# Supplementary material

## Purification of DLPs

DLPs from simian rotavirus SA11-4F were purified from infected monkey kidney (MA104) cells using the protocol described previously[1] with some modifications. Briefly, the infected cell supernatant was treated with 20 mM EDTA to remove the outer capsid layer from intact virions. The resulting DLPs were then concentrated by pelleting through a cushion of 35% sucrose (w/v) in Tris-buffered saline (TBS) and then purified by two rounds of isopycnic centrifugation in cesium chloride (CsCl). The DLPs, which banded at a density of 1.38 g/cm3, were collected from the CsCl gradient and dialyzed exhaustively against TBS. Purified particles were stored at 4°C in the presence of 1μg $ml^{-1}$ protease inhibitors aprotinin and leupeptin. Isolated DLPs were electrophoresed on a 7.5% SDS-polyacrylamide gel (**Supplementary Fig. 2a**) to assess purity. The gel was stained with GelCode Blue (Pierce Biotechnology, Inc., Rockford, IL).

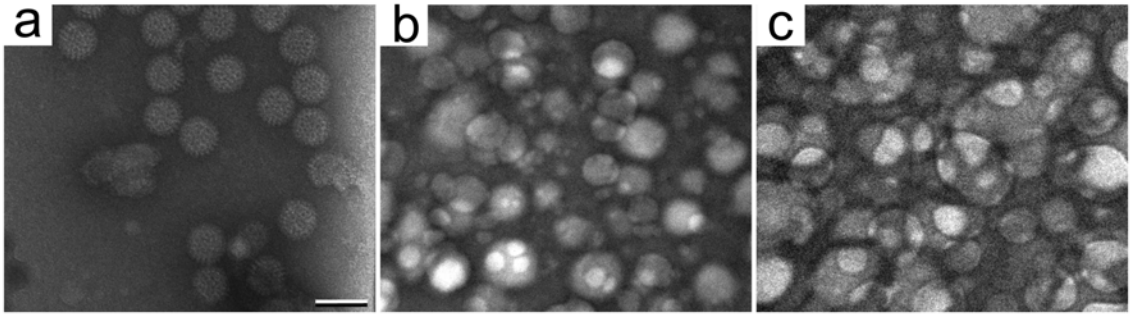
## Preparation of Affinity Capture devices

Affinity Capture devices were prepared using silicon nitride microchips (Protochips, Inc., Raleigh, NC) having dimensions of 2.60 mm x 2.00 mm and a 50-nm thick central window, 500 μm x 50 μm. Microchips were heated in a petri dish for 90 min at 150°C and cooled to room temperature prior to use. Lipid mixtures composed of varying amounts of Nickel-nitrilotriacetic acid (Ni-NTA) lipids (5% for negative stain, 20% for *in situ* and 50% for cryo) and 1,2-dilauryl-phosphatidylcholine (DLPC) filler lipids (Avanti Polar Lipids, Alabaster, Al) were reconstituted in chloroform (1 mg $ml^{-1}$) and cast over 15-μl aliquots of Milli-Q water placed on a piece of parafilm that could easily fit inside of a humid petri dish. The dish was sealed and incubated on ice for 60 min. The smooth-side of the silicon nitride chips were placed on top of the lipid layers for at least 1 min after which time the chips were gently lifted up from the parafilm. The procedure effectively transfers the lipid layers onto the chips.[2]

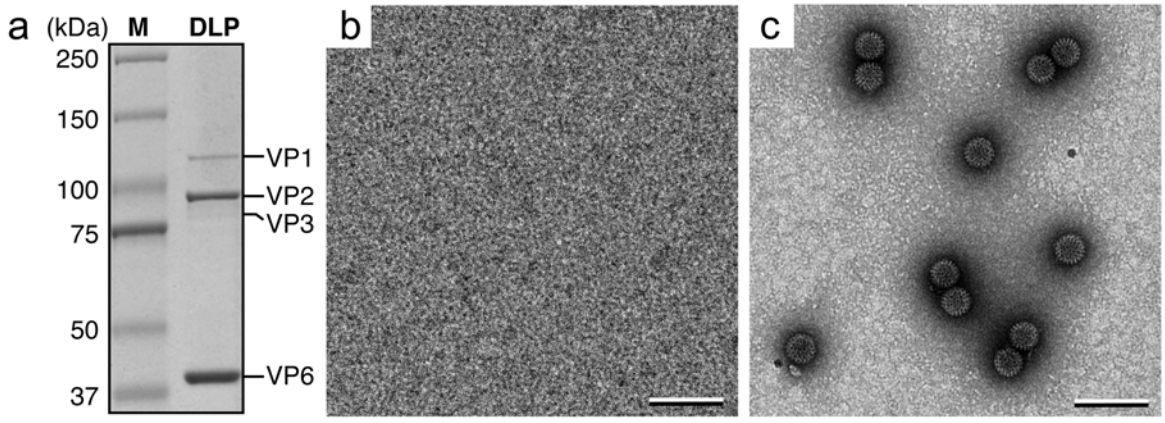
## EM data collection and image processing routines

All specimens were examined under low-dose conditions (~ 5 electrons / $Å^2$) using a FEI Spirit BioTwin TEM (FEI Company, Hillsboro, OR) equipped with a tungsten filament and operating at 120 kV. Images were recorded on a FEI Eagle 2k HS CCD camera with a pixel size of 30-μm at a nominal magnification of 30,000x for a final sampling of 10 Å per pixel. Negatively stained specimens were recorded at a defocus value of -1.5 μm and defocus values for *in situ* and cryo specimens varied between -1 μm to -3 μm. We collected 38 images of negatively stained specimens yielding 624 individual particles (i.e. 624 x 60-fold symmetry for icosahedral particles, equates to 37440 particles if lacking symmetry). The selected particles were used for image processing routines in the SPIDER software package[3] described in the **Results and discussion** section. Likewise, 26 and 58 images were collected for *in situ* and cryo specimens yielding 600 and 572 particles, respectively (i.e. 600 or 572 x 60 for icosahedral particles, equates to 36,000 and 34,320 particles if lacking symmetry). The selected particles were used for alignment and averaging routines. The RELION software package[4] was used to calculate 3D reconstructions and overall resolution of the liquid and frozen specimens as described in the **Results and discussion** section.

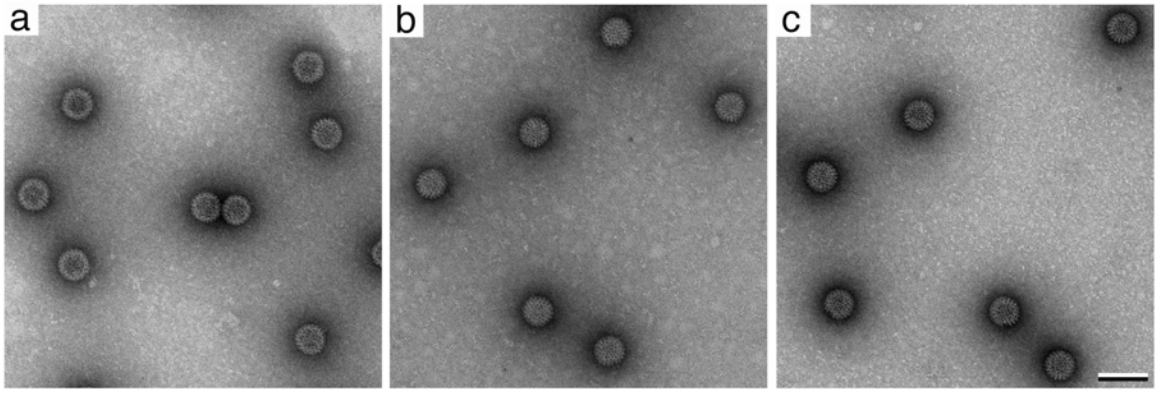
Electronic Supplementary Material (ESI) for Lab on a Chip

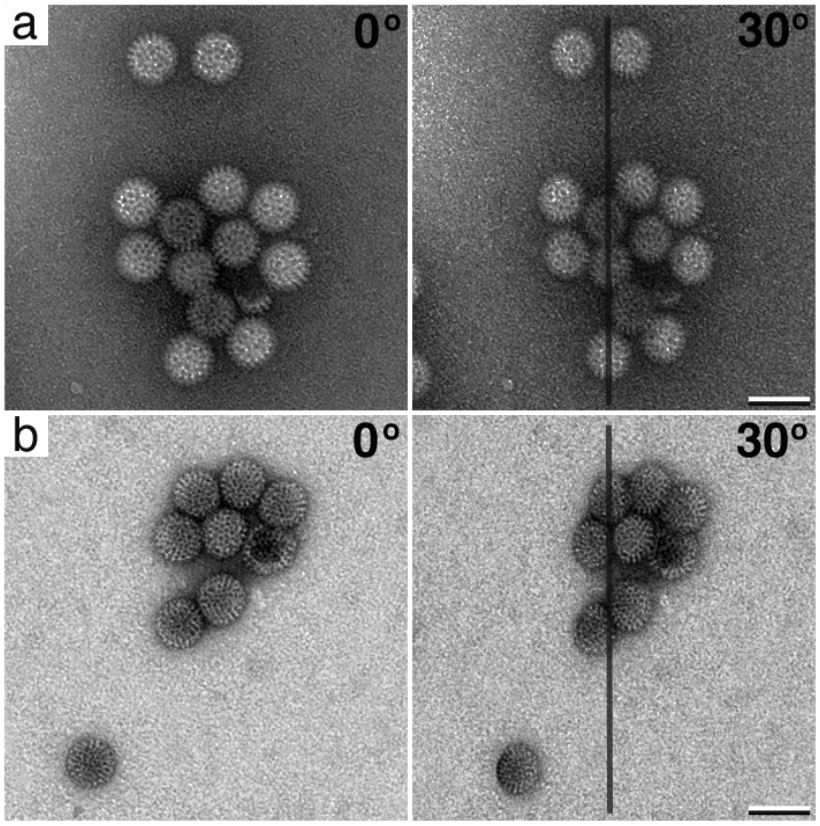

**Supplementary Fig. 1.** Bubble formation in liquid specimens upon continuous exposure to the electron beam at 5 electron / $Å^2$ for 2 min (**a**), 5 min (**b**) and 10 min (**c**). Scale bar is 100 nm.



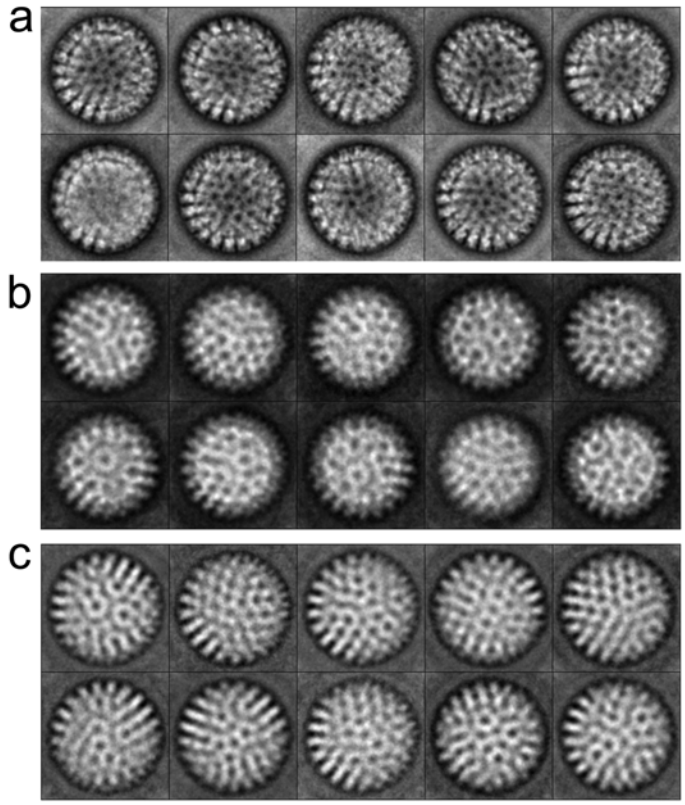

**Supplementary Fig. 2.** Purified DLPs assessed on a 7.5% SDS-PAGE gel (**a**) stained with GelCode Blue contain capsid proteins: VP1, VP2, VP3 and VP6. (**b**) Protein A-labeled silicon nitride devices lacking antibodies failed to recruit DLPs. Scale bar is 200 nm. Purified DLPs in buffer solution (pH, 7.5) were incubated with antibody-decorated microchips were examined by negative staining using **(c)** 0.2% uranyl formate. Specimens were air-dried at room temperature before inserting into the EM. Scale bar is 200 nm.




**Supplementary Fig. 3.** DLPs incubated at a pH of 7.2 for 1 minute (**a**), 30 minutes (**b**) and 60 minutes (**c**) while prepared with 0.2% uranyl formate. Scale bar is 100 nm.




**Supplementary Fig. 4.** EM images of DLPs in liquid **(a)** do not show flattening or major distortion upon tilting. This is in contrast to DLPs prepared by negative staining **(b)** that are flattened upon air-drying. Scale bars are 100 nm.




**Supplementary Fig. 5.** Projections averages of DLPs calculated from specimens prepared in (**a**) negative stain, (**b**) liquid and (**c**) vitreous ice. Contrast of the ice averages (**c**) is inverted for ease of comparison. Individual panels are 110 nm.

## References

1 Bican, P., J. Cohen, A. Charpilienne, and R. Scherrer, *J. Virol.,* 1982*,* **43**, 1113-1117.
2 Degen, K., M. Dukes, J. R. Tanner and D. F. Kelly, *RSC Adv.,* 2012, **2**, 2408-2412.
3 Frank, J., M. Radermacher, P. Penczek, J. Zhu, M. Ladjadj and A. Leith, *J. Struct. Biol., 1996,* **116**, 190-199.
4 Scheres, S. H., *J. Mol. Biol.,* 2012, **415**, 406-418.