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Capturing Enveloped Viruses on Affinity Grids for Downstream Cryo-Electron Tomography Applications

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Enveloped viruses are a large population of known viruses that are human and animal pathogens. Therefore, studies to determine the overall architecture and fine ultrastructure of enveloped viruses are essential. All enveloped viruses are covered by a lipid-coat derived from the host cell's plasma membrane. Due to the processes associated with virus assembly and budding many of them exhibit varying degrees of structural irregularity or pleiomorphism. This structural variability can express itself as slight alterations to the size and internal architecture of the virus, such as with members of the Retroviridae family, to significant variations in the size and shape of the virus, as observed with members of the Orthomyxoviridae and Paramyxoviridae families [1].

Owing to their pleomorphic nature, cryo-electron microscopy (cryo-EM) and cryo-electron tomography (cryo-ET) are the most suitable methods for structural analyses. Both techniques involve the vitrification of highly purified and concentrated viral samples onto EM grids. This preserves the virus in the native state. Unfortunately, the structural heterogeneity of enveloped viruses negatively impacts the success of standard viral purification methods used for the production of highly concentrated and purified samples. Enveloped virus purification presents other challenges, specifically, the inclusion of host-membrane derived vesicles, the complete destruction of the viruses, or the disruption of the internal architecture of individual virus particles. Enveloped virus purification methods commonly involve chemical precipitation (PEG) and high-density gradients (sucrose or glycerol) for the separation of viruses from cell debris. However, these steps can alter virus structure, impact the titer, as well as artificially select for one subtype of particle morphologies.

Recently, "Monolayer Purification" and "Affinity Grid" methods were introduced to the EM field in order to apply His-tagged protein purification methods directly to the EM grid [2]. Affinity grids are EM grids coated with a lipid layer that contains a large percentage of non-functionalized lipids combined with a variable percentage of Ni-NTA (Nickel-nitrilotriacetic acid) moiety lipids [3]. The Ni-NTA active group binds directly to His-tagged proteins. Using His-tagged Protein A and a protein-specific antibody enables this technique to be used on non-His tagged targets. This approach has been applied to the structural analysis of the ribosome from crude cell extracts [4], entire RNA processing pathways [5], and the development of an *in situ* biological TEM imaging platform [6].

Here we illustrate the application of affinity grid technologies for the purification and capture of pleiomorphic-enveloped viruses directly to EM grids for both conventional TEM and cryo-EM/cryo-ET studies. We examined affinity grids for the selective capture of human immunodeficiency virus (HIV) virus-like particles (VLPs), measles virus (MeV), influenza A, and Newcastle disease virus (NDV). We applied both Ni-NTA/His-tagged protein A/antibody and direct Ni-NTA/His-tagged viral protein methods for selectively adhering viruses to the affinity grid lipid layer. The application of affinity grid methods may prove essential for the gentle and selective purification of enveloped viruses directly onto EM grids for ultrastructural analysis and provide novel prospects for imaging viruses that have been historically difficult to produce and purify by conventional methods.

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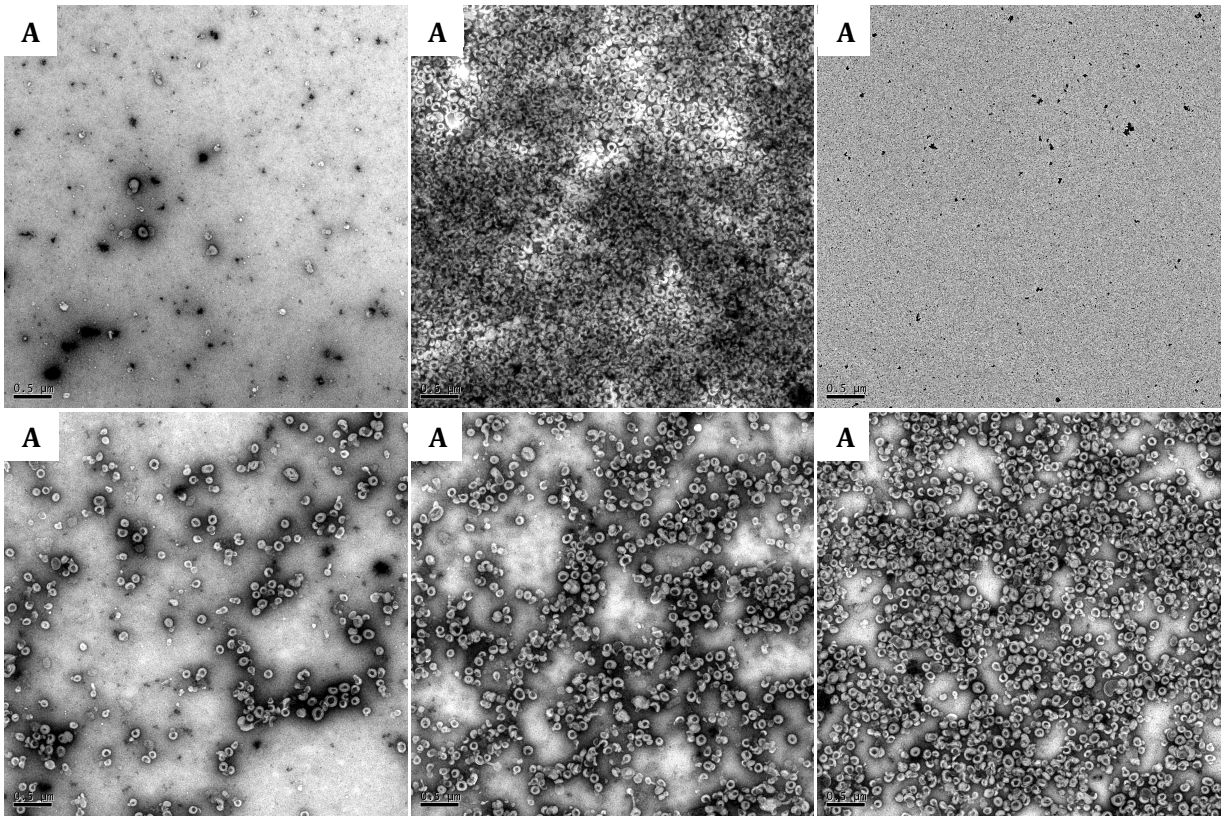

Figure 1. HIV CD84 virus-like particles (VLPs). Negative stained unpurified (A) and purified (B) HIV CD84 VLP sample on carbon coated conventional EM grids. Panel C shows the affinity monolayer with His-tagged Protein A and anti-HIV Env antibody. Panels D, E, and F illustrate that increasing the amount of Ni-NTA in the affinity monolayer increases the number of captured HIV CD84 VLPs (D -2%, E -20%, F -50% Ni-NTA).

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