used in this work, (called *4P*)[17] was derived from decoys composed of lowest-energy conformations, so it should be able to identify near-native conformations from a large decoy set based on the conformational docking energy. It was also shown to be suitable to fold medium-sized proteins although it performed better on the α- and α- + β- than on the β-proteins.[17] However, the *4P* forcefield did not include any thermal effects because it was derived from lowest-energy conformations. Therefore, for some proteins it revealed difficulties to reproduce canonical molecular dynamics.[24] The other two sets of weights used here were *07*αβ[25] and *09*.[26] Both were derived in order to include temperature dependence in the forcefield. The *07*αβ set was derived for an αβ-protein (another set *07*α was derived for an α-protein, but has not been used in this work).[25] The *09* set was derived for an α-protein.[26] Both were used here at a temperature of 300 K.

Minimization with UNRES

UNRES energy minimizations have been applied to docking orientations generated by FTDock (see next section). As the UNRES energy is calculated in internal coordinates, geometric constraints to the minimization can be easily included. Thus, in order to restrict a given degree of freedom, one only needs to cancel the corresponding gradient. For instance, when minimizing the side chains alone, only the corresponding interacting terms were calculated, with a dramatic reduction in computational times. One limitation of the original description is that, as the chain is constructed with fixed separation between C^{α}s (Figure 1) the distance between the last residue of the receptor and the first one of the ligand is restricted during a minimization. To allow for extra mobility, we included two dumb residues (D_1 and D_2) between receptor C-term and ligand N-term (Figure 1). This is a more rational way to include better sampling of the orientation and translational space between the molecules. These dumb residues were only included between receptor and ligand molecules, not between chains in cases of multi-chain receptor or ligand so that the whole receptor or ligand can move at once. In cases of missing residues, in the X-ray unbound structures, we have not used any restraints to keep the conformation of the residues next to the missing ones (to avoid unwanted flexibility in cases of missing large residue segments, it would be desirable to model the missing residues).

Minimization was performed with the *m1qn3* package, which uses a limited memory quasi-Newton approximation of the Hessian and a line-search strategy. It has been proven to be specially suited to solve large scale minimization problems.[27] As a termination criterion we used both gradient convergence (a fraction smaller than 10^{-20} between final and initial gradients) and a limited number of steps (70 was the number of iterations and 150 the number of simulations).

Rigid body sampling and pyDock scoring

Initial sets of 10,000 rigid-body docking conformations were generated using FTDock.[28] We used electrostatics and a grid size of 0.7 Å, which were previously shown to be the best conditions for energy-based scoring with pyDock.[29] For comparison purposes, we used one of the most competitive scoring functions for protein–protein docking, pyDock,[30] which calculates the interaction between receptor and ligand based on: i) truncated and linearly screened electrostatic term; ii) truncated and weighted Van der Waals term; and iii) an accessible surface area (ASA)-based desolvation energy term with atomic parameters previously optimized for docking.[31]

Benchmark

To test the reliability of the methods described in this work, we used a standard benchmark for structural prediction of protein–protein complexes.[32] It is composed of 84 complexes that have available X-ray crystal structures for the bound and the unbound subunits. We always used the unbound subunits for all docking calculations in this work. In order to avoid biased results (some unbound structures in the benchmark were oriented as in the complex, which can make it easier for FTDock to find near-native docking solutions in some cases), the initial structures were randomly oriented before any calculation (a minor random deviation from the exact bound orientation helps to avoid any bias). In addition, we checked here that the final accepted solutions in the docking results did not arise from initial random orientations serendipitously close to the correct complex structure. Moreover, we checked that similar sampling results were obtained with FTDock when using different initial random orientations (data not shown).

Besides, in order to try several parameterization and sampling methods, most of the tests were done over a subset of this benchmark. This subset was composed by nine complexes: three antibody/antigen complexes, three enzyme/inhibitor case, and three classified as "other". All of them were described as rigid body in the original benchmark.

Success rates were defined as the percentage of test cases in which at least one acceptable docking solution is found with rank lower or equal than a given value when ranking the sampled conformations according to the different scoring functions. A near-native or acceptable docking solution was defined as the one that, after superimposing the receptor molecules of the reference and the corresponding structure, had a root-mean-square deviation (RMSD) value of less than 10 Å between both ligand C^{α} atoms.

Results and discussion

Side-chain optimization of docking poses

The first test was analyzing the capabilities of the UNRES forcefield (in different conditions) for optimizing the conformation of the side-chains in rigid-body docking solutions. For that, we tried the three different sets of weighting factors *4P*, *07*αβ, and *09* (described in Methods) on a sub-set of cases of the *benchmark* for which rigid-body docking poses were generated by FTDock (using the unbound subunits). Each docking pose was described by the UNRES approach and only the side-chains of both molecules were allowed to move. The three forcefield conditions above described were applied, using the total energy of the system. The final scoring energy after minimizing the side chains is given by the corresponding UNRES forcefield, considering either the interaction energy between receptor and ligand (inter) or the total energy of the system (total). The results are shown in Figure 2.

As can be seen, all conditions place a near-native solution within the top 1000 docking orientations in around 80% of the cases. However, when we focus on the success rates for the lowest-ranked docking orientations, there are some clear differences. First, in all conditions, the use of interaction energy seems better than the total energy. Then, we can see that although the *4P* forcefield gives better results for the top 10 docking predictions, the *07*αβ forcefield is more consistent and gives better results for most ranking values up to top 100 (this forcefield yields the best results especially at top 1, and top 20).

Side-chain optimization: results in full benchmark

Following the previous test, we decided to evaluate the results of the most promising forcefield (*07*αβ) on the total of 68 cases of the benchmark that had at least one near-native solution within the FTDock-generated docking sets (using the unbound subunits). The results are shown in Figure 3. The first striking result is that success rates of the minimized docking poses are worse than when scoring the rigid-body docking poses with pyDock. The reason could be that the energy function of the UNRES forcefield might not be useful to disregard false positives that may have very different interfaces. In order to test this, for each case we considered

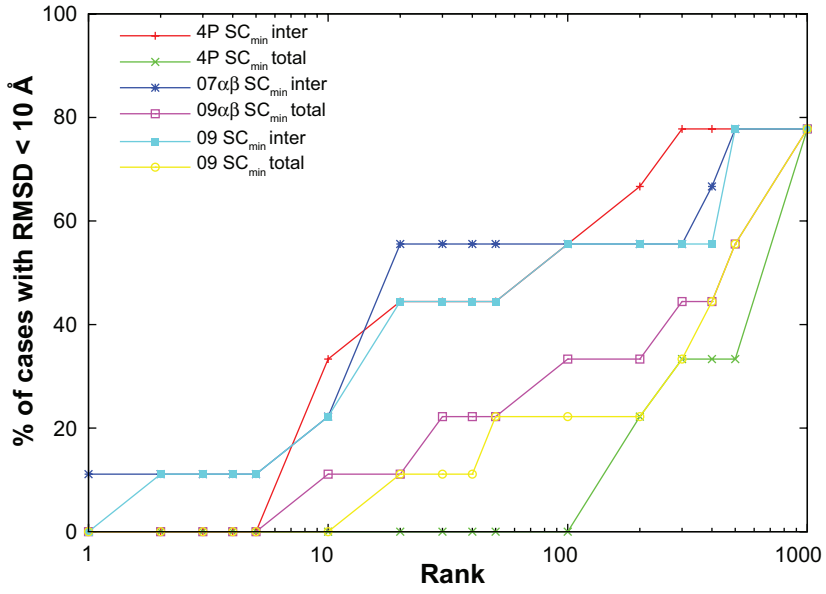

Figure 2 Success rates (eg, percentage of cases with at least a near-native solution with rank lower or equal than that indicated in axis) of the side-chain minimization with different UNRES forcefields on a subset of nine benchmark cases.
Notes: For each forcefield setup, the interaction energy between receptor and ligand (inter) or the total energy of the system (total) has been used for the final scoring as indicated.
Abbreviation: RMSD, root mean square deviation.

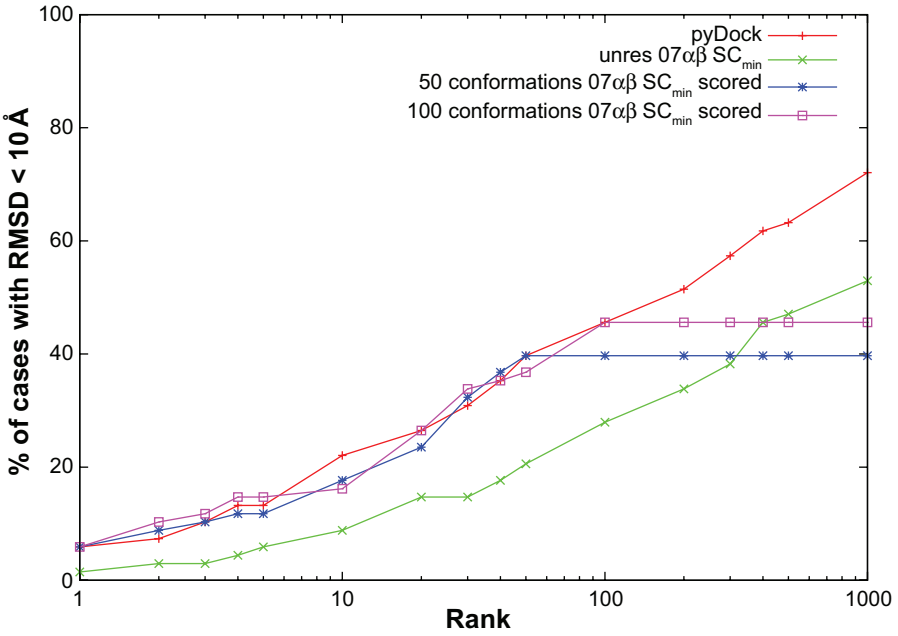

Figure 3 Results for the 68 complexes of the benchmark that had at least one near-native conformation generated by FTDock.
Notes: Success rates (eg, percentage of cases with at least a near-native solution with rank lower or equal than that indicated in axis) for *07*αβ UNRES interaction energy after side-chain minimization are shown (green line). For comparison, success rates for pyDock scoring of the rigid-body docking poses are also shown (red line). When only the best 50 or 100 docking poses as sorted by rigid-body pyDock are considered, the success rates for scoring them with *07*αβ UNRES after side-chain minimization are shown in blue and magenta lines, respectively.
Abbreviation: RMSD, root mean square deviation.

only the top 50 or 100 conformations as sorted them by pyDock before minimization, and then they were ranked by the UNRES potential after minimization of the side-chains. The success rates were practically the same as the pyDock values (for top 10 the UNRES success rates were slightly worse than for pyDock, but for top 2, top 3, top 4 or top 5, the UNRES success rates were slightly better than pyDock). This indicates that the UNRES forcefield after side-chain minimization can be used for the scoring of docking poses once most of the false positives have been removed.

It is interesting to analyze the results of the UNRES minimization in the complete docking sets, on the complexes grouped by type. In Figure 4 we can see the results for three types of complexes (as defined in the original benchmark): enzyme-inhibitor, antibody-antigen, and "other" types of cases. We can see that for the top 10 docking poses, the results are practically the same for all types of complexes. This is in contrast with the results by pyDock (also shown for comparison). The scoring by pyDock clearly works better for enzyme-inhibitor cases, and clearly worse for "other" type of cases, in line with what has been previously observed.[29] These results indicate that the UNRES scoring is providing results that are independent on the type of complex. However, the UNRES results are similar to pyDock ones for the "other" type, and it is clear that UNRES scoring does not particularly recognize the characteristics that make certain types of complexes (enzyme-inhibitors and antibody-antigens) to be better identified by pyDock. The positive aspect is that UNRES is not biased towards certain types of complexes (at least for the low-rank values), as the parameters have not been specifically derived from structural databases of protein–protein complexes (where some complex types are over-represented). We could speculate that any general future improvement in the forcefield (both minimization and scoring) might be beneficial for all docking cases (including the difficult ones, as in the "other" type), although this remains to be seen.

Global energy minimization: backbone and side-chain optimization

We also evaluated the results of applying global energy minimization with UNRES (including backbone, side-chain and rotation/translation ligand movements) to the refinement that rigid-body docking poses. In Figure 5 are shown the results with the different UNRES forcefields. As can be seen, although the success rates for the top 10 docking poses are the same as with side-chain only optimization, for the rank values above 10 the results are worse. Since the global minimization was performed after side-chain minimization, perhaps the conformations did not evolve further and hence did not improve. However, it was necessary to run global

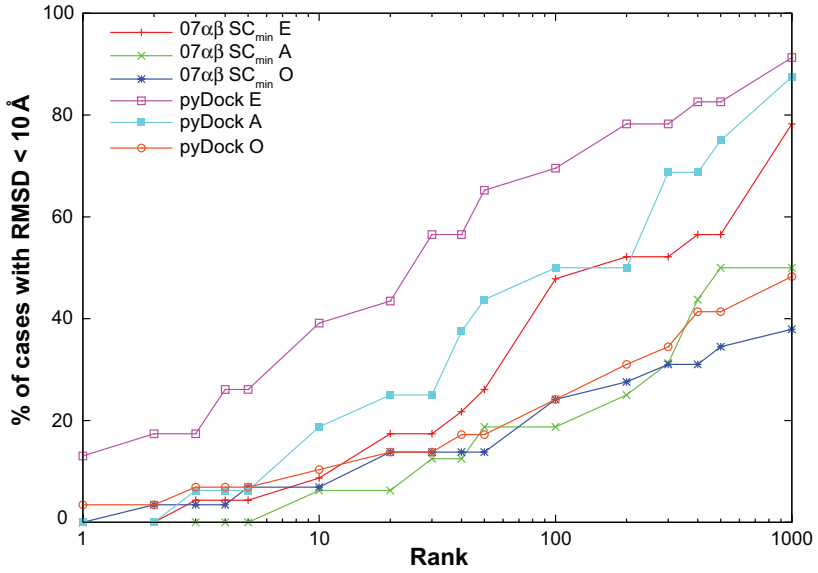

Figure 4 Success rates (eg, percentage of cases with at least a near-native solution with rank lower or equal than that indicated in axis) on groups of cases classified according to complex type, for the 68 complexes of the benchmark that had a near-native conformation generated by FTDock.
Notes: The success rates of *07*αβ UNRES forcefield after side-chain minimization are shown for enzyme-inhibitors (red line), antibody-antigen (green line) and "other" type of cases (blue line). For comparison, the success rate of pyDock scoring of rigid-body docking poses are shown for the same groups of cases (magenta, cyan and orange lines, respectively).
Abbreviation: RMSD, root mean square deviation.

minimization after side-chain optimization, otherwise there were problems of instability (some docking orientations unfolded when global minimization was directly applied after rigid-body docking; probably due to an excessive number of clashes in the rigid-docking geometries).

In some cases the globally refined docking structures significantly improved after the UNRES global energy optimization. For instance, in the case of the 1D6O/1IAS complex (PDB 1B6C), the distribution of docking orientations after side-chain and global energy minimization improved the

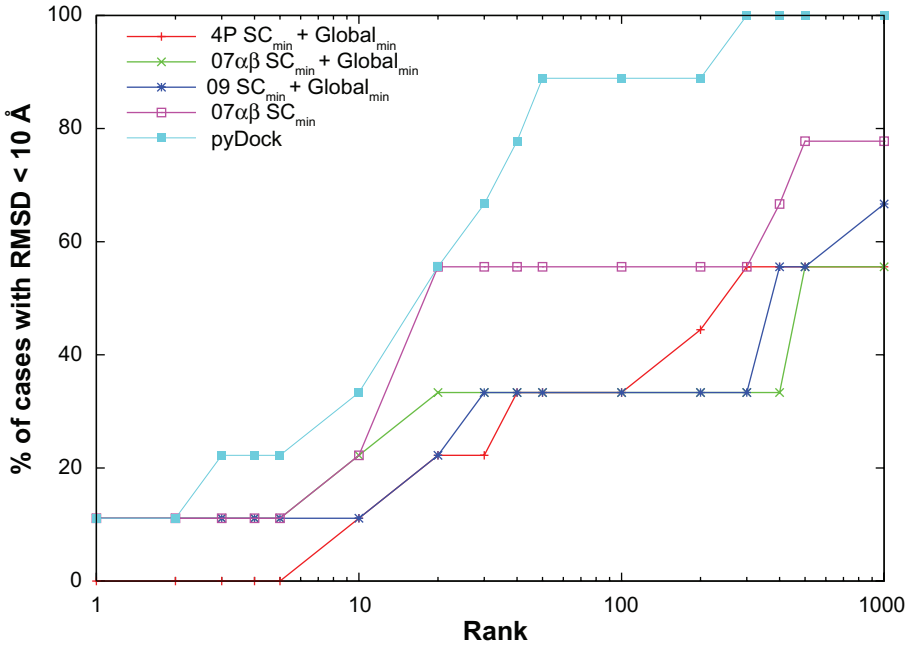

Figure 5 Success rates (eg, percentage of cases with at least a near-native solution with rank lower or equal than that indicated in axis) for the subset of nine benchmark cases when performing full energy minimization of each FTDock-generated conformation after side-chain minimization, with the UNRES forcefields *4P* (red line), *07*αβ (green blue) and *09* (blue line). For comparison, the success rates for only side-chain minimization with the UNRES *07*αβ forcefield is shown in magenta, and the rigid-body docking scoring with pyDock in cyan.
Abbreviation: RMSD, root mean square deviation.

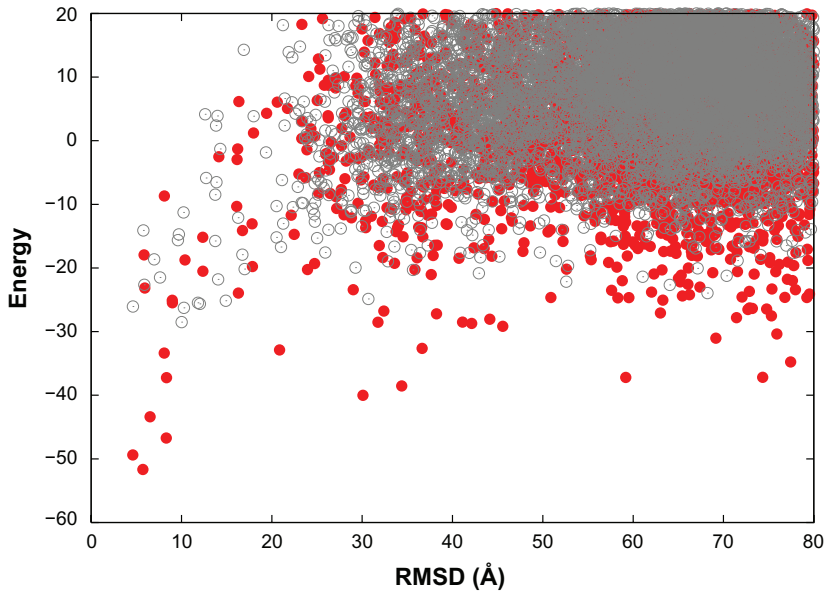

Figure 6 Energy landscape for the 1B6C complex before (pyDock values in gray) after side chain minimization and global energy minimization (ie, including backbone and side-chains) with UNRES forcefield *07*αβ (in red).
Notes: A nice funnel can be seen for the docking poses closest to the reference structure (lowest RMSD values).

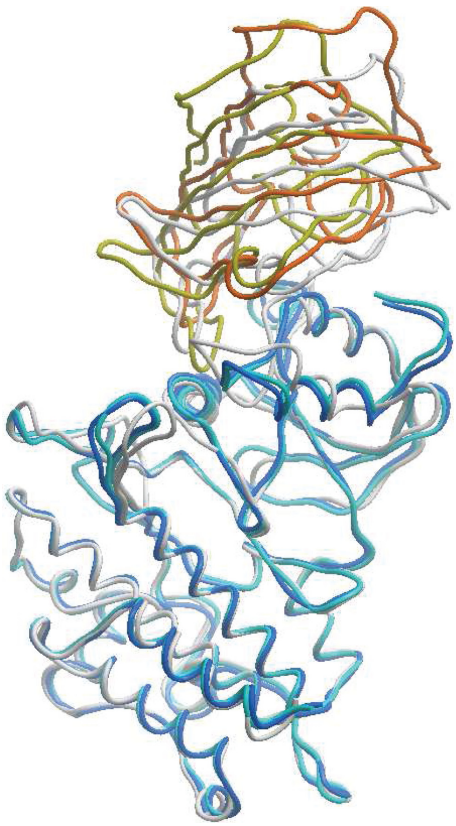

Figure 7 Docking and UNRES refinement results for 1B6C complex (reference in white). The lowest energy solution after global minimization (ie, including backbone and side-chains) is shown (receptor in orange, ligand in blue).
Notes: The same solution before minimization is also shown (receptor in yellow, ligand in cyan). This conformation went from 11.8 Å to 5.9 Å ligand RMSD after minimization (and from rank 5 by pyDock, to rank 1 by UNRES). For clarity, the 1D6O/1IAS reference complex molecule is shown in white (the biggest molecule, defined as the ligand in the original benchmark, was superimposed).

docking landscape. As can be seen in Figure 6, the docking energy landscape has a nice funnel shape in which the lowest-energy docking conformations are the closest to the reference state in terms of RMSD. In this case, the lowest-energy docking solution after side-chain and full energy minimization was a near-native docking solution (rank 1; 5.9 Å ligand RMSD with respect to the reference complex structure; Figure 7), which before minimization was not even considered as a near-native solution by our standards (11.8 Å ligand RMSD; ranked 5 by pyDock). Other conformations, not considered near-native solutions by our standards before minimization, also had better RMSD after full minimization (14.9 to 8.04 Å, final rank after minimization: 366; 10.1 to 5.9 Å, final rank after minimization: 49; and 12.0 to 9.0 Å, final rank after minimization: 30).

Conclusions

We have implemented and tested a version of the UNRES forcefield for the use in the refinement of protein–protein docking orientations. The results indicate an improvement on the geometry of the docking solutions in some cases, although in general, the scoring did not improve with respect to rigid-body pyDock function. The fact that the UNRES scoring gives similar results for all types of complexes indicates that there is no bias towards specific, overrepresented types of complexes, and can be the basis for developing better unbiased scoring methods.

Acknowledgments

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Disclosures

The authors report no conflicts of interest relevant to this research.

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