Supplementary Information Salomons et al.

Storing Liquid Chromatographic Separations on Surface Energy Traps: Decoupling the LC and the Mass Spectrometer

OT-2 Slide Holders:

The Opentrons OT-2 pipetting robot is designed to work with the 96 well plate systems common in molecular biology. As such, the bed of the robot has 11 slots for well plates and well-plate-shaped objects. To allow the robot to work with glass slides, we designed and 3D printed a series of slide holders to mount the slides to the robot bed. The designs of the slide mounts can be seen in SI Figure 1 below. The base of the mounts matches the dimensions of a standard 96 well plate, which allows them to fit into the slots on the bed of the OT-2. The glass slides then fit snugly into the top of the slide holder.

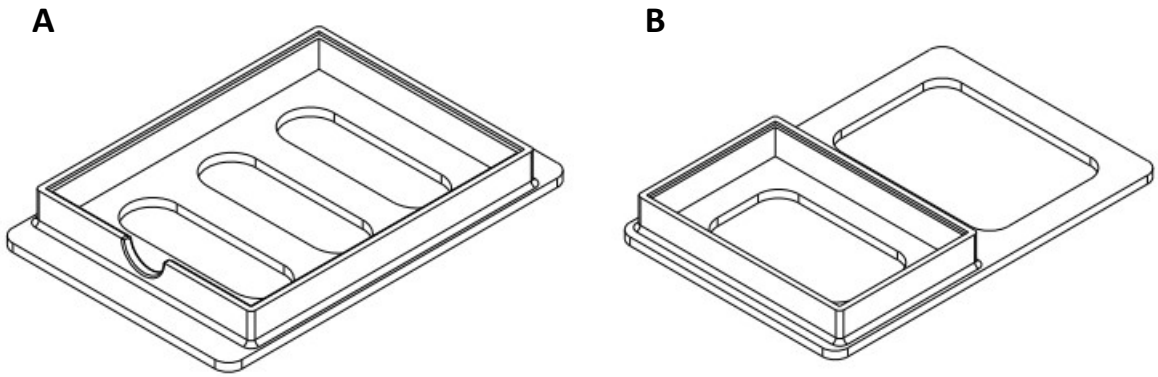

Figure S1: Technical drawings of the slide holders used to mount the SET arrays to the bed of the OT-2 pipetting robot. The 4"x3" slide holder is pictured in A, while the 50mmx75mm (2"x3") slide holder is pictured in B. The footprint of each slide holder matches a standard 96 well plate.

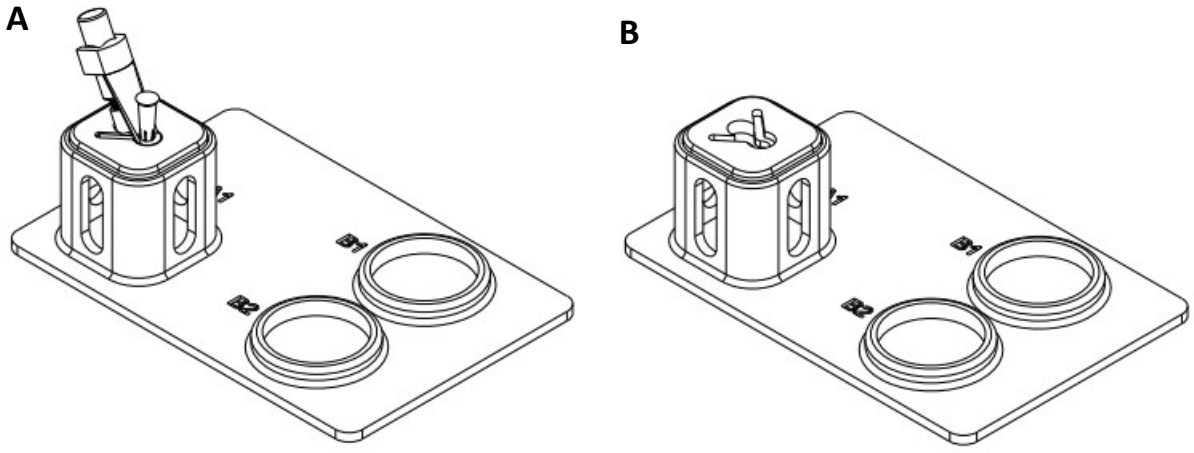

Figure S2: Technical drawings of the writing head holder with (A) and without (B) the writing head. This piece protects the writing head from damage when it is not attached to the robot's pipette. Before archiving, the robot picks up the writing head from its

holder, and the eluent tubing is then attached to the writing head manually. The wells marked B1 and B2 hold 20mL glass scintillation vials for collecting waste liquid.

To Access the SETs on the glass slides mounted on the slide holders, the OT-2 needs to know the dimensions of the slide holder and the location of each SET on the glass slide (this is also true for standard well plates etc.). To do this the OT-2 uses JSON files they call labware definitions, which hold the dimensions of the labware as well as the number, position, size, and shape of each well. The syntax of the labware definition files was reverse engineered by inspecting some of the JSON files that came pre-loaded with the robot, and a python script was written to generate the custom labware definition files for each slide-mount pair. After generation, the accuracy of each file was tested by mounting the slide to the robot and instructing the robot to position the tip of its pipette 1mm above the centre of various SETs. Any inaccuracies were corrected by editing the JSON file by hand, although this was exceedingly rare. The JSON files of the SET array attached to the 4x3 slide holder and the writing head holder can be found in the supplementary information as 4x3 Targets v1.json and Writing Head Stand5.json.

Programming the OT-2 for Deposition:

When the OT-2 is instructed to move to a well in a well plate, it uses the following standard movement profile. First, it raises the pipette up such that the pipette tip clears the top of the well plate, then it moves the pipetting arm horizontally to position the pipette above the target well, then it lowers the pipette so the tip enters the well, stopping 1mm above the bottom of the well. This movement profile is undesirable for our application, so instead we used direct movement commands. In addition, instead of instructing the robot to visit each SET individually, we instructed it to move from the first SET of each row directly to

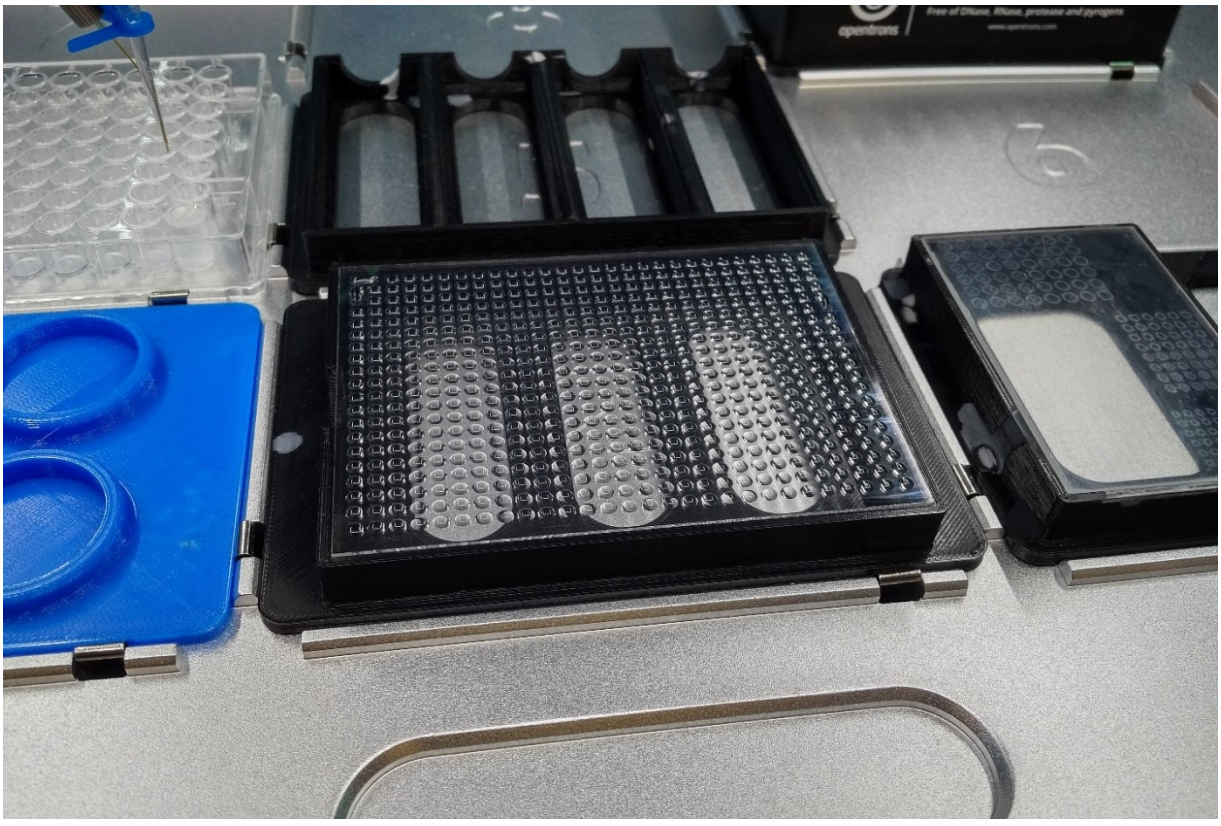

Figure S3: The SET array immediately after archiving a chromatogram. The writing head (visible top left) is parked over a well plate to catch any waste. The archived separation begins at the top left of the slide, and the writing head moves back and forth left to right, right to left, ending at the bottom left of the slide. Left of the SET array (blue) is the holder for the writing head when it is not attached to the robot's pipette; the section that holds the writing head is not visible. Right of the SET array is a 50mmx75mm slide holder with a slide mounted. This slide contains the SET arrays used for the optimization experiments.

the last SET of the row. This ensures a smooth and continuous movement by eliminating the rapid deceleration-acceleration that occurs as the robot arrives and departs from each SET.

To run an archiving experiment, the micro-fractionator needs to be synchronized with the HPLC. However, standard contact-closure synchronization was not possible, due to both the OT-2 pipetting robot and the Agilent 1100 HPLC used not supporting contact closure. This limitation was bypassed by suing an Arduino microcontroller to relay information between the two instruments. A custom cable connecting the Arduino to the HPLC allowed the Arduino to listen to the HPLC. Once the injection occurred, the Arduino would relay the information to the OT-2 via USB. The OT-2 protocol scripts are written in Python, which allows communications with the Arduino to be written directly into the protocol scripts. We programmed the OT-2 to hold the writing head over a waste collection well-plate while it waited for a start signal from the Arduino. Once it received the signal it would move at high speed over to the first SET and begin archiving the separation. The python script used for archiivng can be found in the supplementary info as LC Trace Archiving v3.1.py.

The video included in the supplementary information (SI Video 1-Deposition Example.mp4) shows deposition in action. The device shown in the video is an early prototype—not the one shown in the photos or used for the experiments. While the hardware of the final micro-fractionation device is different from that shown in the video, the deposition principle remains the same.

Figure S4: An overhead view of the SET after archiving. Some isolated incidents of cross talk between neighbouring SETs can be observed. These incidents are limited to the left and right edges of the SET array, as well as the upper left corner, where the separation begins. These locations are where the writing head is changing direction and thus unable to maintain a smooth,

consistent motion. Nevertheless, these incidents are rare, and since each SET contains only 0.36s of separation time the integrity of the separation is unaffected.

Ionization Efficiency of Caffeine and Phenacetin:

To verify that the relative ionization efficiencies of caffeine and phenacetin were equal, we infused an equimolar solution of caffeine and phenacetin into a mass spectrometer and recorded the intensities, as seen in figure 5 below. For this experiment, a Q-exactive HFX was used, with an infusion rate of 2.5µL/min and a spray voltage of 3.75kV.

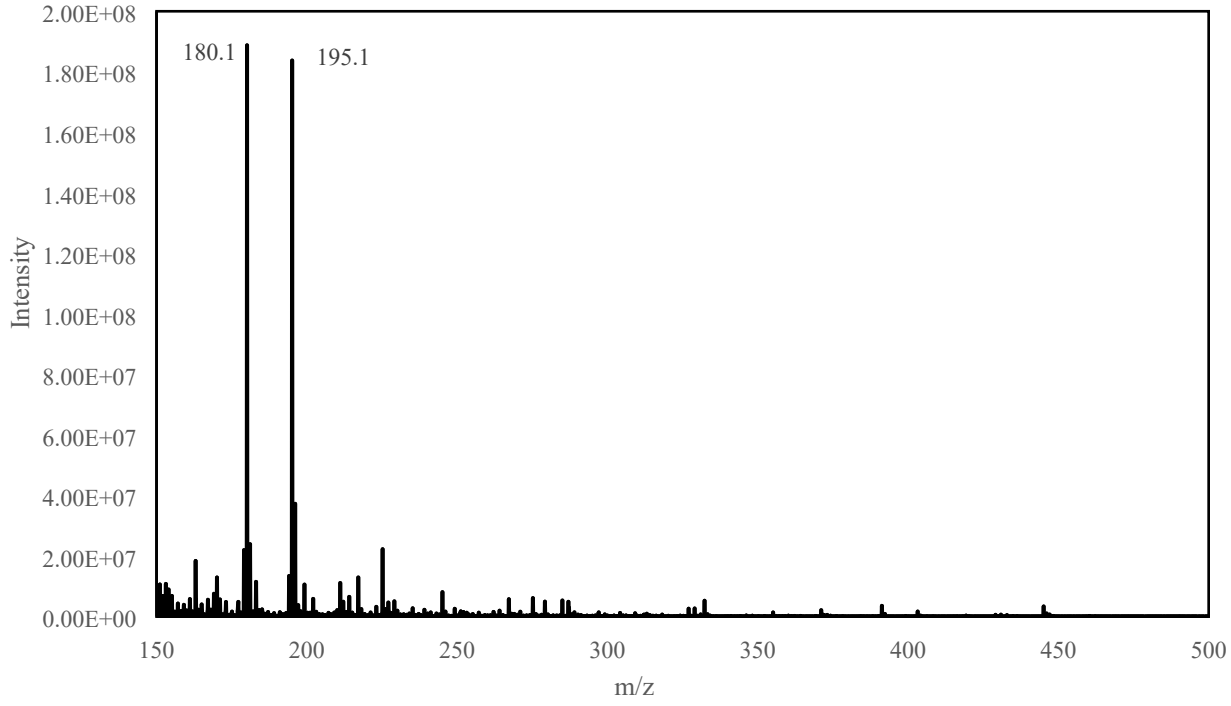

Figure S5: A mass spectrum of caffeine and phenacetin, averaged over 5 minutes of infusion. The intensity of the caffeine peak at 195.1m/z was found to be 97.3% of the intensity of the phenacetin peak at 180.1m/z.