

A cryo-electron micrograph showing a grid of vitreous ice with numerous small, dark, circular protein particles embedded within it. The particles are distributed across the grid, with some appearing in clusters and others in isolation. The background is a dark, textured field of ice.

**Cryoplunge 3 system with GentleBlot  
for cryo-EM**

## Cryoplunge™ 3 system with GentleBlot™

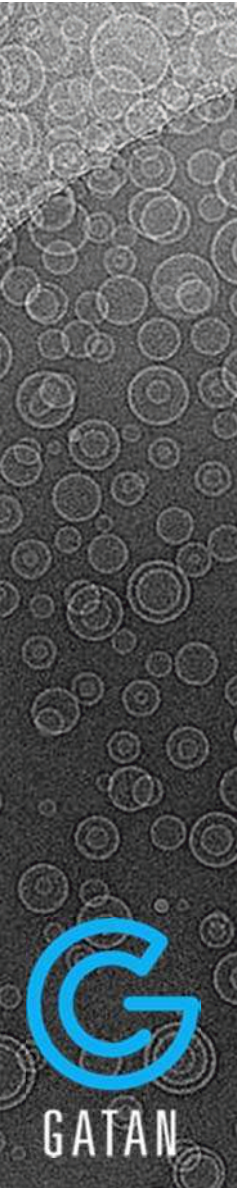
- GentleBlot technology provides very gentle 1- and 2-side blotting for preparing frozen hydrated specimens for cryo-EM
- Ethane temperature controller maintains temperature of the ethane just above its melting point
- Quick disconnect tweezers facilitate handling the frozen hydrated grid
- Protected environment for transferring the frozen hydrated grid within the cryo workstation
- Easy-to-use
- Versatile design
- Consistent, high quality results



Cryoplunge 3 with GentleBlot



Filter paper punch



# Cryoplunge™ 3 system with GentleBlot™



## Cryoplunge™ 3 system with GentleBlot™

- GentleBlot has two components
  - Specialized blot assemblies to manually adjust the pressure applied to the specimen grid during the blotting process
  - Factory preset pneumatic force that provides optimal speed for the blotters to contact the specimen grid



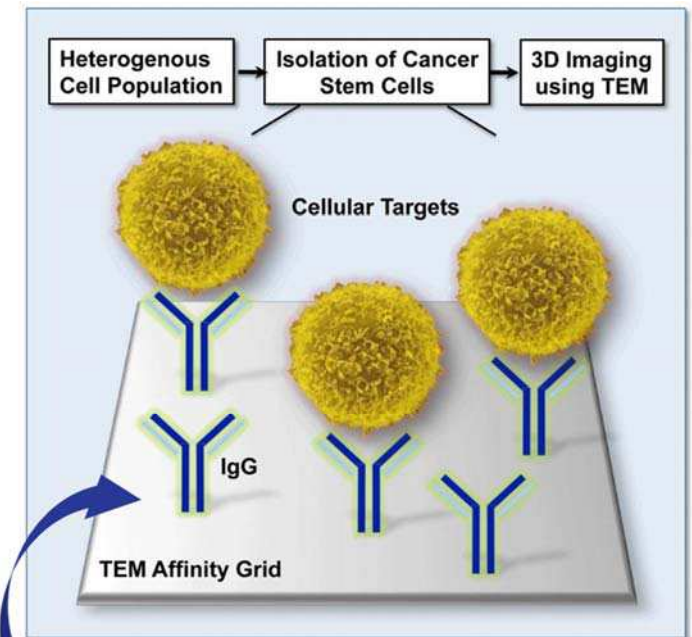


## Cryoplunge™ 3 system with GentleBlot™

- The solution for blotting fragile specimen substrates
  - Cryo-SiN (silicon nitride TEM windows)
  - Continuous carbon foils
  - Large mesh grids/substrates used for tomographic data collection
  - C-flat® or Quantifoil® holey carbon films
  - Any fenestrated grid with a very thin (~5 nm) carbon coat overlay
  - EM affinity grids (affinity capture technology)

# Affinity capture technology

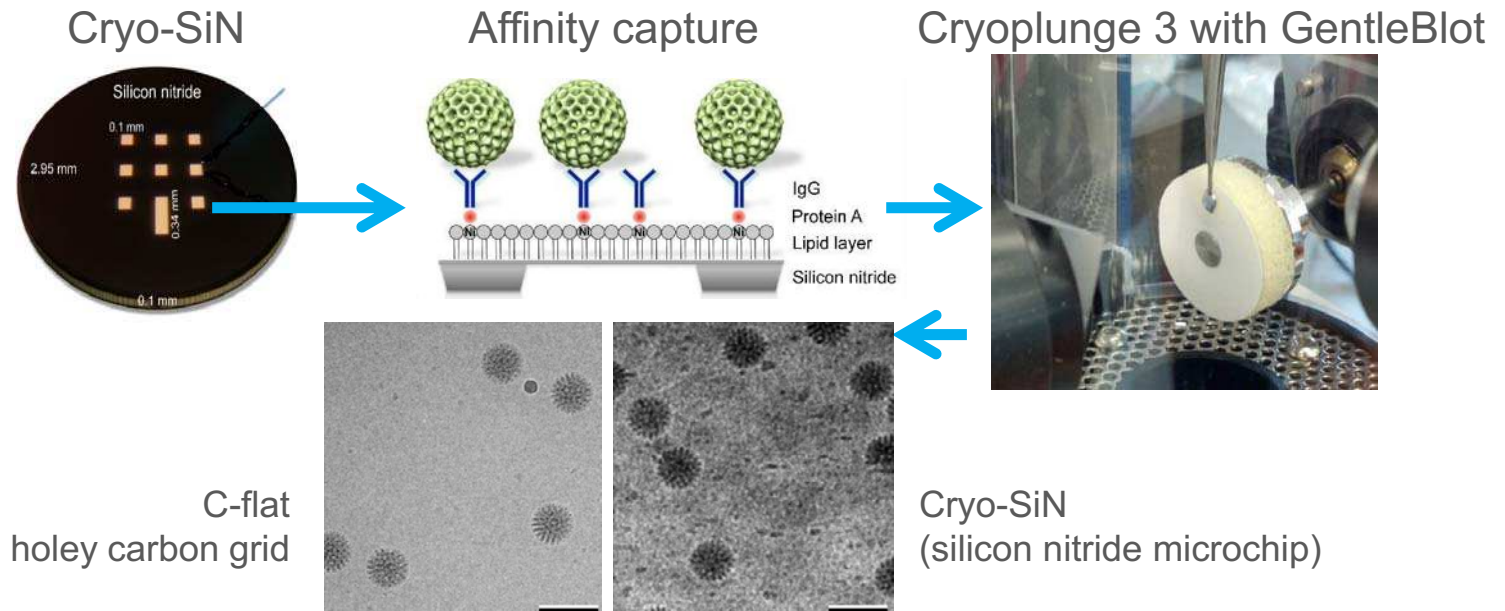
- Affinity capture technology is an innovative platform that features EM affinity grids and affinity capture devices
  - Affinity biofilm (lipid monolayer that is less than  $\sim 15 \text{ \AA}$ ) coats the surface of the entire fenestrated carbon film and does not impair imaging
- Specialized molecular 'tools' are designed to specifically isolate macromolecular assemblies or specific cells of interest
- Allows rapid purification of biological machinery for TEM imaging within minutes
- Provides superior partitioning of the specimen



<http://debkellylab.org/>

Affinity biofilm with specialized molecular Tools capturing specimen of interest.

# GentleBlot—Solution for blotting fragile substrates

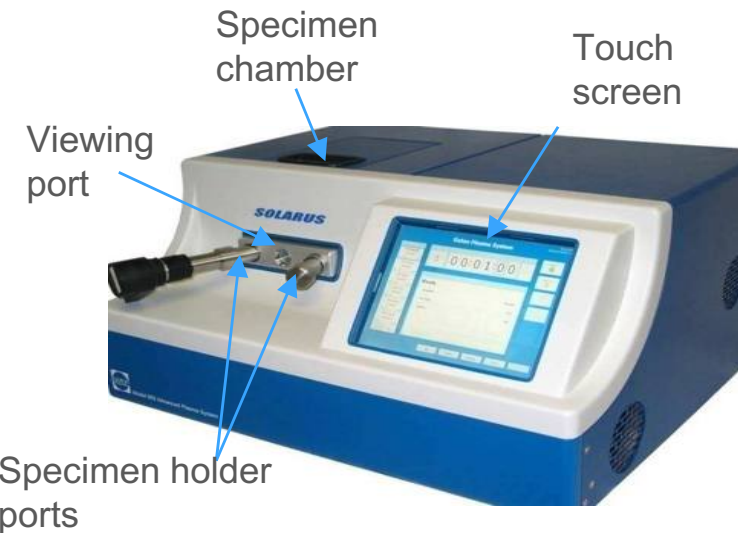


Vitrified inactive rotavirus DLPs on Affinity Capture Substrate. Scale bar is 100 nm. Images courtesy of Dr. Debbie Kelly, VTCRI, Roanoke, VA. USA.

Tanner, J. R.; Demmert, A. C.; Dukes, M. J.; Melanson, L. A.; McDonald, S. M.; et al. Cryo-SiN—An alternative substrate to visualize active viral assemblies. *J Analyt Molecul Tech* 2013;1(1): 6.

## Solarus<sup>®</sup> 950 system for uniformly hydrophilic substrates

- Plasma cleaning of the EM substrate is a key step in the preparation process when a uniformly hydrophilic surface is required
- Solarus 950 system is designed to ionize a hydrogen/oxygen gas mixture (in addition to the standard argon or argon/oxygen mixtures)
  - 50% cooler than cleaning with traditional Ar/O<sub>2</sub> (75%:25%) mixture
  - Gentle cleaning with less sputter damage
  - Uniformly hydrophilic charge



Solarus 950 advanced plasma cleaning system



## Prepare the specimen

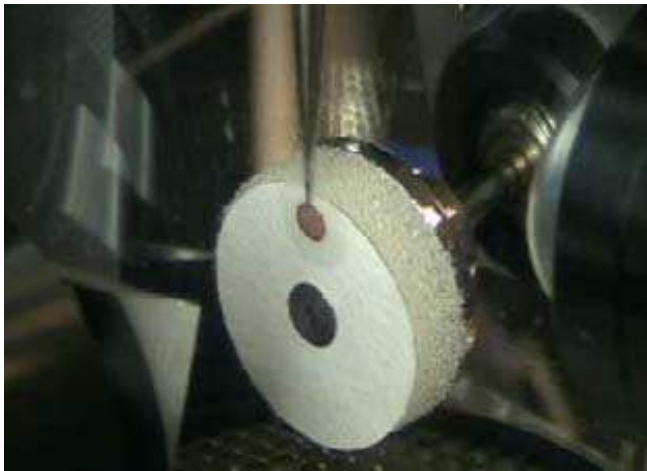
- Optimize the support substrate surface
  - Hydrophilic, hydrophobic, Affinity Capture
- Apply a small aliquot of specimen to surface of substrate
  - 2 – 5  $\mu\text{L}$  depending on the concentration of the specimen
  - Affinity Capture optimizes partitioning of the specimen within the holes of the fenestrated support at low concentration (0.01 mg/mL) and low volume (2  $\mu\text{L}$ )



Apply the specimen

## Blot the specimen

- Blot with filter paper until only a thin fluid layer ( $\sim 100$  nm) is left on the support substrate



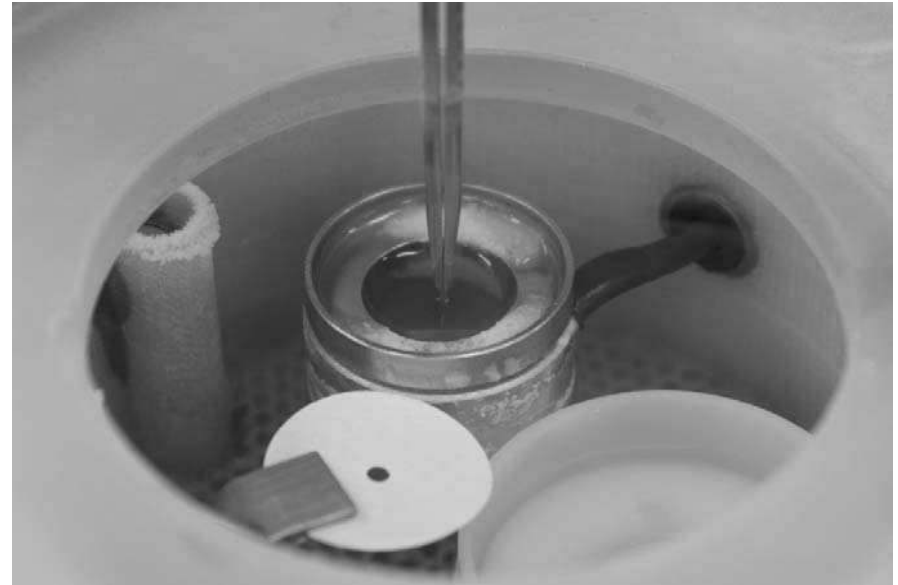
1-side blotting



2-side blotting

## Plunge the specimen

- Plunge the thin fluid layer into a suitable cryogen of high heat capacity (liquid ethane), which results in instantaneous freezing of the specimen in a layer of non-crystalline (vitreous) ice



Plunge freeze the specimen grid into liquid ethane

## Transfer the frozen hydrated specimen

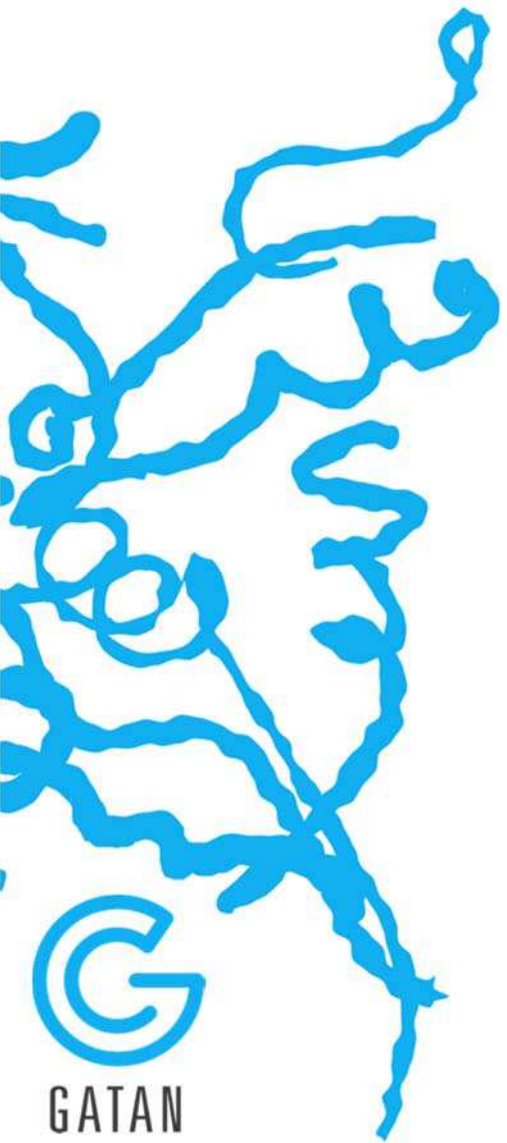
- Cryo-transfer the frozen hydrated specimen for high-resolution data collection in the TEM



Transfer frozen hydrated specimen from the workstation of Cryoplunge 3 system



Load the frozen hydrated specimen into the cryo-transfer holder



## Results

# 140x map of frozen hydrated grid—2-side blot

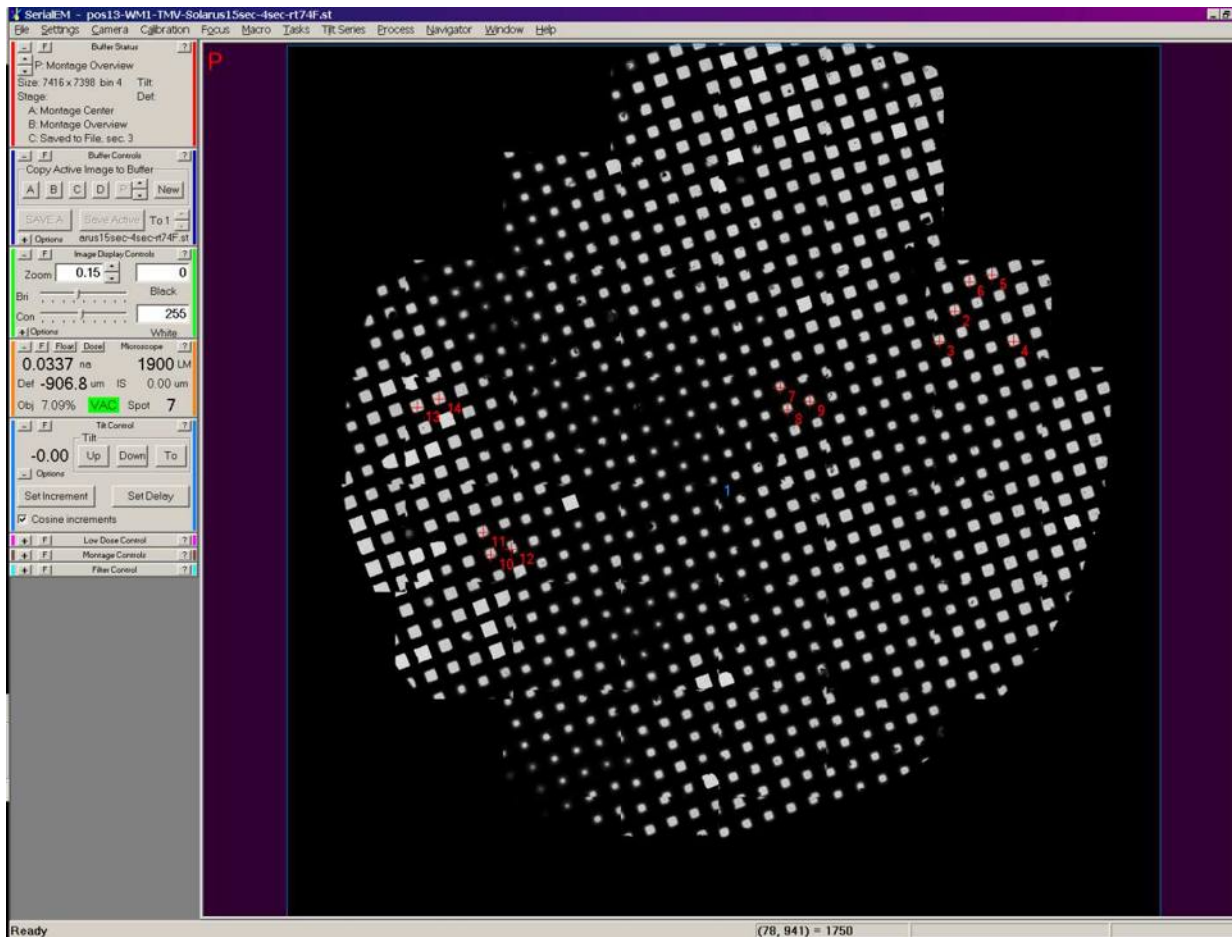
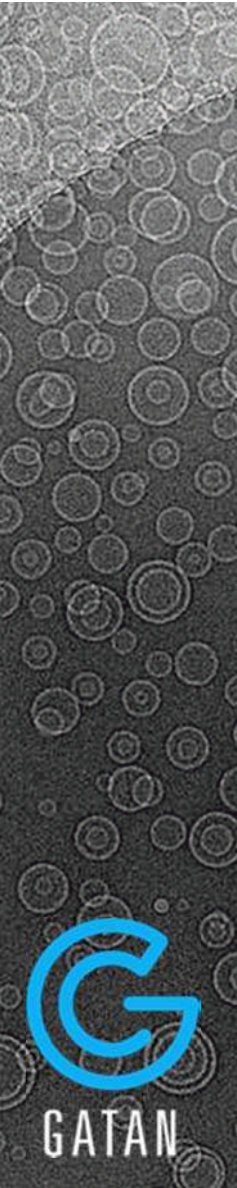
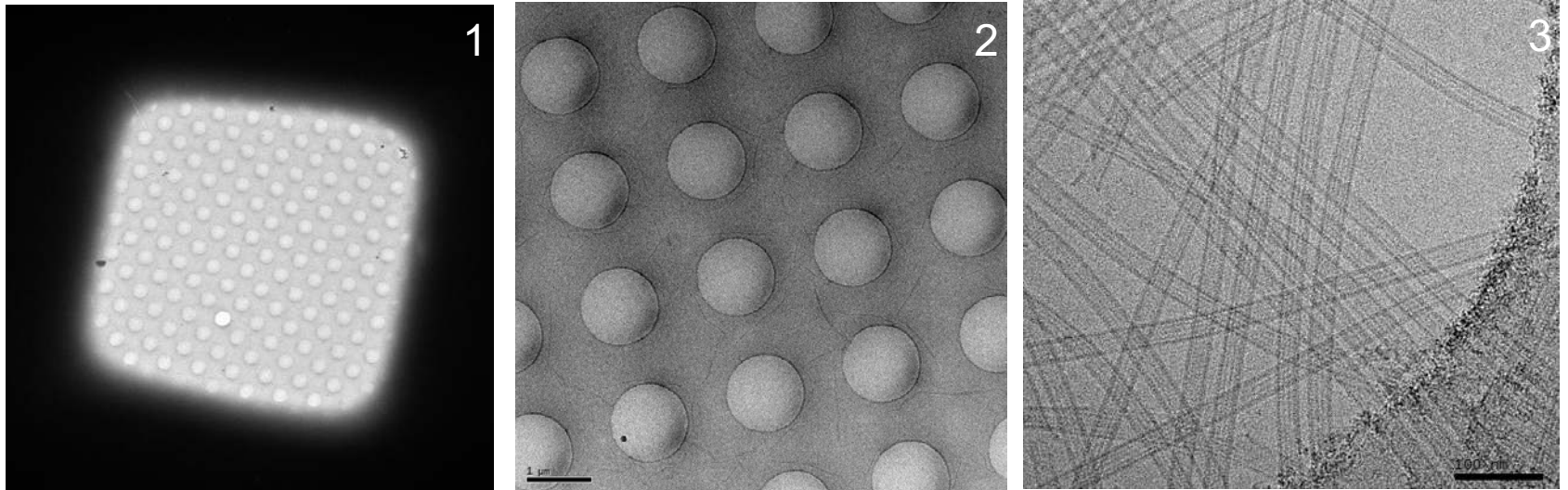


Image courtesy of Dr. Chen Xu, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA, USA.



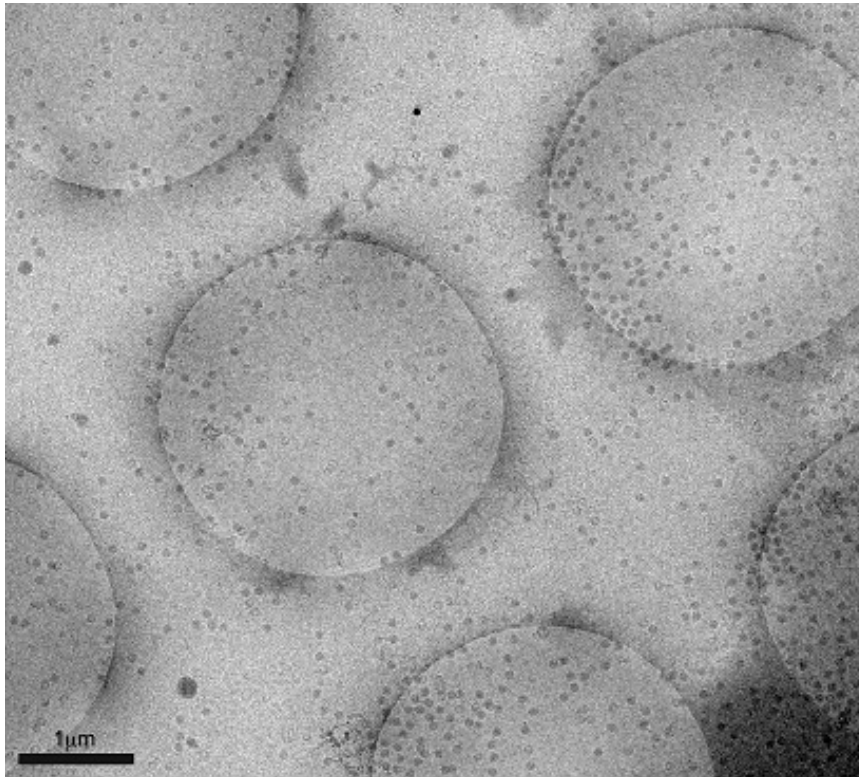
## Frozen hydrated microtubules



The three images above are an example of the high quality frozen hydrated preparations produced with Cryoplunge 3 system. (1) Image of a single grid square; TEM magnification  $\sim 900\times$ , electron dose  $0.01\text{ e}^-/\text{\AA}^2$ . (2) Higher magnification image of a portion of the grid square;  $4700\times$ ,  $0.1\text{ e}^-/\text{\AA}^2$ . (3) Image of part of one hole;  $59\times$ ,  $20\text{ e}^-/\text{\AA}^2$ . The frozen-hydrated microtubule specimen was prepared on Quantifoil<sup>®</sup> R1.2/1.3 macro machined holey carbon grids which were plasma cleaned using the Solarus 950 advanced plasma cleaning system for 15 s at 50 W using hydrogen and oxygen plasma. All images were recorded on an FEI Tecnai F30 TEM with a 626 70° Single tilt liquid nitrogen cryo-transfer holder and an UltraScan<sup>®</sup> 4000 camera.

Images courtesy of Dr. Chen Xu, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA, USA.

## Frozen hydrated double-layered particles



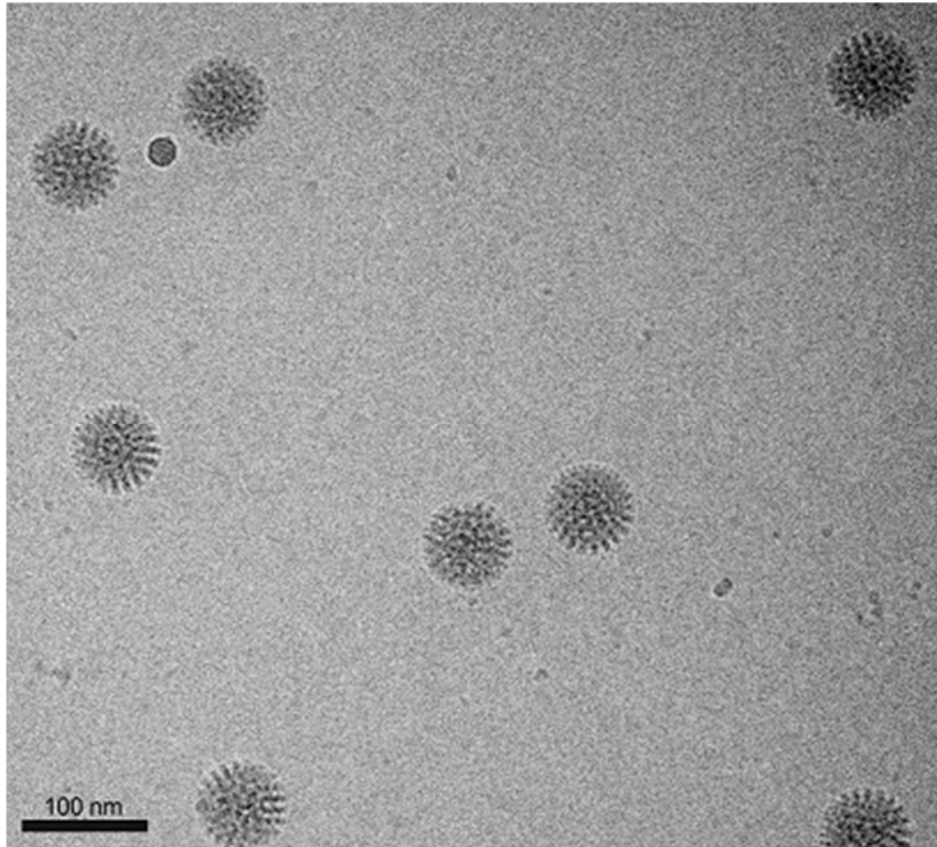
Superior partitioning of the specimen as they attached to the affinity capture biofilm in the holes on the support film

- Image of frozen-hydrated rotavirus double-layered particles (0.01 mg/mL concentration, 2  $\mu$ L volume) prepared on affinity grids decorated with His-tagged protein A and antibodies against the outer capsid protein, VP6
- Specimen was prepared using Cryoplunge 3 system with GentleBlot on Quantifoil 2/1 support film
- Specimens were examined under low dose conditions at 120 kV.
- Image was recorded at 6000x using a dose of  $\sim 5 \text{ e}^-/\text{\AA}^2$

Image courtesy of Dr. Debbie Kelly, Virginia Tech Carilion Research Institute, Roanoke, VA, USA.

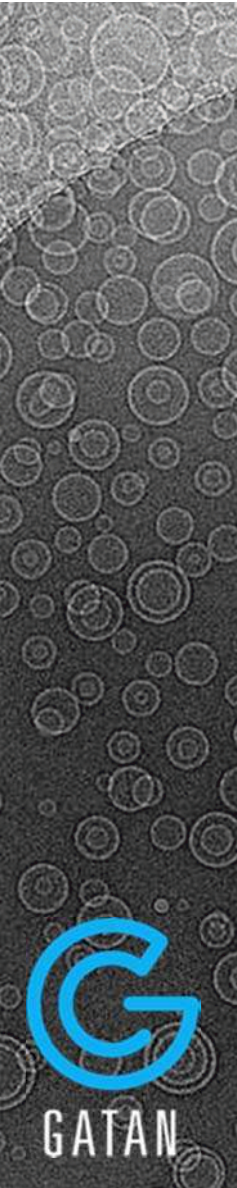


## Frozen hydrated double-layered particles

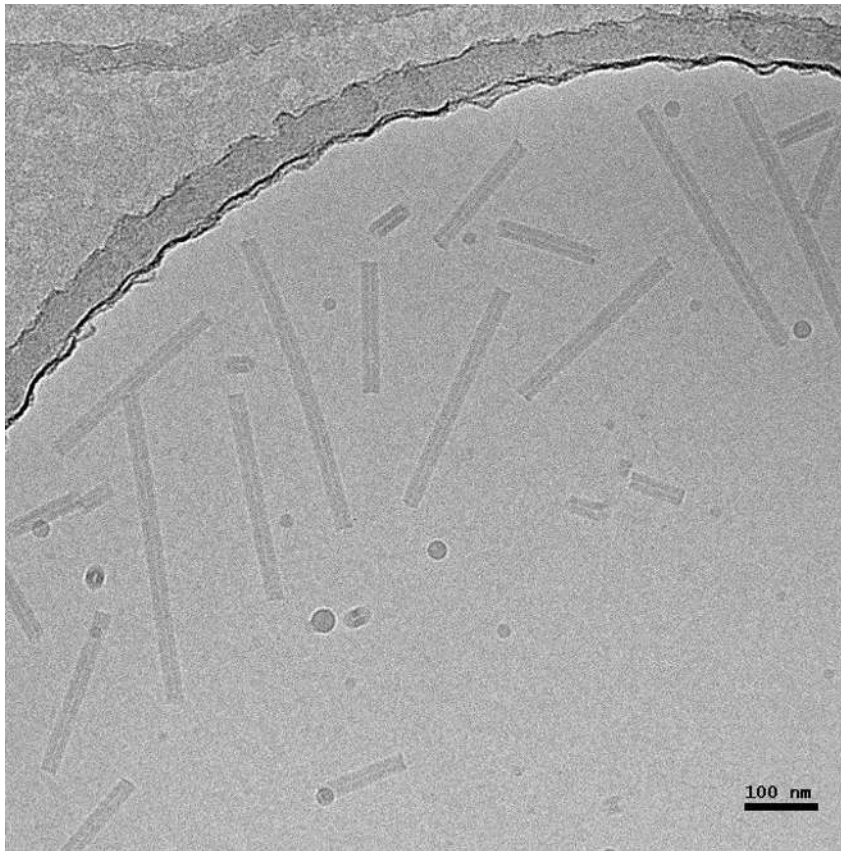


- Image of frozen-hydrated rotavirus double-layered particles (0.01 mg/mL) prepared on affinity grids decorated with His-tagged protein A and antibodies against the outer capsid protein, VP6
- Specimen was prepared using Cryoplunge 3 system with GentleBlot on C-flat 2/1 grids, Protochips, Inc.
- Specimens were examined under low dose conditions at 120 kV
- Image was recorded at 50,000x using a dose of  $\sim 5 \text{ e}^-/\text{\AA}^2$

Image courtesy of Dr. Debbie Kelly, Virginia Tech Carilion Research Institute, Roanoke, VA, USA.



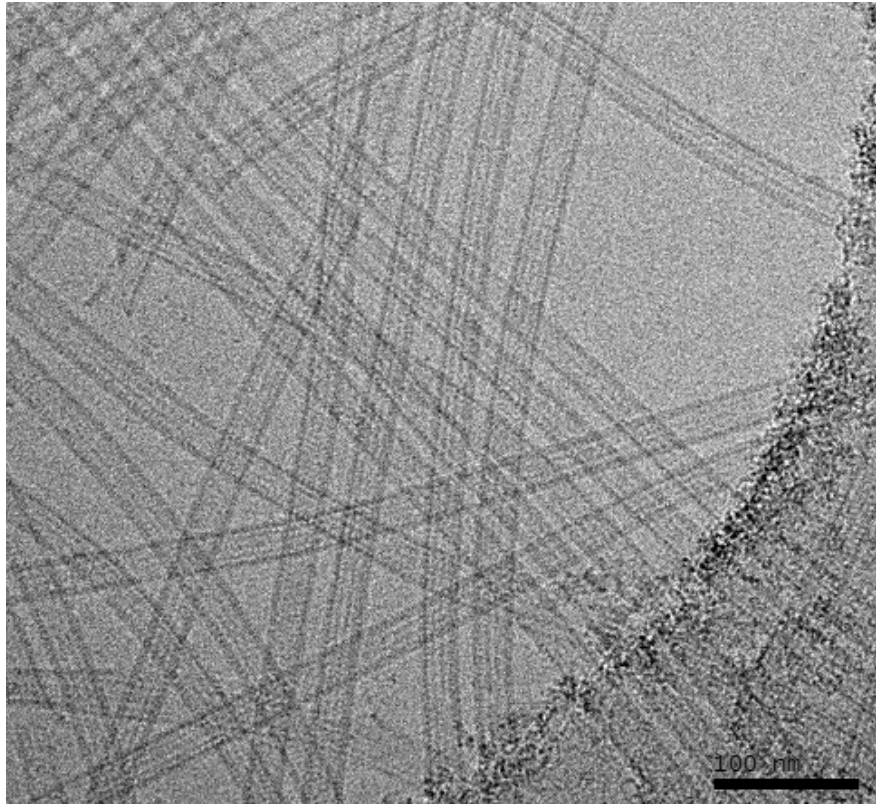
## Frozen hydrated tobacco mosaic



- Frozen hydrated tobacco mosaic (TMV) sample prepared using Cryoplunge 3 system
- Image was recorded at TEM magnification of 59kx at 300 keV and an electron dose of  $20 \text{ e}^-/\text{\AA}^2$  using a model 626 Liquid nitrogen cryo-transfer holder and UltraScan 4000 camera
- Sample was prepared on Quantifoil 1.2/1 specimen support, using Solarus 950 advanced plasma cleaning system prior to freezing

Image courtesy of Dr. Chen Xu Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA, USA.

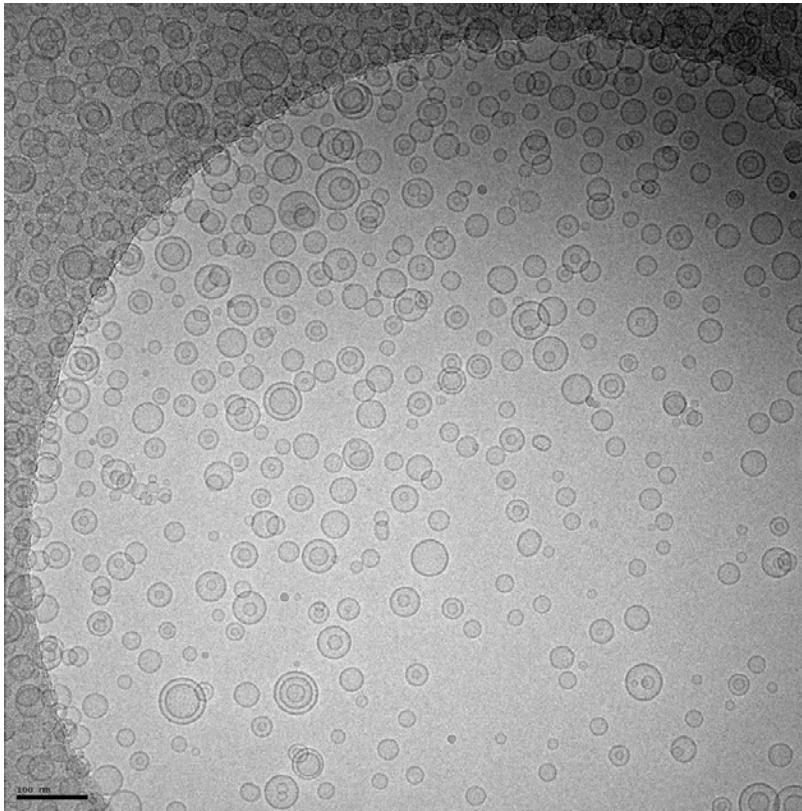
## Frozen hydrated microtubules



- Frozen hydrated microtubule sample prepared using Cryoplunge 3 system
- Image was recorded at 300 keV using a model 626 Liquid nitrogen cryo-transfer holder and UltraScan 4000 camera
- Sample was prepared on Quantifoil 1.2/1 specimen support, using Solarus 950 advanced plasma cleaning system prior to freezing

Image courtesy of Dr. Chen Xu, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA, USA.

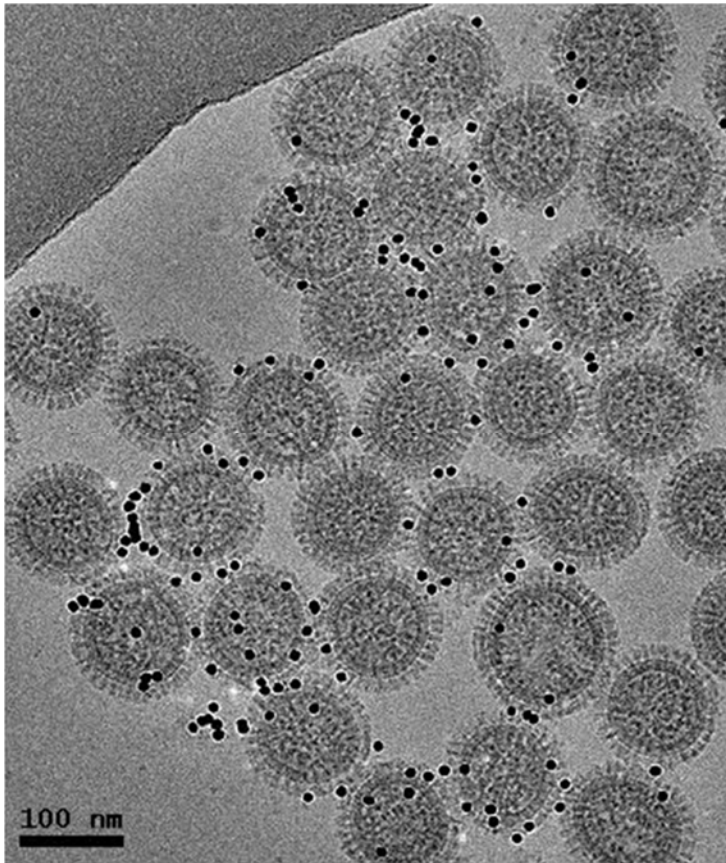
## Frozen hydrated lipid vesicles



- Frozen hydrated lipid vesicles prepared using Cryoplunge 3 system
- Image was recorded at TEM magnification of 25kx at 200 keV and electron dose of  $\sim 20 \text{ e}^-/\text{\AA}^2$  using model 910 Multi-specimen single tilt cryo transfer holder and UltraScan 4000 camera
- Sample was prepared on Quantifoil specimen support, using Solarus 950 advanced plasma cleaner prior to freezing

Image courtesy of Jessica Goodwin and Htet Khant, National Center for Macromolecular Imaging, Baylor College of Medicine, Houston, TX, USA.

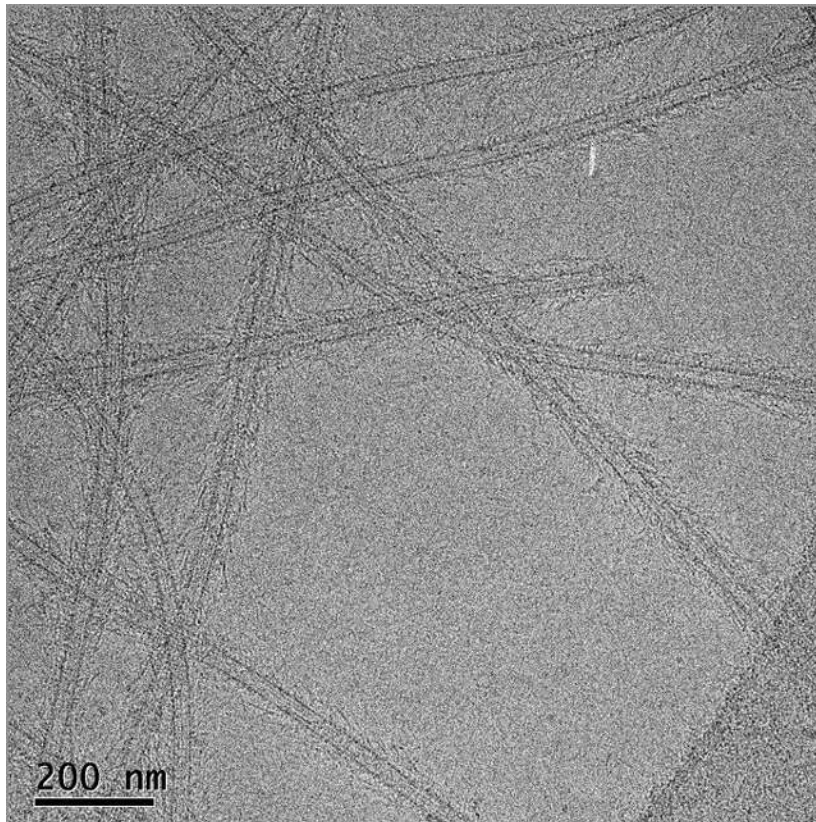
## Frozen hydrated influenza virus



- Frozen hydrated influenza virus prepared using Cryoplunge 3 system
- Gold particles were added as fiducial markers for cryo-electron tomography
- Zero loss image was recorded at TEM magnification of 50kx and electron dose of  $\sim 20$   $e^-/\text{\AA}^2$  at 300 keV using UltraScan 4000 camera
- Sample was prepared on Quantifoil (2/2) specimen support, using Solarus 950 advanced plasma cleaning system prior to freezing

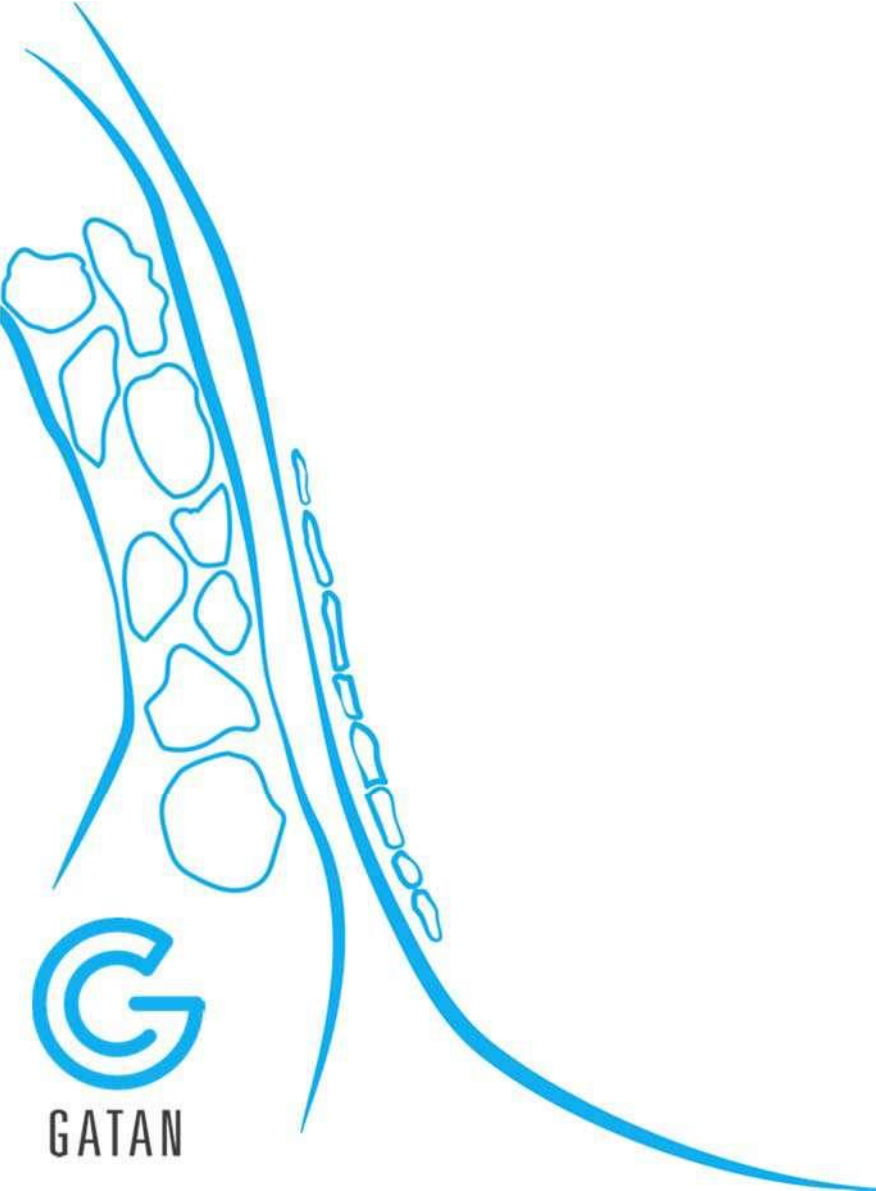
Image courtesy of Dr. Ruben Diaz-Avalos, New York Structural Biology Center, NY, USA.

## Frozen hydrated Ndc80 complex decorated microtubules



- Frozen-hydrated image of the Ndc80 complex decorated microtubules: magnification 30kx, electron dose 40 e<sup>-</sup>/Å<sup>2</sup>
- Specimens were prepared on CFlat Protochips, Inc. CF-2/1-4C-50 holey carbon grids which were plasma cleaned using the Solarus 950 advanced plasma cleaning
- Frozen hydrated preparations produced using Cryoplunge 3 system
- Image recorded at 100 kV on a TEM equipped with a model 626 70° Single tilt liquid nitrogen cryo-transfer holder

Image courtesy of Dr. Elizabeth Wilson-Kubalek, Cell Biology, The Scripps Research Institute, La Jolla, CA, USA.



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