

Supplementary Information for:

## Ultra-high capacity microfluidic trapping of giant vesicles for high-throughput membrane studies

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Movie S1: Confocal time series of typical loading of GUVs into trap number 1. Flow rate 10  $\mu\text{l}/\text{min}$ . Scale bar: 50  $\mu\text{m}$

Movie S2: Confocal time series showing GUVs flowing by traps without side-posts. Flow rate 10  $\mu\text{l}/\text{min}$ . Scale bar: 50  $\mu\text{m}$

Movie S3: Confocal time series showing unloading of GUVs using the syringe operating in injection mode at a flow rate of 20  $\mu\text{l}/\text{min}$ . Scale bar: 50  $\mu\text{m}$

Movie S4: Confocal time series of the quenching of fluorescein fluorescence inside GUV as the external solution pH is changed from 11 to 5, with a flow rate of 5  $\mu\text{l}/\text{min}$ . Scale bar: 50  $\mu\text{m}$

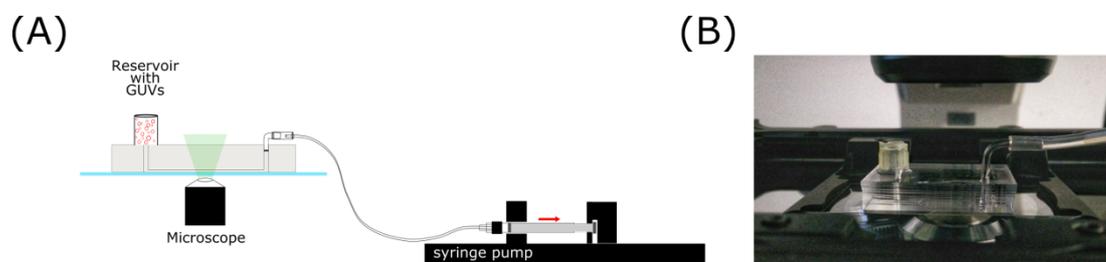


Figure S1. Operation and loading of the microfluidic chip with GUVs. (A) Schematic representation of the chip imaged with an inverted confocal microscope. GUVs are loaded into the reservoir and introduced into the device using a syringe pump operating in withdraw mode. B) Photograph of chip on the microscope stage.

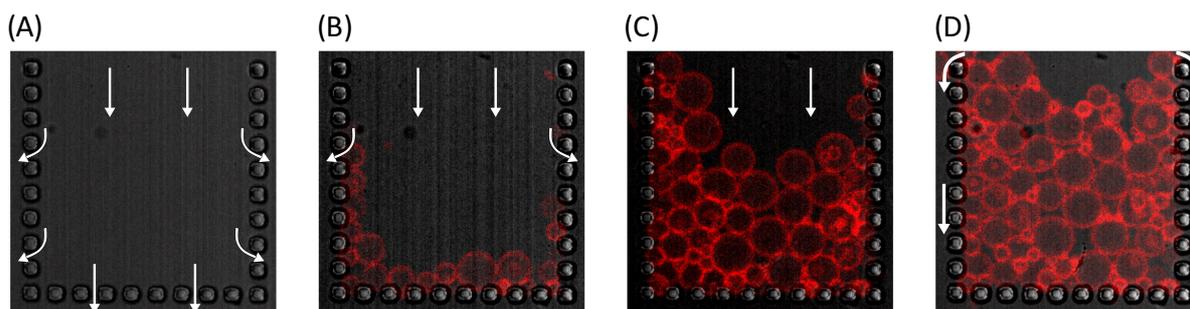


Figure S2. Confocal time series taken from Movie S1, with arrows indicating the major flow lines as vesicles enter the trap. A) The gaps between the posts at the bottom and sides allow the initial entrance of vesicles as the solution is able to flow between them. B) The first row of vesicles blocks most of the flow at the bottom, but the side posts still allow entrance of new vesicles. C) Vesicles continue to fill the trap in this manner. D) Once completely occupied, the majority of the flow is diverted around the trap.

### S3. Comparison of trapping performance with and without the side-posts

To quantitatively compare the efficiencies of the device with and without the side-posts, we flushed in GUV suspension volumes of 100, 500 and 2000  $\mu\text{l}$  and acquired images of all 17 traps within a channel and across three different channels. Data were then obtained on the average number of vesicles per trap and the % occupancy. From the data shown in the Figure S3, it is clear that after 100  $\mu\text{l}$  volume, either with or without the side posts, many traps remain unfilled with the last few traps (12 to 17) having less than 20% occupancy. Interestingly, the filling of the traps without the side-posts gradually decreased from the first to the last trap. This highlights the need for the side-posts as each subsequent trap is increasingly less efficient. After flushing of 500  $\mu\text{l}$  solution, most of the traps with the side-posts have  $\sim 60 - 90\%$  occupancy but without they have less than  $\sim 40\%$ . For this volume, the average number of trapped vesicles was approximately 2.5 times higher with the side-posts. Finally, after the addition of 2000  $\mu\text{l}$ , all of the traps with side-posts had  $> 90\%$  occupancy whereas without more than half of the traps remained unfilled. Importantly, for all volumes, the occupancy and average number of GUVs is higher with the addition of the side-posts. After 2000  $\mu\text{L}$  the total number of vesicles (per channel) without side-posts is 1349, which is similar to 959 trapped with the side-posts after just 100  $\mu\text{l}$ . This suggests the inclusion of the side-posts increases the efficiency by approximately 20 times. In other words, approximately 20 times more GUV suspension is wasted without them. This highlights the need to include the side-posts, especially with time sensitive samples which require fast loading and imaging of multiple GUVs for statistics. It should be noted that we were not able to completely fill the device without them.

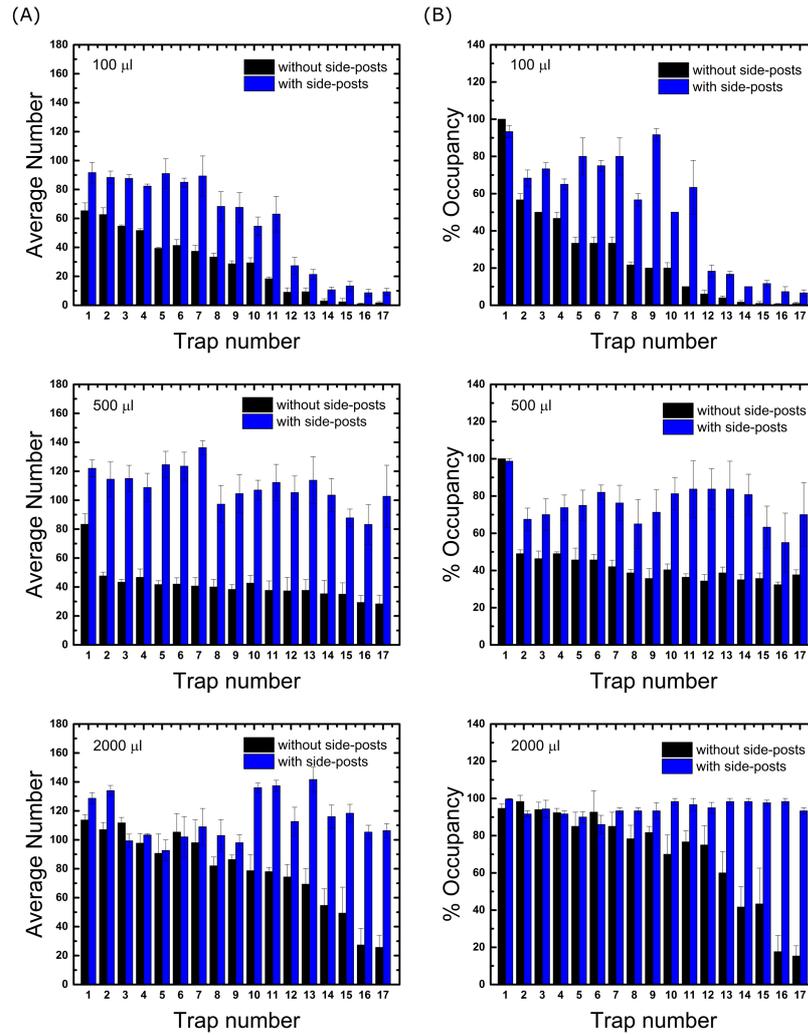


Figure S3 - Comparison of trap filling efficiencies. Plots of (A) the average number of GUVs and (B) the % occupancy with and without the side-posts for sample volumes of 100, 500, and 2000 µl. The loading flow rate was 10 µl/min. Mean values are from three different channels and error bars are taken from the standard deviation of the mean.

$$Trapping\ efficiency \approx 100 \times \frac{volume\ containing\ 23,000\ GUVs\ (0.71\ mL)}{volumed\ flushed\ through\ to\ capture\ 23,000\ GUVs\ (2\ mL)} \quad (S1)$$

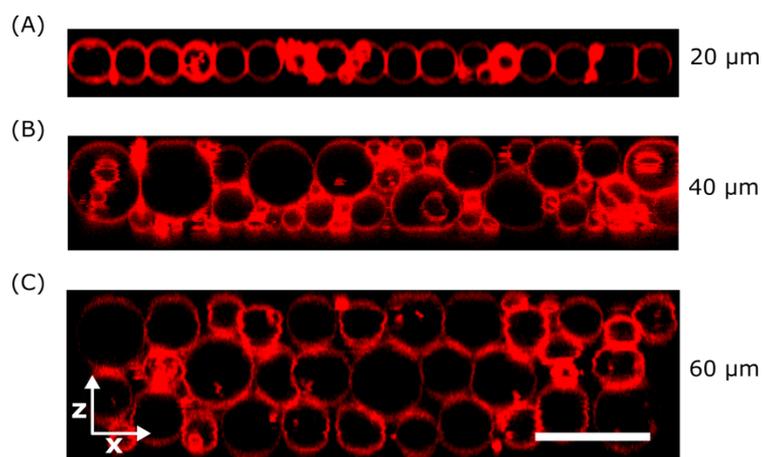


Figure S4. Layering GUVs in traps in 3-D. Confocal side-view renderings of layers of vesicles with a A) 20  $\mu\text{m}$ , B) 40  $\mu\text{m}$  and C) 60  $\mu\text{m}$  high channels. Scale bar: 50  $\mu\text{m}$ .

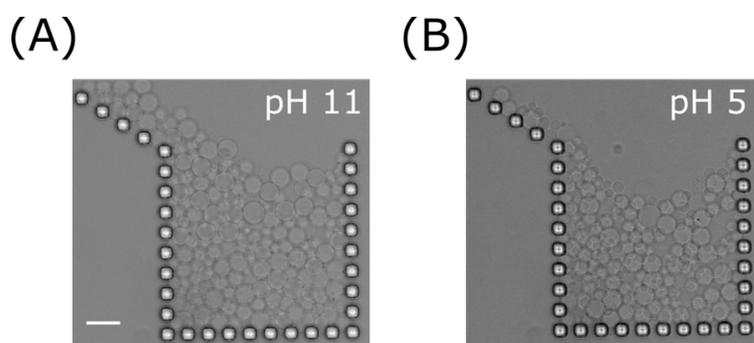


Figure S5. Bright-field transmitted light images of the same GUVs, first at pH 11 (A) and afterwards at pH 5 (B).

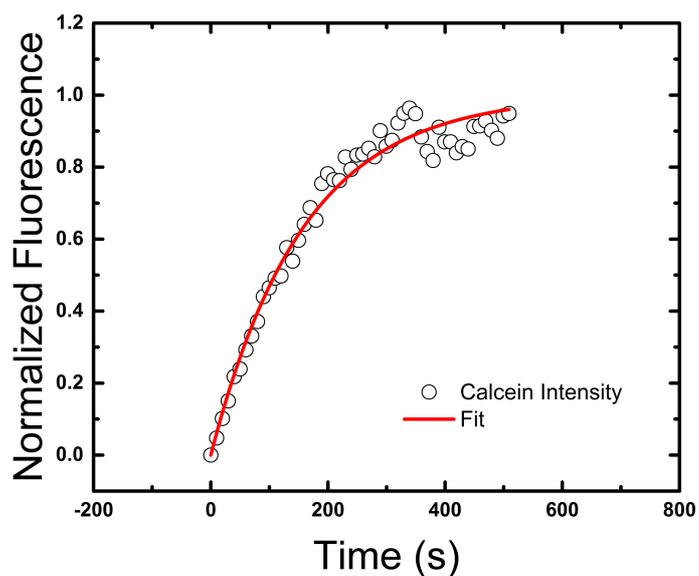


Figure S6. Example rate of calcein influx from a single vesicle. The data is fitted with Equation 1.