FINAL SCRIPT: APPROVED FOR FILMING

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Project Page Link: https://review.jove.com/account/file-uploader?src=20488118

Title: Incorporating Target Protein Structure Flexibility and Dynamics in Computational Drug Discovery Using Ensemble-Based Docking Analysis

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Author Questionnaire

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

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

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 21
Number of Shots: 39

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Saharuddin Bin Mohamad:** Our research focuses on applying computational techniques to design more effective drugs, aiming to accelerate drug discovery and ultimately improve treatment outcomes and patients' quality of life.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What research gap are you addressing with your protocol?

1.2. **Ahmad Fadhlurrahman Ahmad Hidayat:** Current computer-aided drug design often overlooks target protein flexibility. The discussed protocol addresses this gap by incorporating multiple protein conformations derived from molecular dynamics simulations.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.11*

What research questions will your laboratory focus on in the future?

1.3. **Ahmad Fadhlurrahman Ahmad Hidayat:** Ensemble-based drug design improves accuracy by considering protein flexibility. We aim to integrate artificial intelligence for faster, personalized, and more effective drug discovery in the future.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.4. **Ahmad Fadhlurrahman bin Ahmad Hidayat:** I believe that publishing in Jove helps researchers to share more knowledge regarding techniques and protocols. The use of video introduces a distinct notion for publication, in contrast to traditional black and white manuscripts.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

AUTHOR: Please deliver the testimonial in both Malay and English
Videographer: Please capture the testimonial in both Malay and English

Protocol

2. Root-Mean-Square Deviation (RMSD)-Based Clustering Analysis

Demonstrator: Ahmad Fadhlurrahman Ahmad Hidayat

2.1. To begin, launch the Avogadro software on a computer system **[1]**.

2.1.1. WIDE: Talent launching the Avogadro software on a computer system.

2.2. For cluster analysis, type the command given on-screen **[1]**. When prompted, type **1** for the protein group to calculate least squares fit and root mean square deviation or RMSD *(R-M-S-D)*, then type **1** again for system output **[2]**.

2.2.1. TEXT ON PLAIN BACKGROUND:
Command: gmx cluster –s md.tpr –f md_center.xtc –g cluster.log –sz cluster-size.xvg –clid clus-id.xvg –cl cluster.pdb –cutoff 1.0

2.2.2. SCREEN: 67174_SCREEN_2.2-(1).mp4 00:33-00:40

2.3. Open the **cluster-size.xvg** *(cluster-size-dot-x-v-g)* file **[1]**. If the number of clusters is **low**, **increase** the RMSD cutoff value. Alternatively, if the number is **high**, **decrease** the cutoff **[2]**.

2.3.1. SCREEN: 67174_SCREEN_2.3.mp4 00:05-00:09

2.3.2. SCREEN: 67174_SCREEN_2.3.mp4 00:18-00:49
Video Editor: Please speed up if necessary

2.4. Perform grace analysis with the command shown on-screen using different RMSD cutoff values as needed **[1-TXT]**.

2.4.1. SCREEN: 67174_SCREEN_2.4.mp4 00:00-00:19
AND
TEXT ON PLAIN BACKGROUND:
Command: xmgrace totalenergy.xvg

Video Editor: Please play both shots side by side

2.5. Now, open the **Chimera** software and search for **cluster.pdb** *(cluster-dot-P-D-B)* **[1]**. Click on **Presents** and **Publication 1 (silhouette, rounded ribbon)** *(Publication-1-silhouette-rounded-ribbon)* for visual representation **[2]**.

2.5.1. SCREEN: 67174_SCREEN_2.5.mp4 00:00-00:14

2.5.2. SCREEN: 67174_SCREEN_2.5.mp4 00:15-00:22

2.6. ~~Navigate to **File, Save Image** and click on **Save** to export the rendered image **[1].**~~ Sequentially click **Select, Chain, (no ID)** *(No-I-D)* followed by **cluster.pdb (#10)** *(cluster-dot-P-D-B-Number-ten)*, then **Select** and **Invert (selected models)** *(invert-selected-models)* **[2]**. Go to **Actions** then press **Atoms/Bonds** *(Atoms-Bonds)* and click on **delete** to isolate the chain **[3]**.

2.6.1. SCREEN: 67174_SCREEN_2.6.mp4.
NOTE: Shot not provided

2.6.2. SCREEN: 67174_SCREEN_2.6.mp4. 00:00-00:11

2.6.3. SCREEN: 67174_SCREEN_2.6.mp4. 00:12-00:16

2.7. Now select **File,** click on **Save PDB** *(Save-P-D-B)* and press **Save** to save the file. Name the file **cluster1.pdb** *(Cluster-one-dot-P-D-B)* **[1-TXT]**.

2.7.1. SCREEN: 67174_SCREEN_2.7.mp4 00:00-00:16
TXT: Repeat for clusters 2 to 4

2.8. For ensemble-based docking, launch the **AutoDock Tools** software to open it **[1]**. Place files **cluster1.pdb** and **ligand.pdb** into a new folder **[2]**.

2.8.1. SCREEN: 67174_SCREEN_2.8.mp4 00:02-00:11

2.8.2. SCREEN: 67174_SCREEN_2.8.mp4 00:12-00:26

2.9. Now click on **File, Preferences** and **Set**. In the pop-up, paste the folder address into the **Startup Directory** field and click **Set [1]**. Click the **blue folder icon**, choose **cluster1.pdb**, and click **Open [2].**
2.9.1. SCREEN: 67174_SCREEN_2.9.mp4 00:06-00:22
2.9.2. SCREEN: 67174_SCREEN_2.9.mp4. 00:28-00:33

2.10. Go to **Edit ,** then press **Charges**, **Add Kollman Charges**, and click **OK [1]**.
2.10.1. SCREEN: 67174_SCREEN_2.10.mp4 00:00-00:07

2.11. Click on **Grid, Macromolecules, Choose**, select **cluster1** in the **Choose Macromolecules** box, then press **Select Molecules.** Click **OK** to generate a modified **AutoDock4 macromolecule** file **[1]**. Save it as **cluster1.pdbqt** *(cluster-1-dot-p-d-b-q-t)* **[2]**.
2.11.1. SCREEN: 67174_SCREEN_2.11.mp4 00:00-00:08
2.11.2. SCREEN: 67174_SCREEN_2.11.mp4 00:08-00:17

2.12. Next, empty the workspace by clicking **Edit** then press **Delete** and **Delete All Molecules**, before clicking **Continue [1]**. Then press **Ligand, Input, Open**. When a **Ligand file for Autodock4** folder appears, select **ligand.pdb**, and click **Open** and then **OK [2]**.
2.12.1. SCREEN: 67174_SCREEN_2.12.mp4. 00:00-00:06
2.12.2. SCREEN: 67174_SCREEN_2.12.mp4. 00:07-00:16

2.13. Now choose **Ligand, Torsion Tree** and **Detect Root** to define the torsional flexibility of the ligand **[1]**. Go to **Ligand,Output , Save as PDBQT** *(Save-As-P-D-B-Q-T)* and save the **Formatted Autotors Molecules** folder as **ligand.pdbqt [2]**.
2.13.1. SCREEN: 67174_SCREEN_2.13.mp4 00:00-00:06
2.13.2. SCREEN: 67174_SCREEN_2.13.mp4 00:07-00:24

2.14. After emptying the workspace, open the **cluster1.pdbqt** file by clicking on **Grid, Macromolecules** and **Open**, then press **Yes** and **OK [1]**. Navigate to **Grid** again and press the **Set Map Types** and choose **Open ligand.** Select and **Open ligand.pdbqt [2].**
2.14.1. SCREEN: 67174_SCREEN_2.14.mp4 00:07-00:17
2.14.2. SCREEN: 67174_SCREEN_2.14.mp4 00:18-00:26

2.15. Now navigate to the **Grid Box** option under Grid. In the **Grid Options** box, set **number of points** in the **x, y, and z dimensions** to **120**, and **spacing** to **0.375** angstrom. Leave the center settings as default **[1]**. Then click **File** and **Close Saving Current [2]**.
2.15.1. SCREEN: 67174_SCREEN_2.15.mp4 00:00-00:28
2.15.2. SCREEN: 67174_SCREEN_2.15.mp4 00:30-00:35

2.16. Go to **Grid, Output** and press **Save GPF** *(Save-G-P-F)*. When the **Grid Parameter Output** file appears, enter **grid.gpf** *(grid-dot-G-P-F)* as the file name, and click **Save [1]**. Next, click on **Run** and **Run AutoGrid.** At **Parameter Filename** tab, click **Browse.** Open the **grid.gpf** file **[2].**
2.16.1. SCREEN: 67174_SCREEN_2.15.mp4 00:00-00:14
2.16.2. SCREEN: 67174_SCREEN_2.15.mp4 00:15-00:24

2.17. Now, **Browse** through the **Program Pathname.** Search for **autogrid4.exe** and click **Open** and **Launch [1].** Sequentially click on **Docking** followed by **Macromolecules** and **Set Rigid Filenames**. When a **PDBQT Macromolecules file** appears, select **cluster1.pdbqt**, and click **Open [2]**.
2.17.1. SCREEN: 67174_SCREEN_2.17.mp4 00:00-00:08
2.17.2. SCREEN: 67174_SCREEN_2.17.mp4 00:38-00:48

2.18. **Choose** the **Ligand** from the **Docking** menu. When the **Choose Ligands** box appears, select **ligand**, and click on **Select Ligand**, then press **Accept** ~~in **the AutoDpf4 Ligand Parameter** dialog~~ **[1]**. Now, navigate to **Genetic Algorithm** from **Docking**. When the **Genetic Algorithm Parameters** box appears, set **GA** *(G-A)* **Runs** to **100**, and click **Accept [2].**
2.18.1. SCREEN: 67174_SCREEN_2.18.mp4 00:00-00:11
2.18.2. SCREEN: 67174_SCREEN_2.18.mp4 00:12-00:21

2.19. Click on **Docking, Output, Lamarckian GA(4.2)** *(La-Mark-Eeyan-G-A-Four-Point-Two)* **[1].** When an **Autodock4.2 GALS** *(Auto-Dock-Four-Point-Two-Gals)* **Docking Parameter Output** file appears, name it as **docking.dpf** *(docking-dot-D-P-F)*, and click **Save [2]**.

2.19.1. SCREEN: 67174_SCREEN_2.19.mp4 00:00-00:08
2.19.2. SCREEN: 67174_SCREEN_2.19.mp4 00:08-00:17

2.20. Now press **Run** and **Run AutoDock**. A **Run Autodock** box will appear **[1]**. At **Parameter Filename** click **Browse.** When an **autodock4 Parameter** file appears, select **docking.dpf** and click on **Open [2].** At **Program Pathname** click **Browse**. An **autodock4** file will appear. Search for **autodock4.exe** *(Auto-dock-Four-dot-E-X-E)* and click **Open** followed by **Launch [3].**
2.20.1. SCREEN: 67174_SCREEN_2.20.mp4 00:00-00:05
2.20.2. SCREEN: 67174_SCREEN_2.20.mp4 00:06-00:10
2.20.3. SCREEN: 67174_SCREEN_2.20.mp4 00:10-00:20

2.21. Delete all molecules as demonstrated previously and repeat the process for all cluster files **[1].**
2.21.1. SCREEN: 67174_SCREEN_2.21.mp4 00:47-00:55, 00:00-00:17

Results

3. Representative Results

3.1. The chemical structure and the 3D structural representation of flavokawain B *(Flavo-Ka-Vain-B)* and lysozyme at the initial state before molecular dynamics simulation was obtained **[1]**.

3.1.1. LAB MEDIA: Figure 8 A and B

3.2. The total energy of the protein structure was stable during the simulation **[1]** and root mean square deviation stabilized after 20 nanoseconds **[2]**. Root mean square fluctuation revealed high flexibility in regions between residues 40 to 50, 60 to 80, and 100 to the end **[3]**.

3.2.1. 1 LAB MEDIA: Figure 9A

3.2.2. 1 LAB MEDIA: Figure 9B. *Video Editor: please highlight portion of graph going up until 20 nanosecond mark*

3.2.3. LAB MEDIA: Figure 9C. *Video editor: Please highlight the three peak regions of the line graph that occur between 40–50, 60–80, and 100–end along the x-axis.*

3.3. A total of 15 structural clusters were obtained from root mean square deviation-based clustering of 10,001 trajectory frames, with the largest cluster containing 5,818 members **[1]**. Superimposed conformations of all clusters showed visible structural variations among the trajectories **[2]**.

3.3.1. LAB MEDIA: Table 1. *Video editor: Circle the top row where cluster number 1 is listed with 5,818 members.*

3.3.2. LAB MEDIA: Figure 10

3.4. Molecular docking of flavokawain B with the representative structures of the top 4 clusters showed consistent binding at the same site across all conformations **[1]**, with cluster 2 showing the lowest binding energy of minus 29.37 kilojoules per mole **[2]**.

3.4.1. LAB MEDIA: Figure 11. *Video editor: Highlight the green structures in all four panels (A–D)*

3.4.2. LAB MEDIA: Table 2. *Video editor: Circle the row with cluster number 2*

3.5. Electrostatic surface mapping confirmed identical binding sites in all cluster conformations, with flavokawain B nested in the same pocket region **[1]**.

3.5.1. LAB MEDIA: Figure 12. *Video editor: Highlight the orange parts in all 4 blue molecules*

3.6. Detailed interaction analysis showed flavokawain B binding was stabilized by several surrounding residues including Ala-31 *(Alanine-Thirty-one)*, Glu-35 *(Glutamine-thirty-five)*, Leu-56 *(Leucine-Fifty-Six)*, Gln-57 *(Gamma-carboxy-glutamic acid-Fifty-seven)* ,

Ile-58 *(Isoleucine-fifty-Eight)*, Ala-95 *(Alanine-Ninety-Five)*, Ile-98 *(Isoleucine-Ninety-Eight)*, Trp-108 *(Tryptophan-one-zero-Eight)*,Val-109 *(Valine-one-zero-nine)*,Ala-110 *(Alanine-one-ten)*, Trp-111 *(Tryptophan-one-eleven)*, and Arg-114 *(Arginine-one-fourteen)* **[1]**.

3.6.1. LAB MEDIA: Figure 13B. *Video editor: Please sequentially highlight each green and purple circle for corresponding VO narration*

Pronunciation Guide

1. **Ensemble**

- Pronunciation link: https://www.merriam-webster.com/dictionary/ensemble
- IPA: /ɑnˈsɑːmbəl/
- Phonetic Spelling: ahn-sahm-buhl

2. **Conformations**

- Pronunciation link: https://www.howtopronounce.com/conformations
- IPA: /ˌkɑːnfɔːrˈmeɪʃənz/
- Phonetic Spelling: kawn-for-may-shunz

3. **Avogadro**

- Pronunciation link: https://www.howtopronounce.com/avogadro
- IPA: /ˌævəˈgɑːdroʊ/
- Phonetic Spelling: av-uh-gah-droh

4. **RMSD (Root-Mean-Square Deviation)**

- Pronunciation link: https://www.howtopronounce.com/rmsd
- IPA: /ˌɑːrˌɛmˌɛsˈdi/
- Phonetic Spelling: ar-em-es-dee

5. **Xmgrace**

- Pronunciation link: https://www.howtopronounce.com/xmgrace
- IPA: /ˌɛksɛmˈgreɪs/
- Phonetic Spelling: eks-em-grayce

6. **Chimera**

- Pronunciation link: https://www.merriam-webster.com/dictionary/chimera
- IPA: /kaɪˈmɪrə/
- Phonetic Spelling: kai-mih-ruh

7. **AutoDock**

- Pronunciation link: https://www.howtopronounce.com/autodock
- IPA: /ˈɔːtoʊˌdɑːk/
- Phonetic Spelling: aw-toh-dawk

8. **PDBQT**

- Pronunciation link: https://www.howtopronounce.com/pdbqt
- IPA: /ˌpiːdiːbiːkjuːtiː/
- Phonetic Spelling: pee-dee-bee-kyoo-tee

9. **Flavokawain B**

- Pronunciation link: https://www.howtopronounce.com/flavokawain
- IPA: /ˌflævoʊˈkɑːweɪn/
- Phonetic Spelling: flah-voh-kah-wayn

10. **Lysozyme**

- Pronunciation link: https://www.merriam-webster.com/dictionary/lysozyme
- IPA: /ˈlaɪsəˌzaɪm/
- Phonetic Spelling: lai-suh-zyme

11. **Alanine**

- Pronunciation link: https://www.merriam-webster.com/dictionary/alanine
- IPA: /ˈæləˌniːn/
- Phonetic Spelling: al-uh-neen

12. **Glutamine**

- Pronunciation link: https://www.merriam-webster.com/dictionary/glutamine
- IPA: /ˈgluːtəˌmiːn/
- Phonetic Spelling: gloo-tuh-meen

13. **Leucine**

- Pronunciation link: https://www.merriam-webster.com/dictionary/leucine
- IPA: /ˈluːˌsiːn/
- Phonetic Spelling: loo-seen

14. **Isoleucine**

- Pronunciation link: https://www.merriam-webster.com/dictionary/isoleucine
- IPA: /ˌaɪsoʊˈluːˌsiːn/
- Phonetic Spelling: eye-soh-loo-seen

15. **Tryptophan**

- Pronunciation link: https://www.merriam-webster.com/dictionary/tryptophan
- IPA: /ˈtrɪptəˌfæn/

- Phonetic Spelling: trip-tuh-fan

16. **Valine**

- Pronunciation link: https://www.merriam-webster.com/dictionary/valine
- IPA: /ˈveɪˌliːn/
- Phonetic Spelling: vay-leen

17. **Arginine**

- Pronunciation link: https://www.merriam-webster.com/dictionary/arginine
- IPA: /ˈɑːrdʒəˌniːn/
- Phonetic Spelling: ar-juh-neen

18. **Electrostatic**

- Pronunciation link: https://www.merriam-webster.com/dictionary/electrostatic
- IPA: /ɪˌlɛktroʊˈstætɪk/
- Phonetic Spelling: ih-lek-troh-sta-tik

19. **Molecular Dynamics**

- Pronunciation link: https://www.howtopronounce.com/molecular-dynamics
- IPA: /məˈlɛkjələr daɪˈnæmɪks/
- Phonetic Spelling: muh-lek-yuh-lur dai-na-miks

20. **Docking**

- Pronunciation link: https://www.merriam-webster.com/dictionary/docking
- IPA: /ˈdɑːkɪŋ/
- Phonetic Spelling: daw-king