

Submission ID #: 72146

Scriptwriter Name: Sri Lakshmi Priya

Project Page Link: <https://review.jove.com/account/file-uploader?src=22004578>

Title: Stabilizing Protein and Protein-RNA Condensates in Low-Melting Agarose Hydrogels for Quantitative Biophysical Measurements

Authors and Affiliations:

Nina C. Kathe, Leonidas Emmanouilidis, Mihajlo Novakovic, Frédéric H.-T. Allain

Institute of Biochemistry, ETH Zürich

Corresponding Authors:

Nina C. Kathe	nina.kathe@bc.biol.ethz.ch
Frédéric H.-T. Allain	allain@bc.biol.ethz.ch

Email Addresses for All Authors:

Nina C. Kathe	nina.kathe@bc.biol.ethz.ch
Frédéric H.-T. Allain	allain@bc.biol.ethz.ch
Leonidas Emmanouilidis	leonidas_emmanouilidis@protonmail.com
Mihajlo Novakovic	mihajlo.novakovic@bc.biol.ethz.ch

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? **NO**
- 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: 10

Number of Shots: 23

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. **N. Kathe:** Our research focuses on developing a standardized agarose embedding protocol that immobilizes biomolecular condensates in three dimensions while preserving their dynamic properties.
 - 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. **N. Kathe:** Condensates tend to move, fuse, or stick to surfaces, which makes certain measurements difficult, whereas this agarose embedding protocol addresses these limitations.
 - 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. **N. Kathe:** This protocol allows researchers to image and quantify condensates over large time scales while preserving dynamic behavior.
 - 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. **N. Kathe:** The biggest challenge when performing this protocol is getting both the agarose temperature and the timing of the embedding right.
 - 1.4.1. 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?~~

- 1.5. **N. Kathe:** Once embedded, condensates can be studied by a wide range of microscopy techniques and other biophysical methods, such as NMR.
 - 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.*

2. Preparation of Agarose Stock Solution

Demonstrator: N. Kathe

- 2.1. To begin, weigh the appropriate amount of low-melting agarose or LMA (*L-M-A*) or regular agarose [1] and add it to a 1.5-milliliter tube containing droplet buffer [2].
 - 2.1.1. WIDE: Talent weighing low-melting agarose or regular agarose. ***Videographer:** Please obtain CU shot as well for this shot*
 - 2.1.2. Talent adding the agarose into the 1.5mL tube containing droplet buffer.
- 2.2. Heat the agarose stock to 80 to 90 degrees Celsius in a heating block or water bath [1] and fully dissolve the agarose by vortexing for approximately 10 minutes [2].
 - 2.2.1. Talent placing the agarose stock into the heating block or water bath
 - 2.2.2. Shot of vortexing the tube containing agarose.
- 2.3. Equilibrate the agarose stock to 37 degrees Celsius for low-melting agarose or 55 degrees Celsius for regular agarose in the heating block or water bath [1]. Verify that the solution remains clear and liquid at this temperature [2]. Check the temperature of the agarose stock with a thermometer [3].
 - 2.3.1. Shot of agarose stock placed in the heating block or water bath for equilibration.
 - 2.3.2. Shot of the agarose solution remaining clear and liquid.
 - 2.3.3. Talent checking the temperature of the agarose stock with a thermometer.

3. Preparation and Gelation of Agarose-Stabilized Condensates

- 3.1. Prewarm all materials that will come into direct contact with the agarose to approximately 37 degrees Celsius by placing them on a heating block or in an incubator for 2 to 3 minutes [1].
 - 3.1.1. Talent placing pipette tips, tubes, and microscopy slides on a heating block or in an incubator for prewarming.
- 3.2. Prepare the agarose stock as previously demonstrated [1].
 - 3.2.1. Talent preparing the agarose stock.

3.3. Gather the required reagents to [1] prepare the droplet buffer, phase-separating components, and additional additives for the samples [2]. Equilibrate all prepared solutions to room temperature [3]. Calculate the required volumes of droplet buffer, agarose stock, phase-separating components, and supplementary additives for the sample [4]. As starting values, use a final agarose concentration of 0.3 to 0.5 percent weight per volume for standard biophysical assays and 0.8 to 1.0 percent weight per volume for long-term stabilization experiments [5].

3.3.1. Talent arranging the reagents on the workbench.

3.3.2. TEXT ON A PLAIN BACKGROUND:

Reagent Preparation:

Buffer A (pH 7.2):

25 mM HEPES

50 mM NaCl

Nucleocapsid protein stock:

60 μ M in Buffer A

Polyadenylic acid stock:

30 μ M in Buffer A

3.3.3. Talent placing the prepared solutions at room temperature for equilibration.

3.3.4. TEXT ON A PLAIN BACKGROUND:

Sample Composition:

Buffer A: 6 μ L

2% Agarose Stock: 5 μ L (Final 0.5%)

Nucleocapsid Protein: 5 μ L (Final 15 μ M)

Poly(A): 4 μ L (Final 6 μ M)

Final Volume: 20 μ L

3.3.5. Talent noting the starting final agarose concentration values for the sample.

3.4. Add the calculated volume of buffer into a prewarmed 1.5-milliliter tube [1]. Add the agarose stock to the same tube [2], followed by any supplementary additives [3] and then the phase-separating components [4].

3.4.1. Talent adding the calculated volume of buffer into a prewarmed 1.5 milliliter tube.

3.4.2. Talent adding the agarose stock to the tube.

3.4.3. Talent adding supplementary additives to the tube.

- 3.4.4. Talent adding the phase-separating components to the tube.
- 3.5. Keep the tube in the heating block or water bath [1], and pipette the mixture up and down a few times, avoiding air bubbles [2].
 - 3.5.1. Talent placing the tube in the heating block
 - 3.5.2. Talent pipetting the mixture up and down while the tube remains in the heating block or water bath.
- 3.6. Using a pipette, quickly transfer the sample onto the microscopy slide [1].
 - 3.6.1. Shot of transferring the sample onto a microscopy slide using a pipette.
- 3.7. Allow the sample to gel at room temperature for 5 to 10 minutes [1]. Measure the sample [2].
 - 3.7.1. Talent placing the microscopy slide at room temperature for gelation.
 - 3.7.2. Talent measuring the sample according to the standard protocol.

Results

4. Results

- 4.1. Droplets formed with N protein and poly A (*Poly-A*) in the presence of 0.5% weight per volume low-melting agarose were roughly the same size [1] as droplets formed without low-melting agarose after 5 minutes at room temperature [2].
 - 4.1.1. LAB MEDIA: Figure 1A,B. *Video editor: In 1A, in the Cy3-poly(A) row, highlight the "no agarose" at "5 min" image. In 1B, highlight the blue bar at 5 min under the no agarose*
 - 4.1.2. LAB MEDIA: Figure 1A,B. *Video editor: In 1A, in the Cy3-poly(A) row, highlight the "+ agarose" at "5 min" image. In 1B, highlight the blue bar at 5 min under the "+ agarose "*
- 4.2. Sixty minutes after sample preparation, agarose-embedded droplets did not change in size [1], whereas condensates without low-melting agarose showed a drastic increase in size due to fusion and surface wetting [2].
 - 4.2.1. LAB MEDIA: Figure 1A,B. *Video editor: In 1A, in the Cy3-poly(A) row, highlight the "+ agarose" at "60 min" image. In 1B, highlight the dark grey bar at 60 min under the "+ agarose "*
 - 4.2.2. LAB MEDIA: Figure 1A,B. *Video editor: In 1A, in the Cy3-poly(A) row, highlight the "no agarose" at "60 min" image. In 1B, highlight the dark grey bar at 60 min under the "no agarose "*
- 4.3. Fluorescence recovery after photobleaching recovery and apparent droplet diffusion were highly similar with low-melting agarose [1] and without low-melting agarose [2].
 - 4.3.1. LAB MEDIA: Figure 1C. *Video editor: Highlight the blue recovery curve labelled "+ agarose".*
 - 4.3.2. LAB MEDIA: Figure 1C. *Video editor: Highlight the grey recovery curve labelled "no agarose".*
- 4.4. Diffusion rates of FUS N-terminal (*fus-N-terminal*) domain or FUS NTD (*fus-N-T-D*) condensates were not detectably influenced by agarose concentrations of 0.25 [1], 0.5 [2], and 1% weight per volume [3].
 - 4.4.1. LAB MEDIA: Figure 1D. *Video editor: Highlight the red data point above between 0.2 and 0.4 on the Agarose % axis.*
 - 4.4.2. LAB MEDIA: Figure 1D. *Video editor: Highlight the red data point between 0.4 and 0.6 on the Agarose percentage axis.*

- 4.4.3. LAB MEDIA: Figure 1D. *Video editor: Highlight the red data point above "1.0" on the Agarose percentage axis.*
- 4.5. N protein plus s2m RNA (*N-protein-plus-S-two-M-RNA*) droplets were stabilized in low-melting agarose for long enough to acquire stochastic optical reconstruction microscopy images [1].
 - 4.5.1. LAB MEDIA: Figure 1E. *Video editor: Highlight the image labelled "STORM".*
- 4.6. Nuclear magnetic resonance spectra of FUS NTD droplets with agarose [1] and without agarose had comparable line widths [2].
 - 4.6.1. LAB MEDIA: Figure 1F. *Video editor: Highlight the black spectrum labelled "no agarose".*
 - 4.6.2. LAB MEDIA: Figure 1F. *Video editor: Highlight the red spectrum labelled "0.5% agarose".*
- 4.7. Agarose-embedded condensates showed higher signal intensity [1] than non-stabilized droplets [2].
 - 4.7.1. LAB MEDIA: Figure 1F. *Video editor: Highlight the red spectrum labelled "0.5% agarose".*
 - 4.7.2. LAB MEDIA: Figure 1F. *Video editor: Highlight the black spectrum labelled "no agarose".*

Pronunciation Guides:

- 1. Agarose
Pronunciation link: <https://www.merriam-webster.com/dictionary/agarose>
IPA: /'ægəˌroʊs/
Phonetic Spelling: ag·uh·rohs
- 2. Low-melting
Pronunciation link: No confirmed link found
IPA: /ˌloʊ'meltɪŋ/
Phonetic Spelling: loh·mel·ting
- 3. Droplet
Pronunciation link: <https://www.merriam-webster.com/dictionary/droplet>
IPA: /'drɑːplət/
Phonetic Spelling: draa·pluht
- 4. Vortexing
Pronunciation link: <https://www.merriam-webster.com/dictionary/vortex>
IPA: /'vɔːrˌtɛksɪŋ/
Phonetic Spelling: vor·tek·sing

5. Equilibrate
Pronunciation link: <https://www.merriam-webster.com/dictionary/equilibrate>
IPA: /ˌiːkwəˈlaɪˌbreɪt/
Phonetic Spelling: ee·kwuh·ly·brayt
6. Celsius
Pronunciation link: <https://www.merriam-webster.com/dictionary/Celsius>
IPA: /ˈsɛlsiəs/
Phonetic Spelling: sel·see·uhs
7. Thermometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/thermometer>
IPA: /θərˈmɑːmɛtər/
Phonetic Spelling: thur·maa·muh·tur
8. Microscopy
Pronunciation link: <https://www.merriam-webster.com/dictionary/microscopy>
IPA: /maɪˈkrɑːskəpi/
Phonetic Spelling: my·kraas·kuh·pee
9. Incubator
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubator>
IPA: /ˈɪŋkjəˌbeɪtər/
Phonetic Spelling: ing·kyuh·bay·tur
10. Reagents
Pronunciation link: <https://www.merriam-webster.com/dictionary/reagent>
IPA: /riˈeɪdʒənt/
Phonetic Spelling: ree·ay·juhnt
11. Phase-separating
Pronunciation link: No confirmed link found
IPA: /ˈfeɪz ˈsepəˌreɪtɪŋ/
Phonetic Spelling: fayz·sep·uh·ray·ting
12. Biophysical
Pronunciation link: <https://www.merriam-webster.com/dictionary/biophysical>
IPA: /ˌbaɪəʊˈfɪzɪkəl/
Phonetic Spelling: by·oh·fiz·ih·kuhl
13. Nucleocapsid
Pronunciation link: <https://www.merriam-webster.com/medical/nucleocapsid>
IPA: /ˌnuːkliəʊˈkæpsɪd/
Phonetic Spelling: noo·klee·oh·kap·sid
14. Polyadenylic
Pronunciation link: No confirmed link found
IPA: /ˌpɑːliˌædəˈnɪlɪk/

Phonetic Spelling: pol·ee·ad·uh·nil·ik

15. Supplementary

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

IPA: /ˌsʌplə'mentri/, /ˌsʌplə'menteri/

Phonetic Spelling: sup·luh·men·tree

16. Gelation

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

IPA: /dʒə'leɪʃən/

Phonetic Spelling: juh·lay·shuhn

17. Condensates

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

IPA: /'kɑːndən'set/

Phonetic Spelling: kon·duhn·sayt

18. Fluorescence

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

IPA: /flʊ'resəns/, /flɔ:'resəns/

Phonetic Spelling: floor·ess·uhns

19. Photobleaching

Pronunciation link: No confirmed link found

IPA: /ˌfoʊtoʊ'bli:tʃɪŋ/

Phonetic Spelling: foe·toe·blee·ching

20. Diffusion

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

IPA: /dɪ'fjuːʒən/

Phonetic Spelling: dih·fyoo·zhuhn

21. Stochastic

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

IPA: /stəʊ'kæstɪk/

Phonetic Spelling: stoh·kas·tik

22. Reconstruction

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

IPA: /ˌriːkən'strʌkʃən/

Phonetic Spelling: ree·kuhn·struk·shuhn

23. Nuclear

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

IPA: /'nuːkliər/, /'njuːkliər/

Phonetic Spelling: noo·klee·ur

24. Magnetic

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

IPA: /mæg'netɪk/

Phonetic Spelling: mag·net·ik

25. Resonance

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

IPA: /'rezənəns/

Phonetic Spelling: rez·uh·nuhns

26. Spectra

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

IPA: /'spektrə/

Phonetic Spelling: spek·truh

27. Agarose-embedded

Pronunciation link: No confirmed link found

IPA: /'ægə,roʊs ɛm'bedɪd/

Phonetic Spelling: ag·uh·rohs em·bed·id