

Submission ID #: 69350

Scriptwriter Name: Sulakshana Karkala

Project Page Link: <https://review.jove.com/account/file-uploader?src=21151798>

Title: Amyloid and the Cross-Beta Architecture

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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**

If **Yes**, we will need you to record using screen recording software.

We recommend using the screen capture program [OBS](https://obsproject.com/). JoVE's tutorial for using OBS Studio is provided at this link: <https://review.jove.com/v/5848/screen-capture-instructions-for-authors?status=a7854k>

As these files are necessary for finalizing your script, please upload all screen-captured video files to your project page as soon as possible:

<https://review.jove.com/account/file-uploader?src=21151798>

- 3. Filming location:** Will the filming need to take place in multiple locations? **NO**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **NO**

Current Protocol Length

Number of Steps: 31

Number of Shots: 56

Introduction

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

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Louise Serpell:** Our research uses X-ray fiber diffraction to elucidate amyloid fibril cross- β structures and uncover their underlying molecular organization.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Louise Serpell:** Protocol uses X-ray fiber diffraction to confirm cross- β patterns and elucidate fibril architecture.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 3.2.1*

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Louise Serpell:** This protocol enables structural analysis of amyloid fibrils, from confirming cross- β architecture to developing detailed molecular models.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Louise Serpell:** Proper sample alignment is critical, as it directly determines the quality and structural detail obtainable from X-ray fiber diffraction data.
 - 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.11.1.*

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Louise Serpell:** This work enables atomic-resolution amyloid modeling and integration with complementary methods, such as AFM, to resolve multiscale structural details.
 - 1.5.1. 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

SCREEN/SCOPE timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Pallavi Sharma) for queries regarding lab media.

2. Alignment of Amyloid Fibrils for X-ray Fiber Diffraction Data Collection

Demonstrator: Louise Serpell

- 2.1. To begin, align the protein or peptide fibrils to produce a bundle whose fiber axes are ideally all parallel [1].
 - 2.1.1. WIDE: Talent arranging materials on the bench to prepare for fibril alignment.
- 2.2. Melt candle wax with a hot plate until it liquifies [1]. Using a glass cutter, cut 1 millimeter borosilicate glass capillaries to a length of about 2 to 3 centimeters [2].
 - 2.2.1. Talent heating candle wax on a hot plate until liquid.
 - 2.2.2. Talent cutting borosilicate glass capillaries with a glass cutter.
- 2.3. Dip the cut capillaries briefly into the liquid wax [1]. Then draw the candle wax up by capillary action to approximately 1 to 2 millimeters within the capillary [2].
 - 2.3.1. Shot of capillary ends being dipped into liquid wax.
 - 2.3.2. Shot of wax being drawn into the capillary by capillary action.
- 2.4. Now use plasticine to attach the sealed capillaries horizontally inside a Petri dish [1]. Maintain a gap of 0.5 to 2 millimeters between the wax-filled ends of the capillaries [2].
 - 2.4.1. Talent attaching the sealed capillaries horizontally in a Petri dish with plasticine.
NOTE: Also still shown in uploaded IMAGE-x.png
 - 2.4.2. Shot showing the measured gap between wax-filled capillary ends.
- 2.5. With a pipette, carefully suspend a 10-microliter droplet of fibril solution between the wax-filled ends of the two capillaries [1]. Cover the Petri dish to protect the sample from dust and evaporate overnight [2].
 - 2.5.1. Talent pipetting a 10 μ L droplet between two capillaries.
 - 2.5.2. Talent placing a cover over the Petri dish.
- 2.6. Then, seal the plate to control the drying environment [1].
 - 2.6.1. Talent sealing the plate.
- 2.7. To align the amyloid fibrils in a glass capillary, fix a 1-milliliter syringe with rubber tubing to the wide end of a 0.7-millimeter diameter siliconized glass capillary [1]. Aspirate 2 to

3 centimeters of fibril suspension into the capillary [2].

2.7.1. Talent assembling syringe, tubing, and glass capillary.

2.7.2. Shot of fibril suspension being pipetted into the capillary. NOTE: 2.7.1 and 2.7.2 are filmed as one shot

2.8. Seal one end of the capillary using melted wax and leave the other end open to allow evaporation [1]. Arrange the capillary vertically and allow it to dry until a dried disk is formed [2-TXT].

2.8.1. Talent sealing one end of the capillary with wax.

2.8.2. Shot of Capillary arranged vertically in a holder. NOTE: 2.8.1 and 2.8.2 as one shot

2.9. Place a high-concentration fibril sample onto a glass slide and cover before air-drying for 24 hours [1].

2.9.1. Talent dispensing fibril sample onto a flat surface.

2.10. ~~With a scalpel, carefully lift the dried fibril film from the surface [1]. Attach the film to a glass capillary tube using glue or plasticine [2].~~

~~2.10.1. Talent lifting the film with a scalpel.~~

~~2.10.2. Shot of the film being attached to a glass capillary. NOTE: Not filmed~~

2.11. Carefully mount the fibril mat on a goniometer head and orient it so that the X-ray beam passes through the edge of the mat and parallel to the plane of the film [1].

2.11.1. Talent mounting the sample on a goniometer head and orienting it.

2.12. Examine the sample under a light microscope equipped with a cross-polariser to observe birefringence [1-TXT].

2.12.1. LAB MEDIA: IMAGE3.png

2.13. Next, attach the capillary fiber sample onto a goniometer head using plasticine [1]. Position the sample in the X-ray beam based on whether it is a fiber bundle, mat, or disk [2].

2.13.1. Talent attaching the capillary to the goniometer head.

2.13.2. LAB MEDIA: IMAGE4.png

AND

TEXT ON PLAIN BACKGROUND:

Sample rotation axis will be around the fiber axis for a fiber bundle

Fibers tend to align across the diameter of the tube in disks **NOTE: Show Figure 2C also**

A mat will have two distinct directions

Video Editor: Please play both shots side by side in a split screen

2.14. Collect a diffraction pattern using in-house X-ray equipment or synchrotron radiation [1].

2.14.1. LAB MEDIA: [IMAGE5.png](#)
-STILL provided uploaded IMAGE-5

3. X-ray Amyloid Fiber Diffraction Data Processing and Unit Cell Determination Using CLEARER

3.1. To perform data analysis, upload the file into the CLEARER (*clearer*) diffraction pattern analysis software [2].

3.1.1. SCREEN: Serpell-SCREENSHOT.MOV: 00:03-00:12

3.2. Navigate to **Diffraction Pattern** and **diffraction setting** to input diffraction settings, including pixel size, X-ray source, and sample to detector distance [1].

3.2.1. SCREEN: Serpell-SCREENSHOT.MOV: 00:13-00:21

3.3. Click on **Image Processing** and **Rotate** to rotate the diffraction image so that the meridian is vertical [1]. Use the **Centering module** by navigating to **Diffraction Pattern** and clicking on **Center Image** to center the image [2].

3.3.1. SCREEN: Serpell-SCREENSHOT.MOV: 00:52-00:56

3.3.2. SCREEN: Serpell-SCREENSHOT.MOV: 01:04-01:08

3.4. Define the meridian and equator axes. Save the centered image for subsequent analysis [1].

3.4.1. SCREEN: Serpell-SCREENSHOT.MOV: 01:22-01:27

3.5. Now specify an appropriate angle along the meridian and equator [1]. Press **Diffraction Pattern** and click on **Radially Average Module** to generate a graph of diffraction signal position versus intensity [2].

3.5.1. SCREEN: Serpell-SCREENSHOT.MOV: 01:35-01:47

3.5.2. SCREEN: Serpell-SCREENSHOT.MOV: 02:13-02:16

3.6. Navigate to the **Peak Find** module from the **Diffraction Pattern** menu to measure and automatically output the diffraction signal [1]. Adjust the peak search width to detect true signals without noise [2].

3.6.1. SCREEN: Serpell-SCREENSHOT.MOV: 03:06-03:20

3.6.2. SCREEN: Serpell-SCREENSHOT.MOV: 03:33-03:45

3.7. Use the **Zoom & Measure** option to verify diffraction signal positions output using **Peak Find [1-TXT]**.

3.7.1. SCREEN: Serpell-SCREENSHOT.MOV: 04:53-05:02

3.8. Explore potential unit cell dimensions using the **Unit Cell Optimization Module [1]**. Input initial estimates based on strong equatorial signals and define the minimum and maximum indices for cell search **[2]**.

3.8.1. SCREEN: Serpell-SCREENSHOT.MOV: 05:43-05:50

3.8.2. SCREEN: Serpell-SCREENSHOT.MOV: 07:12-07:17

3.9. Calculate potential unit cell dimensions and compare the predicted diffraction signal positions with observed reflections. Select the unit cell that best fits the data and the sample **[1]**.

3.9.1. SCREEN: Serpell-SCREENSHOT.MOV: 08:22-08:35

4. Structural Modeling and Validation of Amyloid Fibrils Using CLEARER Fiber Diffraction Simulation

4.1. To construct molecular models, build 3D arrangements of the peptides in a beta sheet configuration that fits the determined unit cell **[1]**. Ensure all symmetry-related components are included **[2]**.

4.1.1. SCREEN: Serpell-SCREENSHOT.MOV: 11: 46-11:53

4.1.2. SCREEN: Serpell-SCREENSHOT.MOV: 13:06-13:06

4.2. Load the PDB (*P-D-B*) model into the **Structure Chain Generator Module** by clicking on **Diffraction Simulation, Structures** and **Structure Chain Generator [1]**. Assign the fiber axis and beam axis, input unit cell dimensions, and then check for steric clashes **[2]**.

4.2.1. SCREEN: Serpell-SCREENSHOT.MOV: 13:46-13:53

4.2.2. SCREEN: Serpell-SCREENSHOT.MOV: 15:09-15:18

4.3. Navigate to **Diffraction Simulation** and press **Fiber Diffraction Simulation** to upload the PDB coordinates into the **Fiber Diffraction Simulation Module [1]**. Input the fiber axis direction, beam orientation, unit cell dimensions, X-ray source, and detector settings, then click **Simulate** to run the simulation **[2]**.

4.3.1. SCREEN: Serpell-SCREENSHOT.MOV: 16:12-16:16

4.3.2. SCREEN: Serpell-SCREENSHOT.MOV: 16-28-16:46

4.4. Ensure the beam axis is perpendicular to the fiber axis [1]. Then navigate to **File** and click on **Save As** to save the simulation settings as .html (*dot-H-T-M-L*) files for later editing [2].

4.4.1. SCREEN: Serpell-SCREENSHOT.MOV: 15:09-15:11.

4.4.2. SCREEN: Serpell-SCREENSHOT.MOV: 18:49-18:56

4.5. Save the simulated diffraction pattern in raw format or as a .tiff file [1]. Compare the simulated pattern with the experimental diffraction pattern [2].

4.5.1. SCREEN: Serpell-SCREENSHOT.MOV: 18:59-19:02

4.5.2. SCREEN: Serpell-SCREENSHOT.MOV: 19:42-19:51

4.6. Measure simulated diffraction signals using the same analysis workflow. Record and compare these values with experimental data [2].

4.6.1. SCREEN: : Serpell-SCREENSHOT.MOV: 20:00-20:33

4.7. ~~Use the **Layers** module from **Diffraction Pattern** to overlay simulated and experimental diffraction patterns and compare the alignment of signal positions [1]. Arrange pattern quadrants for visual comparison of signal positions and relative intensities [2].~~ **NOTE:**
Not provided

~~4.7.1. SCREEN: Activating the layers overlay function.~~

~~4.7.2. SCREEN: Displaying quadrant based comparison of patterns.~~

4.8. ~~Lastly, evaluate the validity of the proposed unit cell and structural model [1]. Iteratively modify the model and unit cell parameters to improve agreement with experimental data [2].~~

~~4.8.1. SCREEN: Assessing model agreement with diffraction data.~~

~~4.8.2. SCREEN: Iterative refinement of model and unit cell parameters.~~

Results

5. Results

5.1. Electron micrographs showed a high density of amyloid fibrils on the grid suitable for preparation of an X-ray fiber sample [1].

5.1.1. LAB MEDIA: Figure 1.

5.2. Stretch-frame aligned fibers produced a characteristic cross-beta diffraction pattern [1] with a meridional reflection at 4.76 angstroms and equatorial reflections arising from chain length, sheet spacing, and protofilament size [2]. An idealized cross-beta diffraction pattern demonstrated that meridional and equatorial reflections correspond to repetitive spacings along the fiber axes [3].

5.2.1. LAB MEDIA: Figure 3 *Video Editor: Please highlight A*

5.2.2. LAB MEDIA: Figure 3 *Video Editor: Please highlight B*

5.2.3. LAB MEDIA: Figure 3. *Video Editor: Please highlight C.*

5.3. A well-centered diffraction pattern was symmetric, with equatorial and meridional peaks overlapping in position [1]. Diffraction peaks were automatically detected and listed using peak-finding analysis [2].

5.3.1. LAB MEDIA: Figure 4 *Video Editor: Please highlight A*

5.3.2. LAB MEDIA: Figure 4 *Video Editor: Please highlight A (right side) and B*

5.4. The detected diffraction peaks were used to identify plausible unit cell dimensions [1]. A structural model was prepared using the identified unit cell and visualized prior to diffraction simulation [2].

5.4.1. LAB MEDIA: Figure 5. *Video Editor: Please highlight A*

5.4.2. LAB MEDIA: Figure 5 *Video Editor: Please highlight B*

5.5. Simulated diffraction data generated from the proposed structural model showed reasonable agreement with the experimental diffraction pattern in both reflection positions and relative intensities [1]. Overlaying simulated and experimental diffraction patterns further demonstrated alignment of corresponding reflections [2].

5.5.1. LAB MEDIA: Figure 6 *Video Editor: Please highlight A then B*

5.5.2. LAB MEDIA: Figure 6C.

1. Fibrils
Pronunciation link: <https://www.merriam-webster.com/dictionary/fibril>
IPA: /'faɪbrəl/
Phonetic Spelling: fy·bruhl
2. Borosilicate
Pronunciation link: <https://www.howtopronounce.com/borosilicate>
IPA: /,bɔːroʊ'sɪlɪkət/
Phonetic Spelling: bor·oh·sil·ih·kut
3. Capillaries
Pronunciation link: <https://www.merriam-webster.com/dictionary/capillary>
IPA: /'kæpə,leri/
Phonetic Spelling: kap·uh·lair·ee
4. Plasticine
Pronunciation link: <https://www.merriam-webster.com/dictionary/plasticine>
IPA: /'plæstə,siːn/
Phonetic Spelling: plas·tuh·seen
5. Microliter
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /'maɪkrə,liːtər/
Phonetic Spelling: my·kruh·lee·tur
6. Amyloid
Pronunciation link: <https://www.merriam-webster.com/dictionary/amyloid>
IPA: /'æmɪ,loɪd/
Phonetic Spelling: am·ih·loyd
7. Siliconized
Pronunciation link: <https://www.howtopronounce.com/siliconized>
IPA: /'sɪlɪkə,naɪzd/
Phonetic Spelling: सिल·ih·kuh·nyzd
8. Scalpel
Pronunciation link: <https://www.merriam-webster.com/dictionary/scalpel>
IPA: /'skælpəl/
Phonetic Spelling: skal·puhl
9. Goniometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/goniometer>
IPA: /,ɡoʊni'ɑːmətər/
Phonetic Spelling: goh·nee·om·uh·tur
10. Cross-polariser
Pronunciation link: <https://www.howtopronounce.com/cross-polariser>
IPA: /krɔːs 'pəʊlə,raɪzər/
Phonetic Spelling: kraws poh·luh·ry·zur
11. Birefringence
Pronunciation link: <https://www.merriam-webster.com/dictionary/birefringence>
IPA: /,baɪrɪ'frɪndʒəns/
Phonetic Spelling: by·rih·frin·juhns

12. Diffraction
Pronunciation link: <https://www.merriam-webster.com/dictionary/diffraction>
IPA: /dɪˈfrækʃən/
Phonetic Spelling: dih·frak·shuhn
13. Synchrotron
Pronunciation link: <https://www.merriam-webster.com/dictionary/synchrotron>
IPA: /ˈsɪŋkrəˌtraːn/
Phonetic Spelling: sing·kruh·tron
14. Meridian
Pronunciation link: <https://www.merriam-webster.com/dictionary/meridian>
IPA: /məˈrɪdiən/
Phonetic Spelling: muh·rid·ee·uhn
15. Equator
Pronunciation link: <https://www.merriam-webster.com/dictionary/equator>
IPA: /ɪˈkweɪtər/
Phonetic Spelling: ih·kway·tur
16. Radially
Pronunciation link: <https://www.howtopronounce.com/radially>
IPA: /ˈreɪdiəli/
Phonetic Spelling: ray·dee·uh·lee
17. Equatorial
Pronunciation link: <https://www.merriam-webster.com/dictionary/equatorial>
IPA: /ˌekwəˈtɔːriəl/
Phonetic Spelling: ek·wuh·tor·ee·uhl
18. Reflections
Pronunciation link: <https://www.merriam-webster.com/dictionary/reflection>
IPA: /rɪˈflekʃən/
Phonetic Spelling: rih·flek·shuhn
19. Steric
Pronunciation link: <https://www.howtopronounce.com/steric>
IPA: /ˈsterɪk/
Phonetic Spelling: ster·ik
20. Quadrants
Pronunciation link: <https://www.merriam-webster.com/dictionary/quadrant>
IPA: /ˈkwɑːdrənt/
Phonetic Spelling: kwod·ruhnt
21. Micrographs
Pronunciation link: <https://www.merriam-webster.com/dictionary/micrograph>
IPA: /ˈmaɪkrəˌɡræf/
Phonetic Spelling: my·kruh·graf
22. Meridional
Pronunciation link: <https://www.howtopronounce.com/meridional>
IPA: /məˈrɪdiˈoʊnəl/
Phonetic Spelling: muh·rid·ee·oh·nuhl

23. Protofilament

Pronunciation link: <https://www.howtopronounce.com/protofilament>

IPA: /ˌprɒtəʊˈfɪləmənt/

Phonetic Spelling: proh·toh·fil·uh·muhnt

24. Symmetric

Pronunciation link: <https://www.merriam-webster.com/dictionary/symmetric>

IPA: /səˈmetrɪk/

Phonetic Spelling: suh·met·rik

25. Plausible

Pronunciation link: <https://www.merriam-webster.com/dictionary/plausible>

IPA: /ˈplɔːzəbəl/

Phonetic Spelling: plaw·zuh·buhl