SAMPLE PREPARATION GUIDE

Poseidon 200™

Affinity Capture Surface Functionalization of Poseidon E-chips for *in situ* Liquid Electron Microscopy

Introduction

An inherent limitation of imaging species in liquid with electron microscopy is that motion occurs when entities are freely diffusing in solution. Long range lateral diffusion, Brownian motion, beam-induced movement and short-ranged diffusion in the Z-direction all contribute to poor structural resolution. This movement can be mitigated by tethering the species to the surface of the membrane that forms the liquid enclosure. In addition to immobilizing particles within the liquid chamber, functionalized E-chips can also enable rapid purification via isolation and concentration of active protein complexes for *in situ* imaging. The method of surface functionalization described in this sample preparation note, termed "Affinity Capture" can enable the capture of species such as a virus, cell surface proteins, or isolate whole cells.

Affinity Capture devices consist of a transparent nickel-nitrilotriacetic acid (Ni-NTA) lipid coating on the surface of the silicon nitride membrane functionalizing the E-chip. The Ni-NTA dispersed throughout the coating can be used in conjunction with Histadine tagged (His-tagged) protein A and antibodies to bind specifically and with high affinity to target protein complexes, thereby tethering them within a fluidic chamber.

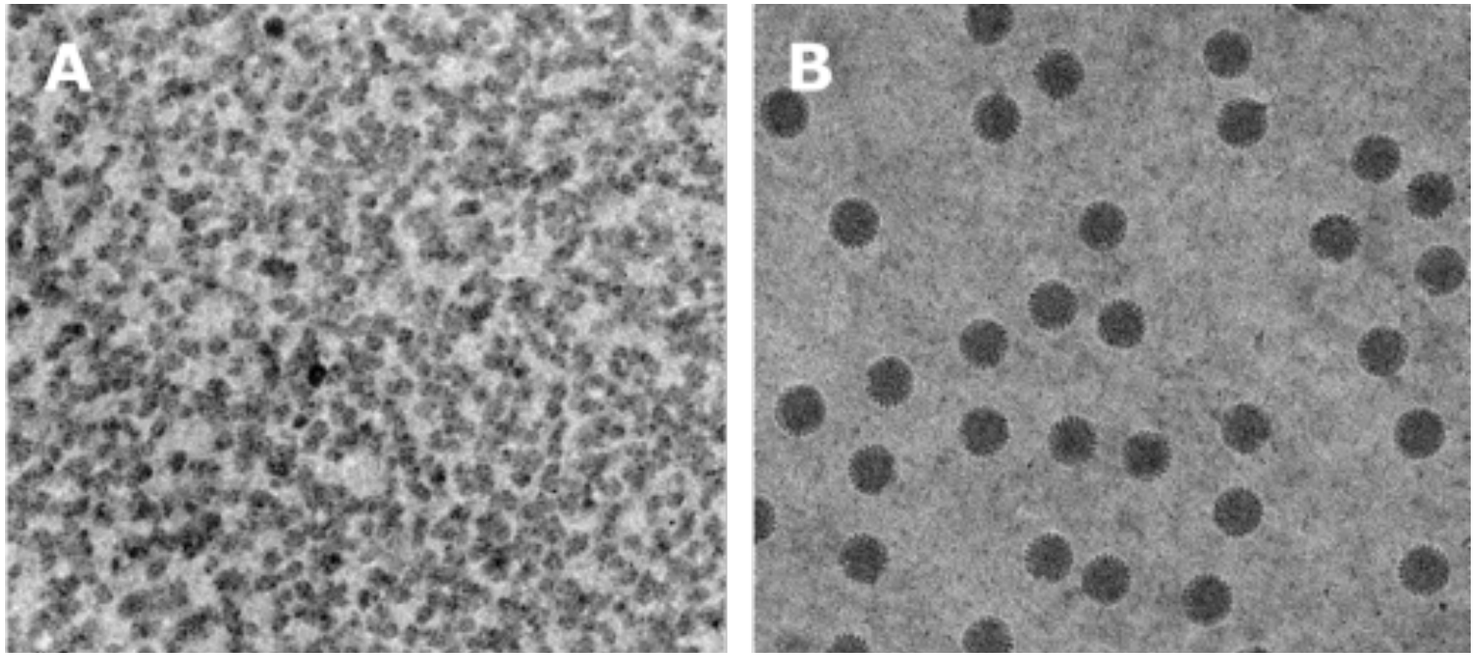

Figure 1: Macromolecular species tethered to the silicon nitride membrane of a Poseidon E-chip using surface functionalization. (A) TEM image of ribosome proteins imaged in 150 nm of buffer. (B) TEM image of double layer rotavirus particles imaged in 150 nm of buffer. TEM images were acquired with a 120 kV acceleration voltage and 1-3 micron defocus for contrast

Discussion

To test whether devices could be used to isolate biological macromolecules within the Poseidon 200 *in situ* TEM flow cell, devices were prepared using 20% Ni–NTA coating. A 500-µL aliquot of *E.coli* cell lysate containing transgenically expressed His-tagged ribosomes, was loaded into the Poseidon 200 specimen flow-holder using a step-motor pumping system that regulated the solution flow rate to be 300 µL per hour. No notable ribosome complexes were present at the initial time point of solution flow. As the lysate solution containing tagged complexes flowed over the device, binding of ribosome proteins was observed

within 5 min, and the surface of the E-chip became saturated shortly thereafter, as shown in Figure 1A.
In the previous example, the species of interest, ribosomes, was able to bind directly to the Ni-NTA in the surface functionalization via a transgenically expressed histidine tag. The utility of the coating can be further increased by employing a histidine-tagged protein A linker (commercially available from Abcam) which can be used to bind a variety of antibodies. This strategy was used to capture rotavirus double-layered particles (DLPs) onto functionalized microchips decorated with antibodies against the viral capsid protein, VP6, as show in Figure 1B.

Preparation of Hydrophobic E-chip Surface

In order for the coating to adhere properly to the E-chip it is necessary for the surface of the E-chip to be hydrophobic, so that the nonpolar lipid tails will adhere to the silicon nitride membrane on the E-chips. The most efficient way to make the E-chip surface hydrophobic is to heat it, in order to drive off any moisture from the surface.

1. Place the clean E-chips membrane side up on a clean glass slide or dish.
2. Place the dish containing the E-chips onto a hot plate heated to 150 °C for 1.5 hours.
3. Remove the dish from the hot plate, and allow the E-chips to cool to room temperature.

Functionalization of E-chips

Note: The prepared solution and the following steps must be carried out on ice in a relatively vibration-free area in order for the Affinity Capture layer to form properly. 1,2-Dilauroyl-phosphocholine and 1,2-dioleoyliminodiacetic acid-succinyl-nickel salt are available for purchase from Avanti Polar Lipids, Inc. The concentration of active binding sites can be tuned by changing the concentration of the Ni-NTA lipids doping liquids. Here we use a 20% Ni-NTA lipid concentration. The rest of the coating consists of

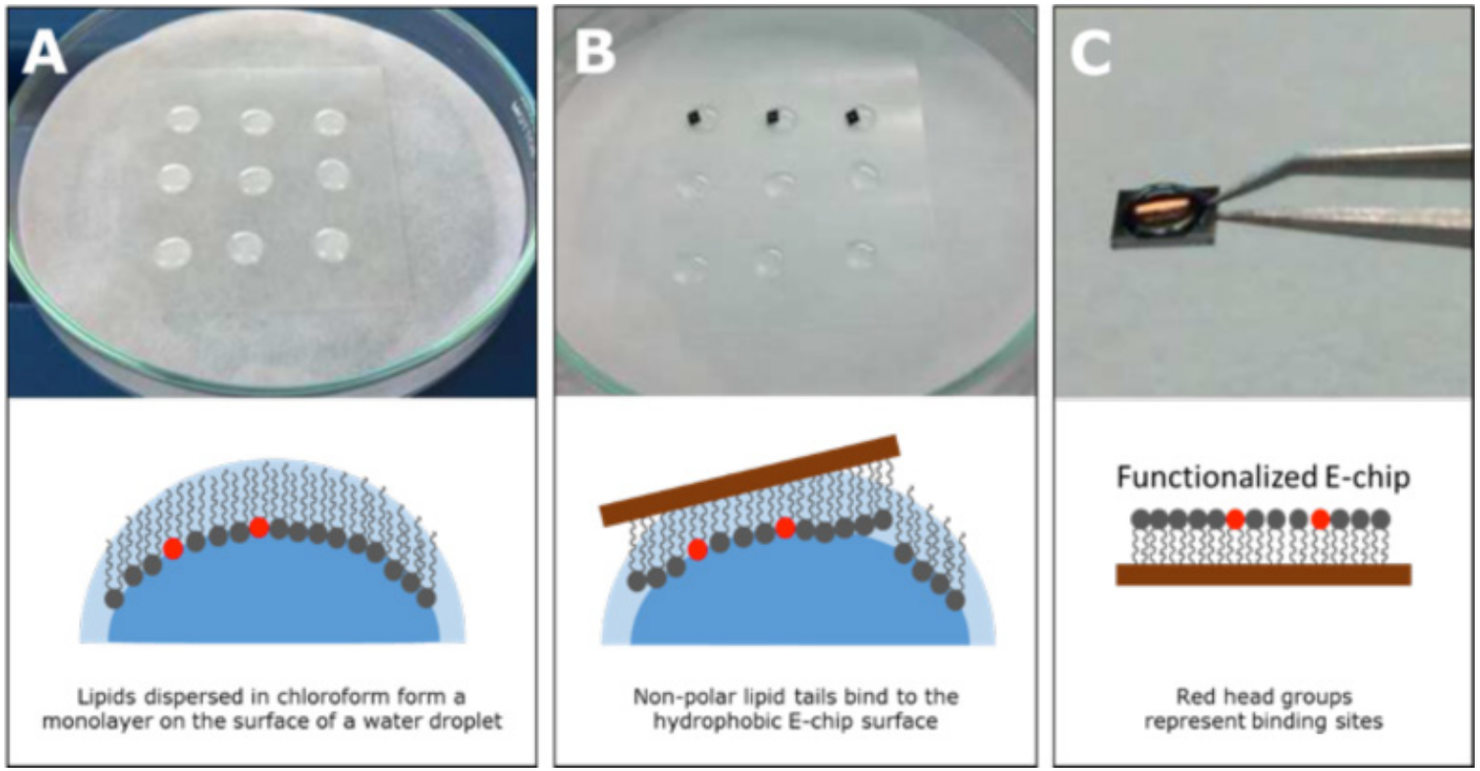

Figure 2: Steps to functionalize the E-chip

filler lipids that spatially disperse the active Ni-NTA lipids. The resulting lipid mixture is dissolved in chloroform, and added onto a droplet of water. The chloroform-lipid mixture will form a thin monolayer film over the water droplet, which can then be transferred to the surface of an E-chip.

1. Using small volume Hamilton glass syringes to measure the components, prepare 40 microliters of solution consisting of the following ratios of components
   - 25% chloroform
   - 55% filler lipid (1 mg/mL of 1,2-Dilauroyl-phosphocholine in chloroform)
   - 20% Ni-NTA lipid (1 mg/mL of 1,2-dioleoyliminodiacetic acid-succinyl-nickel salt in chloroform)
2. Apply a 1 µL aliquot of the mixture over each 15 µL drop of Milli-Q water on a piece of parafilm in a humid petri dish (Fig. 2A). Incubate samples on ice for at least 60 minutes before proceeding to the next step.
3. Place an E-chip with an integrated spacer on top of a monolayer sample, so that the membrane side is the one in contact with the droplet and incubate for 1 minute (Fig. 2B).

Note: If you are using a sample that contains a His-tag, such as the His-tagged ribosomes described above, proceed to step 6.

4. Gently lift the E-chip off of the droplet. Holding the E-chip with a pair of tweezers, add a 3 µL aliquot of your His-tagged Protein A. Incubate for 1 minute at room temperature (Fig. 2C).
5. Blot away the excess drop using Whatman #1 filter paper and immediately add a 3 µL aliquot of antibody solution. Incubate for 1 minute at room temperature.
6. Remove the excess solution using a Hamilton syringe and immediately add a 1 µL aliquot of your sample of choice (against which your antibody is active). Incubate for at least 2 minutes at room temperature.
7. Immediately load the E-chip into the Poseidon holder, ensuring that the surface does not dry out during the transfer.

Applications

Poseidon 200 is compatible with a broad range of materials and biological samples and enables the user to achieve nanometer to atomic resolution imaging of specimens in dynamic liquid environments. The Poseidon TEM holder is available with either 2 or 3 liquid ports and can be easily configured on an experiment-by-experiment basis for flow, mixing or static operation. Thus, in addition to maintaining a hydrated environment, dynamic processes such nucleation, nanoparticle growth, self-assembly, and particle-particle interactions can be observed.

Contact us to discuss Poseidon's full range of capabilities. We can be reached at (919) 341-2612 or contact@protochips.com.

Note: The virus particles used in this study were treated with 20 mM Ethylenediaminetetraacetic acid (EDTA) to remove the outer capsid layer, rendering them non-infectious. Safe handling protocols should always be followed when working with infectious or potentially dangerous biological specimens.

REFERENCE:
Lab on a Chip, 13, 216-219 (2013)
RSC Advances, 2(6), 2408-2412 (2012)

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