

Submission ID #: 71092

Scriptwriter Name: Sri Lakshmi Priya

Project Page Link: <https://review.jove.com/account/file-uploader?src=21683108>

Title: An *In Vitro* Assay for LpxC Degradation by the Membrane-Bound Protease FtsH and Adaptor LapB from *Escherichia Coli*

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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**
- 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: 35

Number of Shots: 58

Interviews

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. **Wei Mi:** *E.coli* regulates lipopolysaccharide synthesis by controlling LpxC degradation. Our research focuses on establishing an in vitro degradation assay for LpxC.
 - 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. **Wei Mi:** This protocol can be applied to quantitatively measure FtsH-mediated proteolysis of water-soluble substrates.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

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

- 1.3. **Rajkanwar Nathawat:** Following this procedure, the proteoliposome system allows systematic investigation of how adaptors, anti-adaptors, and lipid composition influence LpxC degradation.
 - 1.3.1. 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.~~

- 1.4. **Wei Mi:** Future studies can build on this work by adapting this assay to investigate membrane-associated proteolysis in other AAA+ protease systems.
 - 1.4.1. 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.

NOTE: LAB MEDIA/SCREEN timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Sri Lakshmi Priya) for queries regarding lab media.

2. Labeling of LpxC–Atto488

Demonstrator: Rajkanwar Nathawat and Wei Mi

- 2.1. To begin, obtain LpxC (*L-picks-C*), Filamentation temperature-sensitive protein H or FtsH (*F-T-S-H*) and Lipopolysaccharide assembly protein B or LapB (*Lap-B*) proteins [1]. Dilute 0.5 milligram of LpxC in 50 millimolar phosphate buffer at pH 7.6 to a final volume of 225 microliters [2]. Add 25 microliters of 1 molar sodium bicarbonate to adjust the pH to approximately 8.3 [3].
 - 2.1.1. WIDE: Talent walking into the room with a tray of tubes. **Videographer:** Please obtain CU shot as well for this shot.
 - 2.1.2. Talent adding 0.5 mg of LpxC into a tube containing phosphate buffer.
 - 2.1.3. Talent adding sodium bicarbonate into the tube containing LpxC solution.
- 2.2. Prepare a working solution of Atto488 (*AT-toh-four-eight-eight*) by diluting 2 microliters of the previously prepared stock solution with 18 microliters of 0.1 molar sodium bicarbonate buffer to obtain a final concentration of 0.0012 milligram per microliter [1].
 - 2.2.1. Talent pipetting the stock solution into a tube containing sodium bicarbonate buffer.
- 2.3. Immediately add 4 microliters of the Atto488 working solution to the protein solution [1]. After wrapping the tube with aluminium foil, place it at 4 degrees Celsius with gentle agitation at 18 rpm for 2 hours [2].
 - 2.3.1. Talent adding the Atto488 working solution into the tube containing protein solution.
 - 2.3.2. Talent placing the wrapped tube into a refrigerated shaker.
- 2.4. Once the PD-10 (*P-D-ten*) desalting column is equilibrated with 10 milliliters of ultrapure water, add 15 milliliters of phosphate buffer to the column [1].
 - 2.4.1. Talent adding phosphate buffer to the column.
- 2.5. Apply the labeling reaction mixture to the column and allow the mixture to fully enter the resin [1].

- 2.5.1. Close shot of the applying mixture to the column and the mixture entering the resin bed.
- 2.6. Add 5 milliliters of 50 millimolar phosphate buffer to the column and observe two distinct separated bands corresponding to LpxC–Atto488 and free Atto488 dye [1]. Collect fractions from the first band in approximately 0.5 milliliter aliquots [2].
 - 2.6.1. Close shot of two visible bands moving through the column. *Video editor: Please add the label “LpxC–Atto488” to the first top green band and “free Atto488 dye” to the second bottom orange band.*
 - 2.6.2. Talent collecting the first eluted fraction into a tube.
- 2.7. Using SDS-PAGE (*S-D-S-PAGE*), separate the eluted fractions [1]. Under ultraviolet illumination, visualize fluorescence prior to Coomassie Brilliant Blue or CBB (*C-B-B*) staining [2].
 - 2.7.1. Talent loading sample into the SDS-PAGE
 - 2.7.2. Shot of fluorescent protein bands visible under ultraviolet illumination.
- 2.8. Pool the fractions containing LpxC–Atto488 [1]. After concentrating the labeled protein to approximately 2 milligrams per milliliter, using a ~~microvolume spectrophotometer~~ NanoDrop [2], measure the concentration of protein and Atto488 to estimate labeling efficiency [3]. **Notes: The VO is added for the added shot. The sentence numbers are adjusted to accommodate the added shot.**
 - 2.8.1. Talent combining selected fractions into a single tube.
 - 2.8.2. Talent loading samples into a NanoDrop.

Added shot: SCREEN: Screen-2.8.3.png **Videographer’s Notes: 2.8.3 added step, she will be sending screen shot. Need to crop out very top of screen info. I also shot.**
- 2.9. Aliquot LpxC–Atto488 into 1.5 milliliter microcentrifuge tubes [1]. After flash-freezing the samples in liquid nitrogen, store the samples at minus 80 degrees Celsius [2].
 - 2.9.1. Talent dispensing labeled protein into microcentrifuge tubes.
 - 2.9.2. Talent placing the frozen tubes into a minus 80 degrees Celsius freezer.

3. Preparation and Extrusion of Liposomes

- 3.1. Inside a fume hood, transfer 100 milligrams of 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine or POPC (*P-O-P-C*) in chloroform into a glass test tube [1]. After evaporating the solvent under a gentle stream of nitrogen gas, dry it overnight under vacuum [2].
 - 3.1.1. Talent transferring the lipid solution into a glass test tube inside a fume hood.
 - 3.1.2. Talent placing the tube under vacuum for overnight drying.

- 3.2. Add 1.315 milliliters of buffer A containing 20 millimolar Tris-hydrochloride and 100 millimolar sodium chloride to the dried POPC film [1]. Resuspend the lipids by alternating 1 minute of water-bath sonication with 1 minute of vortexing at maximum speed for approximately 15 minutes until lipids are fully dispersed [2].
- 3.2.1. Talent adding buffer A to the dried POPC film.
- 3.2.2. Talent alternating between water-bath sonication and vortexing the lipid suspension.
- 3.3. Aliquot the liposome suspension into 200 microliter portions [1]. Store the aliquots at minus 20 degrees Celsius [2].
- 3.3.1. Talent dispensing the liposome suspension into aliquot tubes.
- 3.3.2. Talent placing the aliquots into a minus 20 degrees Celsius freezer.
- 3.4. After thawing the 200-microliter liposome aliquot in a water bath at room temperature, dilute it with 800 microliters of buffer A to a final volume of 1 milliliter [1].
- 3.4.1. Talent adding buffer A into the tube containing liposome suspension.
- 3.5. Subject the liposomes to three freeze-thaw cycles by alternating between liquid nitrogen and a water bath at room temperature [1].
- 3.5.1. Talent transferring the liposome tube from liquid nitrogen to a room temperature water bath.
- 3.6. After rinsing the syringes, 2 filter supports, and a 200-nanometer polycarbonate membrane with buffer A [1], assemble the mini-extruder [2]. Extrude the liposomes 20 times [3] until they appear transparent [4]. Notes: The VO is added for the added shot. The sentence numbers are adjusted to accommodate the added shot. Videographer's notes: 3.6.1.1 NEW, 3.6.1.2 NEW, 3.6.1.3 NEW, 3.6.1.4 On script now as 3.6.1
- Added shot: Show all the components. Video editor: Label the components: Syringes, Filter Supports, Polycarbonate Membrane
- Added shot: Show the assembly of the mini-extruder
- 3.6.1. Talent passing the liposome suspension back and forth through the membrane using the extruder.
- Added Shot: Shot of liposome in the light before the process, and shot of liposome in the light after the process. Video editor: Label the liposome shot before the process as "Before" and the liposome shot after the process as "After."
- 3.7. Once the 1 milliliter of extruded liposome suspension is divided into three tubes, dilute each tube with 600 microliters of buffer A [1].
- 3.7.1. Talent adding buffer A to the tube containing liposome suspension.

4. Proteoliposome Reconstitution and Analysis

4.1. Prepare 10 percent volume per volume Triton X-100 (*Triton-X-hundred*) by mixing 100 microliters of Triton X-100 with 900 microliters of ultrapure water [1].

4.1.1. Talent adding Triton X-100 into a tube containing ultrapure water.

4.2. After fully dissolving the Triton X-100 in a shaker, using a spectrophotometer, measure the absorbance of the liposome suspension [1] at 540 nanometers [2]. Notes: The VO is revised for the added shot. The sentence numbers are adjusted to accommodate the added shot.

4.2.1. Talent measuring liposome absorbance using a spectrophotometer.

Videographer's notes: 4.2.1 after this step, new step will be added. She will be sending prerecorded video here that needs to be shown before 4.3.1

Added shot: SCREEN: screen-capture-4.3.3.avi: 00:26-00:30

4.3. Add 5 microliter aliquots of 10 percent Triton X-100 sequentially to the liposome suspension [1]. After each addition, gently mix the suspension and measure the absorbance [2] at 540 nanometers [3]. Continue adding Triton X-100 [4] until the absorbance reaches a maximum and then decreases to approximately 60 percent of the peak value [5]. Notes: The VO is revised for the added shot. The sentence numbers are adjusted to accommodate the added shot.

4.3.1. Talent adding Triton X-100 aliquots into the liposome suspension.

4.3.2. Talent measuring absorbance using a spectrophotometer. Videographer's notes: 4.3.2 + 4.3.3 Did not shoot. She will be sending pre recorded video here again of results done in a different lab a different day.

Added shot: SCREEN: screen-capture-4.3.3.avi: 00:58-01:01

4.3.3. SCREEN: screen-capture-4.3.3.avi: 01:28-01:31, 02:36-02:39, 04:34-04:38

Added shot: SCREEN: swelling-titration-curve-4.3.3.pdf Notes: This shot is added as per the author's comment.

4.4. Add 35 microliters of 10 percent Triton X-100 to swell the liposomes, based on the titration curve [1]. Incubate at room temperature for 1 hour with gentle agitation [2].

4.4.1. Talent adding Triton X-100 to the liposome suspension.

4.4.2. Talent placing the liposome tube on a gentle shaker at room temperature.

Author's Notes: 4.4.1 and 4.4.2 are filmed in one shot.

4.5. After washing approximately 2 grams of nonpolar polystyrene adsorbent once with 10 volumes of methanol and once with 10 volumes of ethanol, wash ten times with 10 volumes of ultrapure water [1]. Store the washed adsorbent in buffer A at 4 degrees Celsius [2].

4.5.1. Talent adding ultrapure water to the adsorbent.

- 4.5.2. Talent placing the tube with washed adsorbent in buffer A in the refrigerator.
- 4.6. To each tube of Triton X-100-swollen liposomes [1], add the required reagents [2] and incubate for 1 hour with gentle agitation [3].
- 4.6.1. Close of talent holding the tube and pipette.
- 4.6.2. TEXT ON A PLAIN BACKGROUND:
500 mM TCEP - 3 μ L
FtsH - 22.5 μ g
LapB - 22.5 μ g
Adjust volume to 1 mL with buffer A
- 4.6.3. Talent placing the tubes on a gentle shaker at room temperature.
- 4.7. Remove the detergent [1] by sequential incubation with nonpolar polystyrene adsorbent [2]. After Centrifuging at 400 g for 3 seconds at 4 degrees Celsius, transfer the supernatant to ultracentrifuge tubes [3].
- 4.7.1. Close-up of removing the detergent.
- 4.7.2. TEXT ON A PLAIN BACKGROUND:
40 mg at RT for 0.5 h
40 mg at RT for 1 h
40 g at 4 °C overnight
40 mg at 4 °C for 1 h
- 4.7.3. Talent transferring the supernatant into ultracentrifuge tubes.
- 4.8. Wash the nonpolar polystyrene adsorbent with 1 milliliter of buffer A and combine the wash with the supernatant [1]. Add additional buffer A to a final volume of approximately 6 milliliters [2].
- 4.8.1. Talent adding buffer A to the adsorbent and combining the wash with the supernatant. Videographer's notes: 4.8.1 + 4.8.2 retake for both steps. Noted on video
- 4.8.2. Talent adding buffer A to the tube.
- 4.9. In a fixed-angle rotor, once the ultracentrifuge tubes are balanced, ultracentrifuge the samples at 250000 g at 4 degrees Celsius for 45 minutes to pellet the proteoliposomes [1].
- 4.9.1. Talent placing the tubes into an ultracentrifuge rotor.
- 4.10. After discarding the supernatant, resuspend the proteoliposome pellet in 70 microliters of buffer A supplemented with 1 millimolar tris 2-carboxyethyl phosphine [1].
- 4.10.1. Talent resuspending the proteoliposome pellet in buffer A.

4.11. Once the proteoliposomes are flash-freezed in liquid nitrogen, store them at minus 80 degrees Celsius [1].

4.11.1. Talent storing the frozen proteoliposomes in a minus 80 degrees Celsius freezer.

4.12. After thoroughly mixing the proteoliposome suspension, add 5 microliters of the sample to 5 microliters of 2 times SDS loading buffer [1].

4.12.1. Talent adding the sample into the tube containing SDS loading buffer.

4.13. In a 4 to 20 percent SDS-PAGE gel, load the proteoliposome samples alongside 525 nanograms of FtsH [1].

4.13.1. Talent loading samples and FtsH standard onto the gel. Videographer's notes: 4.13.1 shot early in the day out of order

4.14. After staining the gel with CBB, scan the gel [1]. Quantify the band intensity of FtsH [2] and calculate the volume of proteoliposome suspension equivalent to 525 nanograms of FtsH [3]. Notes: The VO is revised for the added shot. The sentence numbers are adjusted to accommodate the added shot.

4.14.1. Talent scanning the stained gel.

4.14.2. LABMEDIA: 4.14.2.1.tif

Added shot: LABMEDIA: 4.14.2.2.xlsx: Show the second table (columns Q to V) and highlight the cell U8 Notes: This shot was added as the author wants to show 2 LABMEDIA in 1 step.

5. *In vitro* degradation of LpxC–Atto488

5.1. For each concentration of LpxC–Atto488 ranging from 0.5 to 40 micromolar, prepare a 70-microliter *in vitro* degradation reaction containing FtsH and LapB proteoliposomes in degradation buffer and LpxC–Atto488 [1]. In parallel, prepare corresponding control reactions without proteoliposomes for background subtraction [2] and incubate both reactions at the desired temperature [3].

5.1.1. Talent adding FtsH and LapB proteoliposomes into reaction tubes.

5.1.2. Talent adding control reagents into the tube.

5.1.3. Talent placing the tubes into the incubator. Notes: The authors moved it between 5.2.1 and 5.2.2 Videographer's notes: 5.1.3 should go after 5.2.1 to be correct order

5.2. Transfer a 20-microliter fraction into a 1.5 milliliter microcentrifuge tube containing 48 microliters of 5 percent trichloroacetic acid to quench the reaction at 0 minutes [1] and incubate both reactions at the desired temperature [2]. Quench the second

fraction at 10 minutes and the third at 20 minutes [3]. Notes: The VO is added for the moved shot.

5.2.1. Talent transferring the first fraction into a 1.5 mL microfuge tube containing trichloroacetic acid.

5.1.3 Talent placing the tubes into the incubator. Notes: This was placed here as per the author's request.

5.2.2. Talent quenching the remaining fractions at the indicated time points. Author's Notes: Indicate time in the video 10 min for 1st set of shot and the 20 min for 2nd set of shot Videographer's notes: 5.2.2 wants to add graphic for 10min + 20min time frames

5.3. After incubating the samples at 37 degrees Celsius for 30 minutes to precipitate proteins, centrifuge them at 18,800 g at room temperature for 20 minutes [1].

5.3.1. Talent placing the samples into a centrifuge.

5.4. Transfer 50 microliters of supernatant into a tube containing 60 microliters of 500 millimolar Tris-hydrochloride to neutralize the samples [1]. After mixing Mix and transferring 100 microliters to a black 96-well plate using a plate reader, and measure the fluorescence with excitation at 498 nanometers and emission at 520 nanometers [2].

5.4.1. Talent transferring the supernatant into a tube containing Tris-hydrochloride.

5.4.2. Talent placing the 96-well plate into a plate reader. Talent transferring the sample into the black 96-well plate.

Results

6. Results

- 6.1. After successfully labeling LpxC with Atto488, the coincidence of the fluorescent [1] and protein bands indicated that free dye was effectively removed from the labeled LpxC preparation [2].
 - 6.1.1. LAB MEDIA: Figure 1A. *Video editor: Emphasize the bands on line 1 to 6 labelled as LpxC-Atto488.*
 - 6.1.2. LAB MEDIA: Figure 1B. *Video editor: Emphasize the bands on line 1 to 6 labelled as LpxC-Atto488.*
- 6.2. Absorbance at 540 nanometers reached a maximum as Triton X-100 increased [1], then decreased with further Triton X-100 addition [2].
 - 6.2.1. LAB MEDIA: Figure 2. *Video editor: Emphasize the highest point of the graph near 15 microliters Triton X-100 (lies between 10 and 20)*
 - 6.2.2. LAB MEDIA: Figure 2. *Video editor: Emphasize the lowest point of the graph near 35 microliters Triton X-100 (lies between 30 and 40)*
- 6.3. Efficient membrane protein reconstitution was achieved when the absorbance at 540 nanometers decreased to approximately 60% of the peak value [1].
 - 6.3.1. LAB MEDIA: Figure 2. *Video editor: Emphasize the lowest point of the graph near 35 microliters Triton X-100 (lies between 30 and 40)*
- 6.4. Comparable band intensities of FtsH [1] and LapB indicated similar reconstitution efficiencies for both proteins [2].
 - 6.4.1. LAB MEDIA: Figure 3. *Video editor: Emphasize the upper band labeled FtsH in the "FtsH/LapB-PL" lane*
 - 6.4.2. LAB MEDIA: Figure 3. *Video editor: Emphasize the lower band labeled LapB in the "FtsH/LapB-PL" lane*
- 6.5. A darkly stained region at the bottom of the gel likely corresponded to POPC lipids [1].
 - 6.5.1. LAB MEDIA: Figure 3. *Video editor: Circle and emphasize the dark stained region at the bottom of the gel below the protein bands in the "FtsH/LapB-PL" lane*
- 6.6. The fluorescence increase during the first 10 minutes was comparable to the increase during the subsequent 10-minute interval [1].
 - 6.6.1. LAB MEDIA: Supplementary Table 1. *Video editor: Show rows 2 to 10 and column A to I. Emphasize cells G7, H7, I7*

6.7. Proteolytic activity of LpxC by FtsH and LapB increased with increasing LpxC-Atto488 concentration [1].

6.7.1. LAB MEDIA: Table 1. *Video editor: Emphasize the values at the columns repeat 1, repeat 2, repeat 3*

Pronunciation Guides:

1. Filamentation
Pronunciation link: <https://www.merriam-webster.com/dictionary/filamentation>
IPA: /ˈfɪl.ə.mənˈteɪ.ʃən/
Phonetic Spelling: fil·uh·muhn·tay·shuhn
2. Lipopolysaccharide
Pronunciation link: <https://www.merriam-webster.com/dictionary/lipopolysaccharide>
IPA: /ˌlɪp.ɒs.pɑː.liˈsæk.ə.raɪd/
Phonetic Spelling: lip·oh·pol·ee·sak·uh·ryd
3. Phosphate
Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphate>
IPA: /ˈfɑːs.fert/
Phonetic Spelling: fahs·fayt
4. Sodium bicarbonate
Pronunciation link: <https://www.merriam-webster.com/dictionary/sodium%20bicarbonate>
IPA: /ˈsoʊ.di.əm baɪˈkɑːr.bə.neɪt/
Phonetic Spelling: soh·dee·uhm by·kar·buh·nayt
5. Aluminium
Pronunciation link: <https://www.merriam-webster.com/dictionary/aluminum>
IPA: /əˈluː.mə.nəm/
Phonetic Spelling: uh·loo·muh·nuhm
6. Desalting
Pronunciation link: <https://www.merriam-webster.com/dictionary/desalt>
IPA: /diːˈsɒlt/
Phonetic Spelling: dee·sawlt
7. Resin
Pronunciation link: <https://www.merriam-webster.com/dictionary/resin>
IPA: /ˈrez.ən/
Phonetic Spelling: rez·uhn
8. Electrophoresis
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrophoresis>
IPA: /ɪˌlek.troʊ.fəˈriː.sɪs/
Phonetic Spelling: ih·lek·troh·fuh·ree·suhs
9. Ultraviolet
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultraviolet>

IPA: /ˌʌl.trəˈvaɪ.ə.lət/

Phonetic Spelling: ul·truh·vy·uh·luht

10. Coomassie

Pronunciation link: No confirmed link found

IPA: No confirmed American IPA found

Phonetic Spelling: koo·mas·see

11. Spectrophotometer

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

IPA: /ˌspek.troʊ.fəˈtɑː.mə.t̬ə/

Phonetic Spelling: spek·troh·fuh·tah·muh·ter

12. Microcentrifuge

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

IPA: /ˌmaɪ.kroʊˈsen.trəˌfjuːdʒ/

Phonetic Spelling: my·kroh·sen·truh·fyooj

13. Chloroform

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

IPA: /ˈklɔːr.əˌfɔrm/

Phonetic Spelling: klor·uh·form

14. 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine

Pronunciation link: <https://www.merriam-webster.com/medical/phosphocholine>

IPA: No confirmed American IPA found for the complete chemical name

Phonetic Spelling: one·pal·mih·toyl · two·oh·lee·oh·il · ess·en · glys·uh·roh ·
three·fos·foh·koh·leen

15. Phosphocholine

Pronunciation link: No confirmed link found

IPA: No confirmed American IPA found

Phonetic Spelling: fos·foh·koh·leen

16. Sonication

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

IPA: /ˌsɔː.nɪˈkeɪ.ʃən/

Phonetic Spelling: sah·nih·kay·shuhn

17. Liposome

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

IPA: /ˈlaɪ.pəˌsoʊm/

Phonetic Spelling: ly·puh·sohm

18. Proteoliposome

Pronunciation link: No confirmed link found

IPA: No confirmed American IPA found

Phonetic Spelling: proh·tee·oh·ly·puh·sohm

19. Extrusion

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

IPA: /ɪk'struː.ʒən/

Phonetic Spelling: ik·stroo·zhuhn

20. Polycarbonate

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

IPA: /ˌpɑː.li'kɑːr.bə.nɛt/

Phonetic Spelling: pol·ee·kar·buh·nayt

21. Adsorbent

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

IPA: /æd'zɔːr.bənt/

Phonetic Spelling: ad·zor·buhnt

22. Methanol

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

IPA: /'meθ.ə.nɔːl/

Phonetic Spelling: meth·uh·nawl

23. Ethanol

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

IPA: /'eθ.ə.nɔːl/

Phonetic Spelling: eth·uh·nawl

24. Ultracentrifuge

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

IPA: /ˌʌl.trə'sen.trə'fjuːdʒ/

Phonetic Spelling: ul·truh·sen·truh·fyooj

25. Trichloroacetic

Pronunciation link: <https://www.merriam-webster.com/dictionary/trichloroacetic%20acid>

IPA: /ˌtraɪ.klɔːr.ə'siː.tɪk/

Phonetic Spelling: try·klor·oh·uh·see·tik

26. Proteolytic

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

IPA: /ˌproʊ.ti.ə'lɪ.tɪk/

Phonetic Spelling: proh·tee·uh·lit·ik