

FINAL SCRIPT: APPROVED FOR FILMING**Submission ID #: 69942****Scriptwriter Name: Pallavi Sharma****Project Page Link: <https://review.jove.com/account/file-uploader?src=21332358>****Title: Automated Ultradilute Measurements of Self-Association for the Identification of Antibodies with Favorable High-Concentration Solution Properties****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**

- 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. **YES**

Current Protocol Length

Number of Steps: 30

Number of Shots: 57

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. **Marissa Viola-LaBreck:** Our research develops a high-throughput ultradilute assay to identify antibodies with low self-association, enabling early prediction of high-concentration formulation suitability during developability assessment.
 - 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. **Marissa Viola-LaBreck:** Existing methods are low-throughput or material-intensive; this protocol enables rapid, reproducible screening using minimal antibody samples for more candidates.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.6.3*

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

- 1.3. **Marissa Viola-LaBreck:** This protocol supports therapeutic antibody discovery, developability assessment, formulation screening, and early candidate selection.
 - 1.3.1. 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?~~

- 1.4. **Marissa Viola-LaBreck:** This protocol measures antibody self-association to predict high-concentration solution behavior, including viscosity and formulation suitability.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 5.11.1.*

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

- 1.5. **Marissa Viola-LaBreck:** Future studies could improve automation and predictive models while screening larger antibody libraries earlier. This assay may also evaluate Fc-Fc interactions in reverse orientations with single-arm constructs.
 - 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.

FINAL SCRIPT: APPROVED FOR FILMING**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. **Marissa Viola-LaBreck, Senior Scientist [Sanofi]:** (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. **Marissa Viola-LaBreck, Senior Scientist [Sanofi]:** (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 shotlist.

2. Dialyzing Candidate Monoclonal Antibodies

Demonstrator: Marissa Viola-LaBreck

- 2.1. To begin, pre-soak 100 microliter or 300 microliter dialyzers in 10 millimolar histidine buffer at pH 6.0 for 5 minutes [1].
 - 2.1.1. WIDE: Talent placing 100 microliter/300 microliter dialyzers into 10 millimolar histidine buffer.
- 2.2. Transfer the prepared samples of L-histidine monohydrochloride monohydrate, L-histidine, and potassium acetate to dialysis cassettes [1]. Submerge the dialysis cassettes in 10 millimolar histidine buffer at pH 6.0 [2], add stir bars to the reservoirs [3], place the reservoirs on magnetic stirring plates, and incubate at 4 degrees Celsius for 2 hours and 15 minutes [4].
 - 2.2.1. Talent transferring the samples into dialysis cassettes.
 - 2.2.2. Talent submerging the dialysis cassettes in the histidine buffer reservoir. **NOTE: 2.2.2 and 2.2.3 were combined**
 - 2.2.3. Talent adding stir bars to the reservoirs.
 - 2.2.4. Talent placing the reservoirs on magnetic stirring plates in a refrigerator.
- 2.3. Perform seven buffer exchanges using 10 millimolar histidine buffer at pH 6.0 every 2 hours and 15 minutes or conduct four overnight exchanges for thorough salt removal [2].
 - 2.3.1. Talent replacing the buffer in the dialysis reservoir.
- 2.4. Once the samples are retrieved, transfer them to clean matrix vials [1] and store at 4 degrees Celsius until the colloidal self-interaction nanoparticle spectroscopy or CS-SINS (C-S-S-I-N-S) assay is performed [2].
 - 2.4.1. Talent transferring the samples into clean matrix vials. **NOTE: Add missing media title card**
 - 2.4.2. Talent placing the matrix vials into refrigerated storage.
- 2.5. Measure the sample concentrations [1] and record the polydispersity information after dialysis [2]. Calculate the corrected concentrations by dividing the raw absorbance values at 280 nanometers by the absorbance of a 1-milligram-per-milliliter solution at 280 nanometers [2]. **NOTE: The VO is adjusted for the additional shot**

Added shot at/before 2.5.1 of plate being placed into the reader.

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2.5.1. SCREEN: The recorded polydispersity information is being recorded. **NOTE: 2.5.1 and 2.5.2 combined in files C3497**

2.5.2. Talent calculating the corrected concentrations from the absorbance measurements.

2.6. Prepare the liquid-handler input file with the well addresses and concentrations in micrograms per milliliter, including the controls [1].

2.6.1. SCREEN: The liquid-handler input file with the well addresses, concentrations, and controls are being prepared. **NOTE: 2.6.1 unsalted (C3498)**

3. Desalting the Capture Monoclonal Antibody

3.1. Centrifuge 10-milliliter desalting columns at 1,000 *g* for 2 minutes to remove the storage buffer. Use two columns for every 4 milliliters of capture monoclonal antibody [1]. Discard the flow-through after centrifugation [2].

3.1.1. Talent placing the 10 milliliter desalting columns into the centrifuge.
Videographer: Please film multiple reusable shots to use them later

3.1.2. Talent discarding the flow-through from the collection vessel.

3.2. Wash the columns three times with 1 milliliter of 20 millimolar potassium acetate buffer at pH 4.3 [1] and centrifuge the columns again [2].

3.2.1. Talent adding 20 millimolar potassium acetate buffer to the desalting columns.

3.2.2. *Reuse 3.1.1.*

3.3. Add 4 milliliters of capture monoclonal antibody to each column [1] and elute into a fresh 50-milliliter tube by centrifugation at 1,000 *g* for 2 minutes [2].

3.3.1. Talent adding the capture monoclonal antibody solution to the desalting columns.

3.3.2. Talent placing the desalting columns over a fresh 50 milliliter tube and loading them into the centrifuge.

3.4. Collect the desalted eluate [1] and apply it to a second equilibrated desalting column followed by centrifugation [2].

3.4.1. Talent collecting the desalted eluate from the 15 milliliter tube. **NOTE: 3.4.1 and 3.4.2 combined**

3.4.2. Talent applying the desalted eluate to a second equilibrated desalting column.

3.5. ~~Then, combine all eluted samples [1]. Measure the concentration of the capture monoclonal antibody solution after desalting [1].~~

3.5.1. ~~Talent combining the eluted samples into a single vessel.~~ **NOTE: Not filmed**

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3.5.2. Talent measuring the concentration of the desalted capture monoclonal antibody solution.

3.6. Dilute the capture monoclonal antibody to 0.8 milligrams per milliliter using a 20 millimolar potassium acetate buffer at pH 4.3 and set it aside until needed [1].

3.6.1. Talent diluting the capture monoclonal antibody stock solution with potassium acetate buffer.

4. Concentration and Assembly of Gold Nanoparticles

4.1. Dilute a small aliquot of the poly-L-lysine stock solution to 2.66 milligrams per milliliter with ultrapure water [1].

4.1.1. Talent diluting a small aliquot of the poly-L-lysine stock solution with ultrapure water.

4.2. ~~Prepare 4.8 milliliter aliquots in 5 milliliter tubes using polypropylene microcentrifuge tubes containing 20-nanometer gold nanoparticles [1].~~ Centrifuge the microcentrifuge tubes containing 20-nanometer gold nanoparticles at 21,130 *g* for 6 minutes [2].

4.2.1. ~~Talent dispersing the stock suspension into polypropylene microcentrifuge tubes containing 20-nanometer gold nanoparticles.~~ **NOTE: Not filmed**

4.2.2. Talent placing the tubes into the centrifuge.

4.3. Remove the supernatants without disturbing the pellets and transfer them to a separate conical tube [1].

4.3.1. Talent removing the supernatants and transferring them to a separate conical tube.

4.4. Combine the concentrated pellets and residual volumes in a clean microcentrifuge tube [1]. Determine the nanoparticle volume by pipetting [2] and adjust it using the reserved supernatant [3].

4.4.1. Talent combining the concentrated pellets and residual volumes in a clean microcentrifuge tube. **NOTE: 4.4.1 - 4.4.3 combined in a single shot**

4.4.2. Talent determining the nanoparticle volume by pipetting.

4.4.3. Talent adding the reserved supernatant to adjust the nanoparticle volume.

4.5. Thoroughly mix 1.5 microliters of the poly-L-lysine working solution with 48.5 microliters of the buffer-exchanged capture monoclonal antibody solution [1].

4.5.1. Talent mixing the poly-L-lysine working solution with the buffer-exchanged capture monoclonal antibody solution in a microcentrifuge tube. **NOTE: 4.5.1 and 4.6.1 are combined in a single shot**

4.6. Prepare an equimolar mixture of the concentrated nanoparticle solution and the poly-L-lysine and capture monoclonal antibody solution [1]. Incubate the mixture overnight

at room temperature [2].

4.6.1. Talent combining equal volumes of the concentrated nanoparticle solution and the poly-L-lysine and capture monoclonal antibody solution.

4.6.2. Shot of the mixture kept on the working bench.

5. Liquid-Handler Assay Plate Set Up

5.1. Gather the materials for the assay, such as dialyzed antibodies, the liquid-handler input file with concentrations, conjugated gold nanoparticles, handling tips, plates, and reservoirs [1]. ~~Preload the tips, samples, plates, and reservoirs onto the deck [2].~~

5.1.1. Talent arranging the assay materials on the laboratory bench.

~~5.1.2. Talent loading the tips, samples, plates, and reservoirs onto the liquid-handler deck.~~ NOTE: This shot is moved after 5.2.1.

5.2. Add controls to wells A12 (*A-Twelve*) through D12 (*D-Twelve*) of the sample plate. Leave well H12 (*D-Twelve*) empty for the buffer sample prepared by the liquid handler [1]. Then, preload the tips, samples, plates, and reservoirs onto the deck [2].

5.2.1. Talent dispensing the positive and negative controls into wells A12 through D12 of the sample plate.

5.1.2. Talent loading the tips, samples, plates, and reservoirs onto the liquid-handler deck. NOTE: This shot is moved here as per authors request

5.3. Open the **CS-SINS Method** and start the program [1]. Load the liquid-handler input file when prompted and enter the number of samples [2]. Review the two subsequent calculation-check windows, confirm the values, and proceed with the run [3].

5.3.1. SCREEN: CS-SINS Method being opened. The program is being started.

5.3.2. SCREEN: Input file prompt appearing. The liquid-handler input file being loaded. Number of samples being entered. NOTE: Dont use take 1 or 2 from 5.3.2 (contains sensitive materials) use take 3 (C3525)

5.3.3. SCREEN: First calculation-check window being reviewed. Second calculation-check window being reviewed. Run is being initiated.

5.4. Normalize the samples, including the controls, to 55.5 micrograms per milliliter in a 10 millimolar histidine buffer at pH 6.0 by transferring them from the matrix sample tubes to the PCR plate [1]. Monitor the progress, noting that completed plate areas appear grey and active steps are highlighted in green [2].

5.4.1. Talent transferring samples and controls from the matrix sample tubes to the polymerase chain reaction plate. NOTE: 5.4.1 and 5.5.1 are performed by the machine instead of talent.

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- 5.4.2. SCREEN: Plate layout being updated during processing. Completed plate areas appear grey. Active steps are being highlighted in green.
- 5.5. Prepare a larger-volume dilution of each sample at 11.1 micrograms per milliliter in 10 millimolar histidine buffer by transferring material from the PCR plate to a deep-well block [1].
- 5.5.1. Talent transferring material from the polymerase chain reaction plate to a deep-well block for dilution.
- 5.6. Distribute the nanoparticles evenly between two polypropylene microcentrifuge tubes [1]. Place the tubes, caps open, in the designated tube rack location on the deck [2]. Resume the run and allow the liquid handler to dispense 20 microliters of nanoparticles into each well of the second deep-well block, two wells at a time [3].
- 5.6.1. Talent distributing the nanoparticles between two polypropylene microcentrifuge tubes. NOTE: 5.6.1 was slated as 5.1.1
- 5.6.2. Talent placing the open tubes in the designated tube-rack location on the deck.
- 5.6.3. SCREEN: Run being resumed. Nanoparticles are being dispensed into the wells of the second deep-well block, two wells at a time.
- 5.7. Allow the liquid handler to transfer 192 microliters of sample from the deep-well block containing the 11.1 micrograms per milliliter samples to the second deep-well block and mix thoroughly [1]. Prepare a 4× (Four-Times) CS-SINS mastermix for each sample to achieve a final concentration of 10 micrograms per milliliter after adding 48 microliters of gold nanoparticles [2]. Then, start the 4-hour incubation timer [3].
- 5.7.1. SCREEN: Sample being transferred from the first deep-well block to the second deep-well block. Mixing being performed. NOTE: 5.7.1 and 5.7.2 are combined in a single shot
- 5.7.2. SCREEN: Gold nanoparticles being added. The 4× CS-SINS mastermix being generated for each sample.
- 5.7.3. Talent starting the incubation timer.
- 5.8. Allow the liquid handler to dispense 30 microliters into the source well [1] and then dispense 60 microliters into three of the four wells in each quadrant of a 384-well plate to generate technical triplicates for each sample [2]. Return the remaining 30 microliters to the source plate [3].
- 5.8.1. SCREEN: Material being dispensed into the source well.
- 5.8.2. SCREEN: Material being dispensed into three wells of each quadrant of the 384-well plate to create technical triplicates.
- 5.8.3. SCREEN: Remaining material being returned to the source plate.
- 5.9. Monitor the liquid-handler deck layout as the run progresses [1]. Observe the used tips

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and filled sample wells changing from light to dark grey in real time while active movements are indicated in green [2].

5.9.1. SCREEN: Liquid-handler deck layout being monitored during the run.

5.9.2. SCREEN: Used tips and filled sample wells changing from light to dark grey. Active movements being highlighted in green.

5.10. Remove the plate after completion of the run [1]. Seal it with a minimally adhesive transparent plate seal, then incubate at room temperature for 4 hours [2]. At the 3.5-hour time point, start the dynamic light scattering program to equilibrate the chamber to 20 degrees Celsius [4].

5.10.1. Talent removing the plate from the liquid handler.

5.10.2. Talent sealing the plate with a minimally adhesive transparent plate seal.

5.10.3. SCREEN: Dynamic light scattering program being started. Chamber equilibration to 20 degrees Celsius being initiated.

5.11. Measure the absorbance spectrum of each well from 510 to 570 nanometers in 1-nanometer increments at the 4-hour time point [1].

5.11.1. Talent measuring the absorbance spectrum of the wells using the plate reader.

5.12. Immediately measure the plate using the dynamic light scattering plate reader at the 4.5-hour time point, followed by data analysis [1].

5.12.1. Talent placing the plate into the dynamic light scattering plate reader for measurement.

Results

6. Results

- 6.1. For purified material, 6 of 70 samples scored high-risk above the normalized cutoff value of 0.35 [1], while 64 of 70 samples scored low risk below the cutoff [2].
 - 6.1.1. LAB MEDIA: Figure 6A. *Video editor: Highlight the bars above the green dotted cutoff line.*
 - 6.1.2. LAB MEDIA: Figure 6A. *Video editor: Highlight the bars below the green dotted cutoff line.*
- 6.2. For crude expression samples, 2 of 20 samples scored high-risk above the normalized cutoff value of 0.35 [1], while 18 of 20 samples scored low risk below the cutoff [2].
 - 6.2.1. LAB MEDIA: Figure 6B. *Video editor: Highlight the bars above the green dotted cutoff line.*
 - 6.2.2. LAB MEDIA: Figure 6B. *Video editor: Highlight the bars below the green dotted cutoff line.*
- 6.3. The overall Spearman correlation coefficient between sample values was strongly positive at 0.829 [1].
 - 6.3.1. LAB MEDIA: Figure 6C.

7. Dialyzer
Pronunciation link: <https://www.merriam-webster.com/dictionary/dialyzer>
IPA: /'daɪəˌlaɪzər/
Phonetic Spelling: DY·uh·ly·zer
8. Histidine
Pronunciation link: <https://www.merriam-webster.com/dictionary/histidine>
IPA: /'hɪstəˌdiːn/
Phonetic Spelling: HIS·tuh·deen
9. Monohydrochloride
Pronunciation link: No confirmed link found
IPA: /ˌmɑːnoʊˌhaɪdrəˈklɔːraɪd/
Phonetic Spelling: mon·oh·hy·druh·KLOR·ide
10. Monohydrate
Pronunciation link: No confirmed link found
IPA: /ˌmɑːnoʊˈhaɪdreɪt/
Phonetic Spelling: mon·oh·HY·drayt
11. Potassium
Pronunciation link: <https://www.merriam-webster.com/dictionary/potassium>
IPA: /pəˈtæsiəm/
Phonetic Spelling: puh·TASS·ee·uhm
12. Acetate
Pronunciation link: <https://www.merriam-webster.com/dictionary/acetate>
IPA: /'æsəˌteɪt/
Phonetic Spelling: ASS·uh·tayt
13. Dialysis
Pronunciation link: <https://www.merriam-webster.com/dictionary/dialysis>
IPA: /daɪˈæləsis/
Phonetic Spelling: dy·AL·uh·sis
14. Colloidal
Pronunciation link: <https://www.merriam-webster.com/dictionary/colloidal>
IPA: /kəˈlɔɪdəl/
Phonetic Spelling: kuh·LOY·duhl
15. Nanoparticle
Pronunciation link: <https://www.merriam-webster.com/dictionary/nanoparticle>
IPA: /'nænəʊˌpɑːrtɪkəl/
Phonetic Spelling: NAN·oh·par·tih·kuhl
16. Spectroscopy
Pronunciation link: <https://www.merriam-webster.com/dictionary/spectroscopy>
IPA: /spekˈtrɔːskəpi/
Phonetic Spelling: spek·TROS·kuh·pee

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17. Polydispersity
 Pronunciation link: <https://www.merriam-webster.com/dictionary/polydispersity>
 IPA: /ˌpɑːliˌdiˈspɜːsəti/
 Phonetic Spelling: pol·ee·dih·SPUR·suh·tee
18. Absorbance
 Pronunciation link: <https://www.merriam-webster.com/dictionary/absorbance>
 IPA: /əbˈzɔːrbəns/
 Phonetic Spelling: ub·ZOR·buhns
19. Monoclonal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/monoclonal>
 IPA: /ˌmɑːnəˈkloʊnəl/
 Phonetic Spelling: mon·uh·KLOH·nuhl
20. Antibody
 Pronunciation link: <https://www.merriam-webster.com/dictionary/antibody>
 IPA: /ˈæntɪˌbɑːdi/
 Phonetic Spelling: AN·tee·bod·ee
21. Centrifuge
 Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
 IPA: /ˈsentrəˌfjuːdʒ/
 Phonetic Spelling: SEN·truh·fyooj
22. Eluate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/eluate>
 IPA: /ˈɛljəwət/, /ˈɛljəˌweɪt/, /ˈɛljuət/, /ˈɛljuˌeɪt/
 Phonetic Spelling: EL·yuh·wuht / EL·yuh·wayt
23. Aliquot
 Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquot>
 IPA: /ˈæləˌkwɑːt/
 Phonetic Spelling: AL·uh·kwot
24. Poly-L-lysine
 Pronunciation link: No confirmed link found
 IPA: /ˌpɑːliˌɛlˈlaɪsiːn/
 Phonetic Spelling: pol·ee·EL·LY·seen
25. Ultrapure
 Pronunciation link: No confirmed link found
 IPA: /ˌʌltrəˈpjʊr/
 Phonetic Spelling: uhl·truh·PYOOR
26. Polypropylene
 Pronunciation link: <https://www.merriam-webster.com/dictionary/polypropylene>
 IPA: /ˌpɑːliˈproʊpəˌliːn/
 Phonetic Spelling: pol·ee·PROH·puh·leen
27. Microcentrifuge
 Pronunciation link: No confirmed link found
 IPA: /ˌmaɪkroʊˈsentrəˌfjuːdʒ/
 Phonetic Spelling: my·kroh·SEN·truh·fyooj

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- 28. Equimolar**
 Pronunciation link: <https://www.merriam-webster.com/dictionary/equimolar>
 IPA: /,i:kwə'moulər/, /,ɛkwə'moulər/
 Phonetic Spelling: ee·kwuh·MOH·ler / ek·wuh·MOH·ler
- 29. Conjugated**
 Pronunciation link: <https://www.merriam-webster.com/dictionary/conjugate>
 IPA: /'kɑ:ndʒə,geɪtɪd/
 Phonetic Spelling: KON·juh·gay·tid
- 30. Polymerase**
 Pronunciation link: <https://www.merriam-webster.com/dictionary/polymerase>
 IPA: /pə'limə,reɪs/
 Phonetic Spelling: puh·LIM·uh·rays
- 31. Equilibration**
 Pronunciation link: <https://www.merriam-webster.com/dictionary/equilibration>
 IPA: /ɪ,kwɪlə'breɪʃən/
 Phonetic Spelling: ih·kwil·uh·BRAY·shuhn
- 32. Spearman**
 Pronunciation link: No confirmed link found
 IPA: /'spɪrmən/
 Phonetic Spelling: SPEER·muhn
- 33. Correlation**
 Pronunciation link: <https://www.merriam-webster.com/dictionary/correlation>
 IPA: /,kɔ:rə'leɪʃən/
 Phonetic Spelling: kor·uh·LAY·shuhn
- 34. Coefficient**
 Pronunciation link: <https://www.merriam-webster.com/dictionary/coefficient>
 IPA: /,koʊə'fɪʃənt/
 Phonetic Spelling: koh·uh·FISH·uhnt