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Venom Vesicle Size and Zeta Potential Analysis

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We use this protocol and it's working

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Abstract

This protocol is used for determining the size, concentration, and zeta potential (surface charge) of parasitoid venom vesicles using tunable resistive pulse sensing (TRPS) on the Exoid (Izon). TRPS analysis is conducted using slight modifications of the Izon protocol for the effective measurement of venom vesicles. Completion of the protocol will allow for the characterization of the size and charge profiles of isolated venom vesicles and the determination of vesicle concentration.

Materials

Solutions:

- PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, pH 7.4)
- Measurement Electrolyte: PBS, 0.3% final (v/v) Wetting Solution concentrate (Izon)
- Wetting Solution: Measurement Electrolyte + 1.01% Wetting Solution (v/v, final of 1.31%; Izon)
- Coating Solution: Measurement Electrolyte + 10% (w/v) coating solution powder (Izon)
- Ultra-pure water

Solutions must be filtered fresh per manufacturer recommendations prior to experimentation.

Consumables:

- Nanopore (Izon)
- Low retention pipette tips (Mettler Toledo, cat. 17014341)
- Nylon syringe filter for solutions (WHA99101302, Whatman)
- PES syringe filter for venom samples (Membrane Solutions)
- Calibration particles (Izon)

Instrumentation and laboratory equipment:

- Exoid instrument and software suite (Izon)

Sample Preparation

- 1 Prepare parasitoid wasp venom according to **Bretz and Mortimer, 2025**. The contents of 75 dissected venom apparati will provide sufficient material for size and zeta potential analysis by TRPS.
- 2 Following venom isolation, samples should be dialyzed into PBS.
- 3 Filter the dialyzed venom samples through a PES syringe filter. Select the smallest pore size that will maintain the largest vesicle size observed in the venom.
- 3.1 Maximum vesicle size can be determined by electron microscopy or an initial TRPS run without filtering.

Nanopore Preparation

- 4 Select a nanopore size based on the maximum vesicle size determined in Step 3.1. We will use an NP100 nanopore (appropriate for *Ganaspis hookeri* venom) as an example.
- 5 To prepare the nanopore, follow Izon's software-guided protocol within the Exoid Control Suite (ECS). The stretch of the system is reduced for fitting an NP100 nanopore, then reset to a stretch of 45 mm.
- 6 As a means of opening and moistening the pore, apply 75 μL of wetting solution to the lower fluid cell. Fasten the upper fluid cell to the system, and apply 35 μL of wetting solution to the upper chamber.
- 6.1 To minimize the introduction of bubbles into the system, apply the reverse pipetting technique to all steps involving fluid transfer into the lower and upper fluid cells. To reverse pipette, set your pipette to the desired volume, press the plunger down to the bottom (second) stop, and aspirate the full volume into the tip. Then, press the plunger to the first stop to dispense solution into the Exoid fluid cells. Discard the remaining solution.
- 7 Seat the system's pressure shield and fit the pressure nozzle to the upper fluid cell.
- 8 To open the pore, apply maximum pressure to the system. Use a single, forceful strike with the provided plunger implement against the top of the pressure nozzle. Striking should be forceful enough to generate a steady baseline current, which is indicated by a horizontal trace above 0 nA. A stable baseline current between 10-20 nA is ideal for

smaller nanopores like the NP100, and very little fluctuation of current should be observed for a stable, open pore.

- 9 Follow the ECS Software-controlled wetting protocol to moisten the nanopore.
- 10 Remove the wetting solution and the nanopore from the instrument. Gently clean the nanopore with ultra-pure water from a squirt bottle.
- 11 To aid in nanopore stability with complex biological samples, apply 75 μL of coating solution to the lower fluid cell. Reassemble the upper fluid cell and add 35 μL of coating solution. Re-seat the pressure shield and nozzle.
- 12 Coating is performed for 10 minutes at a current of between 10-20 nA. Following coating, the ECS software will indicate successful coating, or a failure in the process of coating the nanopore, in which case re-coating of the nanopore is required.
- 13 To prepare the nanopore for the next steps of analysis, remove the upper and lower fluid cell coating fluids and gently rinse all components with ultra-pure water.
- 14 After rinsing, reinstall the nanopore. Add 75 μL of measurement electrolyte to the lower fluid cell, and 35 μL of measurement electrolyte to the upper fluid cell.
- 15 Use the guided ECS software steps to characterize the nanopore and determine ideal measurement conditions for your nanopore.

Finalizing Nanopore Characterization

- 16 Following standard ECS-specific nanopore preparation guidelines, dilute CPC100 calibration particles (1.8×10^{13} particle/mL, Izon; or a desired calibration particle dependent on nanopore size) at 1:3000 in measurement electrolyte. Add calibration particles to the upper fluid cell and measure during nanopore setup.
- 17 The following sections will allow you to measure vesicle size, zeta potential, and concentration. The sample prepared in section 1 'Sample Preparation' is sufficient to complete the protocol to measure all parameters. To specifically measure vesicle size and concentration, proceed to step 18 and follow through to step 27.1. To measure vesicle zeta potential and size, proceed to step 28 and follow the protocol to completion.

Vesicle Size and Concentration Analysis using the Exoid

- 18 To begin analysis, collect calibration measurements using CPC100 calibration particles (or desired calibration particles) at a suitable pressure for consistent blockade rate, a

voltage in which stable signal traces are between 100–140 nA, and a suitable stretch for measurement of sample particles.

- 19 Per Izon recommendations, take sample measurements at three pressures: the first pressure, P1, is determined based upon the nanopore characterization. P2 corresponds to P1 minus 200 Pa. P3 corresponds to P1 plus an additional 400 Pa. Stretch and voltage must be maintained constant throughout the entire measurement session for downstream analysis.
- 19.1 For the analysis of venom vesicles, 800–1000 Pa is generally an appropriate pressure for P1.
- 20 To ensure the highest quality assessment of samples, collect a minimum of 500 particle counts at each pressure for the calibration particles and each venom sample.
- 21 Following calibration particle measurement, the sample is removed from the upper fluid cell and the nanopore is rinsed thrice with wetting solution.
- 22 To accurately assess pore quality and remove all previous contaminating sample particles, add 35 μ L of measurement electrolyte solution to the upper fluid cell, and apply maximum pressure following software-guided recommendations for nanopore cleaning. Steps 21–22 should be followed every time a new sample is to be measured by the system.
- 23 Remove the measurement electrolyte and analyze venom samples utilizing the previously determined settings for pressure, voltage, stretch, and particle count threshold.
- 24 To clear any nanopore clogs or bubbles, stop taking measurements and apply firm tapping of the pressure nozzle (as described in step 8) to liberate any stuck particles or bubbles from the nanopore.
- 25 The particle rate should not exceed 1,000 particles/minute. If necessary, dilute the sample to remain below 1,000 particles/minute. An ideal RMS reading of below 10 pA should be maintained.
- 25.1 A dilution factor can be used for analysis within the ECS software if necessary based on original sample concentration.
- 26 To assess venom sample vesicle size and concentration, data are processed within the Izon Data Suite. For accurate analysis, ensure that particle rate increases as a function of increasing pressure within your three calibration datasets corresponding to P1, P2 and P3. This can be assessed using the dynamics plot's "particle rate" graphing function within the software.

27 Next, calibrate your samples. The calibration files corresponding to the identical settings of your sample files will be used in generating size and concentration data. This is performed in the "Multi-pressure calibration" tab.

27.1 Binning can be set within the graph settings, and data can be exported to a CSV file for further analysis.

Zeta Potential and Size Analysis Using the Exoid

28 Within the analysis pane of the ECS software, select [137 mM PBS] for analysis of venom particles. This may be changed dependent upon buffer conditions of sample preparation.

29 For the analysis of venom vesicles, settings for CPC100 calibration particles (or your desired calibration particles) are set using the characterization metrics you obtained from section 2 'Nanopore Preparation' in the ECS as guidelines for analysis.

30 Following the determination of nanopore conditions for voltage and stretch, calibration analysis begins with the determined voltage setting at a pressurized condition of 100 Pa. Next, use a non-pressurized setting at the initial voltage, and analyze with decreasing voltages. These runs are used for building a calibration file using your calibration particles.

31 Following calibration particle measurement, the sample is removed from the upper fluid chamber and the nanopore is rinsed thrice with wetting solution.

32 To accurately assess pore quality and remove all previous contaminating sample particles, add 35 μ L of measurement electrolyte solution to the upper fluid cell, and apply maximum pressure following software-guided recommendations for nanopore cleaning. Steps 31-32 should be followed every time a new sample is to be measured by the system.

33 Venom samples are now ready to be analyzed using the non-pressurized, user-input voltage setting determined by the ECS.

34 For data analysis, analyze calibration particle rates for spread, and calibrate fractions against CPC100 particles under the "charge analysis" section using the "calibrate data sets" menu. Data can be plotted with the Izon Data Suite software using the charge vs size scatterplot, and data can be exported to a CSV file for further analysis.



Protocol references

Nicholas Bretz, Nathan T Mortimer 2025. Isolation of Parasitoid Wasp Venom. **protocols.io**
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