FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 68003
Scriptwriter Name: Debopriya Sadhukhan
Project Page Link: https://review.jove.com/account/file-uploader?src=20740963

Title: Application of I TASSER, trRosetta, UCSF Chimera, HADDOCK Server, and HEX Loria for De Novo and In Silico Design of Proteins

Authors and Affiliations:

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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**

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

Current Protocol Length
Number of Steps: 22
Number of Shots: 07

Introduction

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

REQUIRED:

1.1. **Roberto Álvarez-Martínez:** This research aims to generate structural models of proteins with antimicrobial functions. These models are used to perform specific analyses that help determine the usefulness of each protein as a treatment before in vitro testing.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.3.*

What technologies are currently used to advance research in your field?

1.2. **Roberto Álvarez-Martínez:** Currently, research in this field relies on in-silico procedures for the validation of designed proteins; however, there are no established tools specifically developed to support this part of the process.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 3.*

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

1.3. **Roberto Álvarez-Martínez:** Our future research will focus on the biological network inference and the microbiota interactions between the host and environment on multilayer networks. This work will be supported by mlBioNets, an R package developed by colleagues in the laboratory.

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.

Protocol

2. Predicting 3D Structure Using I-TASSER and trRosetta

Demonstrator: Jimena R-Villarreal

2.1. To begin, visit the I-TASSER *(eye-Tass-er)* server for protein structure and function prediction **[1]**. Submit the molecular target or designed sequence as a FASTA *(fast-uh)* format file, a text file, or by pasting it directly into the input field **[2]**. Assign a unique name to the sequence **[3]** and click **Run I-TASSER** to allow the program to analyze the sequence. **[4]**.

2.1.1. WIDE: Talent (sitting in front of a computer) opening a web browser and navigating to the I-TASSER server. *Videographer: Please record the screen for this shot.*

2.1.2. SCREEN: 68003_screenshot_1.mp4 00:16-00:29.

2.1.3. SCREEN: 68003_screenshot_1.mp4 00:40-00:59. *Video Editor: Blur the author's email ID.*

2.1.4. SCREEN: 68003_screenshot_1.mp4 01:02-01:15.

2.2. To predict the 3D structure using trRosetta *(tee-are roh-zet-uh)*, access the trRosetta server **[1]**. Enter the target receptor sequence as a FASTA format file, a text file, an MSA *(M-S-A)* format file, or paste it directly into the input field on the server **[2]**. After registering using an institutional email, assign a name to the structurally predicted protein **[3-TXT]**.

2.2.1. SCREEN: 68003_screenshot_2.mp4 00:44-00:52.

2.2.2. SCREEN: 68003_screenshot_2.mp4 01:25-01:31.

2.2.3. SCREEN: 68003_screenshot_2.mp4 01:50-01:58. **TXT: A confirmation email will be sent upon successful submission** *Video Editor: Blur the author's email ID.*

2.3. Ensure the option to exclude templates is selected **[1]** and choose **Run trRosettaX-single** *(tee-are roh-zet-uh Ex-Single)* to exclude the use of any homologous sequences and templates **[2]**. Click Submit to initiate the protein structure prediction process **[3]**.

2.3.1. SCREEN: 68003_screenshot_2.mp4 02:03-02:08.

2.3.2. SCREEN: 68003_screenshot_2.mp4 02:09-02:15.

2.3.3. SCREEN: 68003_screenshot_2.mp4 02:20-02:35.

2.4. In the prediction result, verify that the TM-score is a measure of model quality **[1]**. Then, examine contact maps **[2]**, distance maps per amino acid **[3]**, and rotation maps for alpha and beta carbons at angles omega, theta, and phi **[4]**.

2.4.1. SCREEN: 68003_screenshot_3.mp4 00:10-00:27.

2.4.2. SCREEN: 68003_screenshot_3.mp4 00:30-00:41.

2.4.3. SCREEN: 68003_screenshot_3.mp4 00:43-00:48

2.4.4. SCREEN: 68003_screenshot_3.mp4 00:50-01:02.

3. Molecular Docking and Interaction Analysis Using HADDOCK

3.1. Open a web browser and navigate to the HADDOCK *(Haddock)* web server **[1]**. Click on **Submit a new job**, then enter a Job name and the number of molecules **[2].** Upload the PDB structures of the molecules for docking, leave the default settings unchanged, and click on **Next [3]**. Enter active and passive amino acid residues for both Molecule 1 and Molecule 2, and click on **Next [4]**.

3.1.1. SCREEN: 68003_screenshot_4.mp4 00:07-00:12.

3.1.2. SCREEN: 68003_screenshot_4.mp4 00:26-00:47.

3.1.3. SCREEN: 68003_screenshot_4.mp4 00:49-01:16.

3.1.4. SCREEN: 68003_screenshot_4.mp4 01:40-02:17.

3.2. In the **Docking Parameters** section, leave the default settings for all the parameters, such as **Distance restraints**, **Sampling parameters**, **Clustering parameters**, etc, unchanged and click on **Submit** to start the docking process **[1]**.

3.2.1. SCREEN: 68003_screenshot_4.mp4 02:18-03:00. *Video Editor: Speed up the video as needed.*

3.3. Open the results page and review the docking results **[1].** After downloading the ligand-receptor docking file, open a structural visualization software such as UCSF Chimera or PyMOL *(Py-Mol)* **[2]**, upload the docking file, and visualize the structure model **[3]**.

3.3.1. SCREEN: 68003_screenshot_5.mp4 00:08-00:28, 01:28-01:46.

3.3.2. SCREEN: 68003_screenshot_5.mp4 02:05-02:15.

3.3.3. SCREEN: 68003_screenshot_5.mp4 02:16-02:32, 03:31-03:41, 04:59-05:02, 05:20-05:24, 05:35-05:38.

Results

4. Results

4.1. The trRosetta model of the Sodium-hydrogen antiporter revealed a highly ordered tertiary structure composed primarily of alpha helices, forming a tightly packed transmembrane bundle **[1]**. This suggests the protein's functional role as a membrane-embedded ion transporter **[2]**.

4.1.1. LAB MEDIA: Figure 2. *Video Editor: Only show the left image.*

4.1.2. LAB MEDIA: Figure 2. *Video Editor: Only show the left image.*

4.2. The model's reliability is supported by high Local Difference Test values above 80% across the central region **[1]**, with reduced confidence at both termini **[2]**, indicating possible flexibility **[3]**.

4.2.1. LAB MEDIA: Figure 2. *Video Editor: Highlight the entire middle portion of the blue plot (including the dips) where the curve stays high and nearly flat. Make sure the start and end points of this highlighted part are above 80 LDDT, showing good prediction accuracy.*

4.2.2. LAB MEDIA: Figure 2. *Video Editor: Highlight the rest of the portion of the blue plot (the left and right ends that are below 80 LDDT).*

4.2.3. LAB MEDIA: Figure 2.

4.3. Contact and distance maps confirm stable folding, with consistent diagonal and clustered patterns **[1]**.

4.3.1. LAB MEDIA: Figure 3.

4.4. Molecular docking of the designed antifungal peptide into the receptor's extracellular domain results in a stable complex **[1]**, as evidenced by a favorable HADDOCK *(Haddock)* score of minus 73 and a low root-mean-square deviation of 0.7 angstroms **[2]**.

4.4.1. LAB MEDIA: Figure 5. *Video Editor: Highlight the colorful chemical structure inside the dotted red box (not the blue ribbon-like structure).*

4.4.2. LAB MEDIA: Figure 5. *Video Editor: Highlight "HADDOCK score", "-73", "RMSD", and "0.7" from the table.*

4.5. Measured distances confirm close contacts between specific amino acids **[1]**, with the shortest observed between tyrosine 407 *(four-oh-seven)* and cysteine 13 *(thirteen)* **[2]**.

4.5.1. LAB MEDIA: Figure 6. *Video Editor: Highlight the entire Distances column from the table, the green dotted lines, and the corresponding numbers in the image indicating distances.*

4.5.2. LAB MEDIA: Figure 6. *Video Editor: Highlight row 1 from the table and the green dotted line that corresponds to 5.866 A in the image.*

Pronunciation Guides:

1. Antiporter
Pronunciation link:
https://www.howtopronounce.com/antiporter
IPA: /ˈæn.tiˌpɔːr.tər/
Phonetic Spelling: AN-tee-por-ter
2. Tertiary
Pronunciation link:
https://www.merriam-webster.com/dictionary/tertiary
IPA: /ˈtɜrʃiˌɛri/
Phonetic Spelling: TUR-shee-air-ee
3. Helices
Pronunciation link:
https://www.merriam-webster.com/dictionary/helix
IPA: /ˈhiːlɪˌsiz/
Phonetic Spelling: HEE-lih-seez
4. Membrane
Pronunciation link:
https://www.merriam-webster.com/dictionary/membrane
IPA: /ˈmɛmˌbreɪn/
Phonetic Spelling: MEM-brayn
5. Ion
Pronunciation link:
https://www.merriam-webster.com/dictionary/ion
IPA: /ˈaɪˌɑn/
Phonetic Spelling: EYE-on
6. Residue
Pronunciation link:
https://www.merriam-webster.com/dictionary/residue
IPA: /ˈrɛzɪˌdu/
Phonetic Spelling: REZ-ih-doo
7. Hydrogen
Pronunciation link:
https://www.merriam-webster.com/dictionary/hydrogen
IPA: /ˈhaɪdrədʒən/
Phonetic Spelling: HAI-druh-jen

8. Tyrosine
Pronunciation link:
https://www.merriam-webster.com/dictionary/tyrosine
IPA: /ˈtaɪrəˌsiːn/
Phonetic Spelling: TIE-ruh-seen
9. Cysteine
Pronunciation link:
https://www.merriam-webster.com/dictionary/cysteine
IPA: /ˈsɪstiːn/
Phonetic Spelling: SIS-teen
10. Deviation
Pronunciation link:
https://www.merriam-webster.com/dictionary/deviation
IPA: /ˌdiːviˈeɪʃən/
Phonetic Spelling: DEE-vee-ay-shun
11. Chimera
Pronunciation link:
https://www.merriam-webster.com/dictionary/chimera
IPA: /kaɪˈmɪrə/
Phonetic Spelling: kye-MIR-uh