

## BUILDING BLOCKS OF IMMATURE RETROVIRAL PARTICLES: STABLE DIMERIC, TETRAMERIC, AND HEXAMERIC GAG-VIRAL RNA COMPLEXES

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Immature retroviral particles form non-icosahedral paracrystalline lattices comprised of hexameric Gag rings. This array of hexamers is discontinuous, indicating that the Gag lattice is incomplete. Our studies focused on uncovering how the hexameric lattice forms from the initial building blocks of the virus particle—the immature Gag protein and molecules of viral RNA (vRNA). By characterizing early assembly-competent complexes of Gag and subsequent intermediate oligomers formed in the presence of vRNA, we observed the stepwise, ordered addition of subunits to build a hexameric array. The assembly of oligomeric subunits *in vitro* very closely mimics the arrangement of hexamers *in vivo* and arises by a mechanism that precludes the formation of pentamers of Gag.

For these studies, we purified the wild-type Rous sarcoma virus (RSV) Gag protein truncated in protease. In the absence of nucleic acids, Gag exists in a monomer-dimer equilibrium ( $K_d = 20\text{nM}$ ). Binding studies indicate that the dimeric form preferentially interacts with nucleic acids ( $K_d \sim 8\text{-}10\text{nM}$  for binding of a DNA 10-mer). Longer nonspecific RNA and DNA sequences promote the further assembly of Gag to tetramers and hexamers, with a  $K_d$  of  $\sim 12\text{-}16\text{nM}$  for the Gag<sub>4</sub>-nucleic acid complex. When vRNA containing the psi packaging signal was used, larger assemblages were formed by the association of multiple hexamers, with the most stable complexes comprised of 18 and 42 Gag monomers. The molecular masses of the Gag-vRNA complexes were verified by multi-angle laser light scattering and STEM. These data suggest that Gag molecules are added in units of 2 or 6 up to a heptamer of hexamers. We did not find evidence in support of pentameric subunits.

We have recapitulated *in vitro* early assembly intermediates of the Gag hexameric lattice prior to the formation of the complete virus particle. Gag-vRNA complexes follow an ordered series of 6-mer subunit additions prior to forming an immature spherical shell, with the most stable intermediate being a complex of 7 hexamers. This system allows dissection of the earliest molecular steps in immature Gag-vRNA assembly, further informing the rational development of novel structure-based assembly inhibitors.