

Submission ID #: 71078

Scriptwriter Name: Deepthi Das

Project Page Link: https://review.jove.com/files_upload.php?src=21678838

Title: Recombinant Production and Purification of Hydrophobin SC16 from *Escherichia coli* and Monitoring of Its Self-Assembly Using Fluorescence Assays

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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**

Authors: Please create screen capture videos of the shots labeled as SCREEN, create a screenshot summary, and upload the files to your project page as soon as possible:
https://review.jove.com/files_upload.php?src=21678838

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: 12

Number of Shots: 43

Interviews

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

- **Videographer's NOTE: Audio Slates & Video Slates:** For the first half of the protocol shots, I used audio slates (vocally announced the shot numbers before the action started). For the second half, the files include both video slates (physical slate) and audio announcements for easier navigation.
- **Additional Close-Ups (CU):** I captured extra detail shots where steps required more visual explanation. For instance, you will find additional CUs in shots 2.1.1, 3.2.3, and 3.5.1, among others.
- **Microscope Footage:** Please note that we did not shoot any microscope footage today. The special adapter we purchased was not needed for this session.
- **Omitted Shots:** Isabella mentioned that several steps from the protocol are identical to the ones in the SCREEN section, so we didn't reshoot them to avoid redundancy. She will be sending you a separate email with the exact breakdown and details.
- **Testimonial:** Only Raymond was available to record a testimonial. Since there wasn't a specific question provided, I framed a custom question focused on JoVE's value as a visual scientific platform, and his response turned out great.
- **Technical Specs:** As per the handbook, all protocol shots are filmed in 1080p (HD), and all interviews are in 4K. Everything was captured in Sony S-Log, which can be easily converted to Rec.709 in any editing software.

INTRODUCTION:

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

- 1.1. **David Langelaan:** Our research focus is optimizing recombinant production of hydrophobins for diverse applications such as biocompatible films and coatings.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Isabela Drozdowski:** This protocol supports hydrophobin research for industrial coatings, emulsifiers, and environmental applications, including microplastic capture and remediation systems.

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

CONCLUSION:

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

- 1.3. **Raymond He:** This protocol allows researchers to purify hydrophobins in high yields and complete functional assays to ensure that they can self-assemble.
- 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Isabela Drozdowski:** Following this protocol, contact angle assays can be used to monitor surface hydrophobicity after hydrophobin self-assembly on various materials.
- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

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

- 1.5. **David Langelaan:** Future studies may engineer recombinant hydrophobins with enhanced properties tailored for practical industrial and environmental applications.
- 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

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

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. Expression of Hydrophobin SC16 in *Escherichia coli*

Demonstrator: Isabela Drozdowski

- 2.1. To begin, transform SHuffle T7 (*T seven*) Express *Escherichia coli* cells by heat shock at 42 degrees Celsius for 45 seconds using a pET-21 (*P-E-T twenty-one*) expression plasmid encoding H-GB1-SC16 (*H-G-B-1-S-C-16*) [1-TXT]. Inoculate 2 milliliters of lysogeny broth containing 100 micrograms per milliliter of ampicillin with a single colony [2] and incubate overnight at 37 degrees Celsius with shaking [3].
 - 2.1.1. WIDE: Shot of the talent performing heat shock transformation of *Escherichia coli* cells with a plasmid in a laboratory setup. **TXT: H-GB1-SC16: hexahistidine-tagged B1 domain of protein G fused to SC16**
Videographer: Please obtain CU shot as well for this shot.
 - 2.1.2. Talent picking a single colony and inoculating lysogeny broth.
 - 2.1.3. Talent placing the culture in a shaking incubator.
- 2.2. Add 1 milliliter of the overnight culture to 1 liter of lysogeny broth in a 2.8-liter baffled round-bottom flask [1]. Grow the culture at 37 degrees Celsius until the optical density at 600 nanometers reaches approximately 0.6 [2].
 - 2.2.1. Talent transferring overnight culture into a large flask of lysogeny broth.
 - 2.2.2. Talent placing the flask in an incubator and adjusting the rotation speed of cultures in the incubator.
- 2.3. Add isopropyl- β -D-1-thiogalactopyranoside (*isopropyl-beta-D-1-thiogalactopyranoside*) to a final concentration of 0.5 millimolar [1]. Incubate overnight at 20 degrees Celsius while shaking [2]. The next day, centrifuge the tube at 4000 *g* for 20 minutes [3] and pour off the supernatant [4].
 - 2.3.1. Talent adding inducer to the culture flask.
 - 2.3.2. Talent adjusting incubator temperature to 20 °C.
 - 2.3.3. Talent placing culture tubes into a centrifuge.
 - 2.3.4. Talent decanting the supernatant from the pellet.

3. Purification of Hydrophobin SC16

Demonstrator: Raymond He

- 3.1. Resuspend the cell pellet in 30 milliliters of lysis buffer containing 20 millimolar Tris hydroxymethyl aminomethane at pH 8 and 250 millimolar sodium chloride [1]. Incubate the suspension in an 80 degrees Celsius water bath for 15 minutes [2] and mix gently every 5 minutes during incubation [3].
 - 3.1.1. Talent resuspending the pellet in buffer by pipetting or gentle mixing.
 - 3.1.2. Talent placing the tube in a water bath.
 - 3.1.3. Talent gently mixing the tube during incubation.
- 3.2. Centrifuge the cell lysate at 25000 g for 20 minutes at 4 degrees Celsius [1]. Load the supernatant onto a gravity column containing 2 to 3 milliliters of nickel-ion-charged immobilized metal affinity chromatography resin [2]. Wash the column with lysis buffer containing 30 millimolar imidazole [3]. Then, elute protein using 10 milliliters of lysis buffer containing 300 millimolar imidazole [4].
 - 3.2.1. Talent placing lysate into a centrifuge
 - 3.2.2. Talent loading supernatant onto a chromatography column.
 - 3.2.3. Talent adding wash buffer to the column. NOTE: CU is also filmed
 - 3.2.4. Talent collecting eluted protein fractions.
- 3.3. Dialyze the eluted fraction using 3500 kilodalton molecular weight cutoff dialysis tubing [1] against 1 liter of buffer containing Tris and sodium chloride for 3 hours while stirring at 100 rpm [2]. Add thrombin protease at 20 units per milligram fusion protein directly to the dialysis bag [3]. After replacing the buffer, continue dialysis overnight at 4 degrees Celsius [4].
 - 3.3.1. Talent placing the protein fraction into the dialysis bag.
 - 3.3.2. Talent placing the dialysis bag in the buffer placed on the shaker.
 - 3.3.3. Talent adding protease into the dialysis bag.
 - 3.3.4. Talent placing the setup for overnight dialysis.
- 3.4. Reload the dialyzed sample onto the nickel ion-charged immobilized metal affinity chromatography column [1]. Wash with buffer containing 30 millimolar imidazole [2] and elute with buffer containing 300 millimolar imidazole [2].
 - 3.4.1. Talent loading sample onto the column again.
 - 3.4.2. Talent adding wash buffer to the column.
 - 3.4.3. Talent collecting wash and elution fractions.
- 3.5. Analyze collected fractions by SDS PAGE (*S-D-S-page*) with beta mercaptoethanol in the loading buffer [1]. Check for a band at approximately 10 kilodaltons to identify SC16 (*S-C-16*) [2]. Repeat dialysis of the wash fraction against 1 liter of Tris buffer for 3 hours [3]. Measure the absorbance at 280 nanometers to quantify SC16 [4].
 - 3.5.1. Shot of the talent loading samples onto a gel. . NOTE: CU is also filmed
 - 3.5.2. Shot of gel bands indicating protein separation.
 - 3.5.3. Shot of the dialysis of the wash fraction being performed.
 - 3.5.4. Show spectrophotometer reading absorbance values.

4. Endpoint and Kinetic Fluorescence Assays of SC16 Self-Assembly

4.1. Prepare reaction mixtures [1] in triplicate [2]. Add protein and sodium chloride immediately before the assay [3].

4.1.1. Talent preparing buffer mixtures.

4.1.2. TEXT ON A PLAIN BACKGROUND

- 50 micrograms per milliliter SC16
- 50 millimolar sodium chloride
- 20 millimolar each of:
 - MES, Tris hydroxymethyl aminomethane and sodium phosphate
 - pH 6.5

4.1.3. Talent adding protein and NaCl to the reaction mixtures.

4.2. Transfer the mixtures into 2 to 5 milliliter tubes [1]. Rotate the tubes end-over-end at 20 revolutions per minute for 2 hours, ensuring proper mixing upon inversion [2]. Then transfer 100 microliters of each sample into an opaque-bottomed black 96-well plate [3]. Add filtered thioflavin T to a final concentration of 20 micromolar to all samples [4]. Set the microplate reader to the appropriate excitation and emission wavelengths for thioflavin T fluorescence, with a gain of 55 and an integration time of 20 microseconds [5]. Measure and record the fluorescence intensity for each sample [6].

4.2.1. Talent transferring solutions into tubes.

4.2.2. Talent placing tubes on a rotator.

4.2.3. Talent pipetting samples into a plate.

4.2.4. Talent adding filtered thioflavin T into wells.

4.2.5. SCREEN: 71078_screenshot_1.mp4: 00:00-00:12

4.2.6. SCREEN: 71078_screenshot_1.mp4: 00:22-00:28

and

TABLE ON PLAIN BACKGROUND

Sample	ThT Fluorescence (Counts $\pm$ SD)
SC16 without rotation	2679 $\pm$ 177
SC16 after rotation	30315 $\pm$ 2141
Buffer after rotation	665 $\pm$ 888

Video editor: Use split screen to show both the shot side by side

4.3. For kinetic analysis, add 100 microliter reaction mixtures in triplicate in a black 96-well plate [1]. Adjust the buffer pH to 5.5, 6.5, 7.5, or 8.5 before the addition of SC16 and thioflavin T [2].

4.3.1. Talent adding 100 μ L reaction mixtures to wells.

4.3.2. Shot of the talent adjusting pH using acid or base.

4.4. Place the plate in the microplate reader [1]. Set excitation and emission wavelengths to 438 and 494 nanometres, respectively [2]. Program cyclic measurements every 70 seconds, with 60 seconds orbital shaking at 432 revolutions per minute, followed by 10 seconds rest [3]. Then, set integration time to 20 microseconds and gain to 55 [4]. Run for 120 cycles [5].

4.4.1. Talent inserting the plate into the reader.

4.4.2. SCREEN: 71078_screenshot_2.mp4: 00:07-00:17

4.4.3. SCREEN: 71078_screenshot_2.mp4: 00:18-00:38

4.4.4. SCREEN: 71078_screenshot_2.mp4: 00:38-00:47

4.4.5. SCREEN:71078_screenshot_2.mp4: 00:47-00:55

Videographer: Please film shots labelled as SCREEN as a backup

Results

5. Results

5.1. In the first immobilized metal affinity chromatography eluate, a band at approximately 18 kilodaltons corresponding to H-GB1-SC16 was observed before thrombin cleavage [1].

5.1.1. LAB MEDIA: Figure 1. *Video editor: Highlight the lane labeled "E" under "First IMAC" and point to the dark band around the 18 kDa mark labeled "H-GB1-SC16 (18 kDa)"*

5.2. After thrombin cleavage, no protein was observed in the second immobilized metal affinity chromatography flow-through fraction [1].

5.2.1. LAB MEDIA: Figure 1. *Video editor: Highlight the lane labeled "F" under "Second IMAC" and show that there is no visible band in this lane*

5.3. After thrombin cleavage, a band at approximately 10 kilodaltons corresponding to SC16 was observed in the wash fraction [1].

5.3.1. LAB MEDIA: Figure 1. *Video editor: Highlight the lane labeled "W" under "Second IMAC" and point to the band around the 10 kDa mark labeled "SC16 (10 kDa)"*

5.4. In the second immobilized metal affinity chromatography eluate, residual SC16 at approximately 10 kilodaltons [1] and a smaller band at approximately 8 kilodaltons corresponding to H-GB1 were observed [2].

5.4.1. LAB MEDIA: Figure 1. *Video editor: Highlight the lane labeled "E" under "Second IMAC" and point to the band around the 10 kDa mark labeled "SC16 (10 kDa)"*

5.4.2. LAB MEDIA: Figure 1. *Video editor: In the same "E" lane under "Second IMAC," point to the lower band around the 8 kDa mark labeled "H-GB1 (8 kDa)"*

5.5. Successful purification of SC16 was confirmed by the presence of the expected molecular weight band [1] and separation from the H-GB1 fusion partner following thrombin cleavage [2].

5.5.1. LAB MEDIA: Figure 1. *LAB MEDIA: Figure 1. Video editor: Highlight the "W" lane under "Second IMAC" and point to the band labeled "SC16 (10 kDa)"*

5.5.2. LAB MEDIA: Figure 1. *Video editor: Highlight the "E" lane under "Second IMAC" and point to the separate lower band labeled "H-GB1 (8 kDa)"*

5.6. SC16 samples showed increasing fluorescence intensity over time under agitation across all tested pH conditions [1].

5.6.1. LAB MEDIA: Figure 2. *Video editor: Highlight the black SC16 curves in all four panels labeled pH 5.5, pH 6.5, pH 7.5, and pH 8.5 as they rise over time*

5.7. Suboptimal kinetic self-assembly data obtained using linear shaking showed little change in fluorescence intensity over time for SC16 samples [1].

5.7.1. LAB MEDIA: Figure 3. *Video editor: Highlight the black SC16 line across the graph showing it stays nearly flat from start to end*

5.8. The thioflavin T fluorescence intensity increased 11-fold after 2 hours of rotation in SC16 samples compared to non-rotated protein-containing controls [1].

LAB MEDIA: Table 1. *Video editor: Highlight the SC16 row showing the value after 2 hours of rotation and then highlight the SC16 row showing the value without rotation*

1. Escherichia coli
Pronunciation link: <https://www.merriam-webster.com/dictionary/Escherichia%20coli>
IPA: /ˌɛʃəˈrɪkiə ˈkoʊ.laɪ/
Phonetic Spelling: esh·uh·rik·ee·uh koh·ly
2. Plasmid
Pronunciation link: <https://www.merriam-webster.com/dictionary/plasmid>
IPA: /ˈplæz.mɪd/
Phonetic Spelling: plaz·mid
3. Lysogeny
Pronunciation link: <https://www.howtopronounce.com/lysogeny>
IPA: /laɪˈsɑːdʒəni/
Phonetic Spelling: ly·soj·uh·nee
4. Ampicillin
Pronunciation link: <https://www.merriam-webster.com/dictionary/ampicillin>
IPA: /ˌæmpəˈsɪlɪn/
Phonetic Spelling: am·puh·sil·in
5. Optical Density
Pronunciation link: <https://www.merriam-webster.com/dictionary/optical>
IPA: /ˈɑːptɪkəl denˈsɪti/
Phonetic Spelling: op·tih·kuhl den·suh·tee
6. Nanometers
Pronunciation link: <https://www.merriam-webster.com/dictionary/nanometer>
IPA: /ˈnænə.mi.tər/
Phonetic Spelling: nan·uh·mee·tur
7. Isopropyl-β-D-1-thiogalactopyranoside
Pronunciation link: <https://www.howtopronounce.com/isopropyl-beta-d-1-thiogalactopyranoside>
IPA: /ˌaɪsoʊˈproʊpəl ˌbɛrtə diː wʌn ˌθaɪoʊɡəˌlæktəʊpɪˈrænəsəɪd/
Phonetic Spelling: eye·soh·proh·pul bay·tuh dee one thy·oh·guh·lak·toh·pih·ran·uh·side
8. Millimolar
Pronunciation link: <https://www.howtopronounce.com/millimolar>
IPA: /ˌmɪləˈmoʊlər/
Phonetic Spelling: mil·uh·moh·lur
9. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /ˈsentrəˌfjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
10. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /suːˈpɜːnətənt/
Phonetic Spelling: soo·pur·nuh·tuhnt
11. Hydrophobin
Pronunciation link: <https://www.howtopronounce.com/hydrophobin>
IPA: /ˌhaɪdrəˈfoʊbɪn/
Phonetic Spelling: hy·druh·foh·bin

12. Hydroxymethyl
 Pronunciation link: <https://www.howtopronounce.com/hydroxymethyl>
 IPA: /haɪˌdrɑːksiˈmɛθəl/
 Phonetic Spelling: hy·drok·see·meth·uhl
13. Aminomethane
 Pronunciation link: <https://www.howtopronounce.com/aminomethane>
 IPA: /əˌmiːnoʊˈmɛθeɪn/
 Phonetic Spelling: uh·mee·noh·meth·ayn
14. Chromatography
 Pronunciation link: <https://www.merriam-webster.com/dictionary/chromatography>
 IPA: /ˌkroʊməˈtɑːɡrəfi/
 Phonetic Spelling: kroh·muh·taa·gruh·fee
15. Imidazole
 Pronunciation link: <https://www.howtopronounce.com/imidazole>
 IPA: /ɪˈmɪdəˌzoʊl/
 Phonetic Spelling: ih·mid·uh·zohl
16. Dialyze
 Pronunciation link: <https://www.merriam-webster.com/dictionary/dialyze>
 IPA: /ˈdaɪəˌlaɪz/
 Phonetic Spelling: dye·uh·lyz
17. Kilodalton
 Pronunciation link: <https://www.howtopronounce.com/kilodalton>
 IPA: /ˌkɪləˈdɔːltən/
 Phonetic Spelling: kil·uh·dawl·tuhn
18. Protease
 Pronunciation link: <https://www.merriam-webster.com/dictionary/protease>
 IPA: /ˈproʊtiˌeɪs/
 Phonetic Spelling: proh·tee·ays
19. Thrombin
 Pronunciation link: <https://www.merriam-webster.com/dictionary/thrombin>
 IPA: /ˈθrɑːmbɪn/
 Phonetic Spelling: throm·bin
20. Absorbance
 Pronunciation link: <https://www.merriam-webster.com/dictionary/absorbance>
 IPA: /əbˈzɔːrbəns/
 Phonetic Spelling: ub·zor·buhns
21. Spectrophotometer
 Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrophotometer>
 IPA: /ˌspektrəfoʊˈtɑːmɛtər/
 Phonetic Spelling: spek·truh·foh·tom·uh·tur
22. Fluorescence
 Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
 IPA: /flʊˈresəns/, /flɒˈresəns/
 Phonetic Spelling: flor·ess·uhns; flu·ress·uhns

23. Thioflavin

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

IPA: /ˌθaɪoʊˈfleɪvɪn/

Phonetic Spelling: thy·oh·flay·vin

24. Micromolar

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

IPA: /ˌmaɪkroʊˈmoʊlər/

Phonetic Spelling: my·kroh·moh·lur

25. Orbital

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

IPA: /ˈɔːrbɪtəl/

Phonetic Spelling: or·bih·tuhl