Electronic Supplementary Material (ESI) for Lab on a Chip.

Electronic Supplementary Information (ESI)

A combinatorial droplet microfluidic device integrated with mass spectrometry for enzyme screening

Authors

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1. Supplementary Methods

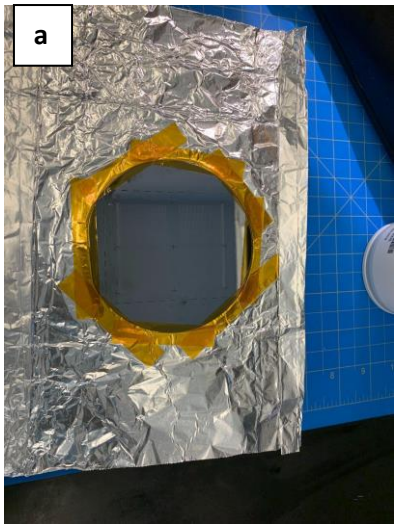
Chip Master and Microwell Array Chip Fabrication

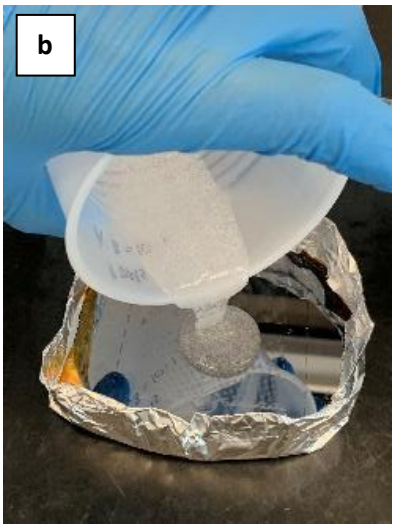
Microfluidic Chip Master Fabrication

1. Clean Si wafer (4", p-type, <100>, 0-100 Ω-cm, UniversityWafer Inc.) with a solvent wash.
   - Rinse with acetone, isopropanol, and DI water. For better results, ultra-sonicate the wafer in the solvents for 3-5 minutes and use ultrapure (18 MΩ) water. For a deep clean, use piranha etch.
   - Remove leftover water by blow-drying the wafer with nitrogen and a dehydration bake at 150°C for 10 min.
2. Spin-coat negative photoresist (SU-8 2075, MicroChem) on pre-treated wafer. Refer to the MicroChem Nano™ SU-8 2000 manual to customize the recipe.
   - Secure the wafer in an appropriately sized chuck.
   - Ramp up to 500 rpm at 100 rpm/s and dwell for 10 sec while dispensing the photoresist.
   - Ramp up to 1700 rpm at 300 rpm/s and dwell for 30 sec.
   - Ramp down to 0 rpm at 300 rpm/s.
3. Soft bake the coated wafer at 65°C for 5 min and at 95°C for 25 min, followed by 5 min cooling.
4. Exposure
   - Align the photomask (transparency or chrome-gold) with the wafer using a mask aligner (OAI Model 204).
   - Expose the wafer with a near UV (350 - 400 nm) source for 8 sec (at least 260 mJ/cm^2 dosage).
5. Bake the exposed wafer at 65°C for 5 min and at 95°C for 10 min, followed by 5 min cooling.
6. Develop the resist with SU-8 developer for 10 min. Rinse with isopropanol and DI water followed by drying with a gentle stream of nitrogen.
7. Hard bake at 175°C for 10 min. This step is optional but recommended.
8. Silanization of SU-8 master to prevent PDMS adhesion. This step should be conducted in a solvent hood or a glovebox.
   - The wafer was placed (SU-8 features facing up) in a desiccator along with a vial of ~200 µl Trichloro(1H,1H,2H,2H-perfluorooctyl)silane.
   - The desiccator was connected to the vacuum line and left undisturbed for 30 minutes to allow the silanizing agent to evaporate and form a thin layer on the master.

Microwell Array Chip Fabrication

1. PDMS molding:
   - Mix PDMS ("Part A") and curing agent ("Part B") in 10:1 weight ratio in a clean disposable cup; ~20g of PDMS per mold. PDMS sourced from Dow (SYLGARD™ 184 Silicone Elastomer Kit, material # 4019862).
   - Create a "well" around the SU-8 master mold with aluminum foil. Pour mixed liquid PDMS onto a SU-8 master mold (Fig. S1.1b-c). SU-8 master mold is silanized (Millipore Sigma catalog #448931) to make the surface hydrophobic to minimize permanent PDMS bonding to the mold.
   - Degas for 30 minutes to remove bubbles in a vacuum desiccator (Fig. S1.1d).





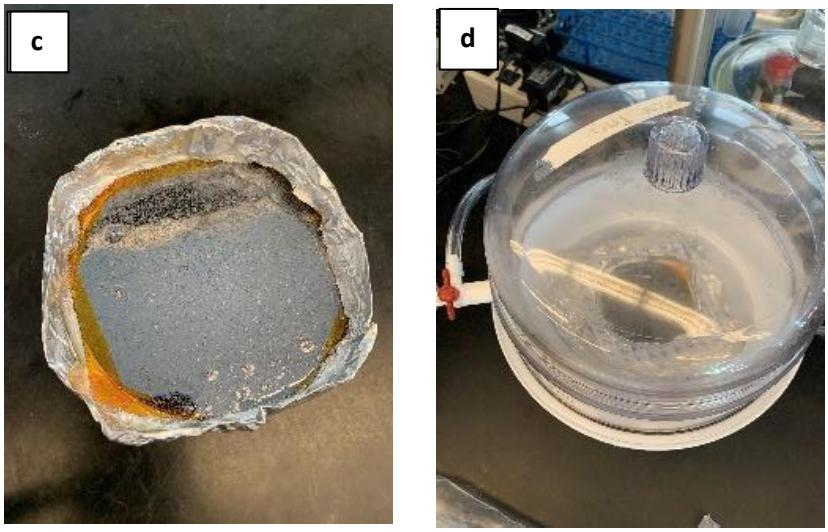


Figure S1.1

2. Assemble with clean glass slides using magnets

- Cut a glass slide into 25.4 mm W x 35mm L x 1mm T using a diamond scriber. (Note: Any commercial glass slides 1" W x 2" L x 1mm T can be used).
- Glass cleaning: Rinse a glass slide with Acetone, isopropyl alcohol, and distilled water. Dry it or wipe with Kimwipe. Blow the dust with an air duster spray.
- Place & align the glass layer on PDMS-filled mold using the alignment marker on the mold (Fig. S1.2). Note: 4 droplet chips per mold.

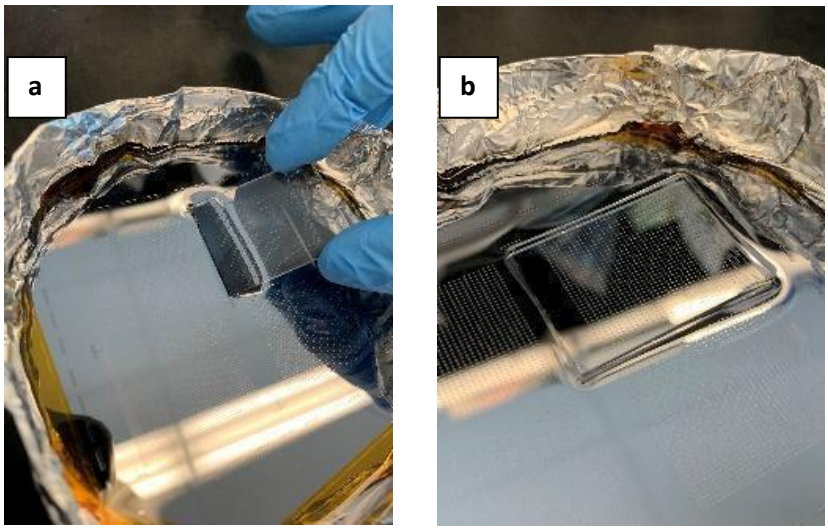


Figure S1.2

- Secure the glass slide with 2 Neodymium magnets (3/16" Thick, 1/4" OD; McMaster-Carr catalog # 5862K109).

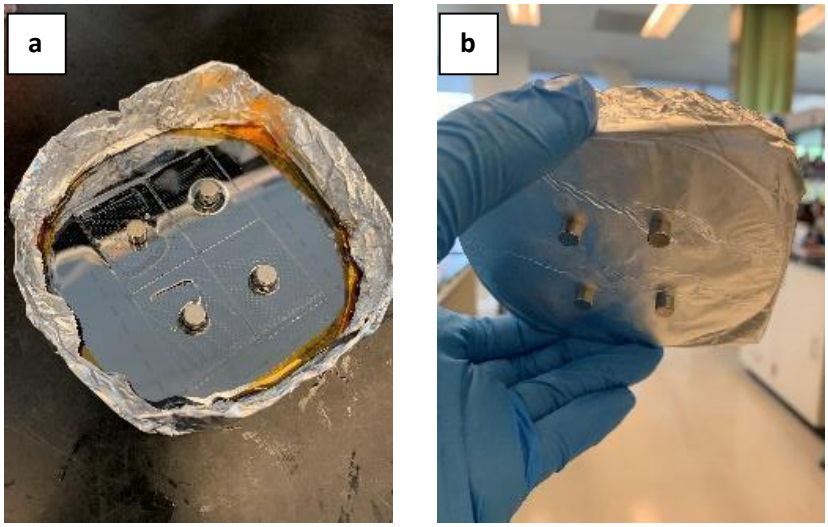


Figure S1.3

- Repeat the steps above for all 4 positions (Fig. S1.3). A total of 8 magnets and 4 glass slides are needed.
- Degas bubbles overnight in a vacuum desiccator. Note that PDMS will be partially cured at RT overnight.

3. Bake/cure PDMS at 80°C for at least 2 hours (Note: Overnight baking is preferred to fully cure)
4. After PDMS is cured, separate the droplet chip (Glass/PDMS) from the master mold
   - Remove magnets
   - Remove the surrounding PDMS from the glass surface. Make sure the top glass surface is free of PDMS.
   - Gently separate/peel the PDMS/glass chip from the mold using a razor blade (Fig. S1.4a). Note: Si master mold is brittle.
   - Trim PDMS (cut ~2mm from both ends to improve sealing against the NIMS chip): The size of PDMS layer should be SMALLER than that of the NIMS chip (Fig S1.4b).

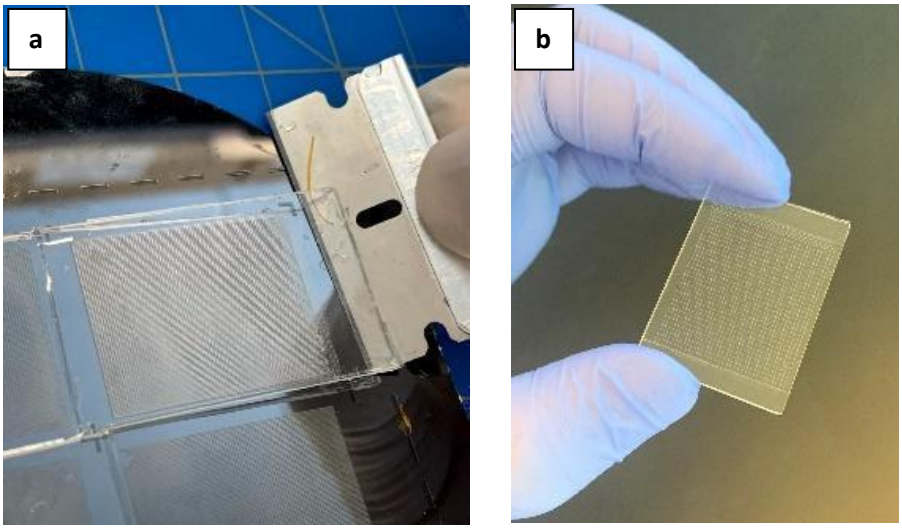


Figure S1.4

5. Assemble NIMS and microfluidic chip with a chip holder

   - Gather 3D print chip holders (top & bottom pieces)
   - Tape the microfluidic chip on the chip holder top piece using foam mounting tape (Fig. S1.5a-c; McMaster-Carr catalog # 76665A86).
   - Secure the chip with Kapton tape (Fig. S1.5d; tape from Digi-Key part # 4393-KPT-1/4-ND). This also helps to prevent droplets from getting into the gap.
   - Tape a small NIMS chip (~1" x 1" = ¼ of a regular NIMS chip) on the chip holder bottom piece using Kapton tape (Fig. S1.5e).
   - After assembly, should have a top and bottom chip holder with the PDMS chip and the NIMS chip, respectively (Fig. S1.5e).

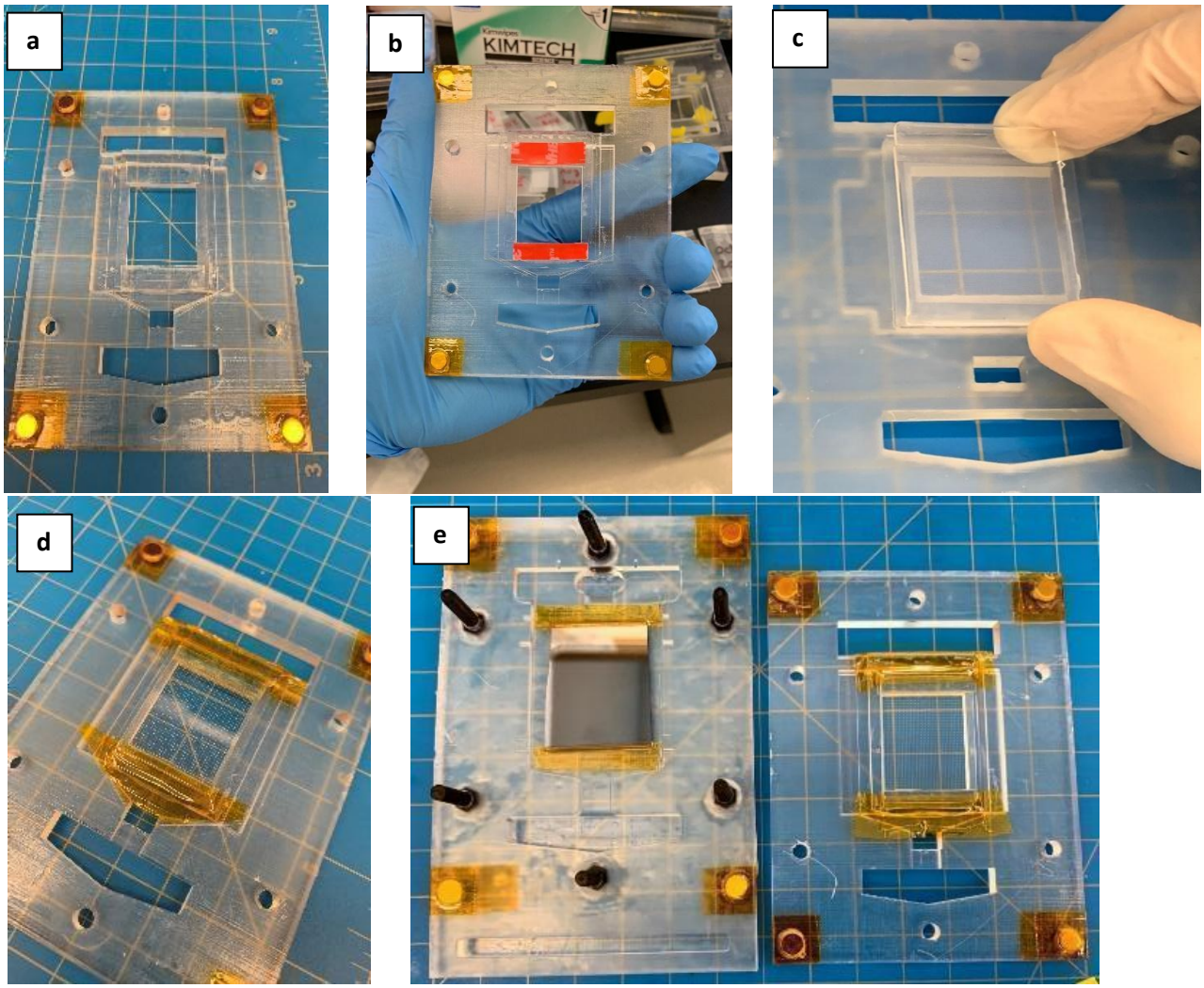


Figure S1.5

- Assemble two chip holder pieces (Top & Bottom) and adjust the gap using wingnuts (McMaster-Carr catalog # 94924A300). See Fig. 1Aii. Note: Test the gap by flowing the oil through the gap to ensure sufficient carrier oil is trapped (covering ¾ of the droplet chip surface).

Running Droplet Assays

See also supplemental videos 1 and 2.

1. Generate droplets using BIO-RAD's QX200 Droplet Generator (catalog # 1864002).
   - Load a new cartridge (BIO-RAD DG8, catalog # 1864008) with sample and carrier oil; 20µl of sample and 70µl of surfactant-added oil (HFE Novec 7500 + 008-Fluorosurfactant from RAN Biotechnologies) in their corresponding wells. It can generate 8 types of droplets each time.
   - After 2 minutes of droplet generation, pool droplets into a new centrifuge tube to prepare droplet cocktail.
   - (Optional) visual inspection of generated droplets under the Stereomicroscope: Droplet quality check with INCYTO c-chips (hemocytometer chip, item # DHCF012, 20µl chamber).

2. Load droplet cocktail (~50 µl each) into the microfluidic system using a pipette (use a 200 µl pipette tip). Wiggle around the chip holder to rapidly fill microwells with droplets. Pipette extra surfactant-free oil to spread out the droplets if needed.

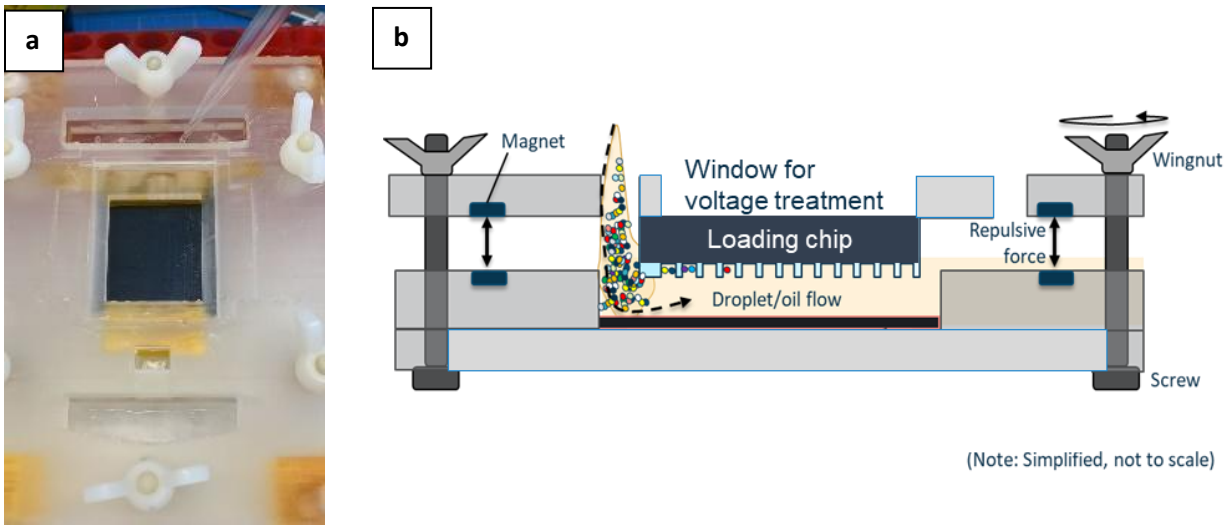


Figure S1.6

3. Rinse away unbound droplets with surfactant free oil.
4. Once all microwells are occupied with droplets, seal the droplet chip against the NIMS surface by tightening (hand-tight) the wingnuts. Note: Be careful not to crack the glass.

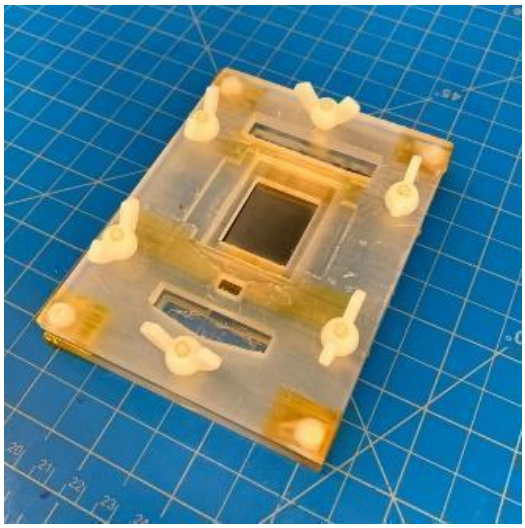


Figure S1.7

5. Merging droplets using an AC voltage treater (BD-20AC hand-held corona treater from Electro Technic Products, catalog # 12051A):
   - Sweeping across the glass (from the top; directly to the glass layer) for 15-20 seconds (Fig. S1.8a).

- Confirm complete merging under the stereomicroscope (Fig. S1.8b [before] and Fig. S1.8c [after]).

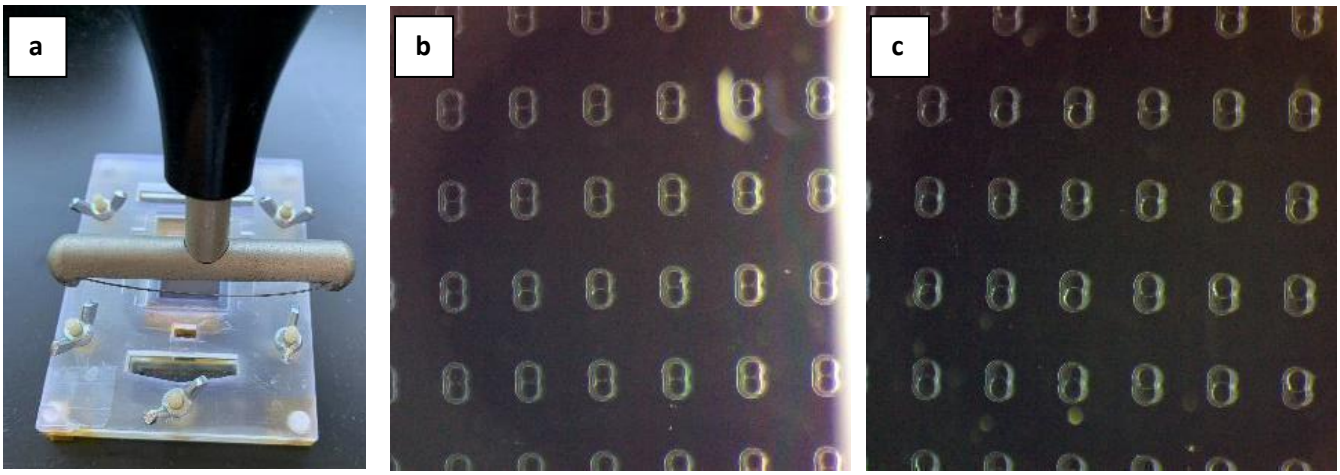
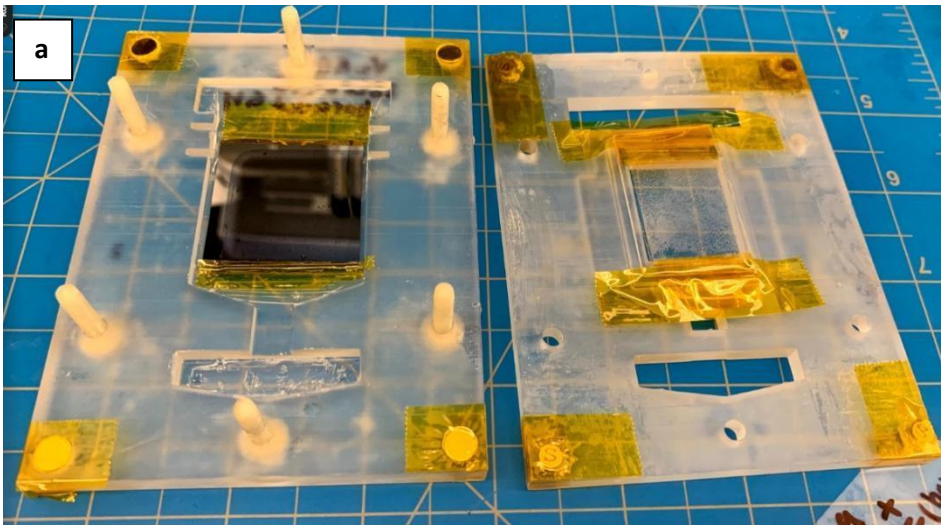


Figure S1.8

6. Droplet enzyme reaction will start immediately upon droplet merging: Incubate the whole system at room temperature (or up to 37°C) overnight. Usually, the reaction will last for approximately two to three hours, then dry out to leave an array of reaction products on the NIMS surface
7. Separate the NIMS chip for MS imaging after the sample & oil have dried out (Fig. S1.9a-b).
    - Unscrew wingnuts and carefully separate the chip holder top (containing the PDMS/glass chip) from the chip holder bottom. Remove the NIMS chip from the chip holder bottom.
    - Pipette calibration solution (~0.25uL) onto the NIMS chip in one to two small squares near the edge (e.g., Anaspec peptide 1 solution). Clearly mark the region of interest with metallic sharpie to quickly locate the region of interest under the MALDI camera (Fig. S1.9c).



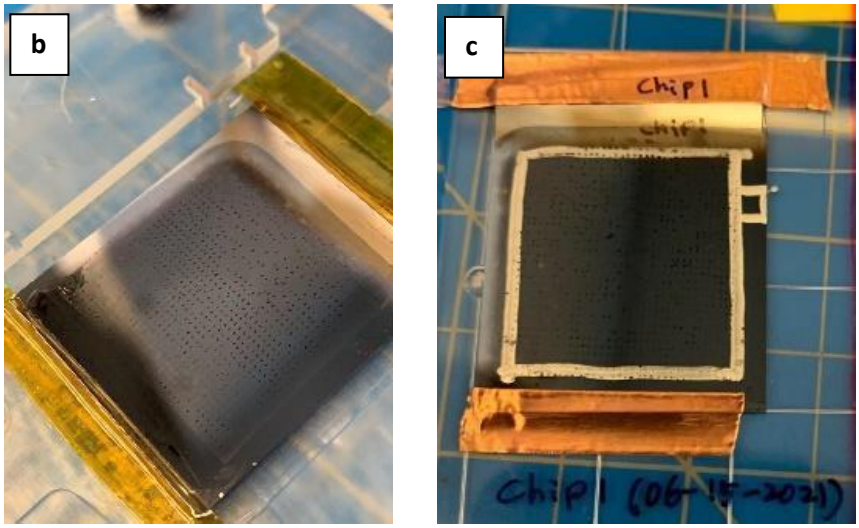


Figure S1.9

2. Supplemental Figures

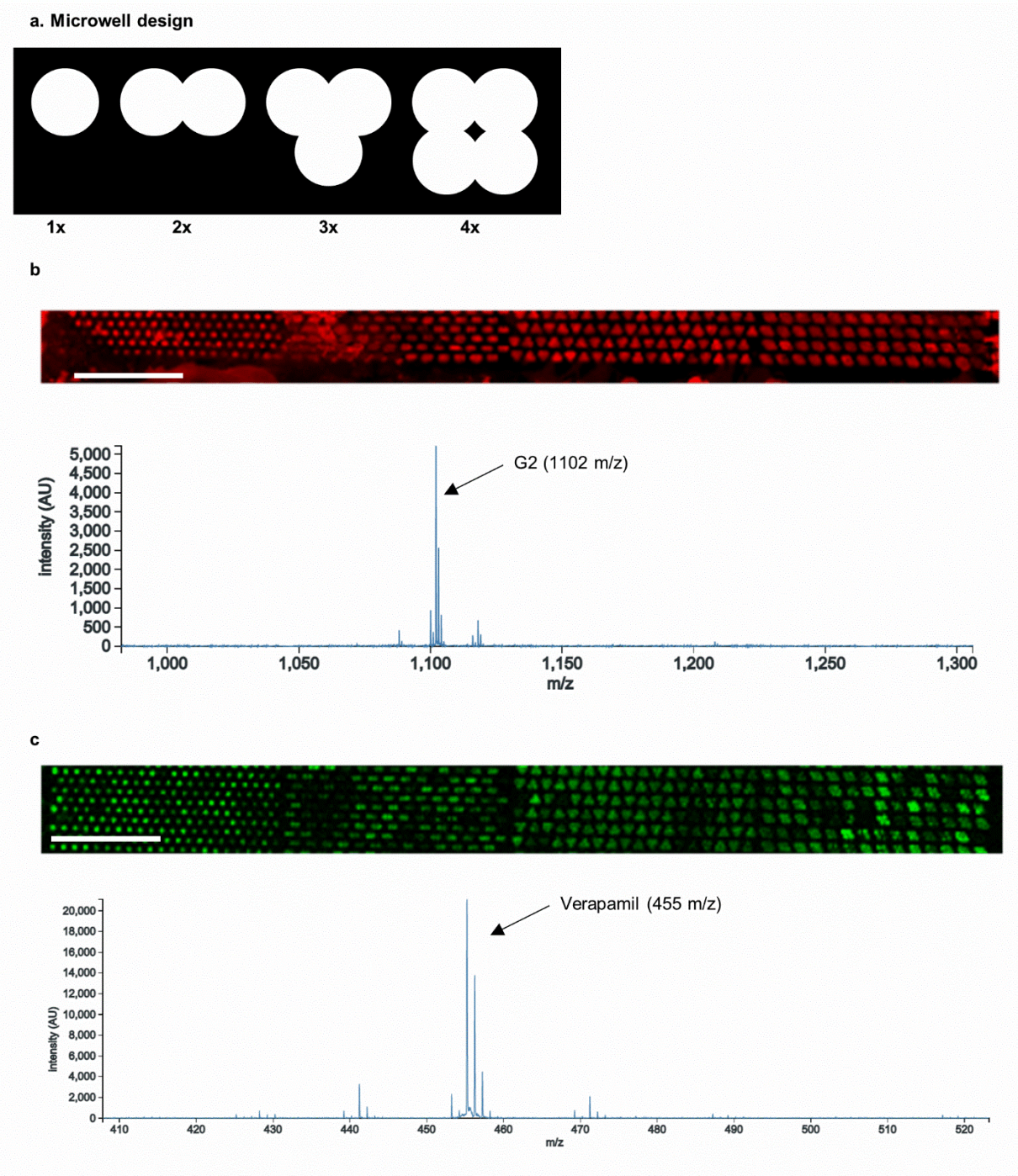
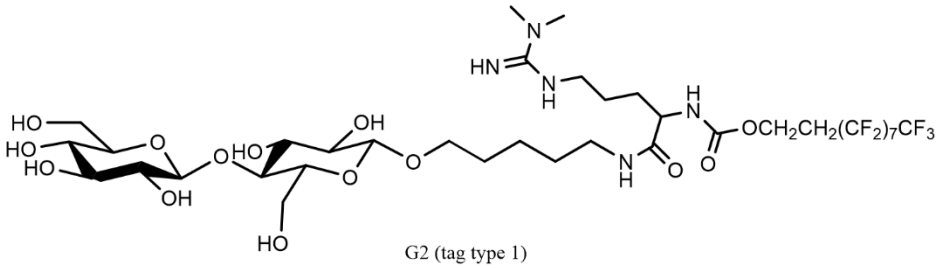
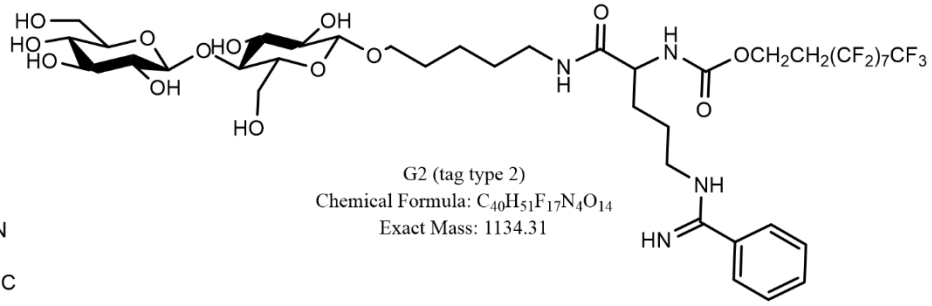


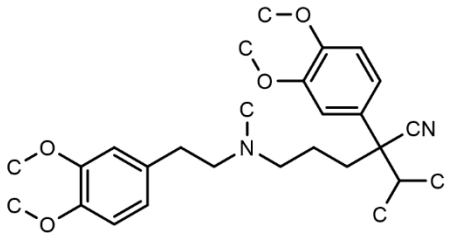
Figure S2. Droplet Array Printing and MS Imaging. **a**) Microwell design. Depositing and imaging of substrate droplets **b**) Verapamil and **c**) cellobiose with NIMS tag. A prior chip design was used in which microwells were closer together than in final design. Scale bar = 5 mm.



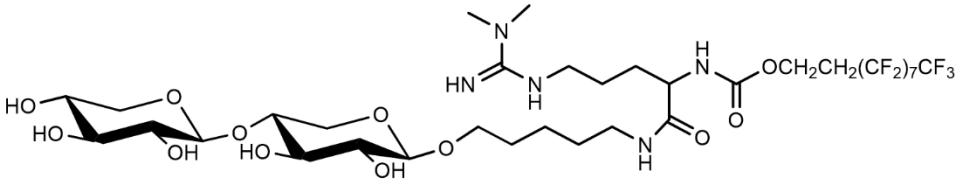
G2 (tag type 1)
Chemical Formula: $C_{36}H_{52}F_{17}N_5O_{14}$
Exact Mass: 1101.32



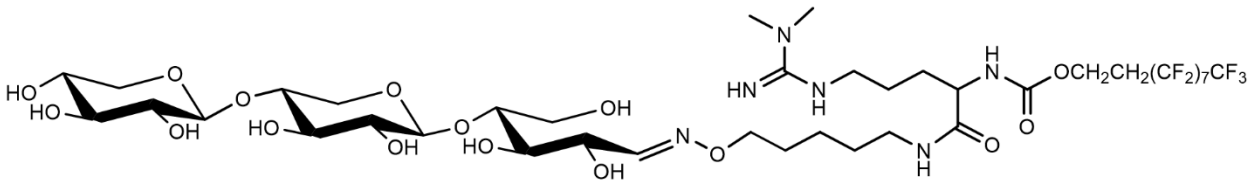
G2 (tag type 2)
Chemical Formula: $C_{40}H_{51}F_{17}N_4O_{14}$
Exact Mass: 1134.31



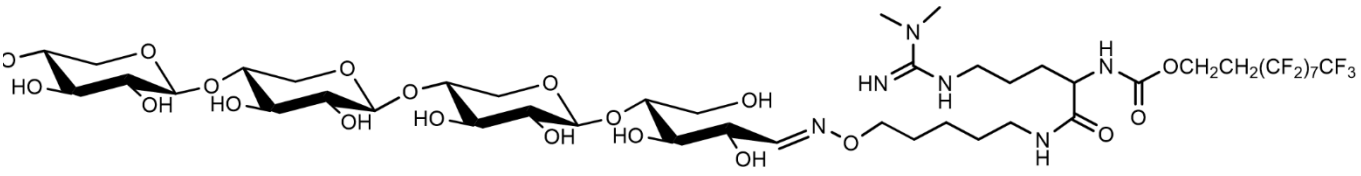
Verapamil
Chemical Formula: $C_{27}H_{38}N_2O_4$
Exact Mass: 454.28



X2
Chemical Formula: $C_{34}H_{48}F_{17}N_5O_{12}$
Exact Mass: 1041.30



X3
Chemical Formula: $C_{39}H_{57}F_{17}N_6O_{16}$
Exact Mass: 1188.36



X4
Chemical Formula: $C_{44}H_{65}F_{17}N_6O_{20}$
Exact Mass: 1320.40

Figure S3. Verapamil and NIMS-tagged substrates used in this study. G2, cellobiose; X2, xylobiose; X3, xylotriose; X4, xylotetraose.

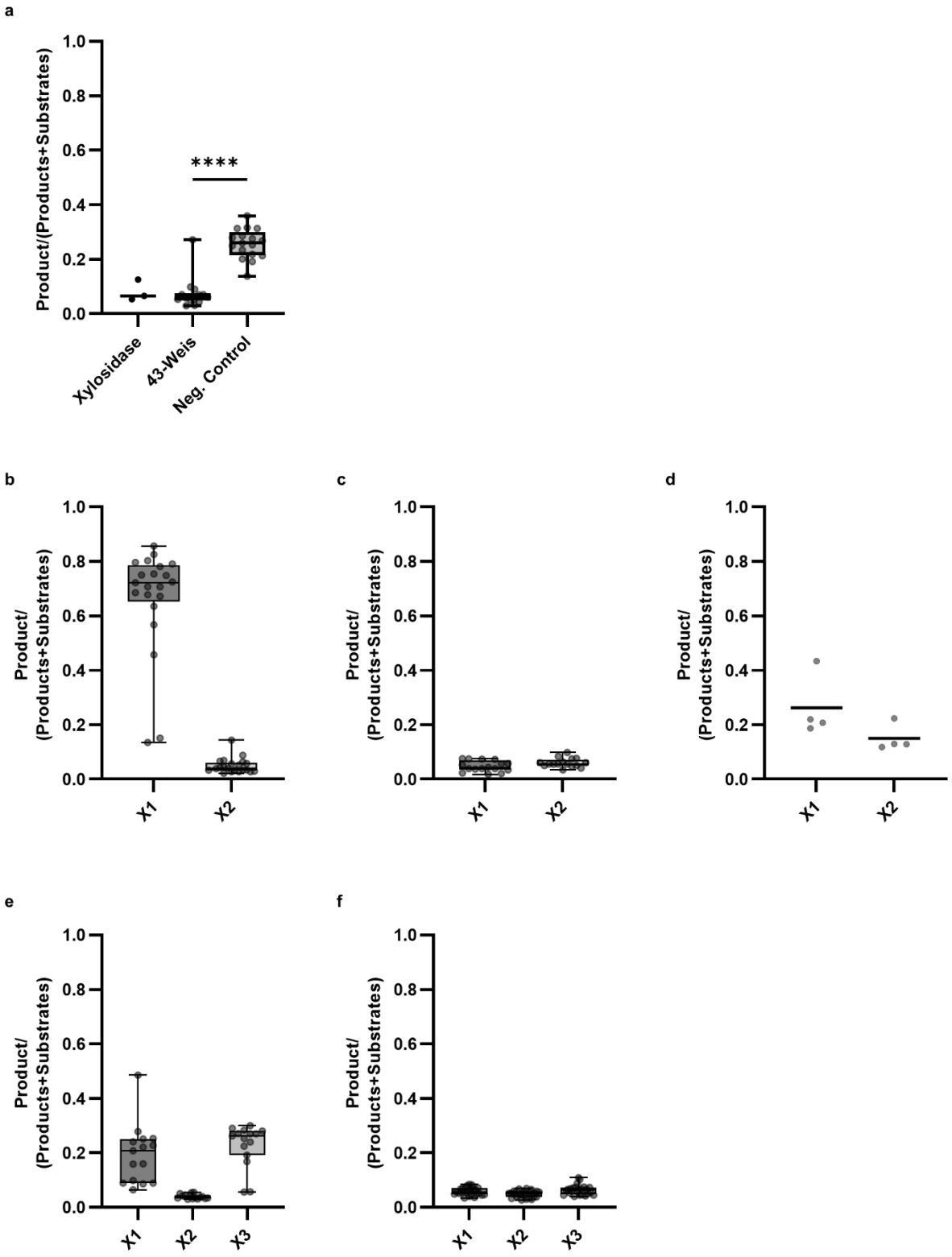
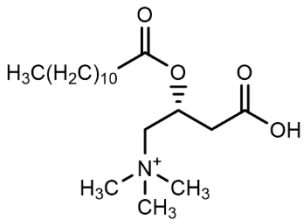
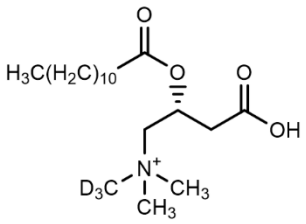


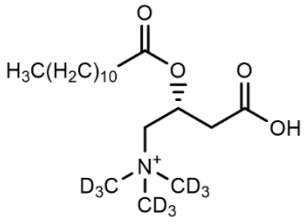
Figure S4. Specific products of reactions shown in Figure 3c-e. **a**) G2 reactions (G1 is the only product), **b**) X3 reaction products for 43-Weis, **c**) X3 reaction products for negative control (glucosidase), **d**) X3 reaction products for commercial xylosidase, **e**) X4 reaction products for 43-Weis, and **f**) X4 reaction products for negative control (glucosidase). Boxes show the 25th to 75th percentiles. The line within the box indicates the median. Whiskers indicate maximum and minimum. Statistics only shown for **a**, in which the negative control is compared to GH43-Weis; ****, P<0.0001. Xylosidase samples are shown as scatter plots (horizontal line indicates mean) due to low sample size (<5). Y-axes show the product peak intensities divided by the sum of the products and substrate peak intensities.



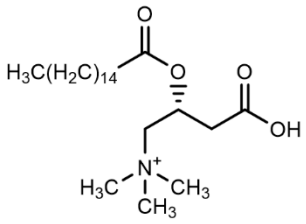
DC-1
Chemical Formula: $C_{19}H_{38}NO_4^+$
Exact Mass: 344.28



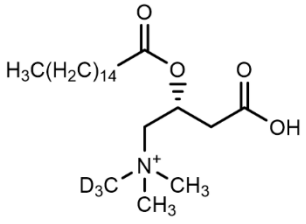
DC-2
Chemical Formula: $C_{19}H_{35}D_3NO_4^+$
Exact Mass: 347.30



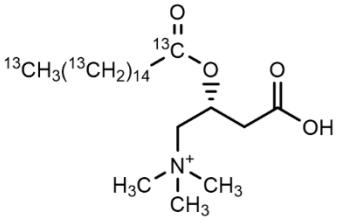
DC-3
Chemical Formula: $C_{19}H_{29}D_9NO_4^+$
Exact Mass: 353.34



PLC-1
Chemical Formula: $C_{23}H_{46}NO_4^+$
Exact Mass: 400.34



PLC-2
Chemical Formula: $C_{23}H_{43}D_3NO_4^+$
Exact Mass: 403.36



PLC-3
Chemical Formula: $C_7{}^{13}C_{16}H_{46}NO_4^+$
Exact Mass: 416.40

Figure S5. Structures of mass spectrometry barcodes used in this study. DC-1, L-Carnitine:HCl O-Dodecanoyl (unlabeled); DC-2, L-Carnitine:HCl, O-Dodecanoyl (N-Methyl-D_3); DC-3, L-Carnitine:HCl, O-Dodecanoyl (N,N,N-Trimethyl-D_9); PLC-1, L-Carnitine:HCl, O-Palmitoyl (unlabeled); PLC-2, L-Carnitine:HCl, O-Palmitoyl (N-Methyl-D_3), and PLC-3, Palmitoyl-$^{13}C_{16}$-L-carnitine hydrochloride (from Sigma).

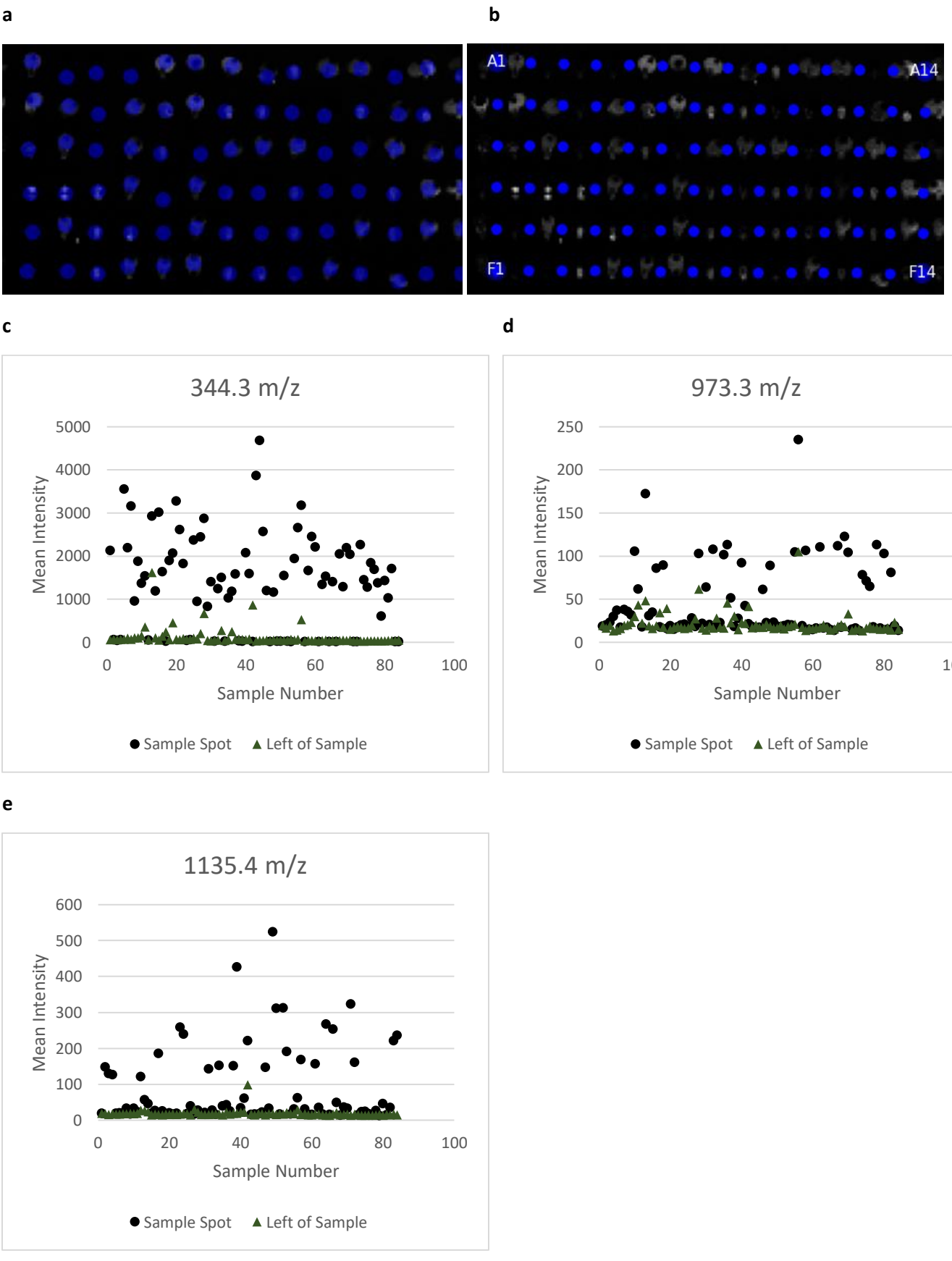


Figure S6. Signal intensity of target compounds on sample spots and in between samples spots for chip shown in Figure 2.. **a**) OMAAT mask used for sample spots. **b**) OMAAT mask used for areas to the left (i.e., in between) sample spots. **c**) Signal intensities for 344.3 m/z ion. **d**) Signal intensities for 973.3 m/z ion. **e**) Signal intensities for 1135.4 m/z ion.

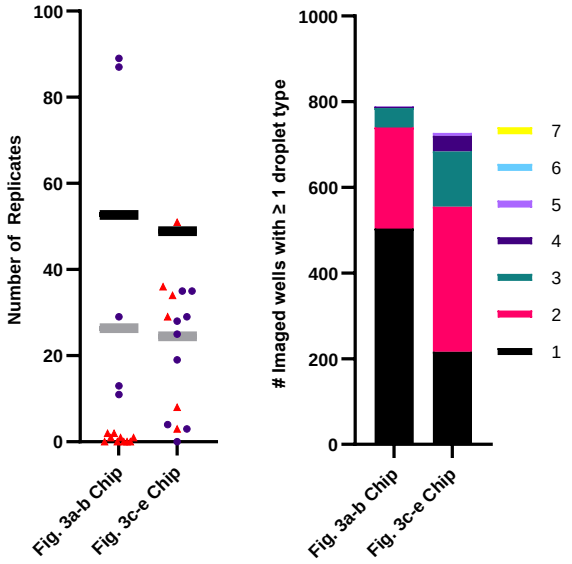


Figure S7. Replicate counts for chip experiments shown in Fig. 3, based on analysis of OpenMSI images. Only microwells with a signal (i.e., at least one droplet type detected) are included. **a**) Numbers of replicates for pairwise combinations. Theoretical number of replicates based on the number of wells imaged, the number of inputs, and either 100% loading efficiency (black bars) or 50% loading efficiency (grey bars). Theoretical numbers do not account for the greater proportion of substrate droplets were used (the number of substrate droplets was equivalent to the number of non-substrate droplets, to ensure most wells received substrate). The number of actual replicate counts for enzyme/substrate or control/substrate combos (blue circles) or non-interesting combinations (e.g., enzyme/enzyme; red triangles) are shown. **b**) Number of spots with 1 or more droplet types.

3. Supplementary Tables

Supplementary tables can be found at this website: https://github.com/biorack/2023_Drop-NIMS.git. Supplementary tables S1-6 provide the raw data, the possible combinations of droplets, and the numbers of replicates for Chip 1 (Tables S1-3; summary data are in Figures 3a-c in the main manuscript) and Chip 2 (Tables S4-S6; summary data are in Figures 3d-e in the main manuscript). Table descriptions are included below.

Table S1. Data exported from OpenMSI with OMAAT for the first chip. The chip was analyzed with OMAAT in two parts (top half and bottom half). Spot locations correlate to the spots on the OMAAT mask. The mean intensities are given for ions of interest (see Materials and Methods). The presence of a droplet was determined by the presence of a target ion above 100 au (1 indicates present; 0 indicates not present). The relative proportion of products are shown (e.g., the proportion of tagged glucose = G1/(Gtag+G1+G2)). The data for this chip are shown in Figure 3a-b.

Table S2. All possible combinations for the first chip. Data for relevant combinations (i.e., enzyme + substrate) are shown in Figure 3a-b.

Table S3. The number of data points for each treatment shown in Figure 3a-b. Since the same droplet contained both X2 and G2 substrates, the number of replicates is the same for both Figure 3a and 3b.

Table S4. Data exported from OpenMSI with OMAAT for the second chip. The chip was analyzed with OMAAT in two parts (top half and bottom half). Spot locations correlate to the spots on the OMAAT mask. The mean intensities are given for ions of interest (see Materials and Methods). The presence of a droplet was determined by the presence of a target ion above 100 au (1 indicates present; 0 indicates not present). The relative proportion of products are shown (e.g., the proportion of tagged glucose = G1/(Gtag+G1+G2)). The calculations for products of X3 and X4 droplets are designated "X3 oligo" and "X4 oligo," respectively. The data for this chip are shown in Figure 3c-e.

Table S5. All possible combinations for the second chip. Data for relevant combinations (i.e., enzyme + substrate) are shown in Figure 3c-e.

Table S6. The number of data points for each treatment shown in Figure 3c-e.

4. Supplementary Videos

Supplementary Video 1. Operation of Drop-NIMS with droplets containing dye and glass in place of NIMS chip.

Supplementary Video 2. Operation of Drop-NIMS with NIMS chip in place.