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Title: Lipid-Protein Membrane Structure-Function Characterization Using Droplet Interface Bilayers

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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? **Yes**

If **Yes**, can you record movies/images using your own microscope camera?

If yes, please provide the recordings for the shots labeled as SCOPE

If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit.

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye**.

If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

Scope shots: 2.3.2, 2.4.2, 2.7.2, 3.4.2

Videographer: Please film the above-mentioned shots using the scope kit

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? **Yes**

If **Yes**, how far apart are the locations? Two separate laboratory rooms on the same floor in the same building (Shull Wollan Center). Approximate distance between the two lab spaces: 50 m

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: 25

Number of Shots: 55 (36 SC, 4 Scope)

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**

INTRODUCTION:

Videographer's NOTE:

We shot the procedural shots first as the client had prepared items for the shoot that he wanted to use before they "went bad".

I labeled the interview shots in the file name because we scrambled the order of those statements during shooting.

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

- 1.1. **Peter Podar:** In this work, we study how physical and electrical properties of lipid membranes and their environment impact ion conduction in the droplet interface bilayer model system.
 - 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. **Peter Podar:** Traditional patch clamp electrophysiology doesn't probe larger-scale membrane biophysics, but this single platform captures both single-channel activity and mm-sized membrane areas to more extensively study lipid membrane biophysics.
 - 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. **Peter Podar:** This protocol allows us to measure how membrane structure and ion conduction change during electrically driven restructuring of droplet interface bilayers.

- 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **Author's NOTE: During recording, I believe this was recorded under 1.5.1**

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

- 1.4. **Peter Podar:** This platform can support many stimulation protocols in DIBs with ion channels, without ion channels, and even photo-isomerizable channels to probe membrane dynamics and electrical properties.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **Author's NOTE: During recording, I believe this was recorded under 1.3.1**

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

- 1.5. **Peter Podar:** As this tunable platform probes both macro- and microscale biophysics, future work would benefit from using fluorescence to more confidently separate protein channel activity from membrane pore conduction.

- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **Author's NOTE: During recording, I believe this was recorded under 1.4.1**

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.

Preparation of Reagents and Vesicles:

- **Buffer solution**

- 10 mM MOPS, 0.1 M KCl (pH 7.4)

- **Gramicidin A (gA) solutions**

- Stock diluted to ~2.66 μ M and 26.6 nM
- Lipid:Peptide ratios ~1:10⁻⁴ (for ensemble experiments)

- **Lipid–peptide vesicles (DPhPC + gA)**

- ~2.36 mM lipid suspension (DPhPC: 1,2-diphytanoyl-sn-glycero-3-phosphocholine)
- Extruded to ~100 nm unilamellar vesicles
- Used for droplet interface bilayer formation

- **Agarose solution**

- 1% agarose prepared in buffer for electrode coating

2. Formation of Droplet Interface Bilayers (DIBs)

Demonstrator: Peter Podar

2.1. To begin, prepare the chlorinated silver wire electrodes after coating them with agarose [1] and assembling them into a patch-clamp headstage and ground electrode holders for electrophysiological measurements [2].

2.1.1. WIDE: Talent placing the prepared electrodes on the work surface. **Videographer:** Please obtain CU shot as well for this shot.

2.1.2. Close-up of assembling the patch-clamp headstage and ground electrode holders.

2.2. For droplet interface bilayer formation, place a clear-bottom Petri dish on the stage of an inverted microscope [1]. Fill the dish with alkane oil, such as 100 percent hexadecane, to a depth of approximately 5 millimeters [2].

2.2.1. Talent placing a clean, clear-bottom Petri dish onto the inverted microscope

stage.

2.2.2. Talent pouring alkane oil into the Petri dish to the specified depth.

2.3. Using the micromanipulator controls, lower both agarose-coated ball ends into the oil to a depth of approximately 2.5 millimeters below the oil surface, avoiding rapid motion to prevent collisions with the Petri dish [1].

2.3.1. Talent lowering both agarose-coated electrodes into the oil using the micromanipulator.

~~2.3.2. SCOPE: Shot showing the electrodes in the oil, but away from the dish.~~

Videographer's NOTE: Segment 2.3 was not shot per the client

2.4. Adjust the microscope focus until both ball ends and their agarose coatings are in sharp focus [1]. Position the electrodes near the edge of the field of view so that both can be observed simultaneously [2].

2.4.1. Talent adjusting the microscope focus.

2.4.2. SCOPE: JoVE_SCOPE_70628_Step2.4.2-(1).mp4 00:04-00:20 *Video editor: Please speed up to fit the VO and show the entire range indicated here*

2.5. Using a calibrated 2-microliter pipette, aspirate the extruded DPhPC (*D-P-H-P-C*) lipid vesicles doped with gramicidin A [1-TXT]. Slowly dispense 250 nanoliters of the suspension directly onto each agarose-coated ball end using a separate pipette tip for each, without touching the agarose shell [2].

2.5.1. Talent aspirating the lipid-peptide vesicle suspension with a 2-microliter pipette.
TXT: DPhPC: 1,2-diphytanoyl-sn-glycero-3-phosphocholine

2.5.2. Talent dispensing the suspension onto each agarose-coated ball end.

2.6. Allow the droplets to spread over the agarose surface and visibly sag downward due to gravity and reduced surface tension, forming a pendant droplet shape [1]. Observe sagging from the side through the Petri dish wall if it is clear [2].

2.6.1. Talent observing droplets spreading and sagging from the agarose-coated electrode.

2.6.2. Shot of droplet sagging viewed through the side of the Petri dish. **Author's NOTE:** The videographer's camera lens was not high enough focal length to adequately capture this in full detail. Please zoom in (crop-in zoom) the recording to capture just the petri-dish and electrodes in view.

2.7. After 5 minutes, gently nudge the anti-vibration table to assess the droplet [1]. Confirm that the sagging droplets move with a slight delay relative to electrode motion, indicating the formation of fully coated lipid monolayer droplets [2]. If this behavior is not observed, wait an additional 2 to 3 minutes and repeat the assessment [3].

2.7.1. Talent gently nudging the anti-vibration table.

2.7.2. SCOPE: JoVE_SCOPE_70628_Step2.7.2-(1).mp4

2.7.3. Talent setting a timer for 2 minutes and then looking at the setup.

3. Electrical Setup and Bilayer Monitoring

3.1. Ensure the microscope and all other required components [1-TXT] are connected to a common ground [2].

3.1.1. Talent showing the components that are connected to a common ground. **TXT: Components: Anti-vibration table, Faraday cage, amplifier, digitizer, function generator and computer**

Added shot 3.1.2: Zoomed in view of the common ground.

Videographer's NOTE: 3.1.2 Client requested we add a shot to 3.1 to show where the "common ground" wire for all the equipment went into the ceiling to clarify the section

3.2. Arrange the instrument connections according to the manufacturer's setup guide [1] and configure the essential electrical and optical setup, following the detailed instructions for droplet interface bilayer experiments [2 and 3]. ~~Then, connect the headstage and ground electrodes to the patch-clamp amplifier [4].~~

3.2.1. Talent arranging cables and components.

3.2.2. Talent adjusting system connections while looking at a sheet having the schematic.

3.2.3. LAB MEDIA: Figure 1C *Video editor: Show this as an inset*

3.2.4. ~~Talent connecting the electrodes to the patch-clamp amplifier.~~ **Author's NOTE:** This was done along with 2.1.2

3.3. Configure an external function generator or the acquisition software output channel to generate a 10 hertz, 10 millivolt triangle voltage waveform [1]. Confirm the amplitude and frequency on a connected oscilloscope and in the acquisition software [2].

- 3.3.1. ~~SCREEN:~~ Talent setting waveform parameters to 10 hertz and 10 millivolts.
Author's NOTE: Steps 3.3.1 and 3.3.2 were converted to talent shots
- 3.3.2. ~~SCREEN:~~ Talent showing waveform amplitude and frequency on the oscilloscope and acquisition software.
- 3.4. After droplets have sagged for approximately 10 minutes, use the micromanipulators to bring the two droplets into gentle contact [1]. ~~Adjust the electrode positions so that the contact area is initially no more than approximately one quarter of the droplet diameter [2].~~
- 3.4.1. Talent bringing the droplets into contact using micromanipulators.
- ~~3.4.2. SCOPE: Droplets touching with a small contact area.~~
- 3.5. Turn on the 10 hertz, 10 millivolt triangle waveform [1] and monitor the capacitive current response in the acquisition software [2].
- 3.5.1. SCREEN: JoVE_SCREEN_70628_Step3.5.1-(1).mp4.
- 3.5.2. SCREEN: JoVE_SCREEN_70628_Step3.5.2-(1).mp4. *Video editor: Play in loop*
- 3.6. Adjust the droplet contact area by slightly moving the electrodes until the peak-to-peak capacitive current response to the waveform is between approximately 100 and 200 picoamperes [1], or, the contact area is no more than approximately one quarter of the droplet diameter [2].
- 3.6.1. SCOPE: JoVE_SCOPE_70628_Step3.7.2-(1).mp4 .
- 3.6.2. SCOPE: JoVE_SCOPE_70628_Step3.4.2(1).mp4 00:00-00:05
- 3.7. Wait for spontaneous bilayer formation, indicated electrically by an expanding peak-to-peak capacitive current response [1] and optically by an increasing internal reflection with an oval appearance at the contact region [2]. Confirm that pure lipid bilayers display rectangular current plateaus in response to the triangular voltage stimulus [3], while gramicidin A-doped bilayers exhibit sloped plateaus reflecting ensemble ionic conduction [4-TXT].
- 3.7.1. **SCREEN:** JoVE_SCREEN_70628_Step3.6.1.(1).mp4 00:00-00:10
- 3.7.2. SCOPE: JoVE_SCOPE_70628_Step3.4.2..(1).mp4 00:06-00:26
- 3.7.3. SCREEN: JoVE_SCREEN_70628_Step3.6.3..(1).mp4 *Video editor: Highlight the graph on the right half of the screen*
- 3.7.4. SCREEN: JoVE_SCREEN_70628_Step3.6.3..(1).mp4 *Video editor: Highlight the*

graph on the left half of the screen. The text overlay has a ratio **TXT: Lipid: Peptide= $\sim 1:10^{-4}$**

- 3.8. Once stable capacitive or conductive responses are confirmed, turn off the triangle waveform [1] and allow the droplet interface bilayer to equilibrate for at least 15 minutes before applying stimulation protocols [2].

3.8.1. SCREEN: JoVE_SCREEN_70628_Step3.8.1-(1).mp4. 00:00-00:04 (*when VO says once stableare confirmed*) and 00:04-00:06 (*turn off the triangle waveform*)

3.8.2. Talent leaving the setup and setting a 15 min timer.

4. Electrical Stimulation Protocol for Paired-Pulse Facilitation and Depression

- ~~4.1. Open the function generator or acquisition software to deliver voltage pulses of the desired amplitude, duration, and inter-pulse interval for paired-pulse facilitation and paired-pulse depression patterns [1].~~

~~4.1.1. SCREEN: opening the software.~~

- 4.2. In the video capture software, set the frame rate to at least 20 frames per second [1]. Select the appropriate camera and objective lens settings [2].

4.2.1. SCREEN: JoVE_SCREEN_70628_Step4.2-(1).mp4 00:04-00:11

4.2.2. SCREEN: JoVE_SCREEN_70628_Step4.2-(1).mp4 00:12-00:15

- 4.3. Next, click **Acquire** and select **New Protocol** [1]. In the **Acquisition Mode** tab, select **Episodic Stimulation** [2]. Set Runs per Trial and Sweeps per Run to 1 [3]. Adjust the sweep duration to 4 seconds longer than the total experimental duration, allowing a 2-second pre- and post-stimulus baseline [4]. Set the sampling rate to between 5 to 10 kilo Hertz [5].

4.3.1. SCREEN: JoVE_SCREEN_70628_Step4.3through4.7-(1).mp4 00:03-00:07

4.3.2. SCREEN: JoVE_SCREEN_70628_Step4.3through4.7-(1).mp4 00:08-00:09

4.3.3. SCREEN: JoVE_SCREEN_70628_Step4.3through4.7-(1).mp4 00:10-00:12

4.3.4. SCREEN: JoVE_SCREEN_70628_Step4.3through4.7-(1).mp4 00:12-00:15

4.3.5. SCREEN: JoVE_SCREEN_70628_Step4.3through4.7-(1).mp4 00:15-00:17

- 4.4. In the **Inputs** tab, assign the recording channel to the current input [1] and then, in the **Outputs** tab, assign the stimulation channel to the voltage output controlling the electrodes [2].

4.4.1. SCREEN: JoVE_SCREEN_70628_Step4.3through4.7-(1).mp4 00:18-00:21

4.4.2. SCREEN: JoVE_SCREEN_70628_Step4.3through4.7-(1).mp4 00:21-00:22

- 4.5. Open the **Waveform** tab [1]. In **Column A**, set **Type** to **Step**, **First Level** and **Delta Level** to 0 millivolts, and **First Duration** to 2000 milliseconds to define a pre-stimulation baseline [2].

4.5.1. SCREEN: JoVE_SCREEN_70628_Step4.3through4.7-(1).mp4 00:22-00:23

4.5.2. SCREEN: JoVE_SCREEN_70628_Step4.3through4.7-(1).mp4 00:27-00:40

- 4.6. In **Column B** set **Type Pulse** for paired-pulse facilitation [1]. Specify the values for first duration and first level voltage amplitude [2], and set the **Train Rate** to define the inter-pulse interval corresponding to the target duty cycle [3]. Adjust the pulse width to 100 milli seconds [4].

4.6.1. SCREEN: JoVE_SCREEN_70628_Step4.3through4.7-(1).mp4 00:46-00:50

4.6.2. SCREEN: JoVE_SCREEN_70628_Step4.3through4.7-(1).mp4 00:53-00:59

4.6.3. SCREEN: JoVE_SCREEN_70628_Step4.3through4.7-(1).mp4 01:00-01:05

- 4.7. In **Column C** for paired-pulse depression, set **Type** to **Pulse** with the same amplitude and pulse duration [1]. Set the **Train Rate** to achieve the desired lower duty cycle and set the pulse width [2]. Next, in **Column D**, configure the post-stimulation parameters identical to the pre-stimulation baseline [3]. Review the protocol [4] and save it with a descriptive name [5].

4.7.1. SCREEN: JoVE_SCREEN_70628_Step4.3through4.7-(1).mp4 01:09-01:14 and

4.7.2. SCREEN: JoVE_SCREEN_70628_Step4.3through4.7-(1).mp4 01:17-01:25

4.7.3. SCREEN: JoVE_SCREEN_70628_Step4.3through4.7-(1).mp4 01:27-01:34

4.7.4. SCREEN: JoVE_SCREEN_70628_Step4.3through4.7-(1).mp4 01:34-01:37

4.7.5. SCREEN: JoVE_SCREEN_70628_Step4.3through4.7-(1).mp4 02:20-02:24

4.8. Start video recording [1] and immediately start electrical acquisition and stimulation by clicking the **Record** button in each software [2].

4.8.1. SCREEN: JoVE_SCREEN_70628_Step4.2-(1).mp4 00:15-00:16

4.8.2. SCREEN: JoVE_SCREEN_70628_Step4.8through4.9-(1).mp4 00:00-00:01

4.9. During the first 60 seconds of stimulation, observe that the current increases and may reach a plateau [1]. During the last 60 seconds, observe that the current decreases [2]. At the end of the protocol, stop electrical acquisition and video recording simultaneously [3].

4.9.1. SCREEN: JoVE_SCREEN_70628_Step4.8through4.9-(1).mp4 00:05-01:04

Author's NOTE: For 4.9.1 and 4.9.2, because we want to illustrate the temporal evolution of the experiment with the eventual plateau, we want to show the entire experiment sped up instead of in shorter, separated sections. Please speed up the recording and indicate by what factor (e.g., "20x-speed"). May be include a 3 to 4 second pause after 4.9.1 to 4.9.2 to accommodate the video.

4.9.2. SCREEN: JoVE_SCREEN_70628_Step4.8through4.9-(1).mp4 01:04-02:05

4.9.3. SCREEN: JoVE_SCREEN_70628_Step4.8through4.9-(1).mp4 02:10-02:15

4.10. Finally, perform post-processing to extract membrane area and compute normalized current, area, and flux from imaging and electrical data [1].

4.10.1. SCREEN: JoVE_SCOPE_70628_Step4.10.1-(1).mp4. 00:05-00:15

Results

5. Results

5.1. Sequential images captured spontaneous bilayer zipping and area expansion when 2 sagging droplets were brought into contact [1].

5.1.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the time-lapse images labeled $t = 0$ s, $t = 2$ s, $t = 5$ s, $t = 10$ s, and $t = 30$ s.*

5.2. The bright, inner oval-shaped reflections at the droplet contact were used to estimate bilayer diameter and membrane area over time [1].

5.2.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the bright inner reflection labeled "Internal reflection of membrane area."*

5.3. Paired-pulse facilitation from 0 seconds to 60 seconds [1] and paired-pulse depression from 60 seconds to 120 seconds were delivered using 100-millisecond pulses [2].

5.3.1. LAB MEDIA: Figure 5. *Video editor: Highlight the red 0 to 60 second section*

5.3.2. LAB MEDIA: Figure 5. *Video editor: Highlight the blue 60 to 120 second section.*

5.4. The representative current responses during ON periods [1], and the stimulation patterns were recorded [2].

5.4.1. LAB MEDIA: Figure 5. *Video editor: Highlight the upper current panel.*

5.4.2. LAB MEDIA: Figure 5. *Video editor: Highlight the lower voltage panel.*

5.5. Normalized current for gramicidin A-doped DPhPC bilayers in hexadecane and dodecane-hexadecane oils was shown [1] during paired-pulse facilitation [2] and paired-pulse depression [3].

5.5.1. LAB MEDIA: Figure 6A.

5.5.2. LAB MEDIA: Figure 6A. *Video editor: Highlight the left half showing PPF*

5.5.3. LAB MEDIA: Figure 6A. *Video editor: Highlight the right half showing PPD*

5.6. Normalized membrane area measured at 30 time points showed similar area evolution in both oil conditions despite differing currents [1].

5.6.1. LAB MEDIA: Figure 6B. *Video editor: Highlight the orange and blue area points*

and error bars.

5.7. Normalized flux separated area-independent changes in conductance and was consistent with changes in membrane conduction beyond simple area expansion [1].

5.7.1. LAB MEDIA: Figure 6C.

5.8. Extended paired-pulse depression stimulation for up to 30 minutes produced long-term depression-like behavior in hexadecane membranes [1] but maintained elevated flux in dodecane-hexadecane membranes, consistent with long-term potentiation-like behavior [2].

5.8.1. LAB MEDIA: Figure 6D. *Video editor: Highlight the orange and red C16 traces*

5.8.2. LAB MEDIA: Figure 6D. *Video editor: Highlight the grey and green C12/C16 traces*

1. Chlorinated

Pronunciation link: https://www.merriam-webster.com/dictionary/chlorinated

IPA: /'klɔːrəˌneɪtəd/

Phonetic Spelling: klor·uh·nay·tuhd

2. Agarose

Pronunciation link: https://www.merriam-webster.com/dictionary/agarose

IPA: /'ægəˌroʊs/

Phonetic Spelling: a·guh·rohs

3. Electrophysiological

Pronunciation link: https://www.merriam-webster.com/dictionary/electrophysiology

IPA: /ɪˌlekˌtroʊˌfɪz.i.əˈlɑː.dʒɪ.kəl/

Phonetic Spelling: ih·lek·troh·fiz·ee·uh·laa·juh·kuhl

4. Patch-clamp

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

IPA: /'pætʃ, klæmp/

Phonetic Spelling: pach·klamp

5. Petri dish

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

IPA: /'pi:tri ,dɪʃ/

Phonetic Spelling: pee·tree dish

6. Hexadecane

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

IPA: / ,hɛk.sə'deɪ.keɪn/

Phonetic Spelling: hek·suh·day·kayn

7. Micromanipulator

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

IPA: / ,maɪ.kroʊ.mə'nɪp.jə ,leɪ.tə/

Phonetic Spelling: my·kroh·muh·nip·yuh·lay·ter

8. Pipette

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

IPA: /paɪ'pet/

Phonetic Spelling: py·pet

9. DPhPC

Pronunciation link: No confirmed link found

IPA: /di: pi: ɛɪtʃ pi: si:/

Phonetic Spelling: dee pee aitch pee see

10. Diphytanoyl

Pronunciation link: No confirmed link found

IPA: /daɪˌfæʔəˈnoɪl/

Phonetic Spelling: dye·fy·tuh·noyl

11. Glycero

Pronunciation link: No confirmed link found

IPA: /ˈɡlɪsəroʊ/

Phonetic Spelling: glis·uh·roh

12. Phosphocholine

Pronunciation link: [\[https://www.howtopronounce.com/phosphocholine\]](https://www.howtopronounce.com/phosphocholine)(<https://www.howtopronounce.com/phosphocholine>)

IPA: /ˌfɑːsˌfoʊˈkoʊ.liːn/

Phonetic Spelling: fos·foh·koh·leen

13. Gramicidin

Pronunciation link: [\[https://www.merriam-webster.com/medical/gramicidin\]](https://www.merriam-webster.com/medical/gramicidin)(<https://www.merriam-webster.com/medical/gramicidin>)

IPA: /ˌɡræməˈsaɪdɪn/

Phonetic Spelling: gram·uh·sy·duhn

14. Monolayer

Pronunciation link: https://www.merriam-webster.com/dictionary/monolayer

IPA: /'mɑ:nə,leɪə/

Phonetic Spelling: mon·uh·lay·er

15. Bilayer

Pronunciation link: https://www.merriam-webster.com/dictionary/bilayer

IPA: /'baɪ,leɪə/

Phonetic Spelling: by·lay·er

16. Oscilloscope

Pronunciation link: https://www.merriam-webster.com/dictionary/oscilloscope

IPA: /ə'sɪlə,skoʊp/

Phonetic Spelling: uh·sil·uh·skohp

17. Capacitive

Pronunciation link: https://www.merriam-webster.com/dictionary/capacitive

IPA: /kə'pæsətɪv/

Phonetic Spelling: kuh·pas·uh·tiv

18. Picoamperes

Pronunciation link: https://www.merriam-webster.com/dictionary/picoampere

IPA: /,pi:kəʊ'æm,pɪrɪz/

Phonetic Spelling: pee·koh·am·peers

19. Ionic

Pronunciation link: https://www.merriam-webster.com/dictionary/ionic

IPA: /aɪˈɑːnɪk/

Phonetic Spelling: eye·on·ik

20. Facilitation

Pronunciation link: https://www.merriam-webster.com/dictionary/facilitation

IPA: /fəˌsɪləˈteɪʃən/

Phonetic Spelling: fuh·sil·uh·tay·shuhn

21. Depression

Pronunciation link: https://www.merriam-webster.com/dictionary/depression

IPA: /dɪˈpreʃən/

Phonetic Spelling: dih·presh·uhn

22. Episodic

Pronunciation link: https://www.merriam-webster.com/dictionary/episodic

IPA: /ˌɛpəˈsɔːdɪk/

Phonetic Spelling: ep·uh·sod·ik

23. Conductance

Pronunciation link: https://www.merriam-webster.com/dictionary/conductance

IPA: /kənˈdʌktəns/

Phonetic Spelling: kuhn·duk·tuhns

24. Dodecane

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

IPA: /ˈdɒsdəˌkeɪn/

Phonetic Spelling: doh·duh·kayn

25. Potentiation

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

IPA: /pəˌtɛnʃiˈeɪʃən/

Phonetic Spelling: puh·ten·shee·ay·shuhn