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pyDockSAXS: protein–protein complex structure by SAXS and computational docking

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ABSTRACT

Structural characterization of protein–protein interactions at molecular level is essential to understand biological processes and identify new therapeutic opportunities. However, atomic resolution structural techniques cannot keep pace with current advances in interactomics. Low-resolution structural techniques, such as small-angle X-ray scattering (SAXS), can be applied at larger scale, but they miss atomic details. For efficient application to protein–protein complexes, low-resolution information can be combined with theoretical methods that provide energetic description and atomic details of the interactions. Here we present the pyDockSAXS web server (http://life.bsc.es/pid/pydocksaxs) that provides an automatic pipeline for modeling the structure of a protein–protein complex from SAXS data. The method uses FTDOCK to generate rigid-body docking models that are subsequently evaluated by a combination of pyDock energy-based scoring function and their capacity to describe SAXS data. The only required input files are structural models for the interacting partners and a SAXS curve. The server automatically provides a series of structural models for the complex, sorted by the pyDockSAXS scoring function. The user can also upload a previously computed set of docking poses, which opens the possibility to filter the docking solutions by potential interface residues or symmetry restraints. The server is freely available to all users without restriction.

INTRODUCTION

Protein–protein interactions orchestrate the vast majority of biological processes in cell. The atomic level description of these interactions, the so-called interactome (1), gives access to the molecular bases of biological activity and the eventual rational intervention for medical purposes. At present, only a tiny fraction of complexes from the estimated number of all possible protein–protein interactions (2) have an available 3D structure due to the limitations of high-resolution structural biology methods, such as X-ray crystallography or nuclear magnetic resonance (NMR) (3). Fortunately, low-resolution methods, especially small-angle scattering (SAS), are of more general application and could be applied in a high-throughput fashion as compared to X-ray crystallography or NMR techniques (4–5).

Small-angle X-ray scattering (SAXS) is a powerful methodology for the structural and dynamic characterization of biomolecules at low resolution (6–9). Recent advances in SAXS instrumentation and the development of software for the comprehensive interpretation of SAXS data in terms of structure make this technique an optimal tool to address the structural characterization of the interactome. Methods based on rigid-body modeling of SAXS data, such as SASREF (10), can generate structural models for protein–protein complexes by simultaneously fitting multiple SAXS/SANS data using simulated annealing algorithm. However, given that these methods rely exclusively on the SAS data, the resulting models display an inherent degeneracy. In addition, these techniques miss the high-resolution information reporting on the details of intermolecular interactions. Therefore, other strategies are necessary to incorporate the interacting surfaces of the partners to enrich the quality of the resulting models. One such strategy is the use of SAXS data in combination with advanced computational approaches, such as protein–protein dock-

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ing, to generate meaningful models of biomolecular assemblies.

Several docking methods for structural prediction of protein–protein interactions have been reported. These methods are mostly based on rigid-body (or semi-flexible) sampling of the interacting molecules, followed by scoring and/or energy minimization (11–15). Completely automatic docking can provide good models for specific protein–protein interactions (16–20). However, the recent CAPRI experiments (http://www.ebi.ac.uk/msd-srv/capri/) (21–25) have highlighted the limitations of current docking approaches and the necessity of using experimental information to help to identify the correct docking models (20,26).

Computational docking tools can be used to generate a large number of poses that are subsequently filtered and scored based on their capacity to describe the experimental data. This strategy has been applied to specific cases (27–30) and has been implemented and systematically benchmarked in a few computational methods that combine SAXS and docking for the structural modeling of protein–protein complexes, such as pyDockSAXS (31), FoXSDock (32) or HADDOCK (33). Among them, we previously reported the first of such methods, pyDockSAXS (31), which provided a 2-fold increase in the success rate for the prediction of protein complexes as compared to that of the individual approaches based on energy-based docking or SAXS data alone (31). Here, a server that makes pydockSAXS available is described. This server provides comprehensive structural models of biomolecular assemblies using the experimental SAXS curve and the structure of the interacting partners as the only input. This strategy can be efficiently used for the high-throughput resolution of protein complexes at large scale with SAXS data.

MATERIALS AND METHODS

The pyDockSAXS method integrates SAXS data and pyDock energy-based scoring (16) to determine the structure of a protein–protein complex from its components.

This integrative method uses FTDock to generate 10 000 rigid-body docking poses, which are re-scored by a combination of pyDock energy and the χ value defining the goodness of fit to the SAXS data computed with CRYSOL 2.8 (34):

$$\text{pyDockSAXS} = E_{\text{pyDock}} + w_c \cdot \chi_{\text{CRYSOL}}, \qquad (1)$$

where w_c is a parameter that was previously optimized on 62 cases of the protein–protein docking benchmark 2.0, using synthetic SAXS data obtained from the complex structures after adding noise.

The structural modeling capabilities of the server have been validated on 81 complexes of the Protein–Protein Benchmark 4.0 (35) which were not present in the previous training of the scoring function, using SAXS data synthetically obtained from the complex structure after adding noise. We considered only complexes in which the molecular mass did not significantly vary between the unbound and the complex structures, as previously described (31). Figure 1 shows the predictive success rates obtained in this benchmark. The pyDockSAXS server identifies an acceptable docking model (i.e. with ligand RMSD < 10 Å from the reference structure after superimposing the receptor molecules) within the top 10 predictions in 25.9% of the cases (as compared to 13.6% success rate when using energy-based scoring alone) (Figure 1). This is a similar improvement as that previously reported for the stand-alone version on the benchmark 2.0 (31). SAXS-based scoring is sensitive to large conformational changes between the unbound structures (used in docking) and the bound state (from which SAXS data are derived). Indeed, in rigid cases, i.e. those with unbound–bound interface Cα RMSD < 1.5 Å, the pyDockSAXS server improved the top 10 success rate up to 36.5% (as compared to 15.4% when using docking alone). This means that in rigid cases, the SAXS-based scoring is more efficient in identifying the correct docking models. On the other hand, the overall results strongly depend on the quality of the docking poses generated by FTDock. When considering only those rigid cases in which FTDock is able to generate at least a near-native solution with ligand RMSD < 5 Å, the success rate for pyDockSAXS is 47.4% (as compared to 26.3% for docking alone). This observation suggests that future improvements in the docking algorithm used to generate the docking poses will have a strong impact on the predictive capabilities of the server.

We have also successfully validated the server on experimental systems of interest. As an example, we have applied pyDockSAXS to rebuild the structure of the *Alvinella pompejana* Cu,Zn superoxide dismutase homo-dimer (PDB 3F7L), using the X-ray coordinates of one monomer (chain A) (36) and the experimental SAXS data deposited in Biosis database (37). This complex presents a spherical shape, which is challenging for modeling based only on SAXS data (31). Thus, it represents an excellent case to test the robustness of the method. The server finds a near-native docking solution as rank 1, and additional acceptable solutions within the top 10 docking models. Actually, six of the top 10 docking models were within (or slightly above) acceptance criteria in CAPRI (Figure 2). However, the other four docking models (not shown in Figure 2) were significantly far from the correct orientation, which indicates that docking results in blind conditions should always be considered with caution.

As another example, we used the pyDockSAXS server to model the complex between the redox proteins adrenodoxin (Adx) and cytochrome c (Cc) which has been identified as a short-lived encounter complex (38). The authors stabilized the complex by engineering both proteins in order to cross-link them using two cysteine mutants: L80C and V28C from Adx and Cc, respectively. The cross-linked complex was structurally characterized by NMR and SAXS (38). This is a challenging case involving expectedly weak interaction forces given its transient nature. In this type of cases, pyDockSAXS can be easily used to generate models compatible with the experimental SAXS profile and energetically accurate. Using the experimental SAXS data stored in the SASBDB repository (39), and the X-ray structures of Adx (PDB 1AYF) and Cc (PDB 2YCC), the pyDockSAXS server generated many different docking orientations. After manually filtering the results from the pyDockSAXS server to keep only the docking poses with the residues Adx C80 and Cc C28 within 10-Å distance in order to describe the cross-linked complex, a model similar

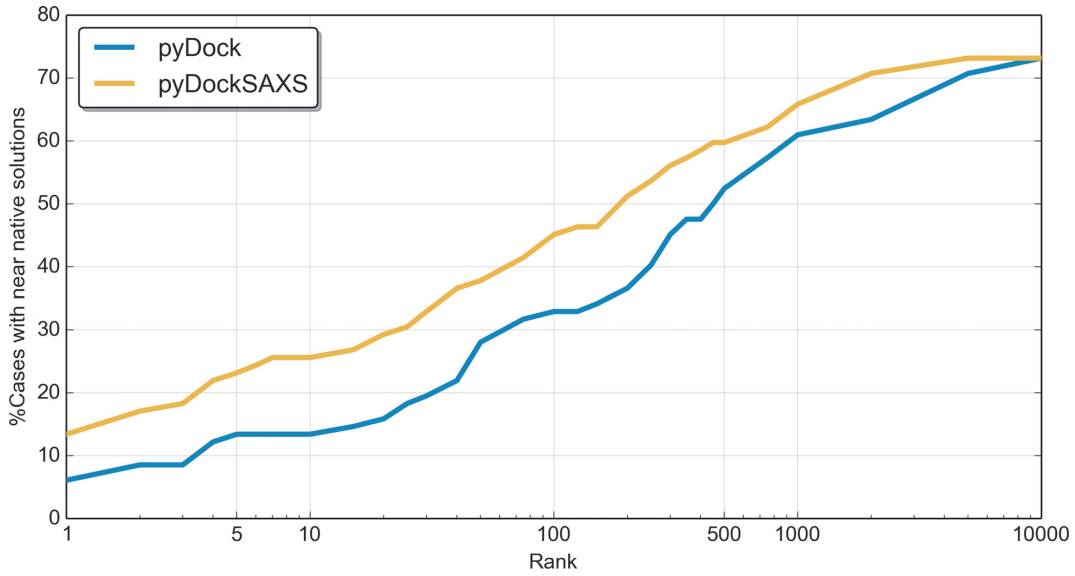


Figure 1. Success rate for pyDockSAXS on a set of 81 cases of protein–protein docking benchmark 4.0 which were not used for training, as compared to that of pyDock alone.

to the NMR structure (PDB 2JQR) was found within the top 10 pyDockSAXS docking poses. The other nine of the top 10 docking poses showed large variability in the mutual orientation between the two molecules. Interestingly, without using the SAXS data, this near-native solution would not have been identified within the top 10 docking poses. This example highlights the capacities of integrating SAXS data with computational docking, and the power that additional residue-specific information has to enrich final solutions. However, while pyDockSAXS provides a reduced set of models that typically includes one or several correct solutions, the existence of high-scoring incorrect models could complicate the identification of the correct assembly.

DESCRIPTION OF THE WEB SERVER

Input

The user is requested to upload the structure files for the two interacting proteins in the Protein Data Bank (PDB) format (40). The choice of molecules as receptor or ligand is arbitrary, although for the sake of efficiency it is recommended to set the receptor as the largest molecule. The user can specify the exact chains that will be included for modeling. Incomplete residues are rebuilt with SCWRL 3.0 (41). At present, cofactors are not considered in the calculations but this possibility will be implemented in future versions of the server. In addition, the server expects a file with the SAXS experimental curve compatible with CRYSOL software. Thus, it should be a plain-text file where the first line is a title ignored by the software and the following lines are composed by three columns of numerical data separated by blanks or commas, which represent momentum transfer, scattering intensity and experimental error, respectively. If experimental errors are not specified, they are automatically estimated by CRYSOL (2% of intensity values). All input file formats are described in the help section of the server.

Users can customize some CRYSOL execution parameters. At present, the available options for CRYSOL calculations implemented in pyDockSAXS are: (i) the use or not of constant subtraction and (ii) to specify different angular units of the SAXS experimental data provided. Other parameters such as the number of spherical harmonics are set to their standard values that have been proven to provide accurate estimation of theoretical SAXS curves.

The option of specifying a rigid-body docking set from previous pyDockWeb (42) executions has also been implemented for the convenience of advanced users. This option allows the user to upload pre-filtered rigid-body docking poses to be evaluated by the server. This could be used to include residue–residue distance restraints based on binding site residues, already implemented in the general pyDockWeb server (26), or to filter manually specific orientations of the complex by the user. This possibility is relevant when residue-specific information is available from other techniques, i.e. NMR, mutagenesis data or bioinformatics tools.

Output and representation of results

After submitting the job for calculation, the user is redirected to the job information and results page. This page is unique for the job and its URL is highly recommended to bookmark, if a contact e-mail address was not provided by the user. The job information and results page is periodically auto-refreshed to provide the user updated information of the status of the submitted job. Once the calculation has finished, the results are shown in this page. The information displayed is (i) an energy table of the top 100 complex orientations predicted and scored by pyDockSAXS (including other relevant energetic terms as pyDock scoring energy and CRYSOL χ^2 value) and available to download as a PDF format file (Figure 2), (ii) a graphical representation of the fitting of the top 10 docking mod-

pyDockSAXS Server

Home Help Status Contact Admin

pyDockSAXS

Protein-protein interactions using SAXS and computational docking

Job (102) information

Created on: 2015-02-04 15:14:14
Status: Calculated

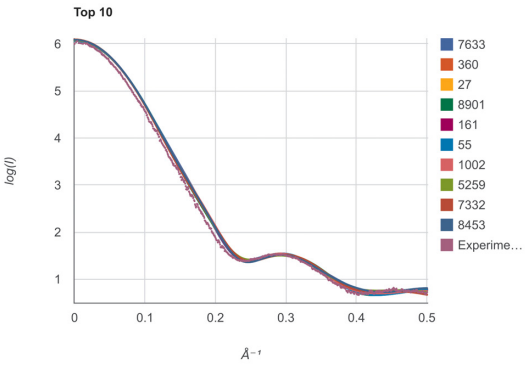
Results:

The compressed results file includes the top 100 complex PDB structures predicted by pyDockSAXS and their corresponding CRYSOL fit curves. Please, refer to the help section for further details.

Download (compressed tar.gz file):

Download the table as a PDF file

Conf	Ele	Desolv	VDW	pyDock	Crysol	pyDockSAXS	RANK
360	-9.367	-7.739	33.111	-13.794	1.967	35.293	1
27	-9.781	-9.536	48.699	-14.447	2.895	45.104	2
8901	-9.439	-13.133	80.302	-14.542	3.307	49.106	3
161	-7.51	-6.083	62.407	-7.352	2.739	50.573	4
55	-5.871	-9.095	35.931	-11.373	3.167	50.913	5
1002	-6.097	-6.462	30.057	-9.554	3.05	51.571	6
5259	-4.748	-12.121	30.637	-13.805	3.641	52.98	7
7332	-5.324	-7.612	18.221	-11.114	3.572	55.035	8
8453	-3.16	-6.973	9.929	-9.141	3.433	55.708	9
7633	-2.55	-2.089	6.583	-3.981	3.519	61.675	10
2617	-12.026	22.27	15.986	11.843	2.144	63.091	11
6181	1.367	-0.59	28.927	3.67	3.009	64.383	12
1835	-3.899	4.391	35.769	4.069	2.975	64.438	13
8972	-10.608	21.704	-16.123	9.484	2.576	65.659	14
6540	-4.505	-1.898	14.17	-4.986	4.131	66.151	15
9608	-9.26	3.318	52.236	-0.718	3.796	67.474	16
5006	-12.422	-8.8	45.087	-16.714	5.918	68.43	17
285	-7.798	-6.628	31.436	-11.283	5.265	69.027	18
3725	-14.771	23.165	-6.683	7.726	3.096	69.31	19
3284	-4.399	-1.201	9.642	-4.635	4.499	69.603	20
5669	-8.762	-4.055	18.502	-10.966	5.337	69.891	21
6829	-11.307	20.663	16.728	11.029	2.959	71.235	22
923	-7.228	24.979	21.961	19.947	2.16	71.386	23
5967	-2.998	4.049	24.827	3.534	3.762	71.42	24
1832	-4.584	-2.76	6.675	-6.677	4.986	71.476	25
806	-9.795	-10.033	57.521	-14.077	6.04	71.94	26
4253	-8.394	-6.059	37.514	-10.701	5.616	72.242	27
6793	-2.783	1.285	6.521	-0.845	4.395	72.53	28
697	-5.591	0.706	11.212	-3.764	4.78	72.757	29
7413	-3.098	-0.75	-6.522	-4.5	4.944	73.323	30
9988	-13.251	19.807	13.307	7.886	3.537	73.71	31
3317	-2.586	6.967	34.201	7.802	3.557	73.812	32
6967	-9.517	-9.599	-1.984	-19.315	7.095	73.913	33
4274	-8.889	23.456	24.71	17.038	2.783	75.426	34
4738	-1.985	7.624	-3.532	5.286	4.066	75.861	35
1007	-7.955	3.404	22.564	-2.295	5.091	76.676	36
1203	-4.903	-2.238	38.973	-3.244	5.3	77.332	37
7905	-1.767	11.974	-8.949	9.311	5.574	91.944	98
9863	-9.49	7.681	25.152	0.706	6.806	92.015	99
3392	-11.64	22.988	76.548	19.002	4.366	92.134	100



Use mouse to zoom in or zoom out. Right click resets view.
Download as image file (including residuals plot)

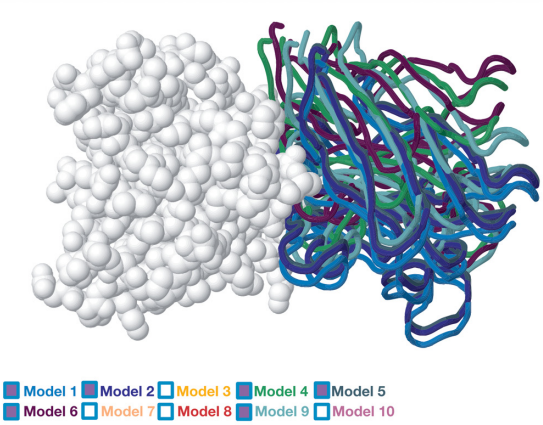


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Figure 2. Output of the pyDockSAXS server showing the results for rebuilding the dismutase oxidase homo-dimer (PDB 3F7L). Models 1 and 2 represent near-native solutions (ligand RMSD < 10 Å). Model 9 would also be acceptable by CAPRI criteria, since interface RMSD < 4 Å. Other models (e.g. 4, 5, 6) have also good interface-RMSD values just above the usual acceptance cutoff.

els to the experimental SAXS data provided and (iii) a JSmol (jsmol.sourceforge.net/) interactive representation of the top 10 models predicted by the server (Figure 2). The output of the server is also available for downloading as a gzip (gzip.org) compressed tar file and includes all the result files organized by folders. Those folders are (i) 'input_data' which include the different input files provided by the user, (ii) 'pydock' with the protein–protein docking information data generated by pyDock method, (iii) 'fit_top10_SAXS' contains the fitting files for the top 10 docking orientations according to CRYSOL χ^2 value and (iv) 'top100' folder, containing the top 100 structures scored by pyDockSAXS in PDB file format (the CRYSOL fit parameters are included in the header of each structure as a 'REMARK' section for user convenience). The organization and format of the result files has been carefully optimized following the feedback provided by community users of the server and it is well described in the 'FAQ and Help' section of the server as well as in the 'README.txt' file included in the compressed results.

Implementation

The implementation of the web server is based on a three-components architecture: (i) a web front end that acts as user input source and makes results available to display and download when job is completed, (ii) a relational database where the job information is stored and (iii) a back end application which periodically polls the database for queued user projects and schedules jobs for parallel calculation of pyDockSAXS using the Slurm batch queuing system (slurm.schedmd.com). The web front end has been implemented using the web2py (www.web2py.com) free and open source web framework, and has been tested in all major modern web browsers. In addition, it adapts fluently to mobile devices screens. The back end application has been written in Python version 2.7 with the use of external libraries as numpy and matplotlib. The relational database has been designed and implemented using MySQL (www.mysql.com). The pyDockSAXS method is part of the pyDock software version 3 and calls internally CRYSOL software to evaluate the fitness of each of the predicted protein–protein complexes to the SAXS experimental data.

The server runs on a multi-user cluster with access to two nodes composed of 16 cores (4 Intel Xeon E5620 Quad Core at 2.4 GHz) and 32 cores (2 AMD Opteron Abu Dhabi 6376 cpus), respectively, with 11 TB of total available disk space and 256 GB of physical memory.

CONCLUSIONS AND FUTURE DEVELOPMENT

The motivation behind the pyDockSAXS web server was to provide access to the scientific community to the efficient pyDockSAXS method, which integrates SAXS experimental data with pyDock protein–protein scoring energy for improved structural predictions of protein–protein complexes.

The pyDockSAXS web server is an on-going project that will implement new features according to the future scientific community feedback. In the next upgrade, cofactors, ions and other non-peptidic molecules will be able to be considered during calculations. We also plan to implement a filter by symmetry, for the use on homo-meric complexes, and an extended input to analyze docking sets from different docking methods.

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