
	<b>SKILLS CENTER STANDARD OPERATING PROCEDURE</b>	<b>A BIOFIZZ</b>  <b>PRODUCTON</b>
<b><i>In-silico</i> Drug Screening 1</b>	<b>Effective Date: 02/15/2021 Module Hours 2</b>	<b>Revision # 1.0 M. Guzie Checked by A. Siclair</b>

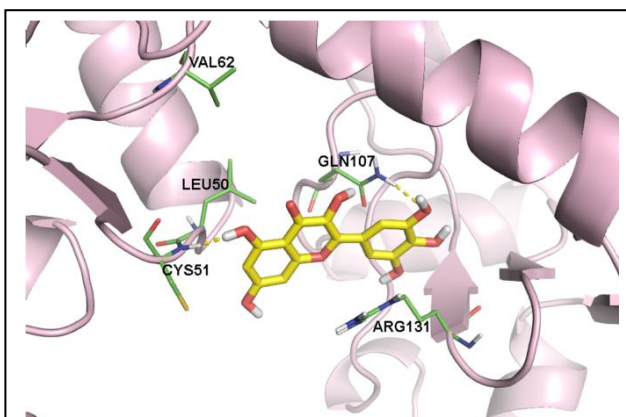
## BACKGROUND

Computer program modeling and analysis has become a critical part of modern biology that has allowed molecular systems to be tested and visualized as a precursor to actual experimentation. Through computer simulation, certain aspects of cellular systems can be analyzed and create predictions on how certain substances will interact with others; from protein-protein interactions, protein-nucleic acid interactions, to ligand-receptor interactions, these modeling systems give scientists clues on the path to find possible therapies and medicines.

Experiments that are conducted via computer simulation are said to be *in-silico*. Programs developed to assess biological systems began to arise about 30 years ago. These techniques have become particularly useful in the design of therapeutic drugs to visualize their interactions with targeted receptors and cells and anticipate possible side effects or undesired interactions. *In-silico* experiments are often conducted alongside in-vitro experiments once a promising drug candidate has been identified, but they also save time and money as a first step to screen possible drugs that are even worth pursuing (Ekins, et al., 2007).



The field of pharmacology has arguably benefited the most from the progression of *in-silico* technology. If a drug of interest is identified it can be visualized in a model system to observe what functional groups are interacting with protein side chains and how the structure is potentially altered; although just a prediction which can be flawed, the insight it gives to drug function can greatly accelerate the drug design process.

Online programs such as SwissDock have made it accessible to the general public to create and use an *in-silico* program that is both relatively easy and user-friendly. This module will provide a methodology for the use of the SwissDock ligand docking program.



Myricetin, a potential anti-cancer drug candidate, potential binding simulation to viral oncoprotein HPV16 E6 using in-silico screening.

### 1. PURPOSE

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The purpose of this procedure is to recognize the importance of in-silico analysis, and how it can be used to screen for potential molecules which bind to a protein of interest as a starting point in drug discovery for example.

## 2. SCOPE

This procedure applies to qualified skills center users.

## 3. RESPONSIBILITY



- 3.1. It is the responsibility of the user to understand and perform the procedure described in this document.
- 3.2. It is the responsibility of the user performing the procedure to fully document any deviations from the written procedure (Send SwissDock simulations results to email.)
- 3.3. It is the responsibility of the user to become trained in the use of this application.

## 4. DEFINITIONS

- 4.1. Ligand: a substance that forms a complex with a biomolecule to serve a biological purpose.
- 4.2. Accession Code: The unique identification code used for biological macromolecules; for proteins, any protein belonging to the Protein Data Bank has a 4-character code called the PDB code.
- 4.3. SwissDock-Cluster: Represents a specific binding site on the target protein (Walboomers et al., 2019).
- 4.4. SwissDock-Element: The different ways in which a ligand can be bound in a certain cluster (Walboomers et al., 2019).
- 4.5. SwissDock-Full Fitness: Calculated “by averaging the 30% most favorable effective energies of its elements, in order to limit the risk of a few complexes penalizing the whole cluster. This effective energy is written as the sum of the total energy of the system and a solvation term” (Grosdidier, et al., 2011).
- 4.6. Gibbs Free Energy ( $\Delta G$ ): gives an indication of the favorability of the reaction-a more negative value indicates a more favorable binding between ligand and target. The Gibbs free energy formula is given by  $\Delta G = \Delta H - T\Delta S$ , where  $\Delta H$  is the change in enthalpy, T is the temperature in Kelvin and  $\Delta S$  is the change in entropy.

## 5. MATERIALS/EQUIPMENT

- 5.1. A computer to perform the procedure.
- 5.2. The SwissDock database.
- 5.3. Chimera Application (optional)

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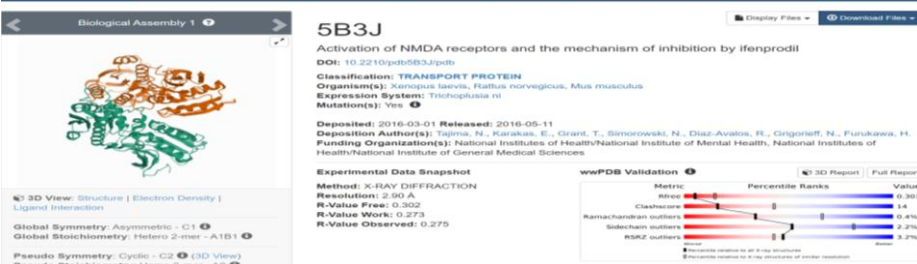
## 6. PROCEDURE

In this procedure, the ligand-docking simulation website SwissDock will be used to assess a ligands interactivity with a target molecule and observe ways in which drugs can be screened for interactivity.

### 6.1. Selecting a Protein/Ligand Pair for Docking

6.1.1. When using SwissDock, both a target and a ligand must be selected for interactivity analysis. Choosing a protein/ligand pair is the first step, whether it be a familiar one, or one requiring further research.



6.1.2. If needing to search some literature to select a combination, the SwissDock site has listed resources available such as the RCSB Protein Database (<https://www.rcsb.org/search>) and the ZINC database for compounds (<https://zinc12.docking.org>). For both target selection and ligand selection, there are databases available below that can be visited to peruse possible reactants. For proteins, the RCSB Protein Data Bank can be used to search through 100,000+ proteins and find their corresponding PDB code: <https://www.rcsb.org/structure/5B3J>. For ligands, either the ZINC database (<https://zinc12.docking.org/>) or Pubchem (<https://pubchem.ncbi.nlm.nih.gov/>) can be used to search through 200 million+ compounds. The ZINC database contains commercially available compounds



6.1.3. Once a target/ligand pair has been selected, visit the SwissDock site at <http://www.swissdock.ch/>.

6.1.4. Go to the “Submit Docking” tab.

6.1.5. Here, two inputs will be available for both target selection and protein selection.

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**Target selection**

Search for targets:

ie. PDB code, protein name, sequence, or URL  
or upload file (max 5MB)

**Ligand selection**

Search for ligands:

ie. ZINC AC, ligand name or category (like scaffolds or sidechains), or URL  
or upload file (max 5MB)

6.1.6. Enter the target protein of interest into the search bar under target selection. This could be the name of the protein, its sequence, an URL, or the PDB code (4 number/letter code found on the PDB website.) An example of the PDB code for an NMDA receptor is shown below (5B3J). Click search and select the correct protein. If the protein is multiple polypeptide chains, there may be a prompt to select one of the chains for analysis. Choose whichever chain, or the chain which has the most binding affinity for the ligand if known. The protein can also be downloaded from the PDB website and uploaded as a file using the “upload file” option.

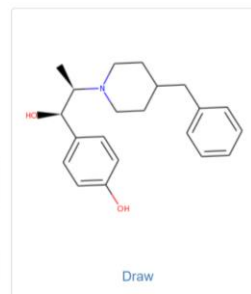
6.1.7. Enter the ligand of interest into the search bar under ligand selection. This could be the ligand name, an URL, or the ZINC accession number (code found on the ZINC database.) An example of the ZINC accession number for ifenprodil is shown below (ZINC14551). Note that there may be multiple forms of a compound due to its stereoisomers on the ZINC database. Click search and select the correct ligand. A file upload can also be used for the ligand.

### ZINC14551 (Ifenprodil)

In: anodyne bb for-sale in-stock world-not-fda



Google Wikipedia PubMed

Added	Availability	Since	Mwt	logP	Download
2004-07-23	In-Stock	2015-08-07	325.452	3.769	↓
Mol Formula	Rings	Heavy Atoms	Hetero Atoms	Fraction sp <sup>3</sup>	Tranche
C21H27NO2	3	24	3	0.43	EHAA

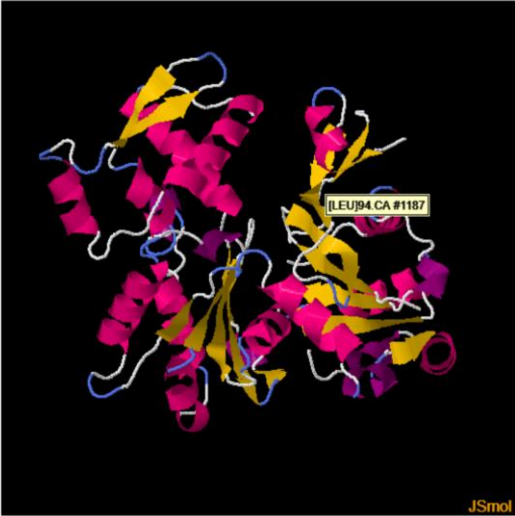


6.1.8. Once both input values have a molecule, fill in the job name and email address information.

6.1.9. Select “Start Docking.”

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6.1.10. It may take 30 minutes or more for the animation to load, but a visualization of the ligand bound protein will appear with several options and values to the right:





Show	Cluster	Element	FullFitness (kcal/mol)	Estimated $\Delta G$ (kcal/mol)
<input type="radio"/>	0	0	-2352.39	-24.15
<input type="radio"/>	0	1	-2352.38	-24.15
<input type="radio"/>	0	2	-2352.38	-24.15
<input type="radio"/>	0	3	-2341.01	-23.79
<input type="radio"/>	0	4	-2337.03	-23.45
<input type="radio"/>	0	5	-2337.03	-23.45
<input type="radio"/>	0	6	-2335.89	-23.19
<input type="radio"/>	0	7	-2335.89	-23.19
<input type="radio"/>	1	0	-2327.94	-13.87
<input type="radio"/>	1	1	-2327.94	-13.87
<input type="radio"/>	1	2	-2327.94	-13.87
<input type="radio"/>	1	3	-2327.94	-13.87
<input type="radio"/>	1	4	-2327.54	-13.81
<input type="radio"/>	1	5	-2327.54	-13.81
<input type="radio"/>	1	6	-2315.53	-13.15
<input type="radio"/>	1	7	-2315.53	-13.15
<input type="radio"/>	2	0	-2325.68	-13.85
<input type="radio"/>	2	1	-2325.67	-13.85
<input type="radio"/>	2	2	-2325.56	-13.84
<input type="radio"/>	2	3	-2325.56	-13.84
<input type="radio"/>	2	4	-2325.56	-13.84
<input type="radio"/>	2	5	-2320.24	-12.57

[Download CSV file](#)  
If predicted binding modes are not displayed, you might suffer from a Jsmol bug we are investigating. In the meantime, please use UCSF Chimera (see below).  
 Binding modes are scored using their FullFitness and clustered. Clusters are then ranked according to the average FullFitness of their elements (see Grosdidier et. al., Proteins, 2007 Jun 1;67(4):1010-25.

Above is the SwissDock binding results for Actin with ATP. Note that ATP isn't shown in the visualization due to a Jsmol bug (see TROUBLE SHOOTING section) but the values still hold. Each different "Show" option that can be selected will show an alternative way of the ligand bound to the protein (although it may be very similar) and thus will have differing values for the remaining results. Chimera is a good application to download the alternative "Show" states and get a better view of the ligand-substrate complex. Note the "Cluster", "Element", "Full Fitness", and " $\Delta G$ " (Gibbs Free Energy) values shown on the right (see DEFINITIONS section for more.) These values are indicative of the overall binding affinity of the ligand as well as favorability of the ligand binding to the protein.

6.1.10. OPTIONAL: Note that proteins/ligands can be uploaded directly after download but can also be opened in Chimera and visualized/customized first and then uploaded. If uploading from Chimera for protein, use a PDB file or ZIP file in the CHARMM format; for ligand, use a ZIP file in the CHARMM format or Mol2 file. For more on Chimera, see Chimera Skills Center Standard Operating Procedure.

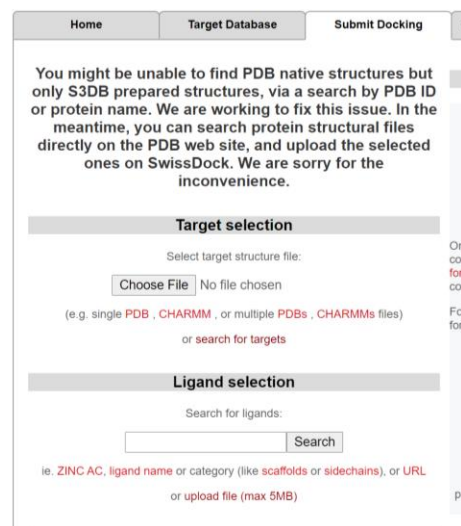
## 7. TROUBLE SHOOTING

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7.1. SwissDock may be experiencing a difficulty in finding PDB files from the database itself.

In this event, you will most likely HAVE to use the Chimera application. If SwissDock is failing to link PDB files from the database, you will have to do so yourself: Download a protein in Chimera, and then upload it onto SwissDock.

7.2. The ligand may not be displayed bound to the target molecule; this is another bug that may be preventing SwissDock from presenting the visual results, but the numerical values are still applicable. Again, Chimera can be used to get a better visualization of the target-ligand complex.





The screenshot shows the SwissDock web interface. At the top, there are navigation tabs: Home, Target Database, and Submit Docking. A message states: "You might be unable to find PDB native structures but only S3DB prepared structures, via a search by PDB ID or protein name. We are working to fix this issue. In the meantime, you can search protein structural files directly on the PDB web site, and upload the selected ones on SwissDock. We are sorry for the inconvenience." Below this, there are two main sections: "Target selection" and "Ligand selection". The "Target selection" section has a "Choose File" button and a "No file chosen" message, with a note that examples include single PDB, CHARMM, or multiple PDBs/CHARMMs files. The "Ligand selection" section has a search box and a "Search" button, with a note that examples include ZINC AC, ligand name, or category (like scaffolds or sidechains), or URL, or upload file (max 5MB).

If predicted binding modes are not displayed, you might suffer from a Jsmol bug we are investigating. In the meantime, please use UCSF Chimera (see below).

7.3. "Target setup failed. Too many ATOM/HETATM records found (16938, max=15000). You may want to truncate your protein." In this event, your protein is too long for the SwissDock docking animation; you can choose to truncate it as it recommends or use a smaller protein.

## 8. REFERENCES

- Ekins, S., Mestres, J., & Testa, B. (2007). *In silico pharmacology for drug discovery: Methods for virtual ligand screening and profiling*. Retrieved from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1978274/>
- Grosdidier, A., Michelien, O., & Zoete, V. (2011). *SwissDock, a protein-small molecule docking web service based ON EADock DSS*. Retrieved from <https://pubmed.ncbi.nlm.nih.gov/21624888/>
- JM. Walboomers, M., Hausen, H., N. Munoz, X., N. Munoz, F., JS. Smith, L., KA. Malecka, D., ML. Verdonk, J. (2019). *Identification of potential binding pocket on viral oncoprotein HPV16 E6: A promising Anti-cancer target for small molecule drug discovery*. Retrieved from <https://bmcmolcellbiol.biomedcentral.com/articles/10.1186/s12860-019-0214-3>

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## 9. MODULE MASTERY TASK

Select a unique protein and ligand and submit docking for the pair; analyze results and corresponding values.

9.1. Select a protein/ligand pair known to bind each other well, such as a familiar drug and its receptor. Give the system a “Job Name” and enter an email address for the results to be sent to for recording. Save the picture of the results and indicate the meaning of the corresponding values (explicitly describe what the cluster, element, and Gibbs Free Energy values indicate), writing a short summary on the results and what it tells you about the overall binding affinity of the complex.

9.2. Select a protein/ligand pair that is not necessarily known to bind each other; maybe it is a pair of personal interest, or molecule you think could serve a purpose of binding another based on your prior knowledge/literature/structural inferences. Do a drug screening of your own! Again, save the picture of the results and indicate the meaning of the corresponding values (explicitly describe what the cluster, element, and Gibbs Free Energy values indicate), writing a short summary on the results and what it tells you about the overall binding affinity of the complex. Did you find a successful drug candidate in your *in-silico* screening process?