Supporting Information

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SI Text

Purification of Myristylated HIV-1 Gag. HIV-1 Gag (clone NL4-3, GenBank accession no. AAB60571.1) was expressed from a codon-optimized gene (Genscript) inserted into pHis-Parallel2 (1), as a fusion protein with a maltose-binding protein-$(His)_6$ tag linked to the C terminus of Gag via a Tobacco etch virus (TEV) protease site. GagMBP$(His)_6$ was expressed in *Escherichia coli* BL21(DE3) for 4 h at 37 °C in LB medium supplemented with 0.1 mM $ZnSO_4$ and 10 mg/L myristic acid. The N terminus of Gag was myristylated through coexpression of *Saccharomyces cerevisiae* N-myristyl transferase, cloned into pRSF-1b (Novagen). Cells were lysed by sonication in lysis buffer (50 mM Tris pH = 8.0, 600 mM NaCl, 20 mM imidazole, 10% glycerol, 50 μM NP40). Cleared lysates were applied to NiNTA resin (QIAGEN), which was washed with 5 resin volumes lysis buffer, followed by 5 volumes lysis buffer containing 1 M NaCl, and 5 volumes of lysis buffer. GagMBP$(His)_6$ was eluted from the NiNTA resin with lysis buffer containing 250 mM imidazole, which yielded soluble aggregates with high 260 nm absorption indicating bound nucleic acid. The protein was applied to a HiTrap Heparin 5 mL column (GE Healthcare) and eluted with a NaCl gradient from 0.1 to 1 M, in buffers containing 50 mM Tris pH = 8.0, 1 mM dithiothreitol, 10% glycerol and 50 μM NP40. The eluted peak was concentrated and applied to a Superose 6 10/300 GL gel filtration column (GE Healthcare) and separated in 50 mM Tris pH = 7.4, 500 mM NaCl, 10% glycerol, 0.1 mM TCEP, and 50 μM NP40, which separated remaining aggregates from monodisperse GagMBP$(His)_6$. Myristylation of the GagMBP$(His)_6$ fusion protein was confirmed by mass spectrometry (Fig. S1*B*). The protein was labeled O/N at 4 °C with Atto 594 maleimide (Sigma-Aldrich) at an engineered cysteine in the linker between the MA and CA domains (Ala120Cys). Labeling was terminated by the addition of DTT, and the buffer was changed to 50 mM Tris pH = 7.4, 500 mM NaCl, 10% glycerol, 0.1 mM TCEP, and 50 μM NP40. The protein was discarded if fluorophore labeling was >100%, which indicates unfolding and labeling of the cysteines in the Zn fingers of the NC domain. The labeled GagMBP$(His)_6$ fusion protein was cleaved by TEV protease on ice at low micromolar concentrations, and finally separated on a Superdex 200 16/60 gel filtration column (GE Healthcare) in 50 mM Tris pH = 8.0, 500 mM NaCl, 10% glycerol, 0.1 mM TCEP, eluting as a monodisperse sample (Fig. S1*A*). The pooled Superdex 200 fractions typically had a concentration of 1–2 μM and were snap frozen on liquid nitrogen in small aliquots without further concentration. Rethawed aliquots were still monodisperse as determined by gel filtration chromatography.

Purification and Fluorophore Labeling of Human Endosomal Sorting Complex Required for Transport (ESCRT) Complexes. ESCRT-I, containing the isoforms VPS37B and MVB12A, was expressed in HEK293 cells from plasmids obtained from Wesley Sundquist (University of Utah, Salt Lake City, UT) (2). ESCRT-I had a Strep-tagged VPS23 subunit, and was initially purified on Strep-Tactin Sepharose (IBA), followed by gel filtration chromatography on a Superdex 200 16/60 column (GE Healthcare), in 50 mM Tris pH = 7.4, 300 mM NaCl, 0.1 mM TCEP. The final protein was concentrated to approximately 2 μM and snap frozen on liquid nitrogen in small aliquots.

ALIX as full-length and ΔPRD (residues 1–702) was expressed in *E. coli* Rosetta2(DE3) at 20 °C O/N in LB medium, with an N-terminal TEV-cleavable $(His)_6$ tag, and purified on NiNTA resin (QIAGEN), followed by gel filtration chromatography on a Superdex 200 16/60 column (GE Healthcare), in 50 mM Tris pH = 7.4, 300 mM NaCl, 0.1 mM TCEP. The $(His)_6$ tag was removed by TEV protease digestion, and repassage over NiNTA captured cleaved $(His)_6$ tags and the $(His)_6$-tagged TEV protease. The final protein was concentrated to approximately 20–60 μM and snap frozen on liquid nitrogen in small aliquots.

The ESCRT-III subunit CHMP4B was expressed as an N-terminal TEV-cleavable $(His)_6$-MBP fusion in *E. coli* Rosetta2(DE3) at 20 °C O/N in LB medium. Cells were lysed by high-pressure homogenization to avoid sonication-induced protein aggregation, and initial purification was carried out on NiNTA resin. The $(His)_6$-MBP-CHMP4B fusion was further purified on a Superdex 200 16/60 column (GE Healthcare), in 50 mM Tris pH = 7.4, 100 mM NaCl, 0.1 mM TCEP. The $(His)_6$-MBP tag was removed by TEV protease digestion at low micromolar concentrations, followed by gel filtration chromatography on a Superdex 75 16/60 column (GE Healthcare), in 50 mM Tris pH = 7.4, 300 mM NaCl, 0.1 mM TCEP. CHMP4B eluted as a monodisperse sample, typically with a concentration of approximately 1 μM. Unconcentrated CHMP4B fractions were kept at 4 °C until use, because freeze-thaw was found to result in loss of material. The other ESCRT-III subunits were purified analogously to CHMP4B, except that the final proteins had higher concentration (approximately 5–20 μM) and could be snap frozen on liquid nitrogen without aggregation or loss of material.

Fluorophore labeling was performed O/N at 4 °C using cysteine-reactive Atto 488 maleimide (Sigma-Aldrich) on engineered N-terminal cysteines (ESCRT-III) or on native surface-exposed cysteines (ESCRT-I, ALIX). Labeling was performed on the fusion proteins before TEV digest and final gel filtration chromatography, so that the monodisperse state of the labeled protein could be ensured. Labeling efficiencies were normally 50–100%.

1. Sheffield P, Garrard S, Derewenda Z (1999) Overcoming expression and purification problems of RhoGDI using a family of "parallel" expression vectors. *Protein Expr Purif* 15:34–39.

2. Morita E, et al. (2007) Identification of human MVB12 proteins as ESCRT-I subunits that function in HIV budding. *Cell Host Microbe* 2:41–53.

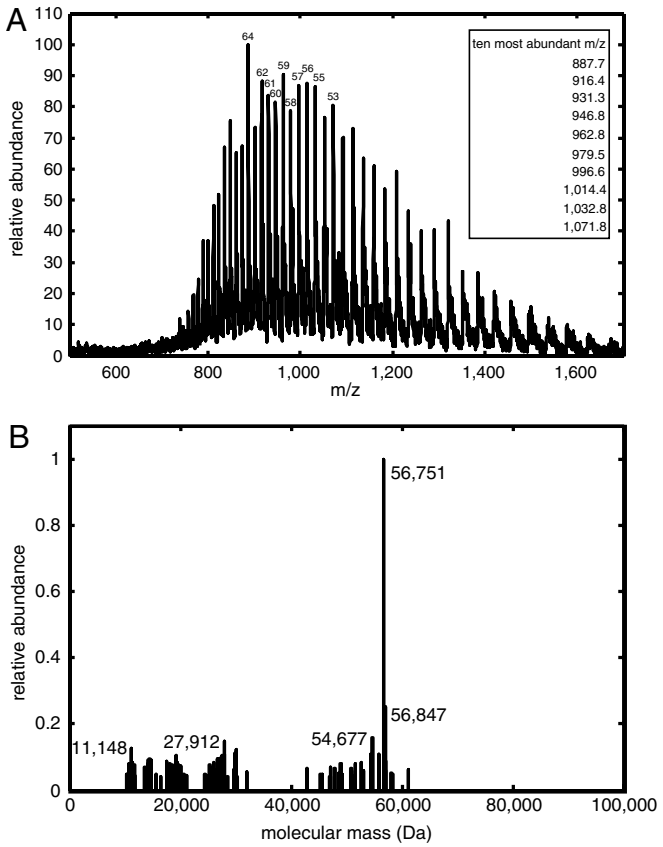

Fig. S1. Mass spectrum of myristoylated Gag. (*A*) *m*/*z* spectrum of HIV-1 Gag after the final gel filtration purification step. The 10 highest *m*/*z* peaks in the spectrum are listed in the inset, and the corresponding *z* value is shown above each peak. (*B*) The deconvoluted *m*/*z* spectrum. The five highest peaks are labeled with their masses. Only one major species is found, with a mass of 56,751 Da. This mass corresponds well with the theoretical mass 56,752 Da of myristoylated Gag (with Ala120Cys mutation and residues LYFQ at C terminus, from TEV protease site), but not with unmyristoylated Gag at 56,542 Da (same construct, without initiator Met).

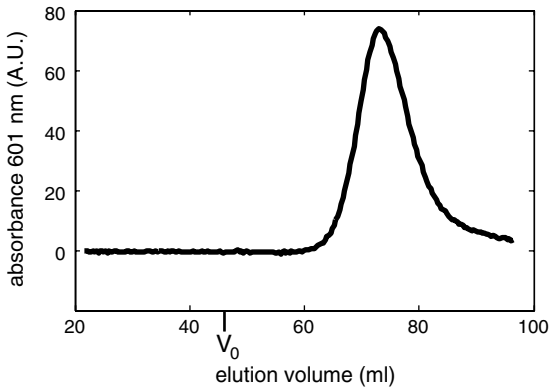

Fig. S2. Size exclusion chromatography of myristoylated Gag. HIV-1 Gag elutes as a monodisperse peak on a size exclusion column. After removal of the MBP$(His)_6$ tag by TEV protease, Atto 594–labeled Gag was applied to a Superdex 200 16/60 size exclusion column. Gag elution was monitored by the fluorophore absorption at 601 nm. V_0 indicates the column void volume.