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Scriptwriter Name: Pallavi Sharma

Project Page Link: <https://review.jove.com/account/file-uploader?src=21511698>

Title: Using Solution NMR to Characterize Biomolecular Condensates Under Biphasic Conditions

Authors and Affiliations:

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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/account/file-uploader?src=21511698>

3. Filming location: Will the filming need to take place in multiple locations? **Yes**

200 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. **Yes**

Current Protocol Length

Number of Steps: 42

Number of Shots: 17

Introduction

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

Videographer's NOTE: we start with the shoot 1.3.1. The talent expressed not having the necessary time to perform the shooting for work reasons. He offered to do the interview and once finished he left the place. There were not many rooms available to conduct the interview, it was decided to choose the most decent room. (White wall background.) The rests of the shooting It was carried out in order.

INTRODUCTION:

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

- 1.1. **Johannes Schmoll:** We develop NMR methods to characterize biomolecular condensates, key to cellular organization, whose aberrant formation is linked to multiple diseases.
 - 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. **Johannes Schmoll:** Using our methods, we analyze condensate material properties, investigate formation mechanisms, and monitor long-term changes, including pathological fibrillation of biomolecules.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.4.1*

CONCLUSION:

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

- 1.3. **Frederic Allain:** This protocol characterizes biphasic condensates without chemical tagging, where droplet-shaped condensates coexist with a dilute phase, mimicking cellular phase separation.

- 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. **Mihajlo Novakovic**: Our methods, focused on condensate properties, can be complemented by conventional liquid-state NMR to study biomolecular structure, interactions, and dynamics across phases.

- 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. **Mihajlo Novakovic**: Future studies will develop more complex, multi-component condensate models, adapt methodologies for higher complexity, and ultimately enable direct in-cell NMR investigation.

- 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.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

What problem in your field of research does your JoVE article address?

- 1.6. **Mihajlo Novakovic, Oberassistent**, : (authors will present their testimonial statements live)
 - 1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

How does the video format of your article help you to share your work with the broader scientific community?

- 1.7. **Johannes Schmoll, PhD Student**, : (authors will present their testimonial statements live)
 - 1.7.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

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

Videographer's NOTE: For reasons of the procedure, there was no place to stop the filming since the experiment required exact temperatures and exact time for execution. Technically it could only be executed once due to the materials. For this reason the shots were made in 4K to be able to make the appropriate crop without losing quality: total shots 3:
Shot 1: talent doing the whole process.
Shot 2: talent shaking the product and showing the result.
Shot 3: close up of the product result.

2. Biphasic Sample Preparation for REDIFINE Experiments and Their Recording

Demonstrators: Mihajlo Novakovic; Johannes Schmoll

2.1. To begin, prepare the final biphasic sample stabilized by agarose hydrogel [1], mix the protein stocks, RNA stocks, or both with the respective warm agarose buffer in a 1.5-milliliter microcentrifuge tube [2-TXT].

2.1.1. WIDE: Talent at the working bench with all the required reagents placed in front.

2.1.2. Talent mixing the protein stocks, RNA stocks with the warm agarose buffer in a 1.5 milliliter microcentrifuge tube. **TXT: Final protein/RNA concentration: 50-500 μ M** **NOTE: 2.1.2, 2.2.1 and 2.2.2 were recorded by name 2.1.2**

2.2. Immediately transfer the mixture into a 3-millimeter NMR tube with a long glass pipette [1] and allow the agarose to solidify shortly after transfer [2].

2.2.1. Talent transferring the mixture into a 3 millimeter NMR tube with a long glass pipette.

2.2.2. Shot of the solidified agarose in the NMR tube.

2.3. Then, lock, shim, match, and tune the proton channel as usual [1]. Calibrate the 90-degree pulse length on the proton channel using the general calibration procedure [2].

2.3.1. Talent operating the NMR spectrometer console while preparing the instrument for proton channel lock, shim, match, and tune.

Videographer's NOTE: The talent suggested to realise the shot 2.3.1 in 8 steps:

1.wide shot (talent ready to proceed with the machine)

2.close up of the process.(puting the example in the machine)

3.wide shot of the machine in action.

4.talent in the desk: proton channel lock.

5.talent in the desk: Shim.

6.talent in the desk: match.

7.talent in the desk: tune.

8.talent in the desk: Spectrum.

2.3.2. SCREEN: Show the spectrometer software interface while calibrating the 90 degree proton pulse length. NOTE: 2.3.2 to 2.4.2 are filmed by the videographer

2.4. Now, record a one-dimensional water-suppressed spectrum [1] and observe a strong biomolecular signal in the spectrum. If no or very little signal is observed, do not proceed with REDIFINE [2-TXT].

2.4.1. SCREEN: Show the spectrometer software interface while starting acquisition of the one dimensional water suppressed spectrum.

2.4.2. SCREEN: Display the acquired spectrum showing strong biomolecular peaks.

Author's NOTE: For screen captures, we did not go one by one and filled all parameters as it is redundant and long.

2.5. Record a diffusion ordered spectroscopy experiment to array the diffusion gradient strength from 2 to 95 percent in 16 linear steps [1-TXT]. Observe the initial decay of the signal from the dilute phase [2]. Then, observe the condensed phase signal plateau during the decay [3].

2.5.1. SCREEN: Screen Recording 1.mp4: 00:00-end. TXT: **Command Used: xau dosy 2 95 16 l y y'**

2.5.2. SCREEN: Screen Recording 2.mp4: 00:00-00:11, 00:20-00:30

2.5.3. SCREEN: Screen Recording 2.mp4: 00:42-00:53

2.6. To continue acquiring data for the full REDIFINE set, create 9 new stebpgp1s19 (*Steb-P-P-G-P-One-s-Nineteen*) experiments by copying the parameters from the initial experiment that was just run [1]. In each consecutive experiment, change only the d20 (*D-Twenty*) diffusion delay in variable steps to span the range from 100 to 1000 milliseconds [2].

2.6.1. SCREEN: Screen Recording 3.mp4: 00:03-00:08, 00:15-00:20, 00:26-00:32

2.6.2. SCREEN: Screen Recording 4.mp4: 00:00-end

2.7. Run each of these experiments with the suitable command. Use the last n to preserve the receiver gain throughout the 10 experiments [1].

2.7.1. SCREEN: Screen Recording 3.mp4: 00:23-00:32

3. Processing REDIFINE Datasets and Visualizing the Results

- 3.1. Set *qsine (Q-Sine)* with *SSB 2 (S-S-B-Two)* as the window function. Then, extract the fifth slice using the appropriate command [1]. Phase the one-dimensional spectrum [2] and save the phasing parameters to the two-dimensional dataset [3].
 - 3.1.1. SCREEN: Screen Recording 5.mp4: 00:00-00:05
 - 3.1.2. SCREEN: Screen Recording 5.mp4: 00:05-00:10
- 3.2. Now, process the two-dimensional dataset with the *xf2 (X-F-Two)* and perform baseline correction with *abs2 (A-B-S-Two)*. After that, extract the 16 slices with *split2D (Split-Two-D)*. Repeat the similar procedure for the remaining 9 experiments [1].
 - 3.2.1. SCREEN: Screen Recording 5.mp4: 00:13-00:20
- 3.3. Check at least the first 4 to 5 slices to ensure correct phasing and baseline correction. If needed, correct the phasing and baseline manually [1].
 - 3.3.1. SCREEN: Screen Recording 5.mp4: 00:20-00:40
- 3.4. Then, use the suitable MATLAB code for the analysis [1-TXT]. In the code, modify the diffusion times and assign experiments 1 through 10 based on the total number of experiments [2]. Then set *P.g (P-G)* according to the probe gradient strengths [3].
 - 3.4.1. SCREEN: Screen Recording 8.mp4: 00:13-00:22 TXT: LLPS_REDIFINE_fit
 - 3.4.2. SCREEN: Screen Recording 8.mp4: 00:39-00:47
 - 3.4.3. SCREEN: Screen Recording 8.mp4: 00:46-00:54

4. Recording CONDENSE-MT Experiments

- 4.1. Determine the *R1* rate of water by *T1* inversion recovery for quantitative CONDENSE-MT (*Condense-M-T*) analysis [1]. Copy the provided pulse sequence, Bruker parameter set, and *vd (V-D)* list to the respective folders [2]. ~~Load the parameter set *T1_inversion_recovery (T-One-Underscore-Inversion-Underscore_recovery)* and adjust the powers and frequencies to the NMR spectrometer [3].~~ NOTE: The *Vo* is edited for the deleted shot
 - 4.1.1. SCREEN: Screen Recording 6.mp4: 00:00-00:05.
 - 4.1.2. SCREEN: Screen Recording 6.mp4: 00:05-00:10
 - 4.1.3. ~~SCREEN: Show the parameter set *T1_inversion_recovery* being loaded and the powers and frequencies being adjusted.~~ NOTE: Not filmed
- 4.2. After calibrating the proton 90-degree pulse, set *NS (N-S)* to 2, *DS (D-S)* to 0, *d1* to 20 seconds, *SW (S-W)* to 10 parts per million, *TD2 (T-D-Two)* to 16k, and *TD1 (T-D-One)* to

- 24 [1]. ~~Enable a weak gradient along Z to avoid radiation damping effects [3].~~ NOTE: The Vo is edited for the deleted shot
- 4.2.1. ~~SCREEN: Show calibration of the proton 90-degree pulse p1.~~ NOTE: Not filmed
- 4.2.2. SCREEN: Screen Recording 6.mp4: 00:10-00:16
- 4.2.3. ~~SCREEN: Gradient is being enabled along Z.~~ NOTE: Not filmed
- 4.3. For the CONDENSE-MT experiment, copy the provided Bruker parameter set, sequence, and frequency list to the Bruker-specific folders. Then, adjust the frequencies and power according to the spectrometer [1].
- 4.3.1. SCREEN: Screen Recording 6.mp4: 00:16-00:25 NOTE: The Vo is edited for the deleted shot
- 4.3.2. ~~SCREEN: Show the parameter set CONDENSE-MT being loaded.~~ NOTE: Not filmed
- 4.4. Set P1 to the calibrated 90-degree pulse length and P4 to 1 millisecond for the excitation pulse to avoid radiation damping. Set NS to 2, DS to 16, d1 to 4 seconds, SW2 (*S-W-Two*) to 20 parts per million, TD2 to 8k, and TD1 to the length of the frequency list [1]. Set SPNAM10 (*S-P-NAM-10*) to Squa100.1000 (*Squa-Hundred-Thousand*) and set p10 to 5 seconds [2].
- 4.4.1. SCREEN: Screen Recording 6.mp4. 00:27-00:35
- 4.4.2. SCREEN: Screen Recording 6.mp4: 00:36 *Video editor: Freeze frame and highlight SPNAM 10 and p10*
- 4.5. Find the SP10 power that yields 100 hertz irradiations. Adjust the SP10 power of the irradiation pulse accordingly. Run the pseudo two-dimensional experiment with the appropriate command [1].
- 4.5.1. SCREEN: Screen Recording 6.mp4: 00:38-00:55
- 4.6. ~~For agarose data, use the normalized integrals directly for fitting with the appropriate script [1].~~
- 4.6.1. ~~SCREEN: Show the normalized integrals being used for fitting in the CONDENSE_MT_referenceMTpool script that calls the function_1MT function.~~ NOTE: Not filmed

Results

5. Results

- 5.1. The REDIFINE fit determined the diffusion coefficients in the dilute and condensed phases [1], the population of protein in the condensed phase [2], the average droplet size [3], and the interphase permeability for the FUS (*Fus*) N-terminal domain sample [4].
 - 5.1.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the axis labeled " D_{dil} (m^2/s)" and the axis labeled " D_{cond} (m^2/s)" on the spider plot.*
 - 5.1.2. LAB MEDIA: Figure 3A. *Video editor: Highlight the axis labeled " n_{cond} (%)" on the spider plot.*
 - 5.1.3. LAB MEDIA: Figure 3A. *Video editor: Highlight the axis labeled " R_{drop} (μm)" on the spider plot.*
 - 5.1.4. LAB MEDIA: Figure 3A. *Video editor: Highlight the axis labeled " p ($\mu m/s$)" on the spider plot.*
- 5.2. The NMR signal of the FUS N-terminal domain remained detectable in the condensed phase [1].
 - 5.2.1. LAB MEDIA: Figure 3C. *Video editor: Show the spectra panel and focus on the visible signal peaks in the blue trace labeled "Biphasic FUS NTD in agarose".*
- 5.3. No difference in the magnetization transfer profile was observed between the agarose reference and the FUS N-terminal domain biphasic sample [1].
 - 5.3.1. LAB MEDIA: Figure 3C. *Video editor: Highlight the two curves labeled "Agarose reference" and "Biphasic FUS NTD in agarose" showing that the lines overlap across the graph.*
- 5.4. The CONDENSE-MT experiment provided dynamic characterization of repeat-expansion RNA condensates [1].
 - 5.4.1. LAB MEDIA: Figure 3D.
- 5.5. The CONDENSE-MT analysis yielded the proton exchange kinetics between the condensate and bulk water [1]. The CONDENSE-MT analysis also yielded the transverse relaxation time of the condensate [2] and the apparent transverse relaxation rate of bulk water [3].
 - 5.5.1. LAB MEDIA: Figure 3D. *Video editor: Highlight the arrows labeled " $k_{condensate,H2O}$ ~215 per second" and " $k_{H2O,condensate}$ ~0.14 per second".*
 - 5.5.2. LAB MEDIA: Figure 3D. *Video editor: Highlight the label " $T_{2,condensate}$ ~7.9 microsecond".*

5.5.3. LAB MEDIA: Figure 3D. *Video editor: Highlight the label “ $\Delta R_{2,\text{water}} \sim 1.4$ per second”.*

1. Agarose
Pronunciation link: <https://www.merriam-webster.com/dictionary/agarose>
IPA: /'æɡəˌroʊs/
Phonetic Spelling: a·guh·rohs
2. Biphasic
Pronunciation link: <https://www.merriam-webster.com/dictionary/biphasic>
IPA: /baɪˈfeɪzɪk/
Phonetic Spelling: bye·fay·zik
3. Microcentrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/microcentrifuge>
IPA: /ˌmaɪkroʊˈsentrɪˌfjuːdʒ/
Phonetic Spelling: my·kroh·sen·truh·fyooj
4. Spectrometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrometer>
IPA: /spekˈtrəːmətər/
Phonetic Spelling: spek·trah·muh·ter
5. Calibrate
Pronunciation link: <https://www.merriam-webster.com/dictionary/calibrate>
IPA: /ˈkæliˌbreɪt/
Phonetic Spelling: kal·uh·brayt
6. Biomolecular
Pronunciation link: <https://www.merriam-webster.com/dictionary/biomolecular>
IPA: /ˌbaɪoʊməˈleɪkjələr/
Phonetic Spelling: bye·oh·muh·lek·yuh·ler
7. Diffusion
Pronunciation link: <https://www.merriam-webster.com/dictionary/diffusion>
IPA: /dɪˈfjuːʒən/
Phonetic Spelling: dih·fyoo·zhuhn
8. Spectroscopy
Pronunciation link: <https://www.merriam-webster.com/dictionary/spectroscopy>
IPA: /spekˈtrəːskəpi/
Phonetic Spelling: spek·trah·skuh·pee
9. Gradient
Pronunciation link: <https://www.merriam-webster.com/dictionary/gradient>

- IPA: /'greɪdiənt/
Phonetic Spelling: gray·dee·uhnt
10. MATLAB
Pronunciation link: <https://www.howtopronounce.com/matlab>
IPA: /'mæt,læb/
Phonetic Spelling: mat·lab
11. Inversion
Pronunciation link: <https://www.merriam-webster.com/dictionary/inversion>
IPA: /ɪn'vɜːʒən/
Phonetic Spelling: in·vur·zhuhn
12. Irradiation
Pronunciation link: <https://www.merriam-webster.com/dictionary/irradiation>
IPA: /ɪ'reɪdi'eɪʃən/
Phonetic Spelling: ih·ray·dee·ay·shuhn
13. Magnetization
Pronunciation link: <https://www.merriam-webster.com/dictionary/magnetization>
IPA: /,mæɡnətə'zeɪʃən/
Phonetic Spelling: mag·nuh·tuh·zay·shuhn
14. Condensate
Pronunciation link: <https://www.merriam-webster.com/dictionary/condensate>
IPA: /'kɑːndən'seɪt/
Phonetic Spelling: kon·den·sayt
15. Permeability
Pronunciation link: <https://www.merriam-webster.com/dictionary/permeability>
IPA: /,pɜːmiə'bɪləti/
Phonetic Spelling: pur·mee·uh·bil·uh·tee
16. Magnetization
Pronunciation link: <https://www.merriam-webster.com/dictionary/magnetization>
IPA: /,mæɡnətə'zeɪʃən/
Phonetic Spelling: mag·nuh·tuh·zay·shuhn
17. Relaxation
Pronunciation link: <https://www.merriam-webster.com/dictionary/relaxation>
IPA: /,ri:læk'seɪʃən/
Phonetic Spelling: ree·laks·ay·shuhn
18. Kinetics
Pronunciation link: <https://www.merriam-webster.com/dictionary/kinetics>
IPA: /kɪ'netɪks/
Phonetic Spelling: kuh·net·iks
19. Transverse
Pronunciation link: <https://www.merriam-webster.com/dictionary/transverse>
IPA: /'trænz,vɜːs/, /trænz'vɜːs/
Phonetic Spelling: tranz·vurs
20. Condensed
Pronunciation link: <https://www.merriam-webster.com/dictionary/condensed>

IPA: /kənˈdɛnst/

Phonetic Spelling: kuhn·denst