

**Instruções para Tutorial DockThor**

O docking (de um ligante da protease do HIV) será realizada através do webserver do programa DockThor:

<http://dockthor.lncc.br/>

Será realizado o procedimento de docking nativo, no qual emprega-se um ligante que já co-cristalizado com a proteína de interesse e considera-se a conformação proteica observada na presença deste ligante (obtida diretamente do PDB referente ao complexo em questão).

**Preparação inicial de arquivos:**

Utilizaremos o tutorial preparado pela própria equipe do DockThor. No entanto, antes de iniciar o tutorial é necessário preparar os arquivos da proteína e do ligante a ser avaliado. É necessário também definir o ponto central do Grid no qual será realizado o procedimento de docking.

**1. Para preparar o arquivo da proteína:**

- Faça o download do arquivo 1HBV.pdb diretamente no site do PDB:

<http://www.pdb.org/pdb/explore/explore.do?structureId=1hbv>

**Estrutura 1HBV - A CHECK ON RATIONAL DRUG DESIGN. CRYSTAL STRUCTURE OF A COMPLEX OF HIV-1 PROTEASE WITH A NOVEL GAMMA-TURN MIMETIC**

Não é necessária nenhuma preparação adicional. O receptor será preparado automaticamente no site do programa.

**2. Para preparar arquivo do ligante:**

Num editor de texto, abra o arquivo 1HBV.pdb e copie todas as linhas contendo informações sobre o ligante. Estas são identificadas pela coluna HETATM e pelo código do ligante GAN.

HETATM	1523	N1	GAN	A	600	-4.428	16.771	18.898	1.00	49.56	N
HETATM	1524	C2	GAN	A	600	-4.368	17.218	20.328	1.00	48.46	C
HETATM	1525	C3	GAN	A	600	-3.362	18.372	20.475	1.00	51.15	C
						...					
HETATM	1564	C42	GAN	A	600	-12.454	13.101	33.796	1.00	39.47	C

- Salve estas linhas em um novo arquivo: GAN.pdb.

### **3. Definição do ponto central do GRID:**

Para definição do ponto central do GRID, selecione um átomo central no sítio ativo. Para visualizar o sítio ativo, utilize o programa Pymol e crie um objeto contendo o ligante GAN e todos os resíduos a uma distância de até 6 Å ao redor.

Considerando a posição central dos Asp catalíticos no sítio ativo, neste caso selecionei o oxigênio OD1 do Asp25 para definição do ponto central do grid. A partir da linha correspondente a este átomo no arquivo PDB, obtem-se as coordenadas para o GRID (destacadas em negrito abaixo).

ATOM 198 OD1 ASP A 25 **-11.544 21.448 28.812** 1.00 10.03 O

Estas coordenadas serão adicionadas no site do DockThor, no momento de submissão do docking.

**Após a preparação destes arquivos, siga o tutorial a seguir, que foi retirado diretamente do site do DockThor.**

Observações importantes:

- Na preparação da proteína, altere o estado de protonação de Asp25 para AspN1 (conforme indicado no próprio tutorial). O pKa das cadeias laterais dos resíduos na estrutura 1HBV foram calculados utilizando o programa propka3.0. Conforme indicado abaixo, observa-se uma variação significativa no pKa de Asp25, quando comparado a Asp29:

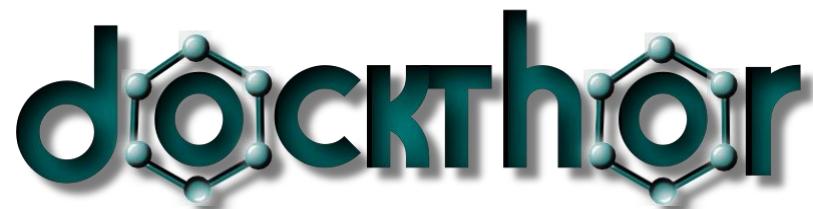
RESI DUE pKa BURI ED

ASP 25 A 5.91\* 100 %  
ASP 29 A 3.77 51 %

Essa variação é decorrente da proximidade espacial entre dois resíduos de aspartato.

- Nenhum cofator será utilizado.

# Portal

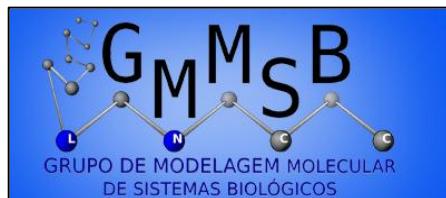


[www.dockthor.lncc.br](http://www.dockthor.lncc.br)

## User Guide Version 1.0

### Contributors

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DockThor® registered mark

# Portal DockThor

## 1. Introduction

The DockThor Portal, developed by the group GMMSB/LNCC, is a free receptor-ligand docking server idealized to facilitate and enable the use of the docking methodology by the academic community. The implemented DockThor® program is a flexible-ligand and rigid-receptor grid based method that employs a multiple solution genetic algorithm along the MMFF94S molecular force field scoring function. The main steps of the ligand and protein set up are available on the DockThor Portal, being possible to change the amino acid residues protonation states and include cofactors (*e.g.* structural water molecules, metals, organic molecules) as rigid entities. The user can also customize the main parameters of the energy grid and the genetic algorithm.

The results of the docking process can be analyzed and sorted automatically. The analysis parameters can also be customized by the user. The DockThor Portal employs the computational facilities provided by the Brazilian SINAPAD (Sistema Nacional de Alto Desempenho) high performance platform.

## 2. Submiting a Docking Job

In the present version 1.0 of the DockThor Portal only protein receptors and pdb type files can be accepted (or pre-prepared DockThor input files \*.in). Other types of receptors (*e.g.* DNA, RNA, another ligand) will be allowed in the next version.

### 2.1 Protein Preparation

1. Click on the **Docking** tab. Then click on the **Protein** tab to open the protein preparation page.

The screenshot shows the DockThor web application. At the top, there's a logo for "dockthor" with the subtitle "A receptor-ligand docking program". To the right of the logo is a 3D molecular model of a protein-ligand complex. Below the logo is a navigation bar with links for "Home", "Docking", "References", "About", and "Support". Underneath this is another navigation bar with tabs for "Protein", "Ligand", "Cofactors", "Docking", and "Results and Analyses". The main content area has a sub-header "1) Submit your protein structure file (.pdb or .in):" followed by a "Upload" button. At the bottom of the page is a footer bar containing logos for various sponsors: GMMSB (Grupo de Modelagem Molecular do Instituto Biológico), SINAPAD, LNCC, inct, FAPERJ, CNPq, Ministério da Ciência, Tecnologia e Inovação, and Governo Federal Brasil.

2. To submit your protein file click on the **Upload** button. So far, it is only possible to upload *Protein Data Bank* (.pdb) type or *DockThor input* (.in) protein files.
  
3. Prepare the protein file applying the basic options clicking on **Prepare**. At this step, all the missing atoms of the residue side chains will be reconstructed. The protein atoms are recognized by the initial .pdb label 'ATOM', Atoms associated with the initial .pdb label 'HETATM' will be ignored. All the atoms are also recognized by their .pdb atom label (*e.g.* CA, CB etc). If the atom label nomenclature is not right the atom will be rebuilt<sup>1</sup>. If two side chain conformations are given for the same residue in the .pdb file, only the first one will be considered by the program.

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<sup>1</sup> To maintain the original H's positions you must use the same H atom .pdb nomenclature used by the DockThor program (please download and examine the generated \*.in file).

The screenshot shows the dockthor homepage. At the top, there is a logo for "dockthor" with the subtitle "a receptor-ligand docking program". Below the logo is a 3D molecular model of a protein. The navigation bar includes links for Home, Docking, References, About, and Support. A secondary navigation bar below it has tabs for Protein (which is selected), Ligand, Cofactors, Docking, and Results and Analyzes.

1) Submit your protein structure file (.pdb or .in):

*Protein file uploaded: 1HBV.pdb*

[Upload](#)

2) Prepare the protein file: [\(View 3D\)](#)

[Prepare](#)

**Attention:** It is only possible to change the protonation states and visualize the protein structure for .pdb type files. All the hydrogen atoms in the original files will be removed. To consider cofactor or water molecules during docking, treat them separately in the Cofactor tab.



4. Set the protonation states of the residues (Asp, Cys, Glu and His are set to the default values - see appendix A). Reprepare your protein file by applying the new protonation states clicking on **Reprepare**.

The screenshot shows the dockthor homepage again. The "Prepare" button is highlighted with a red arrow. A green arrow points down to the "Download prepared files" link. Another red arrow points to the "Residue protonation options (Help)" link, which is expanded to show a list of amino acid residues (ALA, ARG, ASN, ASP, CYS) with their respective protonation states. The "ASP" state for residue ASP\_25 is highlighted with a red arrow and labeled "ASPN1".

5. View the prepared protein file with JSmol clicking on **View 3D**.

6. To download the respective protein files click on **Download prepared files**.

**protein.in** – DockThor receptor input file (contains the MMFF94S atomic type number, the atomic coordinates, the bond connectivities, the atomic partial charges, the pdb type atom, residue and chain labels and the residue and chain numbers).

**resumo.out** – contains the information of the protein preparation process.

**protein\_prep.pdb** – prepared protein file according with the *Protein Data Bank* format (equivalent to the protein.in file for visualization).

**protein.X** – configuration file for each protein chain X. Contains the amino acid residue list and the respective protonation state label.

7. Click **Next** to submit your prepared protein file to docking. It is noteworthy that only the protein file in the *.in* format is necessary to docking.

The screenshot shows the DockThor web application interface. At the top, there is a logo for "dockthor" and a subtext "a receptor-ligand docking program". Below the logo is a molecular structure graphic. The navigation menu includes "Home", "Docking", "References", "About", and "Support". A secondary navigation bar below it includes "Protein", "Ligand", "Cofactors", "Docking", and "Results and Analyzes".

Step 1: "Submit your protein structure file (.pdb or .in)". A file "1HBV.pdb" is uploaded. A green "Upload" button is shown, and a red arrow points to a green "Download prepared files" button with a downward arrow icon.

Step 2: "Prepare the protein file". A "View 3D" button is highlighted with a red arrow. A "Reprepare" button is also present. A red arrow points to the "Download prepared files" button.

Step 3: "Change the protonation state of the amino acids". A "Residue protonation options (Help)" link is shown.

Step 4: "Send the prepared protein file to docking". A large red arrow points to the "NEXT" button.

At the bottom, there are several logos for partners and sponsors: GMMB (Grupo de Modelagem Molecular de Sistemas Biológicos), SINAPAD, LINC/C, INCT, FAPERJ, CNPq, Ministério da Ciência, Tecnologia e Inovação, and the Brazilian government logo.

## 2.2 Ligand Preparation

- To submit a single small molecule<sup>2</sup> to docking, click **Upload**. The formats accepted are those recognized by OpenBabel. For a complete list of these files, click on the [\(Help\)](#) link.

1) Submit your ligand structure file: (Help) ↶

**Upload**



- The uploaded ligand needs to be prepared to generate the respective topology file (.top) to docking. This step comprises the right MMFF94S force field atom type assignment, the atomic partial charges calculation and the assignment of the rotatable chemical bonds. If you want to add hydrogen atoms automatically, just check the **Add hydrogens** box and **Reprepare** the molecule again. The hydrogen atoms will be added through OpenBabel tool at pH = 7.0.
- To view the current structure of your ligand, click **View 3D**.

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<sup>2</sup> In the present portal version, it is only possible to dock one ligand at time. A virtual screening version of the DockThor Portal will be available in the future.

1) Submit your ligand structure file: ([Help](#))

Ligand file uploaded: GAN.pdb

[Upload](#)

2) Prepare the ligand file. Check the box if you want to add hydrogens using OpenBabel. ([View 3D](#))

[Prepare](#)

Add hydrogens



4. You can view the selected rotatable bonds just clicking on the **Rotatable bonds...** button. If you unselect some flexible rotatable bonds click on **Reprepare**.

1) Submit your ligand structure file: ([Help](#))

Ligand file uploaded: GAN.pdb

[Upload](#)

2) Prepare the ligand file. Check the box if you want to add hydrogens using OpenBabel. ([View 3D](#))

[Reprepare](#)  Add hydrogens

[Download prepared files](#)

Rotatable bonds to be flexible during docking (see atom numbers View 3D)

Rotatable bonds: 9	
Atom 1	Atom 2
<input checked="" type="checkbox"/> 6	7
<input checked="" type="checkbox"/> 11	12
<input checked="" type="checkbox"/> 22	23
<input checked="" type="checkbox"/> 29	28
<input checked="" type="checkbox"/> 28	32
<input checked="" type="checkbox"/> 34	35
<input checked="" type="checkbox"/> 35	36
<input checked="" type="checkbox"/> 35	39
<input checked="" type="checkbox"/> 39	41

3) Send the prepared ligand file to docking:

[NEXT](#)

5. If you click on **Reprepare** without unselecting any rotatable flexible bond, the original set of flexible bonds will be restored.
6. To download the prepared ligand files click on **Download prepared files**.
 

***ligand.top*** – DockThor ligand input file. It contains the atom name, the atom number, the MMFF94S atomic type number, the atomic partial charge, the atomic coordinates and the atom valence. It contains also: (i) the atom connectivity; (ii) force field torsional parameters; (iii) selected flexible bonds; (iv) non-bonded intramolecular atom interactions.

***new\_ligand.pdb*** – prepared ligand in the Protein Data Bank format (for visualization). This file is only generated when the **Add hydrogens** option is applied.
7. Click **Next** to send the updated ligand file to docking.

### 2.3 Cofactor and Water Preparation

1. For some protein-ligand complexes it is important to consider cofactor (e.g. NAD, ATP, FAD, Mg, Zn etc.) and/or water/solvent molecules. The DockThor Portal allows the inclusion of one or more cofactor and solvent molecules; they are kept fixed during the docking simulation. Upload each file at a time clicking on **Upload**.

The screenshot shows the DockThor Portal homepage. The header features the DockThor logo and a molecular structure graphic. The navigation bar includes links for Home, Docking, References, About, Support, Protein, Ligand, Cofactors (which is highlighted in blue), Docking, and Results and Analyses. Below the navigation bar, there are two sections: 1) (Optional) Submit your cofactor structure or water structure file(s) with an 'Upload' button, and 2) Send the cofactor/water file(s) to docking with a 'NEXT' button. At the bottom, there are logos for GMMB, SINAPAD, LINCC, INCT, FAPERJ, CNPq, and the Brazilian Ministry of Science, Technology, and Innovation.

2. As well as ligand file, the cofactor and water files (one or more water molecules per file) need to be converted to the topology file. To add hydrogen atoms<sup>3</sup>, check the respective boxes and click **Prepare**.
3. It is also possible to download the prepared files corresponding to each cofactor/water clicking on **Download prepared files**. Each cofactor/water file will generate one link for download.
4. Send the cofactor and/or water to docking by clicking on **Next**.

The screenshot shows the dockthor web interface. At the top, there's a logo and a navigation bar with links for Home, Docking, References, About, Support, Protein, Ligand, Cofactors (which is highlighted), Docking, and Results and Analyses. Below the navigation bar, there are three numbered steps:

- 1) (Optional) Submit your cofactor structure or water structure file(s):
- 2) Prepare the cofactor/water file(s). Check the box of each file if you want to add hydrogens using OpenBabel:  **Attention:** The water hydrogens are not optimized (is better to optimize the water hydrogens before uploading the respective file). Do not include metal ions and water molecules in the same file.
- 3) Send the cofactor/water file(s) to docking:

## 2.4 Docking Configuration

1. The next tab displays the main parameters to run the docking job. It is possible to see the original files for the ligand and protein in **Files uploaded**. Check if they are all right.
2. Fill the **Email** field to receive the link with the results page when the docking job is finished. This field is mandatory.

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<sup>3</sup> Since MMFFLigand does not optimize the hydrogen atoms position, it is recommended to do this previously and do not chose to include hydrogen atoms to these water files.

3. Fill the coordinates of the energy grid center in **Grid center** ( $X_c$ ,  $Y_c$ ,  $Z_c$ ).
4. Fill the **Grid Dimensions** ( $\pm\Delta x$ ,  $\pm\Delta y$ ,  $\pm\Delta z$ ). These values correspond to half of the grid size on each dimension, e.g.  $(X_c-\Delta x) \leq X \text{ Dimension} \leq (X_c+\Delta x)$
5. Select the spatial discretization of the energy grid. This value corresponds to the spacing between the grid points (the default value is 0.25Å). Check if the number of grid points does not exceed the limit allowed (1,000,000) in the **Grid points** box.

The screenshot shows the Dockthor web interface. At the top, there's a logo for "dockthor" and a subtext "a receptor-ligand docking program". To the right is a 3D molecular model of a protein and a ligand.

The navigation bar includes links for Home, Docking, References, About, and Support. Below that is a secondary navigation bar with tabs for Protein, Ligand, Cofactors, Docking (which is selected), and Results and Analyzes.

The main form area contains the following input fields:

- Files uploaded:**
  - Protein: 1HBV.pdb
  - Ligand: GAN.pdb
  - Cofactors: NAD.pdb H2O.pdb MG.pdb
- Email:** userdockthor@drugdesign.com
- Grid center ( $X_c$ ,  $Y_c$ ,  $Z_c$ ):** 3.45, 12.34, -9.76 (with a link to **View grid in 3D**)
- Grid dimensions ( $\pm\Delta x$ ,  $\pm\Delta y$ ,  $\pm\Delta z$ ):** 10, 10, 10 ( $\geq 5\text{\AA}$ )
- Discretization of the Grid (Å):** 0.25
- Grid points:** 531441 (max: 1,000,000)
- Job label:** Test\_Dockthor

Below the form, there's a section for **Genetic Algorithm (GA) Parameters Settings** with a note: "Attention: The program was optimized for the default values - change these values at your own risk."

A large green button labeled "Dock!" is centered below the input fields.

At the bottom, there's a footer with logos for GMMSB, SINAPAD, LINCS, INCT, FAPERJ, CNPq, the Ministry of Science, Technology and Innovation, and the Brazilian Government.

6. To view the grid dimensions and the receptor click **View Grid in 3D**.
7. Choose an identification label for your docking job.
8. Some genetic algorithm (GA) parameters can be modified.

- The GA multisolution algorithm was optimized to deal with highly flexible ligands. We do not recommend changing the standard number of evaluations per run (*i.e.* 1,000,000) or the GA population size (*i.e.* 1,000). **Change these values at your own risk.**
- Each submission job corresponds to 30 independent docking runs. A maximum of 50 docking runs per job is permitted. It is possible to run more independent docking runs submitting more than one job and changing the initial seed. The seed must have a negative value, and for each successive run its value is diminished by one. You should take this into account if you want to submit more than one job in order to obtain more than 50 independent docking runs.



**dockthor**  
A receptor-ligand docking program

Home    Docking    References    About    Support ▾

Protein    Ligand    Cofactors    **Docking**    Results and Analyzes

Files uploaded:

Protein: 1HBV.pdb  
Ligand: GAN.pdb  
Cofactors: NAD.pdb H2O.pdb MG.pdb  
Email: [userdockthor@drugdesign.com](mailto:userdockthor@drugdesign.com)  
Grid center (Xc, Yc, Zc): 3.45 12.34 -9.76 (View grid in 3D)  
Grid dimensions ( $\pm\Delta x$ ,  $\pm\Delta y$ ,  $\pm\Delta z$ ): 10 10 10 ( $\geq 5\text{\AA}$ )  
Discretization of the Grid (Å): 0.25    Grid points: 531441 (max: 1,000,000)  
Job label: [Test\\_Dockthor](#)

→ **Genetic Algorithm (GA) Parameters Settings**

Number of Evaluations:	1000000	(max: 1000000)
Population Size:	1000	(max: 2000)
Number of Runs:	30	(max: 50)
Seed:	-1985	(Negative value)

**Attention:** The program was optimized for the default values - change these values at your own risk.

→ Run your docking job:

→ **Dock!**

9. Run your docking job by clicking on **Dock!**

10. When your docking process is finished an email will be sent at the previously stated e-mail, containing a link to the results page.

### 3. Analyzing the Docking Job

Once the docking job is finished, the user will receive an email with the link to the Results page as below:

*Your docking result is available at*

[http://www.dockthor.lncc.br/index.php?pg=submission&pgs=results&id=Test\\_Dockthor\\_r1rpaa](http://www.dockthor.lncc.br/index.php?pg=submission&pgs=results&id=Test_Dockthor_r1rpaa)

Follow the link in the email to access the Results and Analyses page.

#### 3.1 Results and Analyses

For each docking run the final population is clustered using the total energy and a RMSD = 1.0Å criterion. Only the leaders of each cluster will be used in the final clustering analysis step:

1. It is possible to cluster and sort out the docking solutions according to two criteria: **Total Energy** (intermolecular ligand-receptor + intramolecular ligand energies) or **Interaction Energy** (only intermolecular ligand-receptor energy). The default is **Analysis by total energy**.

1) Perform the clustering and ranking of the docking poses:

Analysis by total energy  
 Analysis by interaction energy

Result Settings

2) (Optional) Submit your reference ligand file for redocking analyses:  
[Upload](#)

3) Analyze your docking results:  
[Analyze](#)

2. Click **Analyze** to perform the docking poses analyses.
3. You can change the clustering criterion (default = 2.0Å) and the number of the best ligand binding modes (default = 20) selected for analyses and visualization. Click on the **Result Settings** button.

1) Perform the clustering and ranking of the docking poses:

- Analysis by total energy
- Analysis by interaction energy

→ [Result Settings](#)

Clustering parameter:  Å  
Number of ligand binding modes:

2) (Optional) Submit your reference ligand file for redocking analyses:

[Upload](#)

3) Analyze your docking results:

→ [Analyze](#)



4. It is possible to view the results interactively on the website. Click on **View results interactively**.

5. To see each docking ligand binding mode (*i.e.* cluster leader) select the corresponding one in the results table. For each solution the system shows the corresponding **Run** of the Genetic Algorithm, the **Model** (number of the cluster leader) of the corresponding GA run, the **Total Energy**, the **Intermolecular Energy** and the **RMSD** (root mean square deviation calculated using the non H atoms) relative to the top ranked pose. This value gives an idea of the conformational difference among the alternative ligand binding modes.

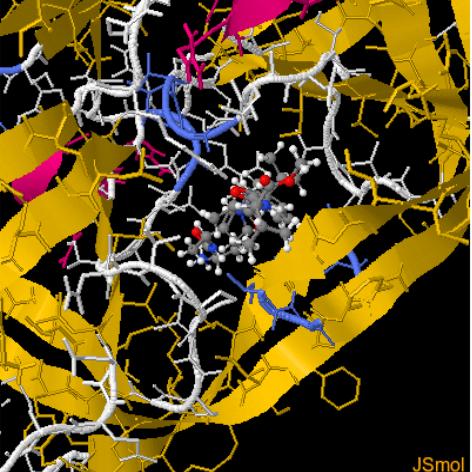
Analysis by total energy  
 Analysis by interaction energy

[Result Settings](#)

2) (Optional) Submit your reference ligand file for redocking analyses:  
[Upload](#)

3) Analyze your docking results:  
[Analyze](#)

 [View results interactively](#)



JSmol

Show Protein

[Reload Visualization](#)

Run	Model	T.Energy (kcal/mol)	I.Energy (kcal/mol)	RMSD (Å)
9	1	62.737	-65.118	0.000
41	2	72.34	-60.251	12.153
27	8	78.246	-57.282	2.042
34	3	78.811	-45.907	7.332
24	6	78.821	-62.773	2.181
29	1	78.893	-60.641	3.592
7	2	79.212	-43.446	8.069
1	1	79.396	-48.391	7.885
11	9	79.467	-57.208	2.221
37	7	81.49	-51.756	2.212

6. It is also possible to upload a reference ligand conformation (.pdb type file) to perform redocking analyses or help the investigation of the distinct binding modes. Then click **Analyze** to perform the docking poses analyses and click on **View results interactively**. The **RMSD** (in the last column) is now calculated relative to the uploaded reference ligand pose. It is possible to hide the protein structure to facilitate visualization.

Analysis by total energy  
 Analysis by interaction energy

[Result Settings](#)

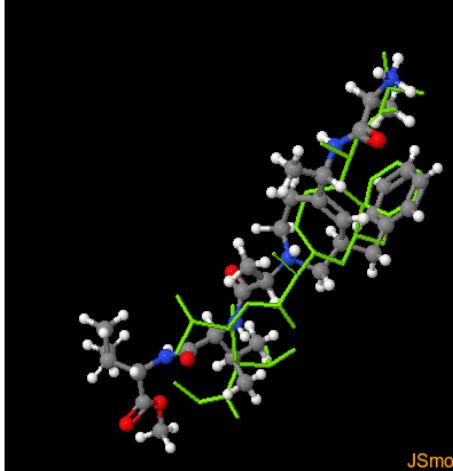
2) (Optional) Submit your reference ligand file for redocking analyses:  
 (Uploaded file: GAN.pdb)

[Upload](#)

3) Analyze your docking results:

[Analyze](#)

[View results interactively](#)



JSmol

[Reload Visualization](#)

Run	Model	T.Energy (kcal/mol)	I.Energy (kcal/mol)	RMSD (Å)
9	1	62.737	-65.118	3.222
41	2	72.34	-60.251	11.418
27	8	78.246	-57.282	3.965
34	3	78.811	-45.907	8.518
24	6	78.821	-62.773	3.541
29	1	78.893	-60.641	1.509
7	2	79.212	-43.446	9.509
1	1	79.396	-48.391	9.003
11	9	79.467	-57.208	3.902
37	7	81.49	-51.756	4.351

Show Protein  Show Reference Ligand

7. To download the docking results, including the clustered conformations, click **Download Results**.

**dockthor.out** – general information about each DockThor run.

**parameters.txt** – general information about the docking parameters used.

**results.out** – total number of clusters obtained (after analyzing all runs).

**out.log** – summary of the clustering analysis for the best cluster leaders (ranking, energies, RMSD).

**out.mol2** – contains the atomic coordinates of the best cluster leaders (multimodel .mol2 type file, sorted out according to the results described in out.log).

**ligand\_run\_X.log** – contains the information of the cluster leaders obtained in run X (using a RMSD criterion of 1.0Å).

**ligand\_run\_X.pdb** – contains the atomic coordinates of the cluster leaders obtained in run X (multimodel .pdb type file).

***protein.in*** – DockThor receptor input file.

***ligand.top*** – DockThor ligand input file.

8. It is always possible to perform other analyses and download the respective files.

## 4. Softwares

The Portal DockThor uses the following programs:

***MMFFLigand***: generates the topology file for the ligand and cofactor files through MMFF94S force field and OpenBabel tools;

***PdbThorBox***: prepares the protein file (adds hydrogen atoms, changes amino acid protonation states, completes missing side chains) with the MMFF94S force field;

***DockThor***: the docking program is a flexible ligand rigid receptor grid based method that employs a multiple solutions genetic algorithm as the search method using the MMFF94S force field as the scoring function.

***Dtstatistic***: clusters and ranks the docking poses according to total or interaction energies.

\* All these programs were developed by the GMMSB/LNCC group.

\*\* Ernesto R. Caffarena, Michel Loos and Isabelle Ortmans also contributed for the PDBTHORBOX development.

## Appendix A

### Residue Protonation States

ASP - Negatively charged aspartic acid (**default**).

ASPN1 - Neutral aspartic acid with a H bonded to the O $\delta$ 1

ASPN2 - Neutral aspartic acid with a H bonded to the O $\delta$ 2

GLU - Negatively charged glutamic acid (**default**).

GLUN1 - Neutral glutamic acid with a H bonded to the O $\epsilon$ 1.

GLUN2 - Neutral glutamic acid with a H bonded to the O $\epsilon$ 2.

CYSH - Neutral cysteine with a H bonded to S (**default**).

CYS - Negatively charged cysteine.

CYSS - Neutral cysteine (disulfide bond).

HIS - Neutral histidine with a H bonded to N $\tau$  (**default**)

HISD - Neutral histidine with a H bonded to N $\pi$ .

HISP - Positively charged histidine.

ARG - Positively charged arginine (**default**).

ARGN1 - Neutral arginine at N $\omega$ <sub>1</sub>.

ARGN2 - Neutral arginine at N $\omega$ <sub>2</sub>.

LYS - Positively charged lysine (**default**).

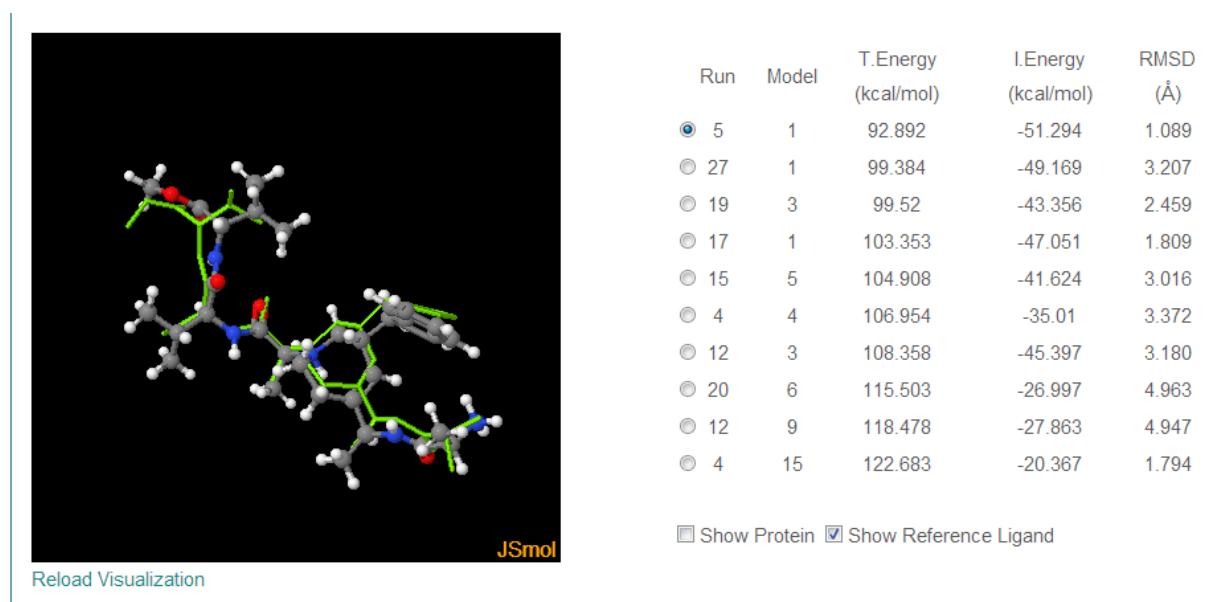
LYSN - Neutral lysine.

## Análise dos resultados:

No site do DockThor é possível visualizar os resultados, através de link enviado por e-mail. Resultados de uma submissão prévia podem ser acessados pelo link:

[http://www.dockthor.lncc.br/index.php?pg=submission&pgs=results&id=docking\\_GAN\\_1HBV\\_1710\\_16\\_ligand\\_iwgvow](http://www.dockthor.lncc.br/index.php?pg=submission&pgs=results&id=docking_GAN_1HBV_1710_16_ligand_iwgvow)

É possível comparar os resultados com o ligante de referência (desde que você acrescente o arquivo contendo a conformação cristalográfica antes de clicar em **Analyze**). Observe se o modo de ligação de menor energia (colorido por átomos) corresponde ao modo cristalográfico (em verde).



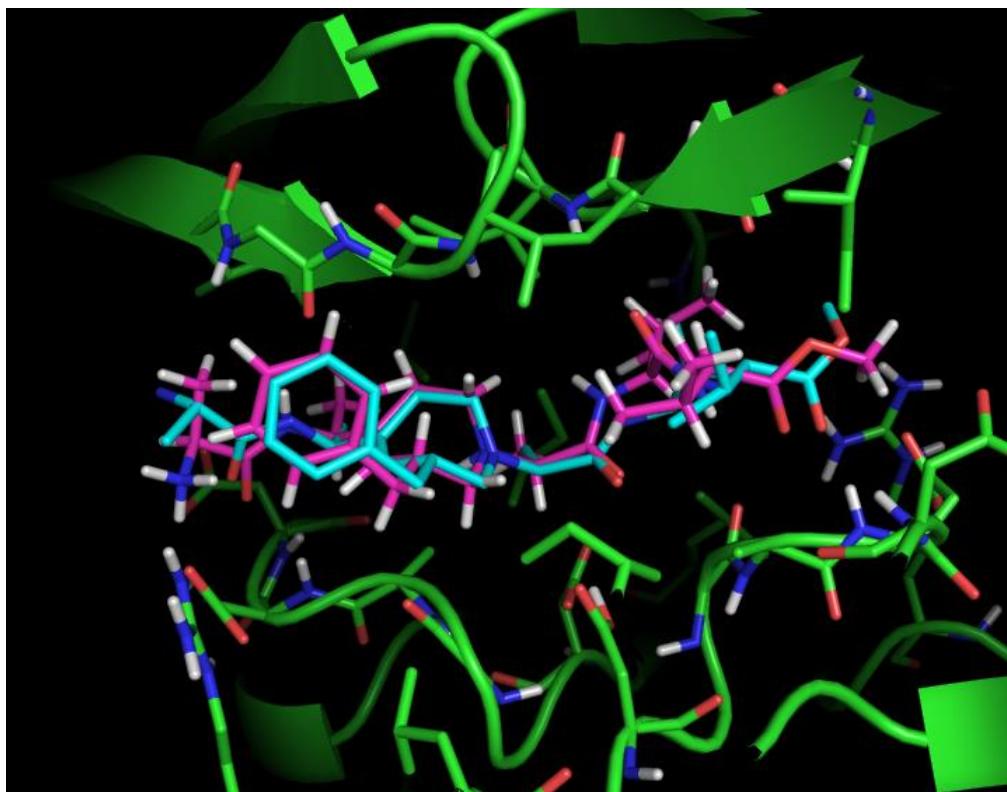
Para analisar cuidadosamente os resultados, abra no Pymol os arquivos:

- protein\_\*\*\*\*\*\_prep.pdb (arquivo da proteína após preparação pelo DockThor)
- GAN.pdb (ligante com coordenadas cristalográficas)
- out.mol2 (conformações de menor energia)

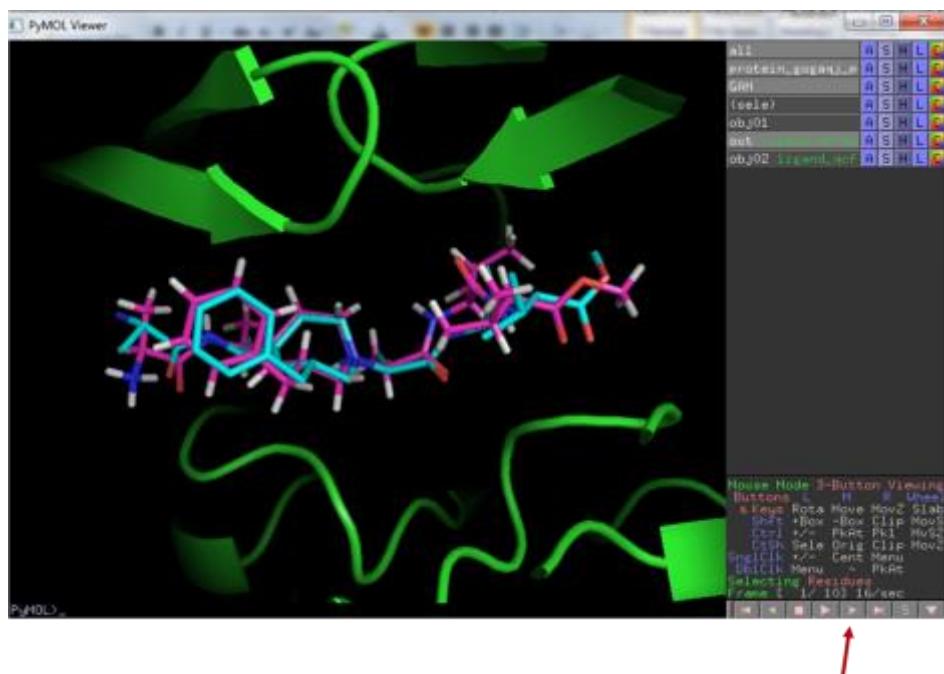
Altere as representações gráficas das proteínas e dos ligantes da maneira que achar mais conveniente para análise. Sugestão de etapas para a análise:

- crie um objeto contendo os resíduos presentes a uma distância de no máximo 6Å do ligante cristalográfico. Represente este objeto como **sticks**, colorindo por átomo.
- represente a proteína como **cartoon**
- represente o ligante GAN.pdb e os resultados de docking como **sticks**, colorindo ambos por átomo, porém colorindo de cores diferentes os carbonos do ligante cristalográfico.
- no menu **Edit > Edit all**, altere o valor de **stick\_radius** de 0.25 para 0.15 e dê enter.

Após todas estas etapas você deverá visualizar uma tela semelhante a mostrada abaixo:



A seguir, alterne entre as conformações de menor energia calculadas por docking, utilizando o botão indicado na figura abaixo:



Meça as distâncias das ligações de hidrogênio entre o ligante cristalográfico e a proteína. Observe se estas interações são preditas nos modos de ligação calculados por docking.