Submission ID #: 61760
Scriptwriter Name: Domnic Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18836848

Title: Capillary Electrophoresis Mass Spectrometry Approaches for Characterization of the Protein and Metabolite Corona Acquired by Nanomaterials

Authors and Affiliations:

Andrew J. Chetwynd[1*], Wei Zhang[2*] Klaus Faserl[3*], James A. Thorn[4], Iseult Lynch[1], Rawi Ramautar[2], Herbert H. Lindner[3]

[1]School of Geography Earth and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham, UK
[2]Biomedical Microscale Analytics, Leiden University, Einsterinweg, Leiden, The Netherlands
[3]Division of Medical Biochemistry, Medical University of Innsbruck, Innsbruck, Austria
[4]AB Sciex UK Ltd, Phoenix House, Lakeside Drive, Warrington, Cheshire, UK

Corresponding Authors:
Andrew J. Chetwynd (a.j.chetwynd@bham.ac.uk)

Email Addresses for All Authors:
w.zhang@lacdr.leidenuniv.nl
klaus.faserl@i-med.ac.at
jim.thorn@sciex.com
i.lynch@bham.ac.uk
r.ramautar@lacdr.leidenuniv.nl
herbert.lindner@i-med.ac.at
a.j.chetwynd@bham.ac.uk

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. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **Yes, Birmingham and Leiden**

   If **Yes**, how far apart are the locations? 1000 Km

Current Protocol Length

Number of Steps: 23
Number of Shots: 55

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Wei Zhang:** This protocol is the first to implement CESI-MS for the characterization of not only the protein corona but also the metabolite corona of nanomaterials.

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

1.2. **Andrew Chetwynd:** CESI offers an orthogonal separation to traditional LC-MS methods and is both highly sensitive and reproducible while using just a few nanoliters of sample.

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

Protocol

2. Biomolecular corona formation and protein corona isolation

Videographer: All steps 2.1-2.10.2 to be filmed in Birmingham

2.1. To begin, split 2 milliliters of the human plasma into 1 milliliter aliquots, one for the metabolite and protein corona and one for plasma metabolome characterization **[1]**.

2.1.1. Talent splitting the plasma into two equal volume aliquots.

2.2. Incubate 1 milligram per milliliter of nanomaterials in the human plasma for 1 hour at 37 degrees Celsius while gently mixing at 500 rotations per minute in a thermomixer **[1]**. Then, pellet the nanomaterials by centrifuging at 4000 *x g* for 15 minutes at 4 degrees Celsius **[2]**.

2.2.1. Talent incubating the nanomaterials in human plasma on thermomixer.

2.2.2. Talent centrifuging the sample.

2.3. Collect the plasma supernatant for metabolite corona analysis **[1]** and retain the nanomaterial-protein corona complex for protein corona characterization **[2]**.

2.3.1. Talent collecting the supernatant.

2.3.2. Talent retaining the pellet.

2.4. Resuspend the pellet in 1 milliliter of 10x PBS buffer **[1]** and vortex vigorously for 2 minutes to remove unbound proteins **[2]**. Centrifuge the solution at 4000 *x g* for 15 minutes at 4 degrees Celsius to pellet the nanomaterials **[3]**, then remove the supernatant carefully without disturbing the pellet **[4]**. *Videographer: This step is important!*

2.4.1. Talent adding 10x PBS to the pellet.

2.4.2. Talent vortexing the solution.

2.4.3. Talent centrifuging the solution.

2.4.4. Talent removing the supernatant.

2.5. Resuspend the pellet in 1 milliliter of ABC buffer **[1-TXT]** and vortex vigorously for 2 minutes to remove unbound proteins and unbound salts **[2]**. Repeat the centrifugation step and discard the supernatant **[3]**. *Videographer: This step is important!*

2.5.1. Talent adding ammonium bicarbonate buffer to the pellet. **TEXT: ABC-ammonium bicarbonate buffer**

2.5.2. Talent vortexing the solution.

2.5.3. Talent discarding the supernatant.

2.6. Dissolve the pellet in 20 microliters of ABC buffer containing 10 millimolar dithiotheritol to reduce protein disulfide bonds **[1]**. Incubate the solution for 30 minutes at 56 degree Celsius **[2]**. *Videographer: This step is important!*

2.6.1. Talent adding ABC buffer containing dithitotheritol

2.6.2. Talent incubating the solution.

2.7. Add 2 micrograms of sequencing grade trypsin to the 20 microliters of ABC buffer containing 0.1% surfactant **[1]** and incubate the digest solution at 37 degrees Celsius for 16 hours **[2]**. *Videographer: This step is important!*

2.7.1. Talent adding trypsin to the solution.

2.7.2. Talent incubating the solution.

2.8. Alkylate the sample by adding 20 microliters of 55 millimolar iodoacetamide in 100 millimolar ABC and incubate at room temperature for 20 minutes **[1]**. *Videographer: This step is important!*

2.8.1. Talent adding ABC with iodoacetamide to the solution.

2.9. Add 20 microliters of 0.1 molar hydrochloric acid to cleave the surfactant and leave the sample for 10 minutes at room temperature **[1]**. *Videographer: This step is important!*

2.9.1. Talent adding HCl to the solution.

2.10. Enrich and desalt the peptides using a C18 packed desalting pipette tip as described in the text manuscript **[1]**. Then, lyophilize the sample and store it at -20 degrees Celsius until analysis **[2]**.

2.10.1. Talent desalting the sample using C18 tips.

2.10.2. Talent storing the sample in -20 refrigerator.

3. Metabolite corona isolation and preparation of the CESI-MS system

Videographer: All of section 3 is to be filmed in Leiden

3.1. Take the control plasma and the supernatant from the nanomaterial plasma incubation and put them on ice **[1]**. Aliquot 50 microliters from each sample into separate vials **[2]** and dilute 10-fold with distilled water **[3]**. After vortexing, move 50 microliters of this diluted sample to new vials **[4]**.

3.1.1. Talent keeping the sample on ice.

3.1.2. Talent aliquoting the sample in a separate vial.

3.1.3. Talent diluting the aliquoted sample.

3.1.4. Talent moving the diluted sample to new vial.

3.2. Add 200 microliters of chloroform, 250 microliters of methanol, and 350 microliters of distilled water **[1]** and vortex vigorously for 2 minutes **[2]**. Centrifuge at 20,800 *x g* at 4 degrees Celsius for 10 minutes **[3]**. *Videographer: This step is important!*

3.2.1. Talent adding the solutions in the mixture.

3.2.2. Talent vortexing the mixture.

3.2.3. Talent centrifuging the sample.

3.3. Take 500 microliters of the supernatant **[1]** and filter it through a 3 kilodalton centrifugal filter for 2 hours at 10000 *x g* at 4 degrees Celsius **[2]**. Dry 430 microliters **[3-added]** of the ultrafiltrate in a speed vac **[4]** and freeze the sample **[5]**. *Videographer: This step is important!*

3.3.1. Talent adding the supernatant in the centrifugal filter.

3.3.2. Talent centrifuging the filter.

3.3.3. Added shot: Talent transferring 430 microliters of the ultrafiltrate to clean vials

3.3.4. Talent adding the filtrate in speed vac NOTE: May still be slated as 3.3.3.

3.3.5. Talent freezing the sample.

3.4. Prior to installation of the capillaries into the CESI *(pronounce 'sesi')* instrument, check that the inlet and outlet ends of the capillaries are intact and that there are no visible breakages in the capillary **[1]**. Then, place a new neutral coated capillary into the instrument following the manufacturer guidelines **[2-TXT]**. *Videographer: This step is difficult!*

3.4.1. Talent checking the capillaries.

3.4.2. Talent placing the capillaries in the instrument. **TEXT: CESI- Capillary electrophoresis**

3.5. Flush the separation capillary in the forward direction with 100 millimolar hydrochloric acid, then with BGE, and finally with distilled water using the conditions described in the text manuscript **[1-TXT]**.

3.5.1. Talent flushing the separation capillary with respective solutions. **TEXT: BGE- Background electrolyte** *Videographer take a single shot of all the flushing process.*

3.6. Then, flush both the separation and conductive capillary with BGE, followed with distilled water, 100 millimolar hydrochloric acid, distilled water again, and finally with BGE **[1]**. Couple CESI to the MS using the nanospray source and adapter **[2-TXT]**.

3.6.1. Talent flushing the separation and the conductive capillary sequentially with the respective solutions. *Videographer take a single shot of all the flushing process.*

3.6.2. Talent connecting the CESI to the MS via nanospray source and adapter. **TEXT: MS- Mass spectrometry**

3.7. Place a new bare fused silica capillary into the CESI instrument **[1]**. Flush the separation capillary in forward direction using 100% methanol **[2]**. Then flush both the separation and conductive capillary with BGE to ensure droplet formation at the outlet ends **[3]**. *Videographer: This step is difficult!*

3.7.1. Talent placing the bare fused silica capillary into the CESI instrument.

3.7.2. Talent flushing the capillary with methanol.

3.7.3. Talent flushing both the capillaries with BGE.

3.8. Then, flush the capillary with distilled water, followed by 0.1 molar sodium hydroxide, distilled water again, and finally with BGE **[1]**. Couple CESI to the ESI-MS using the nanospray source and adapter **[2-TXT]**.

3.8.1. Talent flushing the capillaries with respective solutions.

3.8.2. Talent connecting the CESI to the MS via nanospray source and adapter. **TEXT: ESI- Electrospray ionization**

4. CE-MS for protein corona analysis and CESI-MS for metabolite corona analysis

Videographer: All of section 4 is to be filmed in Leiden

4.1. For protein corona analysis, resuspend the lyophilized samples in 20 microliters of 50 millimolar ammonium acetate at pH 4 **[1]**. Perform CESI-MS analysis for the samples as described in the text manuscript **[2-added].**

4.1.1. Talent adding ammonium acetate to the samples.

4.1.2. Added shot: Talent analyzing prepared samples with CESI-MS NOTE: Use this instead of 4.1.2, 4.1.3, 4.2.1, and 4.2.2. Authors says they are all combined here.

4.1.3. Talent performing hydrodynamic injection of the sample.

4.1.4. Talent performing separation of the sample. **TEXT: Pressure gradient: 0-10 min at 1 psi, 10-35 min at 1.5 psi and 35-45 min at 5 psi using 100 mM, pH 2.9 acetic acid as BGE**

4.2. Collect mass spectra data between 250 and 2000 *mz*- with top-10 data dependent fragmentation acquisition **[1]** and rinse the capillary between samples **[2-TXT]**.

4.2.1. Talent collecting the mass spectra data.

4.2.2. Talent rinsing the capillaries in between the samples. **TEXT: separation capillary: 3 min at 100 psi of 0.1 M HCl and 10 min at 100 psi of BGE; conductive liquid capillary: 3 min at 100 psi**

4.3. To perform metabolite corona analysis, resuspend the nanomaterial-exposed and unexposed plasma samples in 430 microliters of distilled water **[1]** and vigorously vortex for 2 minutes **[2]**. Filter the samples **[3-added]** through a 0.1-micrometer membrane filter **[3]**.

4.3.1. Talent resuspending the NM in distilled water.

4.3.2. Talent vortexing the sample.

4.3.3. Added shot: Talent transferring the samples to the filters

4.3.4. Talent filtering the sample.

4.4. Add 5 microliters of the internal standards as mentioned in the text manuscript to 95 microliters of the filtrate **[1]** and vortex vigorously **[2]**. Centrifuge at 16100 *x g* and 4 degrees Celsius **[3]**.

4.4.1. Talent adding standards to the sample.

4.4.2. Talent vortexing the sample.

4.4.3. Talent centrifuging the sample.

4.5. Perform CESI-MS analysis for the samples as described in the text manuscript **[1-added]**. NOTE: Author deleted most of VO text due to the procedure being automated, but didn't delete the shots. Just show the added 4.5.1.

4.5.1. Added shot: Talent analyzing prepared samples with CESI-MS

4.5.2. Talent injecting the sample.

4.5.3. Talent separating the metabolites.

4.5.4. Talent collecting the mass spectrometer data. **TEXT: Mass Range: 65 - 1000 m/z**

Results

5. Mass spectral analysis of the characterization of the protein and metabolite corona acquired by nanomaterials

5.1. The use of CESI-MS for proteomics **[1]** and metabolomics separation and detection demonstrate good separation windows on both capillaries for each approach, enabling a comprehensive characterization of the biomolecular corona **[2]**.

5.1.1. LAB MEDIA: Figure 1.

5.1.2. LAB MEDIA: Figure 2.

5.2. The proteomic CESI-MS method is capable of distinguishing between an array of proteins and protein concentrations in the corona across a wide range of nanomaterial compositions and can distinguish a unique protein corona for each nanomaterial **[1]**.

5.2.1. LAB MEDIA: Table 1.

5.3. This CESI-MS metabolomics approach enables a quantitative analysis of the metabolite corona and can be used to uncover its unique fingerprints **[1]**.

5.3.1. LAB MEDIA: Figure 3.

5.4. Although this approach passively characterizes the nanomaterial metabolite corona, it is still capable of uncovering interesting insights into the role of metabolites in the biomolecular corona, such as the differential adsorption of isomers **[1]**.

5.4.1. LAB MEDIA: Figure 4.

Conclusion

6. Conclusion Interview Statements

6.1. **Wei Zhang:** This technique makes it possible to characterize both the protein and metabolite corona, allowing for an improved understanding of how the complete biomolecular corona affects nanomaterial uptake by cells.

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