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Title: MALDI-ToF MS Method for the Characterization of Synthetic Polymers with Varying Dispersity and End Groups

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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**

   We will need you to record using screen recording software.

   We recommend using the screen capture program OBS. JoVE's tutorial for using OBS Studio is provided at this link: https://review.jove.com/v/5848/screen-capture-instructions-for-authors?status=a7854k

   Videographer: Please record the computer screen for the shots labeled as SCREEN as back-up

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 13
Number of Shots: 29 (18 SC)

Introduction

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

1.1. **Ashley Miles:** We want to know how polymers react. For this, we use mass spectrometry as it is a fantastic tool to study both the expected reactions, and the other side reactions.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.2.1*

What are the most recent developments in your field of research?

1.2. **Ashley Miles:** In the last ten years, MALDI-ToF MS has advanced to have much more resolution and the fragmentation within MS/MS.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What significant findings have you established in your field?

1.3. **Ashley Miles:** We describe a wide range of reactions including monodisperse, moderately disperse, and highly disperse samples and those with distinct isotopic resolution patterns, for example, polymers with halogenated groups or those prone to metastable ion formation.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

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

Protocol

2. Preparation of MALDI-ToF MS Target Plate

Demonstrator: Ashley Miles

2.1. To begin, prepare the analyte acquisition solution by adding the cation, analyte, and matrix stock solutions into tube A **[1]** and vortex the tube to mix **[2]**. Then, add the cation, calibrant, and matrix stock solutions into tube B and mix thoroughly **[3-TXT]**.

2.1.1. WIDE: Talent adding cation, analyte, and matrix stock solutions into tube A using micropipettes.

2.1.2. Talent vortexing the tube A.

2.1.3. Talent adding cation, calibrant, and matrix stock solutions into tube B and vortexing to mix. **TXT: Cation:Analyte or Calibrant:Matrix stock = 1:5:20 µL**
Video editor: This is a ratio

2.2. Now, pipette 0.5 microliters of the cation, analyte, and matrix sample mixture from tube A onto the MALDI-ToF MS *(mall-dee-toff- M-S)* target plate using the dried droplet method **[1-TXT]**. Then, pipette 0.5 microliters of the calibrant mix from tube B onto an adjacent spot on the target plate **[2]**. Record the exact row and column location of each spot for reference during data acquisition **[3]**.

2.2.1. Talent pipetting the analyte sample mixture from tube A onto the MALDI-ToF MS target plate. **TXT: MALDI-ToF MS: Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry**

2.2.2. Talent pipetting the calibrant sample mixture from tube B onto an adjacent spot.

2.2.3. Talent writing down the row and column positions of each spot in a lab notebook.

2.3. Insert the MALDI-ToF MS target plate into the instrument **[1]** and dock the target plate using the instrument control software **[2]**.

2.3.1. Talent placing the MALDI-ToF MS target plate into the instrument chamber.

2.3.2. Shot of docking the target plate.

3. Selecting a Data Acquisition Method

3.1. Select and open a **method file** in the instrument control software **[1]**. Choose either the **Reflector positive** or **Linear positive** method based on the analyte's mass range **[2]**.

3.1.1. SCREEN: 68455_screenshot_1.mp4 00:03-00:06

3.1.2. SCREEN: 68455_screenshot_1.mp4 00:07-00:20.

3.2. Next, press the **Start** button on the instrument control software to begin MALDI-ToF MS data acquisition **[1]**. Allow the acquisition to complete automatically or press **Stop** when desired **[2]**.

3.2.1. SCREEN: 68455_screenshot_1.mp4 00:27-00:36.

3.2.2. SCREEN: 68455_screenshot_1.mp4 00:37-00:39.

3.3. Then, click **Add** to transfer the MALDI-ToF MS single spectrum to the sum spectrum buffer **[1]**. Reposition the MALDI-ToF MS laser by viewing the camera feed and clicking **anywhere within the same selected well** to target a new position **[2]**. Repeat the MALDI-ToF MS acquisition to continue adding spectra until the results are satisfactory **[3]**.

3.3.1. SCREEN: 68455_screenshot_1.mp4 00:39-00:46.

3.3.2. SCREEN: 68455_screenshot_1.mp4 00:47-00:52.

3.3.3. SCREEN: 68455_screenshot_2.mp4 00:02-00:10 .

4. MALDI-ToF MS Calibration

4.1. Now, select the **calibration sample** on the MALDI-ToF MS target plate **[1]** and perform a new acquisition following the previously described data acquisition steps **[2]**.

4.1.1. SCREEN: 68455_screenshot_3.mp4 00:03-00:10 .

4.1.2. SCREEN: 68455_screenshot_3.mp4 00:23-00:29 .

4.2. Navigate to the calibration tab in the instrument control software and open the appropriate mass control list that corresponds to the selected calibrant **[1]**.

4.2.1. SCREEN: 68455_screenshot_4.mp4 00:10-00:22.

4.3. Match the calibration peaks in the acquired MALDI-ToF mass spectrum with the

reference masses listed in the mass control list **[1]**.

4.3.1. SCREEN: 68455_screenshot_4.mp4 00:23-00:32.

4.4. In the mass control list, select the **mass corresponding to the first calibration peak** observed in the MALDI-ToF mass spectrum **[1]**. Set the reference peak by clicking to the left of the calibrant signal so that the peak is selected **[2]**.

4.4.1. SCREEN: 68455_screenshot_4.mp4 00:34-00:40.

4.4.2. SCREEN: 68455_screenshot_4.mp4 00:41-00:46.

4.5. Then, click **Apply** to assign the calibrant signal as the reference mass **[1]**. Repeat the procedure for all additional calibration peaks by selecting the **next mass of interest** in the mass control list, aligning the corresponding calibrant peak, and applying the reference setting **[2]**.

4.5.1. SCREEN: 68455_screenshot_4.mp4 00:47-00:49.

4.5.2. SCREEN: 68455_screenshot_4.mp4 01:10-01:25.

4.6. For calibrated polymer analyte sample acquisition, locate and select the well containing the cation, analyte, and matrix mixture using the instrument control software **[1]**. Perform MALDI-ToF MS data acquisition using the calibrated method as demonstrated earlier **[2]**. Add an appropriate file name and save the calibrated analyte mass spectrum for further analysis **[3]**.

4.6.1. SCREEN: 68455_screenshot_5.mp4 00:04-00:11.

4.6.2. SCREEN: 68455_screenshot_5.mp4 00:20-00:30.

4.6.3. SCREEN: 68455_screenshot_5.mp4 01:20-01:34.

4.7. Finally, remove the MALDI-ToF MS target plate using the instrument control software **[1]** and clean the MALDI-ToF MS target plate thoroughly for reuse **[2]**.

4.7.1. Shot of the target plate being removed.

4.7.2. Talent wiping the MALDI-ToF MS target plate with appropriate cleaning materials on a lab bench.

Results

5. Results

5.1. The MALDI-ToF MS spectrum of TrisG1$_6$ *(tris-G-1-6)* showed a single peak at 505.239 Daltons, matching its theoretical mass and confirming complete deprotection **[1]**.

5.1.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the peak labeled 505.239 in the spectrum.*

5.2. PDMP polymers with chloride and proton end groups displayed a main peak at 859.426 Daltons **[1-TXT]**, along with sodium-substituted **[2]** and cyclic variants **[3]**.

5.2.1. LAB MEDIA: Figure 5. *Video editor: Highlight the blue peak labeled 859.426*. **TXT: PDMP: Poly(2,2-dimethylpropanoate)**

5.2.2. LAB MEDIA: Figure 5. *Video editor: Highlight the orange peak labeled 881.413 to the right.*

5.2.3. LAB MEDIA: Figure 5. *Video editor: Highlight the green peak labeled 823.448 to the left*.

5.3. The full PDMP distribution spanned 600 to 1600 Daltons with repeat units averaging 100.056 Daltons **[1]**.

5.3.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the peaks from X axis range 600 to 1600*.

5.4. Linear-Poly ethylene brassylate polymers exhibited alpha-hydroxy- omega-propargyl, alpha-propargyl-omega-propargyl and alpha-hydroxy-omega-hydroxy types in three distributions **[1]**.

5.4.1. LAB MEDIA: Figure 8B. *Video editor: Sequentially highlight the three peaks labeled 1700.139, 1694.127, and 1706.133 and their coloured boxes above with green, red and blue data*.

5.5. Azide-functionalized Linear-Poly ethylene brassylate polymers showed peaks for alpha–azido-omega-azido **[1]**, alpha-propargyl-omega-azido types **[2]**, and metastable ions **[3]**.

5.5.1. LAB MEDIA: Figure 10. *Video editor: Highlight the peak labeled 1994.296 in red*.

5.5.2. LAB MEDIA: Figure 10. *Video editor: Highlight the peak labeled 1839.184 in blue*.

5.5.3. LAB MEDIA: Figure 10. *Video editor: Highlight the peak labeled 1815.126 in black*.

5.6. Comb polymers revealed oxidation states with 16 Dalton intervals **[1]** and K-plus adduct shifts confirming quadruple oxidation **[2]**.

5.6.1. LAB MEDIA: Figure 14.

5.6.2. LAB MEDIA: Figure 15.

5.7. MeO-PEG *(Methoxy-Peg)* and unreacted alkynes were identified near 533 to 583 Daltons, validating reaction completeness **[1]**.

5.7.1. LAB MEDIA: Figure 13. *Video editor: Highlight the peaks at 533.309, 577.327 (top figure; with 2 red structures along with the box on the top), 539.263 and 583.306 (bottom figure; blue structures with blue formula inside the box).*

1. analyte
Pronunciation link: https://www.merriam-webster.com/dictionary/analyte How To Pronounce+7How To Pronounce+7Definitions+7How To Pronounce+11Merriam-Webster+11Merriam-Webster+11
IPA: /ˈæn.ə.laɪt/
Phonetic Spelling: AN-uh-lyt

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2. MALDI
Pronunciation link: https://www.pronouncekiwi.com/MALDI Cambridge Dictionary+2Cambridge Dictionary+2Cambridge Dictionary+2How To Pronounce+4pronouncekiwi.com+4YouGlish+4
IPA: /ˈmæl.di/
Phonetic Spelling: MAL-dee

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3. desorption
Pronunciation link: https://www.merriam-webster.com/dictionary/desorption Definitions+4pronouncekiwi.com+4How To Pronounce+4Ask Oracle+1How To Pronounce+1Wikipedia+3Merriam-Webster+3Merriam-Webster+3 *(Note: general pronunciation rules from MW guide)*
IPA: /dɪˈsɔrp.ʃən/
Phonetic Spelling: dih-SORP-shun

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4. ionization
Pronunciation link: https://www.merriam-webster.com/dictionary/ionization Merriam-Webster
IPA: /aɪˌɑn.əˈzeɪ.ʃən/
Phonetic Spelling: eye-AH-nuh-ZAY-shun

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5. calibrant
No confirmed link found
IPA: /ˈkæl.ə.brənt/
Phonetic Spelling: KAL-uh-bruhnt

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6. poly(2,2-dimethylpropanoate)
No confirmed link found
IPA: /ˌpɒl.i (tuːˌtuːˌdaɪˌmɛθ.əl.prəˈpæn.əʊ.eɪt)/
Phonetic Spelling: POL-ee (two-two dye-METH-uhl pro-PAN-oh-ate)

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7. polyethylene brassylate
No confirmed link found
IPA: /ˌpɒliˈɛθ.əˌliːn ˈbræs.ɪ.leɪt/
Phonetic Spelling: pol-ee-ETH-uh-leen BRAS-ih-layt

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8. azide-functionalized
No confirmed link found
IPA: /əˈzaɪd ˈfʌŋkʃ.ə.nə.laɪzd/
Phonetic Spelling: uh-ZYDE funk-shuh-NUH-lyzed

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9. metastable
Pronunciation link: https://www.merriam-webster.com/dictionary/metastable How To Pronounce+2YouTube+2Cambridge Dictionary+2Oxford English Dictionary+15Merriam-Webster+15Wikipedia+15
IPA: /ˌmɛtəˈsteɪbəl/
Phonetic Spelling: MET-uh-STAY-buhl

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10. Daltons
Pronunciation link: https://www.merriam-webster.com/dictionary/Dalton Merriam-Webster
IPA: /ˈdɔl.tənz/
Phonetic Spelling: DOL-tuhnz

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11. MeO-PEG
No confirmed link found
IPA: /ˌmiː.oʊ ˌpɛg/
Phonetic Spelling: MEE-oh PEG

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12. alkynes
No confirmed link found
IPA: /ˈæl.kaɪnz/
Phonetic Spelling: AL-kynz