FastContact: a free energy scoring tool for protein–protein complex structures

P. Christoph Champ and Carlos J. Camacho*

Department of Computational Biology, University of Pittsburgh, Pittsburgh, PA, USA

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ABSTRACT

‘FastContact’ is a server that estimates the direct electrostatic and desolvation interaction free energy between two proteins in units of kcal/mol. Users submit two proteins in PDB format, and the output is emailed back to the user in three files: one output file, and the two processed proteins. Besides the electrostatic and desolvation free energy, the server reports residue contact free energies that rapidly highlight the hotspots of the interaction and evaluates the van der Waals interaction using CHARMm. Response time is ~1 min. The server has been successfully tested and validated, scoring refined complex structures and blind sets of docking decoys, as well as proven useful predicting protein interactions. ‘FastContact’ offers unique capabilities from biophysical insights to scoring and identifying important contacts.

INTRODUCTION

The most intuitive decomposition of the binding free energy involves four terms (1–3): van der Waals (vdW) interactions, electrostatic, hydrophobicity and configurational entropy. The relative contribution of the changes between the bound and free states of these four terms is not the same. For stability (1), the main contributions appear to be electrostatic and desolvation interactions. For refined docked conformations, vdw interactions are expected to balance between the bound and unbound state, as they seemingly do in protein folding (1). This is good news, since it is not yet possible to readily estimate solute–solvent interactions. It should be noted, however, that solute–solute vdw has been shown to be an important consideration for complex refinement (4). Configurational entropy loss upon binding, including rotational and translational degrees of freedom, is always important, rough estimates based on crystal complexes varying between 5 and 15 kcal/mol (2.5–8). For the most part, this entropy depends on the flexibility of the unbound or free state with respect to the bound, with smaller corrections depending on the docking geometry. Since there is no robust estimate of entropy for a given protein, empirical free energy estimates, like ‘FastContact’, are always subject to an entropic correction. Hence, the server is most useful for discrimination between protein–protein docked complexes, and, more generally, for identifying energetically important contacts at the interface.

‘FastContact’, originally published in (9,10) rapidly estimates the electrostatic and desolvation component of the free energy based on a classic distance dependent dielectric 4/r (11) and an empirical contact potential for the desolvation contribution (7) developed using a database of crystal (no complexes) structures from the PDB. Because of the pairwise nature of the empirical interactions, ‘FastContact’ is also able to report the contribution of individual residues and pairs of residues to the free energy. The latter should prove useful for site-directed mutagenesis studies since rankings of these interactions consistently identify the hot spots in the interface.

BRIEF DESCRIPTION OF THE ALGORITHM

The code behind the server was written in Fortran 77 and the server itself was written in PHP. ‘FastContact’ performs a fast computational estimate of the binding free energy between two proteins based on atomic pairwise interactions:

(i) Electrostatic energy: the standard intermolecular Coulomb electrostatic potential with a distance-dependent dielectric constant equal to 4/r, enforcing a minimum atom-to-atom distance separation equal to the sum of their corresponding vdw radii to avoid artificial overlaps.

(ii) Desolvation free energy: knowledge-based contact potential that accounts for hypdophobic interactions, self-energy change upon desolvation of charged and polar atom groups and side-chain entropy loss.

(iii) vdw energy: the standard 6–12 Lennard–Jones potential is evaluated using the program CHARMm (12) as part of the optimization of polar Hydrogens and overlaps.
The first two values (i–ii) can be used to calculate the overall free energy of the protein–protein interactions, assuming solute and/or solvent vDW cancellation between the bound and free proteins, and a correction factor for the configurational entropy loss. The application uses the definition of the atomic composition of each amino acid consistent with CHARMM19 parameters.

USING THE FASTCONTACT SERVER

Required user-input information

Figure 1 shows a snapshot of the input page. The user uploads two Protein Data Bank (PDB) format files (13), one ‘receptor’ and one ‘ligand’, along with their email address. The web server currently makes no distinction between chains; it simply reads in each line in the PDB file starting with an ‘ATOM’ field. The maximum number of residues is limited to 1500. The email address is where the output/results will be sent (as a file attachment). Hydrogen bonds and missing atoms are built and optimized on the uploaded structures using the molecular software CHARMM.

Optional parameters

Range of desolvation interaction. The default range is 6 Å, such that the potential smoothly goes to zero between 5 and 7 Å. This range is suggested for refined models, without overlaps and relatively snugly fit interfaces, e.g. (4,10). The user has the option of changing the range to 9 Å, approaching zero between 8 and 10 Å. This modality is suggested for encounter complexes, e.g. (14,15) for a rigid body docking validation.

Minimization. The default setting for Hydrogen bond optimization and removal of minimal overlaps prescribes a short 3 × 20 ABNR minimization steps with fixed backbone using the program CHARMM and the PARAM19 residue topology file (RTF).

Output format and explanation

The results from a ‘FastContact’ server run are returned to the user via email as a file attachment (with a normal response time of ~1 min). The attached file is a gzipped archive (.tar.gz) containing three results files: (i) the main results file (‘output.txt’); and, the processed (including H-bonds) and renumbered (ii) receptor PDB file (‘protein1’) and (iii) the ligand PDB file (‘protein2’). All of the files are prefixed with the user name (email prefix) and timestamp of the server run for easy reference.

The main source of errors in the output file relates with the format of the input PDB files. For instance, columns usage must strictly follow the PDB standards, and ATOM keyword must describe only protein amino acids. The server cannot minimize the backbone of sequences with gaps, and missing heavy atoms are sometimes not able to be reconstructed by the server. If the server detects an error, it will report a message with possible problems and suggestions.

The main results file (‘output.txt’) returns two components of a free energy function, electrostatic energy and desolvation free energy, and evaluates the solute vDW energy using CHARMM. The latter is sometimes useful to compare between different models (16), but here it is given only as a reference since it is not used in the analysis of contacts. Often vDW energies larger than about ~500 kcal/mol suggest structural overlaps. Although ‘FastContact’ smoothes the potentials to tolerate some limited overlaps, these are, in general, detrimental to the quality of the computational estimates. Figure 2 shows the summary energy output and part of the contact analysis, for the barnase–barstar complex 1BRS. We should caution that, when submitting co-crystallized receptor and ligand structures, the automated minimization implemented in the server leads to an over optimization of the electrostatic contacts of ~10–20% (5). The reason is because the direct electrostatic term used in the server does not have an angular dependence for Hydrogen bonds. Hence, these interactions tend to ‘double-dip’. This effect is compensated when scoring unbound models that always have some built in frustration due to the less optimal backbone and side chain conformations.

DISCUSSION

Critical assessment of protein interactions (CAPRI)

The method implemented in ‘FastContact’ has been successfully applied in the CAPRI experiment both as a free energy filtering procedure of the ‘ClusPro’ server (14) that predicts protein complexes and in protein–protein
refinement (4) (using a 9 and 6 Å desolvation range, respectively). ‘FastContact’ has been instrumental in the success of our group in blind predictions (17,18). In rounds 1 and 2 of CAPRI, Camacho and Gatchell (19) produced some of the best model structures, appropriately distinguishing between near-native and false positive structures for three targets. In rounds 3–5, the automated server ‘ClusPro’ (the only server participating in CAPRI) predicted good models for 5 targets (15), while our manual predictions resulted in good predictions for 6 targets (20) (missing the 3 targets that had a significant structural rearrangement upon binding).

The robustness of our method was further supported by the analysis of the full set of models submitted for CAPRI (rounds 3–5) for the 6 targets that did not undergo a large structural rearrangement upon binding (18). For these targets, we showed that ‘FastContact’ was able to discriminate near-native predictions from docked conformations far from the binding site for 5 of the targets (10), and for all but one of the manual predictions submitted to CAPRI. For instance, Figure 3 shows the re-scoring of models submitted for targets 8 and 12 by 13 different groups around the world. In all cases, ‘FastContact’ correctly identified the near-native conformation, even when the modeler failed to do so.

By splitting the free energy between electrostatics and desolvation, ‘Fastcontact’ also provides immediate insights into the nature of the binding interactions. Namely, negative desolvation is associated with a hydrophobic pocket at the binding site, whereas positive desolvation characterizes mostly polar interfaces. This is important since sometimes electrostatic or desolvation alone could lead to better discrimination than the combination of the two (21,22). The latter is, of course, due to the intrinsic limitations of empirical free energies. In particular, reliable estimates for solvent and entropic interactions are not yet available.

OTHER SERVERS

We are aware of only one server that estimate binding free energies of complex structures: http://sparks.informatics.iupui.edu/czhang/complex.html by the Zhou Lab (8). The server returns a single binding free energy estimate in kcal/mol that was shown to correlate with experimental values (±1.8 kcal/mol) for some 69 crystal structures.

AVAILABILITY

The web server is available freely and without registration at: http://structure.pitt.edu/servers/fastcontact/

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Figure 3. Examples of ‘FastContact’ scoring for a subset of high quality docked models from eight groups for targets 8 and 12 of CAPRI rounds 3–5, from http://capri.ebi.ac.uk/. For each of these targets, we run the models in our server and re-rank the models accordingly. In all cases, the server was able to correctly rank a low RMSD model as the one with the lowest free energy score. For comparison, we also marked with a diamond symbol the model ranked number 1 by the modeler (23–33). (A) target 8; (B) target 12. For a complete analysis of all models please see reference (10).
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