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Three Dimensional Analysis of In Vitro Assembled HIV-1 Gag by Electron Cryotomography

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The major structural elements of retroviruses are contained in a single polyprotein, Gag, which in HIV-1 comprises the matrix (MA), capsid (CA), SP1, nucleocapsid (NC), SP2, and p6 proteins. In the immature HIV-1 virion, the domains of Gag are arranged radially with MA at the membrane and NC-p6 facing the particle center. Once the Gag polyprotein of the immature virus undergoes proteolytic maturation, the MA protein remains associated with the lipid bilayer; a conical core is formed from the reassembled CA protein; and within the core, the RNA genome, NC protein, reverse transcriptase (RT) and integrase (IN) enzymes are localized. Recently, the overall structures of both immature and mature virions have been determined by electron cryotomography (cryo-ET) [1, 2, 3, 4]. Cryo-ET of immature HIV-1 virions, it was observed that the CA domains arranged into sheets of hexamers that surround the virion and are interspersed between patches of disorder [3, 4]. This semi-enclosed particle is unlike the mature core of retroviruses, in which the CA protein under *in vitro* assembly conditions is able to form completely enclosed objects containing both hexamers and pentamers [5].

In order to explore the self-assembly of the HIV-1 Gag polyprotein, we optimized *in vitro* assembly conditions to produce HIV-1 Gag virus-like particles (VLPs). The protein used for the assembly experiments was Δ16-99 Δp6 Gag. The reaction conditions consisted of a final protein concentration of ~1 mg/mL (50 μg Gag), 2 μg yeast tRNA, 20 mM Tris HCl pH 8.0, 100 mM NaCl, and 1 mM DTT. The reaction was incubated at 4 ºC for 2 - 4 hrs. Solutions of the assembled particles were applied to glow discharged EM grids, plunge frozen, and imaged under low-dose cryo-conditions. A majority of the sample consisted of partially enclosed HIV-1 Gag VLPs ~108 nm in diameter (Figure 1, left), however non-enclosed, spiral-shaped structures were also observed (Figure 1, right). In the two assemblies, the measured ring-to-ring spacing of the CA lattice was ~8 nm, which is consistent with the dimensions of the immature HIV-1 virion CA lattice. As with immature HIV-1 virions, we were unable to find pentamers within the lattice, which is inconsistent with what has been observed enclosed objects assembled from the mature CA protein of a related retrovirus [5].

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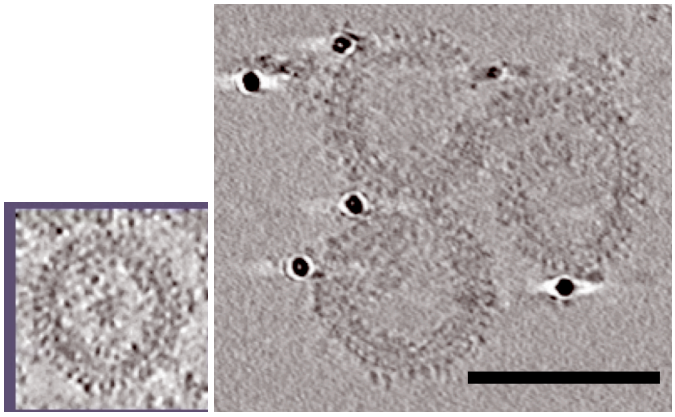

Figure 1. Isolated in vitro assembled HIV-1 Gag VLPs preserved by flash freezing and imaged by cryo-ET. (Left) A 5.2 nm slice through the middle of the reconstruction of a partially 'enclosed' HIV-1 Gag VLP. (Right) A 5.2 nm slice through three HIV-1 Gag assemblies. Uniform spacing is noted within the CA domain (8 nm). Scale bar 100 nm.

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