

FINAL SCRIPT: APPROVED FOR FILMING



Submission ID #: 67145

Scriptwriter Name: Poornima G

Project Page Link: <https://review.jove.com/account/file-uploader?src=20479273>

Title: Structure-Guided Design and Development of Novel Cyclophilin A Inhibitors and Ganoderiol-F Derivatives: An In-Silico Approach

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

✓ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film interviews here: **12/09/2025**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 26

Number of Shots: 56 (55 SC)

Introduction

INTRODUCTION:

NOTE to VO: Please record introductory and conclusion interview statements.

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. This research focuses on designing novel Cyclophilin A inhibitors and Ganoderiol F derivatives to target the cyclin D–CDK (*D-C-D-K*) axis and inhibit cancer cell proliferation.

~~What are the current experimental challenges?~~

- 1.2. Key challenges include achieving optimal ligand-receptor geometric interactions and maintaining stable ligand-protein complexes under dynamic experimental and physiological conditions.

CONCLUSION:

~~What research gap are you addressing with your protocol?~~

- 1.3. Few studies investigate Ganoderiol F derivatives as oncogenic targets; this protocol addresses that gap by designing and validating inhibitors using molecular docking and dynamics analyses.

~~What advantages does your protocol offer compared to other techniques?~~

- 1.4. This protocol integrates multiple computational tools into a unified workflow, ensuring comprehensive ligand–receptor analysis.

~~What questions will future research focus on?~~

- 1.5. Future research will focus on validating the inhibitors in vitro and in vivo and exploring broader applications of Cyclophilin A inhibitors across multiple cancer types.

Protocol

NOTE: SCREEN timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Deepthi Das) for queries regarding lab media.

2. Preparation of the Docking Parameters Using AutoDock 4.2.6

Demonstrator: Elham Mahgoub

- 2.1. To begin, open the 1nmk (*One-n-m-k*) file and locate the amino acid Arginine at position number 55 to record x, y, z coordinates [1].

2.1.1. WIDE: Talent opening the 1nmk file on the computer interface.

- 2.2. In the AutoDock interface, open the **File** menu, select **Read Molecules**, choose the file 1nmk, and click **Open** [1].

2.2.1. SCREEN: 2026-02-01 15-41-21.mp4 01:53 - 02:03, 2:12 - 2:20

- 2.3. Add hydrogen atoms by selecting **Edit**, choosing **Hydrogen**, and clicking **Add**, then choose **Polar only** and confirm with **OK** [1]. Then, go to edit menu again, assign **ad4** (*ad-4*) **atom types** and **Kollman charges** [2].

2.3.1. SCREEN: 2026-02-01 15-41-21.mp4 02:25 - 02:35

2.3.2. SCREEN: 2026-02-01 15-41-21.mp4 02:38 - 02:42, 02:47 - 02:58

- 2.4. Save the prepared receptor as 1nmk.pdbqt (*1 N-M-K-P-D-B-Q-T*) by selecting **File**, choosing **Save**, setting the file type, and confirming the save location [1].

2.4.1. SCREEN: 2026-02-01 15-41-21.mp4 03:10 - 03:25

- 2.5. Next, open the **Ligand** menu, select **Input**, click **Open**, choose 1.pdb (*1 P-D-B*), and click **Open** and when the information window appears review the ligand details [1]. ~~When the information window appears, review the ligand details [2].~~

2.5.1. SCREEN: 2026-02-01 15-41-21.mp4 04:13 - 04:21, 04:29 - 04:46

2.5.2. ~~SCREEN: Display the ligand information window.~~

NOTE: VO merged with [1]

- 2.6. To detect the ligand root, select **Ligand**, then choose **Torsion Tree**, and click **Detect Root**

[1]. Save the ligand as 1.pdbqt (**1 P-D-B-Q-T**) by selecting **Ligand**, choosing **Output**, and clicking **Save as pdbqt** [2].

2.6.1. SCREEN: 2026-02-01 15-41-21.mp4 04:54 - 05:04

2.6.2. SCREEN: 2026-02-01 15-41-21.mp4 05:08 - 05:27

2.7. Now, prepare the receptor grid by selecting **Grid**, choosing **Macromolecule**, selecting **1nmk**, and clicking **Molecule**, confirm the warning, and save as *1nmk.pdbqt* [1]. For setting the map types, select **Grid**, followed by **Set Map Types**, **Ligand**, choose **2**, and finally select **Ligand** again [2].

2.7.1. SCREEN: 2026-02-01 15-41-21.mp4 05:38 – 05:58, 06:01 – 06:10 *Video Editor: Please speed up the footage to match the VO.*

2.7.2. SCREEN: 2026-02-01 15-41-21.mp4 06:23 – 06:31

2.8. Then, define the grid box center by selecting **Grid**, choosing **Grid Box**, and clicking **Centre**, then select an atom and enter the grid box coordinates [1]. Save the grid parameter as a GPF file [2].

2.8.1. SCREEN: 2026-02-01 15-41-21.mp4 06:36 – 07:03 *Video Editor: Please speed up the footage to match the VO.*

2.8.2. SCREEN: 2026-02-01 15-41-21.mp4 07:49 – 08:10 *Video Editor: Please speed up the footage to match the VO.*

2.9. Now, configure docking by selecting **Docking** followed by **Macromolecule**, and **Set Rigid File Name** [1]. Then open *1nmk.pdbqt*, select **Docking**, choose **1**, and load *1.pdbqt* [2].

2.9.1. SCREEN: 2026-02-01 15-41-21.mp4 08:34 – 08:38

2.9.2. SCREEN: 2026-02-01 15-41-21.mp4 08:55 – 09:09

2.10. In the **Docking** menu, select **Search Parameters**, choose **Genetic Algorithm**, review the default parameters, and click **Accept** [1]. Select **Docking**, choose **Output**, click **Lamarckian GA**, and save the docking parameter file as *1nmk.dpf* (**1-N-M-K-D-P-F**) [2].

~~2.10.1. SCREEN: 2026-02-01 15-41-21.mp4~~

2.10.2. SCREEN: 2026-02-01 15-41-21.mp4 09:41 – 09:47, 09:59-10:27

NOTE: 2.10.1 was not captured in the screen recording.

3. Docking Process Using Cygwin-I

- 3.1. In the Cygwin terminal, type `cd c:/` (*C-D-C*) and press **Enter** [1]. Then type `cd project` and press **Enter** again [2]. Next, type `cd molecules` and press **Enter** [3], then type `cd 3` and press **Enter** [4].

3.1.1. SCREEN: 2026-02-01 15-41-21.mp4 10:47 – 11:04

3.1.2. SCREEN: 2026-02-01 15-41-21.mp4 11:07 – 11:09

3.1.3. SCREEN: 2026-02-01 15-41-21.mp4 11:13 – 11:15

3.1.4. SCREEN: 2026-02-01 15-41-21.mp4 11:19 – 11:21

- 3.2. Type the auto-grid command to generate grid maps and press **Enter** to execute AutoGrid [1]. After the execution, type `tail -f 1nmk.glg` (*tail-F-1N-M-K-G-L-G*) and press **Enter** to view the log progress [2].

3.2.1. SCREEN: 2026-02-01 15-41-21.mp4 12:21 – 12:24

3.2.2. SCREEN: 2026-02-01 15-41-21.mp4 12:37 – 12:52

- 3.3. Type the command for molecular docking simulation to generate docking log file and press **Enter** to start AutoDock [1]. Then, type `tail -f 1nmk.dlg` (*tail F-1-N-M-K-D-L-G*) and press **Enter** to monitor the docking process [2].

3.3.1. SCREEN: 2026-02-01 15-41-21.mp4 13:40 – 13:42

3.3.2. SCREEN: 2026-02-01 15-41-21.mp4 13:50 – 13:54

- 3.4. After creating all DLG files, go to folder 1 and open the DLG file in WordPad [1]. Press **Ctrl + F** (*control plus F*), type the search keyword, and press **Enter** three times to reach the RMSD table [2].

3.4.1. SCREEN: 2026-02-01 15-41-21.mp4 14:52 – 14:54

3.4.2. SCREEN: 2026-02-01 15-41-21.mp4 14:55 – 15:20

- 3.5. Now, open a new Microsoft Excel window [1] and create three columns titled Number, Minimum Binding Energy and Run [2]. Copy the binding energy and corresponding run information for each molecule from the RMSD table [3].

NOTE: 3.5.1 and 3.5.2 were not captured in the screen recording.

~~3.5.1. SCREEN: Show opening Excel and labeling the three columns.~~

~~3.5.2. SCREEN: Zoom on column headers "Number," "Minimum Binding Energy," and "Run."~~

3.5.3. SCREEN: 2026-02-01 15-41-21.mp4 15:40 – 15:43

- 3.6. Compile the top 20 molecules in a spreadsheet to include columns for serial number, minimum binding energy, and run [2].

NOTE: VO merged as it is shown in a single shot.

3.6.1. SCREEN: 2026-02-01 15-41-21.mp4 15:55 – 16:04

4. Cygwin-ii for Top 20 Molecules

- 4.1. In the Cygwin terminal, navigate to the specific molecule's folder within the project directory [1]

4.1.1. SCREEN: 2026-02-01 15-41-21.mp4 17:00 – 17:16

- 4.2. Type the command to extract docked ligand coordinates from DLG and trim the data [1]. Press **Enter** once more to finalize, confirming the creation of the 1nmk_run.pdbqt (*run-P-D-B-Q-T*) file [2].

4.2.1. SCREEN: 2026-02-01 15-41-21.mp4 17:22 – 17:41

4.2.2. SCREEN: 2026-02-01 15-41-21.mp4 17:55 – 18:01

- ~~4.3. Type the command for cutting the extra columns and press **Enter** to generate the final PDB file [1].~~ Verify that the 1nmk_run.pdb file is created in the same folder [2].

~~4.3.1. SCREEN: Show typing and executing the full command in the terminal.~~

4.3.2. SCREEN: 2026-02-01 15-41-21.mp4 19:42 – 19:58

NOTE: 4.3.1 was not captured in the screen recording.

- 4.4. Next, open the **PyMOL 2.5** (*pie-mol-2 point 5*) software and the autodock from the desktop [1] and open the receptor file to select the receptor structure for visualization [2].

4.4.1. SCREEN: 2026-02-01 15-41-21.mp4 24:12 – 24:38

4.4.2. SCREEN: PyMOL Section.mp4 0:04 – 0:30

- 4.5. Now, open the corresponding ligand configuration file for the chosen molecule [1] and display the loaded ligand in the 3D workspace [2].

4.5.1. SCREEN: PyMOL Section.mp4 0:35 – 0:49

4.5.2. SCREEN: PyMOL Section.mp4 0:50 – 0:54

- 4.6. Select **Hide Everything** from the menu [1]. Import the file to Maestro to visualize the structure and then click on **Ligand Interaction** to get the ligand interaction diagram [2].

4.6.1. SCREEN: PyMOL Section.mp4 0:55 – 0:59

4.6.2. SCREEN: PyMOL Section.mp4 1:23 - 1:56

5. UCSF Chimera Software for Visualization of H-Bond

- 5.1. Open the **UCSF Chimera** software from the desktop [1]. Select **File** followed by **Open**, navigate to drive c:/Project/Molecules (*C, project and molecules*), select the file 1nmk.pdb (*1-N-M-K-P-D-B*), and click **Open** [2].

5.1.1. SCREEN: 2026-02-01 15-41-21.mp4 30:27 – 30:30

5.1.2. SCREEN: 2026-02-01 15-41-21.mp4 30:36 – 30:55

- 5.2. ~~From within UCSF Chimera, select the appropriate drive where the molecular files are stored [1] and open the corresponding protein structure in the program window [2]. From the menu, select **Residue**, and choose **UNL Zoom** to focus on the UNL residue region [3].~~ NOTE: Shots [1] and [2] are covered in the previous shot itself. No separate action is needed.

~~5.2.1. SCREEN: Show selecting the storage drive and loading the protein file.~~

~~5.2.2. SCREEN: Display the 3D molecular structure of the protein appearing in the Chimera workspace.~~

5.2.3. SCREEN: 2026-02-01 15-41-21.mp4 30:58 – 31:02

- 5.3. Now, select **Zone**, then press **OK** to confirm [1]. Next, select **Action**, choose **Label**, then select **Residue**, and choose **Name + Specifier** to label the residues [2]. **Open Tools**, select **Structure Analysis**, and then click **Find H-Bond** [3]. In the default H-bond settings

Commented [DD1]: Since this software got stuck, author used another software. Therefore the screen captures do not correspond to the given text.

window, check the boxes for **Color H-bonds that do not meet precise criteria** and **Only find H-bonds with selected [4]**.

5.3.1. SCREEN: 2026-02-01 15-41-21.mp4 31:03 – 31:09

5.3.2. SCREEN: chimera.mp4 0:30 - 0:36

5.3.3. SCREEN: chimera.mp4 0:37 - 0:43

5.3.4. SCREEN: chimera.mp4 0:44 - 0:52

5.4. Clear the zone selection to begin a new survey **[1]**. Then, select the zone parameters and zoom into the molecular display and observe the blue and orange lines representing hydrogen bonds **[2]**. Finally, examine the visualized structure and count the total number of visible hydrogen bonds **[3]**.

5.4.1. SCREEN: chimera.mp4 1:08 - 1:13

5.4.2. SCREEN: chimera.mp4 1:14 - 1:40

5.4.3. SCREEN: chimera.mp4 1:41 - 1:49

Results

6. Results

6.1. The docking conformation of the ligand-receptor complex was visualized using PyMOL 2.5, showing 109-hydroxy-6-hydroxymethyl bound within the active pocket of the 1nmk receptor [1].

6.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the Labels "HIS 54.A" and "LYS 151.A"*

6.2. The 45-hydroxy-mangiferonic acid ligand formed hydrogen bonds with the 1nmk receptor, particularly with Arginine 55 at a distance of 1.53 angstroms [1].

6.2.1. LAB MEDIA: Figure 2B. *Video editor: Zoom in on the label "ARG 55.A CG-CD 1.533Å,"*

6.3. Two-dimensional structural analysis illustrated the amino acids with which the ligands interacted through hydrogen bonding [1].

6.3.1. LAB MEDIA: Figure 2C and D

6.4. Molecular dynamics simulations confirmed the stability of the ligand-receptor complex through the analysis of the radius of gyration over 1000 picoseconds [1].

6.4.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the 'Rg' trace.*

6.5. The RMSD graph showed consistent stabilization of the complex after 1 nanosecond of simulation, indicating steady protein-ligand binding [1].

6.5.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the gradual plateauing of the curve after 0.8 mark on the X-axis.*

6.6. The average density of the system was approximately 1,019 kilograms per cubic meter, confirming equilibrium under NVT conditions [1] and the pressure of the system remained stable during NPT equilibration for 100 picoseconds [2].

6.6.1. LAB MEDIA: Figure 3C.

6.6.2. LAB MEDIA: Figure 3D.

6.7. The temperature stabilized around 300 Kelvin during the NVT phase, confirming

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successful thermal equilibration [1].

6.7.1. LAB MEDIA: Figure 3E.

6.8. The ligand-protein complex trajectory box was visualized after the simulation, showing the distribution of water molecules surrounding the docked complex [1].

6.8.1. LAB MEDIA: Figure 3F. *Video editor: Highlight the blue dumbbell shaped central region*