PELE web server: atomistic study of biomolecular systems at your fingertips

Armin Madadkar-Sobhani[1,*] and Victor Guallar[1,2,*]

[1]Joint BSC-IRB Research Program in Computational Biology, Barcelona Supercomputing Center, Barcelona, Spain and [2]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

ABSTRACT

PELE, Protein Energy Landscape Exploration, our novel technology based on protein structure prediction algorithms and a Monte Carlo sampling, is capable of modelling the all-atom protein–ligand dynamical interactions in an efficient and fast manner, with two orders of magnitude reduced computational cost when compared with traditional molecular dynamics techniques. PELE's heuristic approach generates trial moves based on protein and ligand perturbations followed by side chain sampling and global/local minimization. The collection of accepted steps forms a stochastic trajectory. Furthermore, several processors may be run in parallel towards a collective goal or defining several independent trajectories; the whole procedure has been parallelized using the Message Passing Interface. Here, we introduce the PELE web server, designed to make the whole process of running simulations easier and more practical by minimizing input file demand, providing user-friendly interface and producing abstract outputs (e.g. interactive graphs and tables). The web server has been implemented in C++ using Wt (http://www.webtoolkit.eu) and MySQL (http://www.mysql.com). The PELE web server, accessible at http://pele.bsc.es, is free and open to all users with no login requirement.

INTRODUCTION

With modern advances in molecular target therapies, it is becoming essential to elucidate the atomic mechanism for protein–ligand dynamical recognition. Achieving such detailed information requires not only the description of the ligand's induced fit, but also of its migration. Ligand binding, for example, can be impeded by mutations in the entrance pathway/channel. These challenges demand techniques capable of an atomic dynamical study with microsecond time scale resolution. Computer simulations offer such comprehensive information, although traditionally limited to sampling a fraction of the conformational space.

A large amount of work has been devoted to develop docking software (and servers) capable of modelling static protein–ligand complexes (1–5). Achieving a dynamical view of the process is considerably more challenging. Within the last 5 years, however, there has been a great effort in developing specialized software and hardware to perform microsecond molecular dynamics (MD) and reveal the biophysics behind molecular associations (6–9). Unfortunately, these remarkable developments still require a significant computational cost: days/weeks of hundreds of processors (on expensive hardware units). To circumvent this problem, we have designed PELE, an acronym for Protein Energy Landscape Exploration, which is a combination of Monte Carlo stochastic approach with protein structure prediction methods (9). PELE has shown to provide accurate ligand-induced fit (10,11) and migration results (12,13), and to reproduce the conformational sampling in microsecond MD trajectories with two orders of magnitude reduced computational cost (14). Using PELE with an inexpensive multicore machine (16–32 processors), for example, we can map the unbiased ligand migration from the solvent to the active site in an overnight period of time (10). Access to this technology will be a big advantage for many scientists working in different areas.

Briefly, PELE's heuristic approach generates trial moves based on protein and ligand perturbations followed by side chain sampling and global (or local) minimization. Protein perturbation is based on a combination of normal modes obtained in an anisotropic network model (ANM) (11), where a constrained minimization is used with a force placed on each alpha carbon (or all heavy atoms). Normal mode analysis techniques are currently widely used, and web servers exist which are capable of quickly compute the modes (12,13). Ligand perturbation includes a displacement plus sampling of its

dihedrals (and dihedrals of those side chains in direct contact). After a final minimization using the OPLS-2005 force field and a generalized Born implicit solvent (where explicit water can be included), the trial move is accepted or rejected based on a Metropolis criterion. The collection of accepted steps forms a stochastic trajectory. The whole procedure also has been parallelized using the Message Passing Interface (MPI) to take advantage of High Performance Computing (HPC) facilities wherever available.

In this communication, the PELE web server is presented, which provides a free, accessible and user-friendly interface for using PELE from everywhere with the Internet access. To the best of our knowledge, there are no other web servers that can do ligand-induced fit and migration (entrance and escape) studies in a timely manner achievable by our web server today.

MATERIALS AND METHODS

PELE methodology

The PELE algorithm combines Monte Carlo moves, protein ANM perturbations, rotamer library side-chain optimizations, truncated Newton minimizations and Metropolis acceptance tests. As illustrated in Figure 1, PELE's heuristic algorithm involves consecutive iteration of three main steps: localized perturbation, side chain sampling and minimization. The main steps are described below in a nutshell; the detailed method and its initial tests have been published elsewhere (14–16).
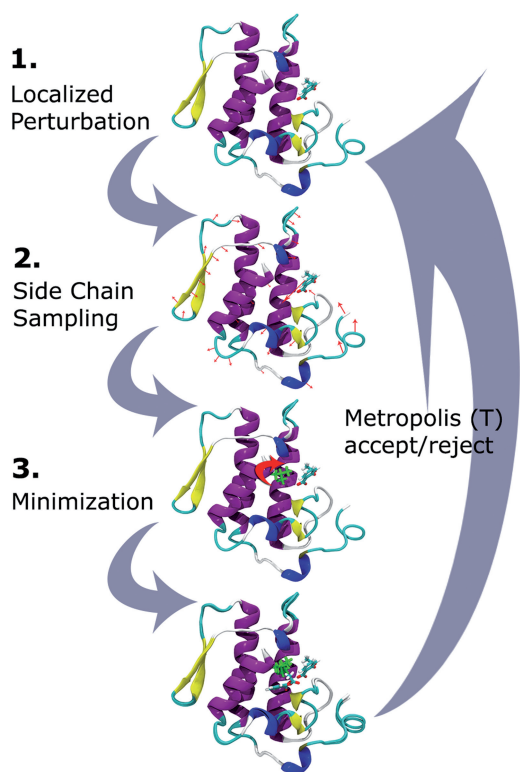
Figure 1. Flowchart of the PELE methodology illustrating the three iterative main steps.

Localized perturbation

After an energy calculation for the initial structure, the procedure begins with the generation of a perturbation in the system. In studies of ligand diffusion or ligand-induced fit, the perturbation starts with a random translation and rotation of the ligand. Some ligands can be treated as rigid bodies; hence, only three rotational and three translational degrees of freedom are required. Flexible ligands, on the other hand, cannot be adequately described as a single rigid unit. The perturbation in this case includes additional degrees of freedom from the dihedral angles of rotatable bonds. Next, a series of steric filters are applied to determine whether there is any contact between the ligand and the backbone of the protein. If any such contacts are found, the perturbation is rejected. Using this method, hundreds of perturbations are generated within seconds, and the one with the best energy is selected.

In addition to the ligand, the perturbation might include the backbone of the protein (or the backbone surrounding the ligand) by performing a minimization, where the alpha-carbons are driven to a new position derived from a small displacement in a low frequency mode (the lowest eigenvectors) from an ANM approach, a simple model for normal mode analysis (11). This is accomplished by a quick minimization with a harmonic constraint on each displaced alpha-carbon. The magnitude of the displacement, the nature of the eigenvector and the strength of the constraint are user-adjustable variables. Such a procedure aims to incorporate the global motion of the protein.

Side chain sampling

The algorithm continues by optimizing all side chains local to the ligand using a rotamer library (17,18) at rotamer resolution of 10, 20 or 30 degrees. The sampling algorithm uses steric filtering and clustering to reduce the number of rotamers to be minimized. For the protein, the energy of all side chains is computed before and after each ANM perturbation. In this way, at the side chain sampling step, the most excited side chains, i.e. those having a larger increase in energies as a consequence of the ANM move, can be optimized.

Minimization

Finally, the last step involves the minimization of a user-defined region, including at least all residues local to the atoms involved in the two previous steps, using the truncated Newton minimizer, the OPLS-2005 force field and a surface generalized Born (SGB) implicit solvent. The minimization is intended to locate a local minimum after the initial perturbation and side chain rearrangements introduced in the previous steps.

These three steps devise a move, which is accepted, defining a new minimum, or rejected on the basis of a Metropolis criterion for a given temperature:

$$\begin{cases} \Delta V < 0 \\ e^{\frac{-\Delta V}{K_B T}} < R \end{cases}$$

That means by a decrease of the potential surface, $\Delta V < 0$, or by satisfying the second criterion, where K_B is the Boltzmann constant, T the temperature chosen for

the simulation and R is a random number with a [0, 1] range. The result of this procedure is a series of local minima forming a stochastic trajectory. Optionally, the algorithm can be biased to attain different goals by adjusting different reaction coordinates where geometry or energy parameters are prioritized. For example, the motion of a ligand can be constrained within a distance from the active site, etc. Furthermore, the procedure has been parallelized using the MPI communications protocol, with the option of interchanging coordinates between different trajectories. Whenever any trajectory is significantly further along a given reaction coordinate(s) than any of the other trajectories, the trailing trajectory is abandoned and restarted from the position of the leading trajectory. This allows an efficient sampling of the configurational space towards one defined objective: entrance/escape of the ligand, optimization of the protein–ligand binding energy, etc.

PELE benchmarks

Although this article aims to introduce the PELE web server, we will give here a short description of previous benchmark studies. Several applications have described exit and entry pathways, comparing its accuracy with experimental data. In our first study (14), we obtained the migration for Cytochrome P450 camphor, myoglobin and the fatty acid-binding proteins. Further studies compared PELE's migration pathways in truncated haemoglobin with kinetic experimental data for the wild-type and the W8F mutant (19). More recently, a comprehensive study on 97 difficult induced fit cases, including cross- and apo-docking, has shown the capabilities of PELE in docking refinement (15). Furthermore, the study underlined the goodness of the OPLS interaction energy when scoring docking poses within one ligand. Binding site search and induced fit docking has been performed, in collaboration with experimental studies, in aryl-alcohol oxidase (20,21), in mTOR kinases (22), in anti-apoptotic Bcl-2 receptors (23) and in different globins (10). The mTOR and Bcl-2 studies illustrate the combination of PELE with docking scoring techniques to discriminate different ligands. At the level of protein dynamics, in absence of ligand, we have compared PELE's conformational search with microsecond MD simulations in ubiquitin and with metadynamics calculations on T4 lysozyme (16). The results clearly show a good agreement in the sampling of PELE with that of more sophisticated simulations.

DESCRIPTION OF THE WEB SERVER

Input

Detailed information about how to submit new jobs and format of the input can be found in the Help section of the web server. Here, only the most important items are described.

The only mandatory input file that must be provided by the user is the structures, i.e. protein(s) and possibly ligand(s), waters and ions with all hydrogen atoms added, in the PDB format (http://www.wwpdb.org/docs.html). Then a control script can be made based on the

selected ready-made script or uploaded by the user. The PELE program reads all the parameters for a simulation run as directives written in a control script with .con as its file extension. There are five optimized ready-made scripts, each with a detailed description, to perform routine tasks: (i) protein local motion; (ii) normal mode exploration; (iii) ligand binding refinement; (iv) unconstrained ligand exploration and binding site search; and (v) driving the ligand. By selecting any of these scripts, only few parameters should be entered (default values are also provided). Additionally, advanced users can upload a custom-made script instead of those mentioned above or edit the generated script before submission. Hovering the pointer over each parameter shows a descriptive text about its purpose. For scripts requiring a ligand perturbation, chain ID and residue number of the ligand in the PDB file must also be specified. Multiple ligands can be included in the input file, although only one can be perturbed at the present time.

There are some prerequisites for the PDB input file. (i) The server does not add any hydrogen atoms or modify any protonation state, i.e. the user should know the system and decide the best protonation state for both the protein and the ligand. The PDB2PQR (24) and MolProbity (25) servers can be used to add hydrogen atoms to proteins in an intelligent way by optimizing the hydrogen-bond network. (ii) To recognize the right protonation state, the user should change the name of some residues in the PDB file as follows: HIS/HIE/HIP for delta, epsilon and protonated histidine, respectively. LYS->LYN, ARG->ARN for neutral lysine and glutamic, respectively; ASP->ASH, GLU->GLH for protonated aspartic and glutamic, respectively; CYS-> CYT, CYS->CYX for deprotonated negative and neutral cysteine, respectively. (iii) The protein structure must be complete, without any missing residues or atoms. Again, the PDB2PQR server can be used for adding missing side-chain atoms (24). (iv) Water residues (if present) should be named SPC or HOH and defined as HETATM records.

There is a possibility to upload a sample PDB file for each ready-made script as test runs, optimized for getting typical results achievable by each script. By checking the contents of these PDB files and the corresponding generated control files, users may gain more insights about how to prepare their own PDB and control files.

It is possible to assign more CPUs or higher wall clock for each submitted job in the Job Settings panel. The default values are 4 CPUs and 24 h, respectively, with 16 CPUs and 72 h as the upper limits. By tuning these values, it is possible to reduce the pending time of a job in case of heavy load of compute cluster (see Implementation section below). As an option, user can also select additional metrics (e.g. RMSD, surface area, interatomic distances, etc.) to be appeared in the output file for each step.

Output and representation of results

On successful submission of a job, user automatically is redirected to a result page for monitoring the progress of the computations and viewing the results in real time. This

page can be bookmarked for a later correspondence and will remain valid for 10 days after initial submission. A typical simulation run takes only few hours (depending on the number of steps and CPUs selected for the job). PELE is capable of reproducing, in ~100 h of CPU time, the ligand escape and entry pathways, and in ~10 h of CPU time, the active site induced fit.

The first part of the results is an interactive graph that plots the selected metrics (listed as columns in the metrics data, i.e. second part of the results below) on the fly (Figure 2). Once a job starts to run in the compute cluster and log file is in place, the interactive graph is produced and updated as the job progresses and structures can be downloaded from the trajectories. The default graph plots total energy of the system over the trajectory for all the CPUs, but the user can change it at any moment (Figure 3A). For instance, it can be changed to plot ligand binding energy versus the RMSD from the native structure (Figure 3B).

The second part of the results is a table of all the selected metrics separated by the CPU number. Here, based on the information presented in the plot (e.g. structure with the lowest binding energy), user can select one or more structures from the trajectories and download them as a PDB file for further modelling purposes. The whole trajectory file (or files in case of having more than one processor) containing all the sampled conformations can be downloaded from the files section, which is the third and last part of the results. This file can be used as an input for interactive viewers, e.g. VMD (http://www.ks. uiuc.edu/Research/vmd/), or PyMol (http://www.pymol. org) to animate ligand/protein motions. In the Examples section of the web server, there are several animations prepared in this way.

Implementation

The PELE web server has been implemented in C++ using Wt (http://www.webtoolkit.eu), a C++ library for developing web applications and MySQL (http://www. mysql.com) as back-end database. The original PELE code has been implemented in Fortran; nevertheless, reimplementation of the old code as a C++ library is a work in progress. Jobs submitted by the web server are transferred to a dedicated compute cluster consisting of 144 cores (AMD Opteron 2.4 GHz) at the time of this writing and will be queued based on their requested wall clock and number of CPUs. In this way, several submitted jobs can be run in parallel based on the available CPUs.

Community help as public forums, bug tracking and feature request are available through PELE's Redmine site (http://pele.bsc.es/redmine), which has been implemented using Redmine (http://www.redmine.org), a free and open source, web-based project management and bug-tracking system.
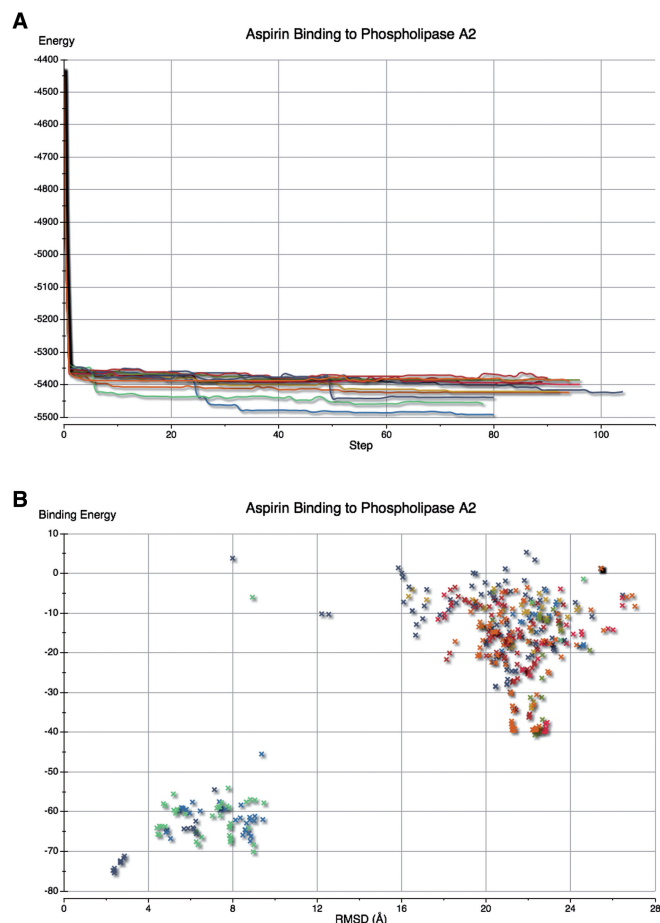
Aspirin binding to phospholipase A2 as a ligand migration test case

As a simple test case for ligand migration, we consider the non-biased aspirin binding to phospholipase A2. This test
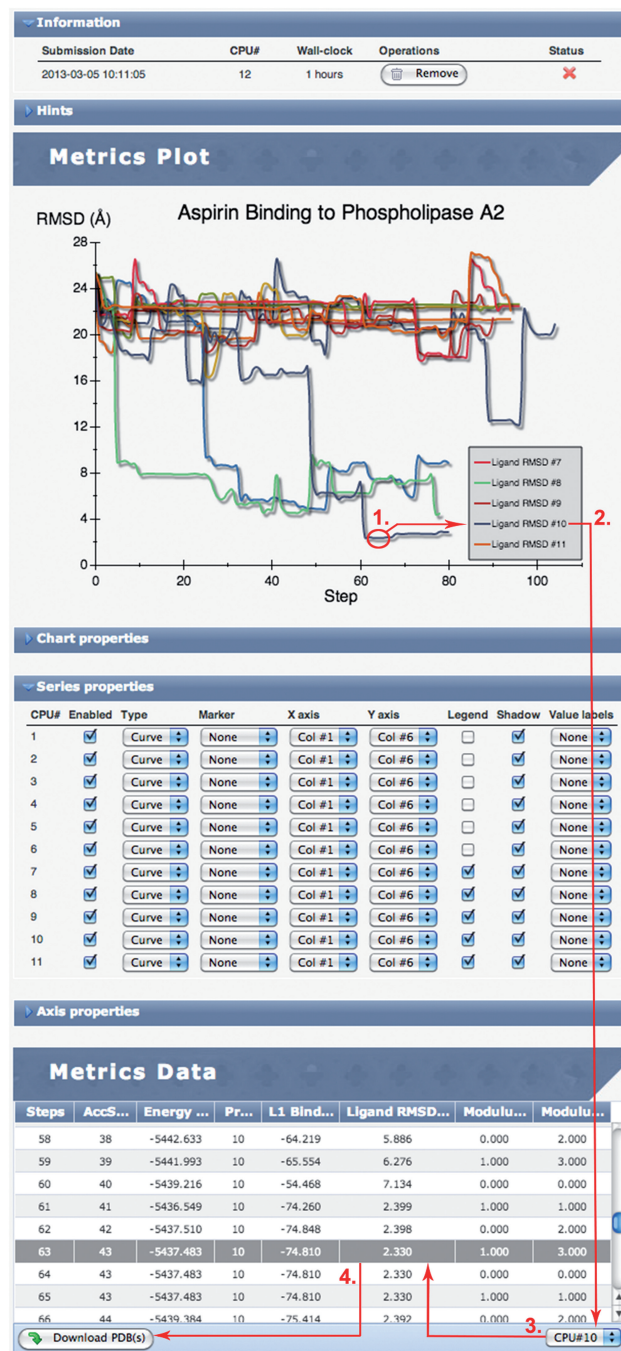
Figure 2. Screenshot of the results obtained after 1 h, using ready-made script #4 (unconstrained ligand exploration and binding site search) exploiting 12 CPUs, for aspirin binding to phospholipase A2 test case (PDB ID: 1OXR). The four steps drawn in red demonstrate how the interactive graph can be used to recognize the processor that found the lowest RMSD from the native structure (CPU#10), and subsequently, how to use that information for selecting and downloading the corresponding structure (Step 63) from the trajectory.

case was first showed by Mike Kuiper, currently a computational scientist at the Victorian Life Science Computation Initiative, at the 2011 Annual Structure-Based Drug Design conference in Boston. By running MD on 256 cores in an IBM Blue Gene Supercomputer

Figure 3. Two samples of what can be obtained using the interactive graph for a submitted job. (**A**) Graph showing the total energy of the system for all the CPUs over the trajectory, obtained for the aspirin binding to phospholipase A2 test case (PDB ID: 1OXR) after 1 h. (**B**) Graph showing the scatter plot of the ligand binding energy versus the RMSD from the native structure for the same system as (A). As can be seen, the structure with the lowest RMSD from the native found by the CPU number 10, also corresponds to the lowest binding energy.

for several days, he could observe spontaneous binding in the active site, reproducing within 2 Å the native crystal structure (PDB ID: 1OXR) (26). As starting system he used four ionized aspirins all placed randomly away from the protein. Although in terms of size and ligand flexibility this is an easy system, similar results have been obtained using MD for more complex systems (9).

Starting with a structure where aspirin (only one copy of the ligand) is located in the bulk solvent, with a 27 Å ligand RMSD to the crystal bound structure, PELE is capable of reproducing the bound crystal structure in less than a 2 h × 8 processors run. Each processor performs an independent search where it combines large and small translations of the ligand. If a native structure is provided, information of the bound structure is only used to compute the RMSD for comparison purpose. Figure 3A shows the evolution of the energy along the simulation (with an initial drop due to the lack of a previous minimization of the crystal). Figure 3B shows

the binding energy against the ligand RMSD to the bound crystal structure. Binding energies are obtained by computing the protein–ligand interaction energy for each local minima produced by PELE. Notably, the simulation is capable of reproducing the bound structure and, more importantly, of identifying it based on the protein–ligand interaction energy. The binding mechanism can be reproduced, for example, from the compilation of different local minima. In the Example section of the server, there is a movie of the binding event for this test case, where the presence of explicit water molecules and a calcium ion in the active site can be observed.

Thus, remarkably, a free search of the binding process from solution can be accomplished within an all atom model in only few hours of CPU. More difficult test cases, such as the binding in the Src kinase recently performed by the D. E. Shaw Research group (9), would require only 10–20 CPU hours on 24 processors. Certainly this constitutes a breakthrough when compared with MD studies, allowing for screening of several compounds in a timely manner. This test case has been made as the default 'Sample PDB' for the 'Unconstrained ligand exploration and binding search' ready-made script (14,15).

Nuclear hormone receptors as a ligand refinement test case

Another ready-made script, which we believe of large utility, is the 'Ligand binding refinement'. This application allows the refinement of initial docking poses where we expect significant protein and ligand reorganization (induced fit). The script will produce small ligand translations coupled to large rotations within a given radius from the active site, ligand RMSD change, etc. The script might be also useful, for example, in refining the structure after a free ligand migration (the first test case above) or after driving the ligand from outside to the active site. These more broad searches might use larger translation and/or steering towards the active site; they might not include the fine sampling required to refine the structure within the active site cavity. As an example, we include here the refining of a benzoxazin derivative from the mineralcorticoid ligand-binding domain receptor (PDB ID: 3VHV) (27). After placing the ligand outside and running a free search, the ligand reached a structure with a 7 Å ligand RMSD from the bound crystal (Figure 4A). Within 2 h × 16 processors using the refining script, we reached a 0.9 Å structure (Figure 4B). Furthermore, the bound structure is clearly identified by the binding energy (Figure 4C). This test is currently the 'Sample PDB' for the 'Ligand binding refinement' ready-made script (14,15).

We should emphasize here that the ready-made scripts could benefit (decreasing the simulation time significantly) from a system-specific parameter tuning. This is particularly true for the ligand refinement case, where reproducing large (or rare) induced fit events might be hard (in agreement with the difficulty in accurately evaluating the binding free energy). By system-specific parameters, we refer to possible promoting modes,
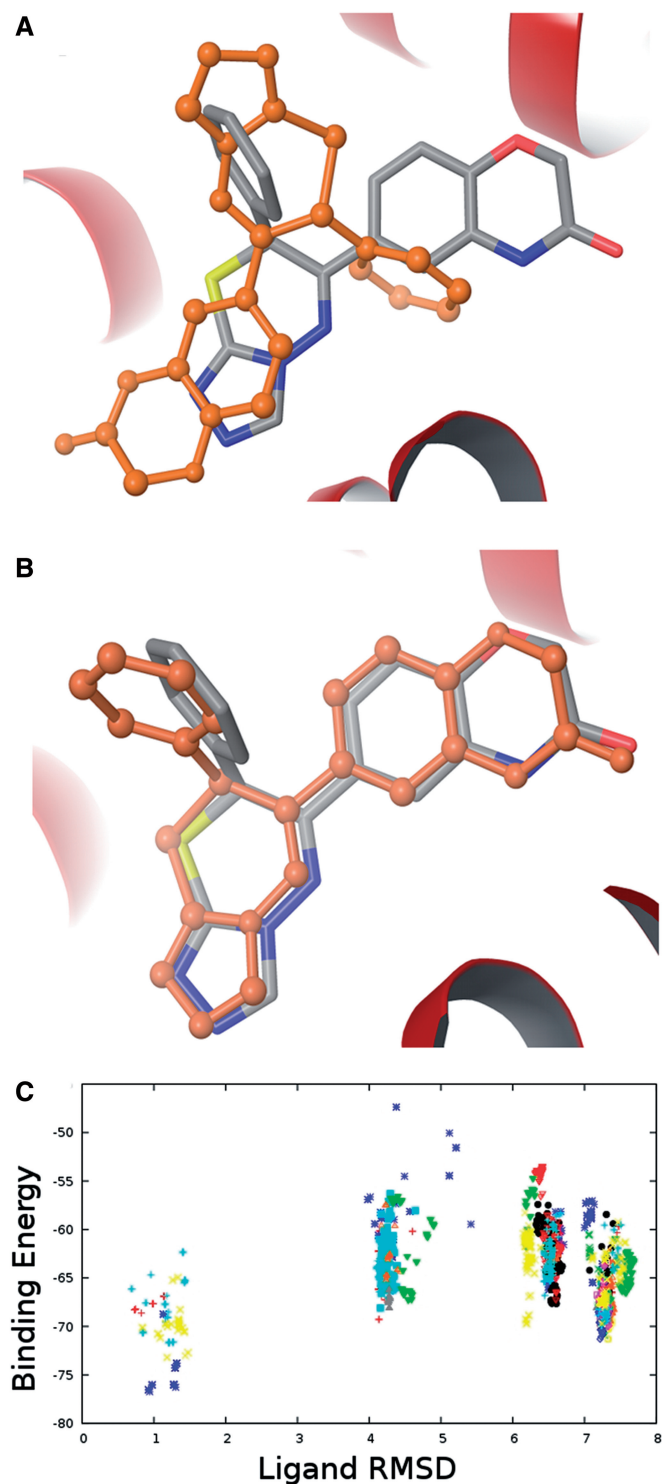
Figure 4. Results obtained from the active site refinement test case. (**A**) Initial structure after ligand dynamics with a 7 Å ligand RMSD from the bound crystal (PDB ID: 3VHV). (**B**) Refined structure produced by PELE with 0.9 Å ligand RMSD. (**C**) Binding energy against the ligand RMSD along the refinement simulation. Ligand RMSDs are computed after alpha-carbon proteins alignment.

ligand partial charges and rotamers, protein protonation states, etc. Many tips are provided in the Examples section of the web server.

CONCLUSIONS AND FUTURE DEVELOPMENT

The motivation behind the PELE web server was to provide, to the large community of scientists especially those who work on molecular target therapies, a fast and accurate tool capable of obtaining an atomic detailed mechanism of the protein–ligand induced fit, of its recognition process and of the ligand migration. Understanding these aspects is essential for the accurate prediction of protein–ligand interactions and plays an important role in rational drug design, including, but not limited to, bypassing drug resistance induced by protein mutations.

PELE, our novel technology based on protein structure prediction algorithms and a Monte Carlo sampling, is capable of describing the all-atom dynamical interactions between proteins and ligands with two orders of magnitude reduced computational cost. As PELE is a versatile and complex program that supports numerous directives and options in the control script, this web server makes the whole process of running PELE simulations easier and more practical by minimizing input file demand, providing user-friendly interface and producing abstract outputs (e.g. interactive graphs and tables). We are confident that PELE web server will be of high interest and practical utility for a wide range of scientists working in structural bioinformatics, computational chemistry and biology, molecular biophysics, rational drug design and related fields.

The PELE web server is an on-going project, and our intention is to improve it constantly. The following are the highlights of the most important features considered for the future versions:

- Visualization of the results using a WebGL (http://www.khronos.org/webgl/)-based molecular viewer.
- Interactive addition of hydrogen atoms and assignment of protonation state using the molecular viewer.
- Fixing missing residues and side chains.
- Support for the nucleic acids, i.e. DNA/RNA.
- CUDA/OpenCL implementation of the PELE code for GPUs.

REFERENCES

1. Verdonk,M.L., Cole,J.C., Hartshorn,M.J., Murray,C.W. and Taylor,R.D. (2003) Improved protein-ligand docking using GOLD. *Proteins*, **52**, 609–623.
2. Friesner,R.A., Banks,J.L., Murphy,R.B., Halgren,T.A., Klicic,J.J., Mainz,D.T., Repasky,M.P., Knoll,E.H., Shelley,M., Perry,J.K. *et al.* (2004) Glide: a new approach for rapid, accurate docking

and scoring. 1. Method and assessment of docking accuracy. *J. Med. Chem.*, **47**, 1739–1749.

3. Morris,G.M., Ruth,H., Lindstrom,W., Sanner,M.F., Belew,R.K., Goodsell,D.S. and Olson,A.J. (2009) Software news and updates AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J. Comput. Chem.*, **30**, 2785–2791.

4. Mashiach,E., Nussinov,R. and Wolfson,H.J. (2010) FiberDock: a web server for flexible induced-fit backbone refinement in molecular docking. *Nucleic Acids Res.*, **38**, W457–W461.

5. Macindoe,G., Mavridis,L., Venkatraman,V., Devignes,M.D. and Ritchie,D.W. (2010) HexServer: an FFT-based protein docking server powered by graphics processors. *Nucleic Acids Res.*, **38**, W445–W449.

6. Mura,C. and McCammon,J.A. (2008) Molecular dynamics of a KB DNA element: base flipping via cross-strand intercalative stacking in a microsecond-scale simulation. *Nucleic Acids Res.*, **36**, 4941–4955.

7. Buch,I., Giorgino,T. and De Fabritiis,G. (2011) Complete reconstruction of an enzyme-inhibitor binding process by molecular dynamics simulations. *Proc. Natl Acad. Sci. USA*, **108**, 10184–10189.

8. Sadiq,S.K., Noé,F. and De Fabritiis,G. (2012) Kinetic characterization of the critical step in HIV-1 protease maturation. *Proc. Natl Acad. Sci. USA*, **109**, 20449–20454.

9. Shan,Y., Kim,E.T., Eastwood,M.P., Dror,R.O., Seeliger,M.A. and Shaw,D.E. (2011) How does a drug molecule find its target binding site? *J. Am. Chem. Soc.*, **133**, 9181–9183.

10. Lucas,M.F. and Guallar,V. (2013) Single vs. multiple ligand pathways in globins. A computational view. *Biochim. Biophys. Acta*, pii, S1570-9639(13)00046-0.

11. Atilgan,A.R., Durell,S.R., Jernigan,R.L., Demirel,M.C., Keskin,O. and Bahar,I. (2001) Anisotropy of fluctuation dynamics of proteins with an elastic network model. *Biophys. J.*, **80**, 505–515.

12. Suhre,K. and Sanejouand,Y.H. (2004) ElNémo: a normal mode web server for protein movement analysis and the generation of templates for molecular replacement. *Nucleic Acids Res.*, **32**, W610–W614.

13. Krüger,D.M., Ahmed,A. and Gohlke,H. (2012) NMSim web server: integrated approach for normal mode-based geometric simulations of biologically relevant conformational transitions in proteins. *Nucleic Acids Res.*, **40**, W310–W316.

14. Borrelli,K.W., Vitalis,A., Alcantara,R. and Guallar,V. (2005) PELE: protein energy landscape exploration. A novel Monte Carlo based technique. *J. Chem. Theory Comput.*, **1**, 1304–1311.

15. Borrelli,K.W., Cossins,B. and Guallar,V. (2010) Exploring hierarchical refinement techniques for induced fit docking with protein and ligand flexibility. *J. Comput. Chem.*, **31**, 1224–1235.

16. Cossins,B.P., Hosseini,A. and Guallar,V. (2012) Exploration of protein conformational change with PELE and meta-dynamics. *J. Chem. Theory Comput.*, **8**, 959–965.

17. Jacobson,M.P., Friesner,R.A., Xiang,Z. and Honig,B. (2002) On the role of the crystal environment in determining protein side-chain conformations. *J. Mol. Biol.*, **320**, 597–608.

18. Xiang,Z., Steinbach,P.J., Jacobson,M.P., Friesner,R.A. and Honig,B. (2007) Prediction of side-chain conformations on protein surfaces. *Proteins*, **66**, 814–823.

19. Guallar,V., Lu,C., Borrelli,K., Egawa,T. and Yeh,S.R. (2009) Ligand migration in the truncated hemoglobin-II from Mycobacterium tuberculosis: the role of G8 tryptophan. *J. Biol. Chem.*, **284**, 3106–3116.

20. Hernández-Ortega,A., Borrelli,K., Ferreira,P., Medina,M., Martínez,A.T. and Guallar,V. (2011) Substrate diffusion and oxidation in GMC oxidoreductases: an experimental and computational study on fungal aryl-alcohol oxidase. *Biochem. J.*, **436**, 341–350.

21. Hernández-Ortega,A., Ferreira,P., Merino,P., Medina,M., Guallar,V. and Martínez,A.T. (2012) Stereoselective hydride transfer by aryl-alcohol oxidase, a member of the GMC superfamily. *ChemBioChem*, **13**, 427–435.

22. Espona-Fiedler,M., Soto-Cerrato,V., Hosseini,A., Lizcano,J.M., Guallar,V., Quesada,R., and Pérez-Tomás,R. (2012) Identification of dual mTORC1 and mTORC2 inhibitors in melanoma cells: prodigiosin vs. obatoclax. *Biochem. Pharmacol.*, **83**, 489–496.

23. Hosseini,A., Espona-Fiedler,M., Soto-Cerrato,V., Quesada,R., Pérez-Tomás,R. and Guallar,V. (2013) Molecular interactions of prodiginines with the BH3 domain of anti-apoptotic Bcl-2 family members. *PLoS One*, **8**, e57562.

24. Dolinsky,T.J., Czodrowski,P., Li,H., Nielsen,J.E., Jensen,J.H., Klebe,G. and Baker,N.A. (2007) PDB2PQR: expanding and upgrading automated preparation of biomolecular structures for molecular simulations. *Nucleic Acids Res.*, **35**, W522–W525.

25. Davis,I.W., Leaver-Fay,A., Chen,V.B., Block,J.N., Kapral,G.J., Wang,X., Murray,L.W., Arendall,W.B. III, Snoeyink,J., Richardson,J.S. *et al.* (2007) MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. *Nucleic Acids Res.*, **35**, W375–W383.

26. Singh,R.K., Ethayathulia,A.S., Jabeen,T., Sharma,S., Kaur,P. and Singh,T.P. (2005) Aspirin induces its anti-inflammatory effects through its specific binding to phospholipase A2: crystal structure of the complex formed between phospholipase A2 and aspirin at 1.9 Å resolution. *J. Drug Target.*, **13**, 113–119.

27. Hasui,T., Matsunaga,N., Ora,T., Ohyabu,N., Nishigaki,N., Imura,Y., Igata,Y., Matsui,H., Motoyaji,T., Tanaka,T. *et al.* (2011) Identification of benzoxazin-3-one derivatives as novel, potent, and selective nonsteroidal mineralocorticoid receptor antagonists. *J. Med. Chem.*, **54**, 8616–8631.