SUPPORTING INFORMATION

Insights into the oligomeric structure of the HIV-1 Vpu protein

Saman Majeed[1], Oluwatosin Adetuyi[1], Peter P. Borbat[2], Md Majharul Islam[1], Olamide Ishola[1], Bo Zhao[3], Elka R. Georgieva[1*]

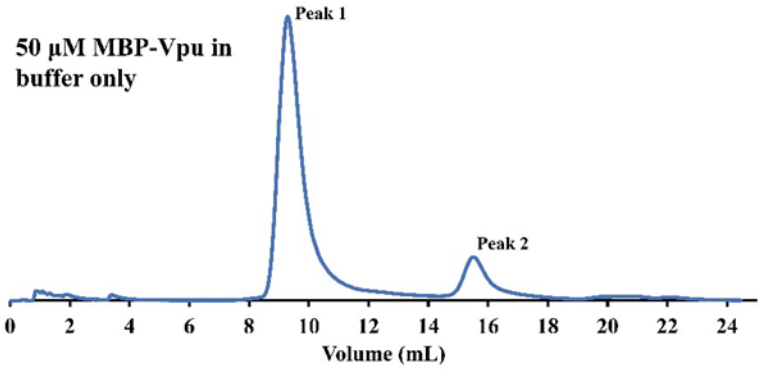
[1]Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409

[2]Department of Chemistry and Chemical Biology and ACERT, Cornell University, Ithaca, NY 14853

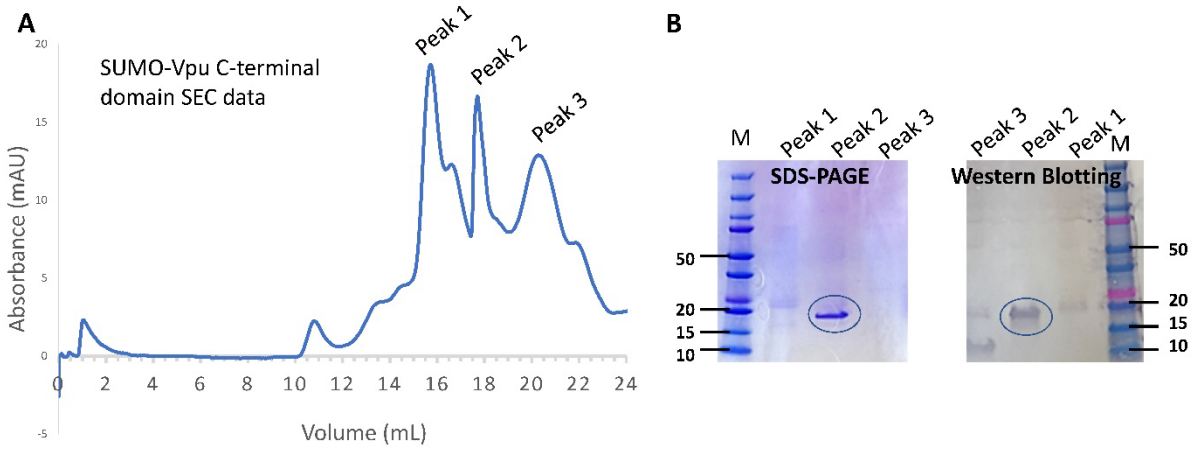
[3]College of Arts & Sciences Microscopy (CASM), Texas Tech University, Lubbock, TX 79409

*To whom the correspondence should be addressed: Elka R. Georgieva, Department of Chemistry and Biochemistry, Texas Tech University

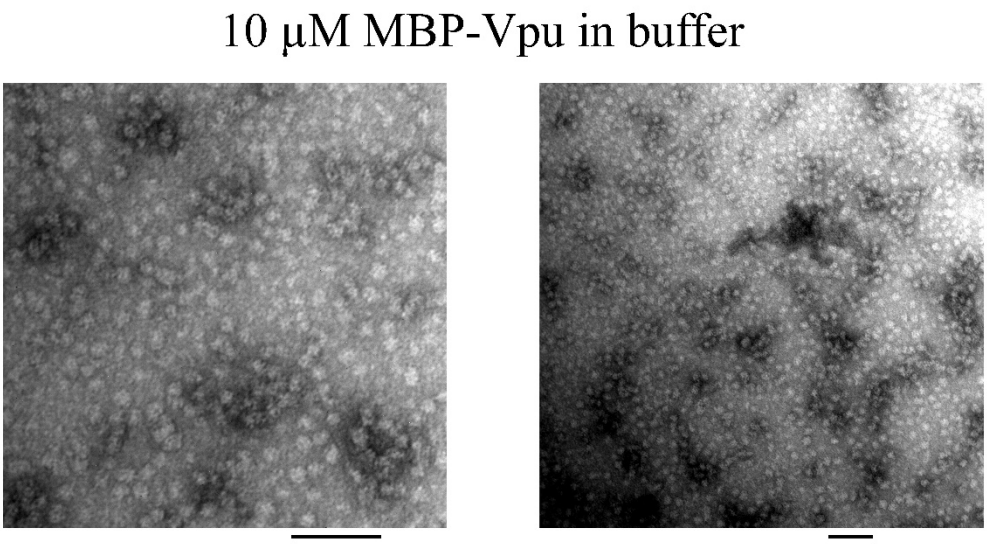
e-mail: *elgeorgi@ttu.edu*



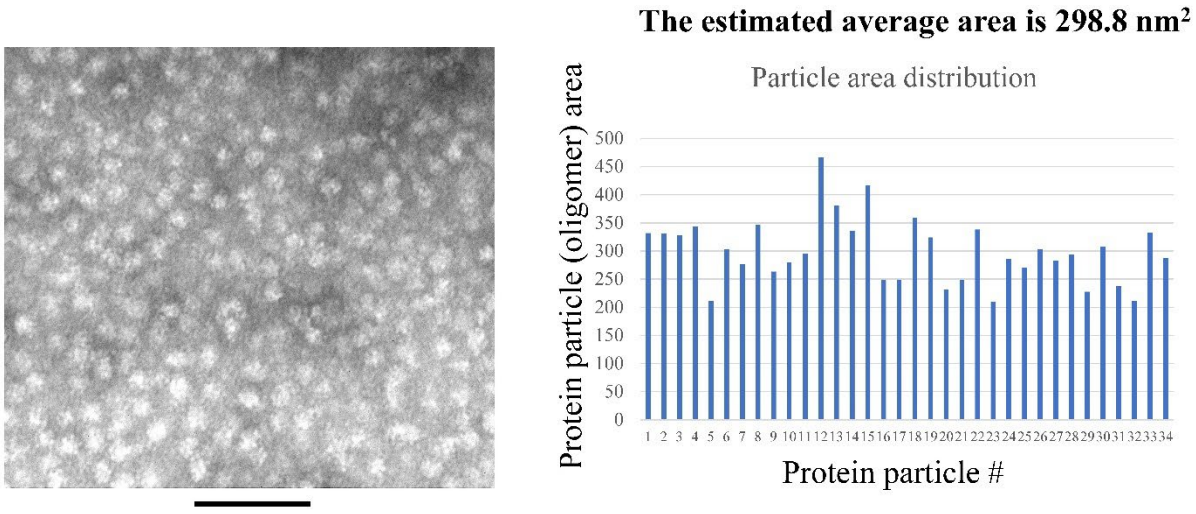
Supporting Figure 1. SEC data for MBP-Vpu in solution—results from second experiment identical to those in Figure 4 (main text) are shown**.** Two elution peaks (Peak 1 and Peak 2) with substantially different intensities were observed. Both Peak 1 and Peak 2 contained MBP-Vpu as confirmed by SDS-PAGE and WB (data not shown).



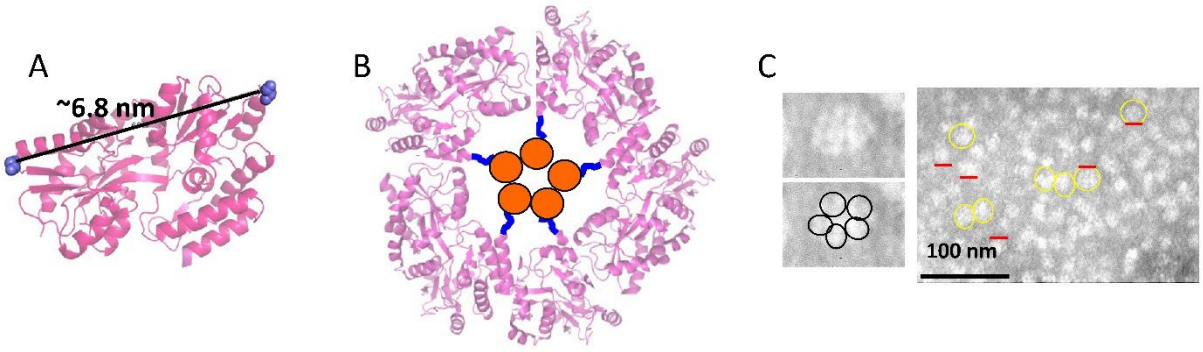
Supporting Figure 2. Vpu C-terminal is monomeric in solution. (A) SEC chromatogram. (B) SDS-PAGE and western blotting of SEC fractions in (A). Vpu C-terminal domain was expressed in *E. coli* as fusion construct with His_{x8}-SUMO tag and purified by using consecutively Ni^{2+}- and Co^{2+} - affinity chromatography, and SEC. The fractions corresponding to the three SEC elution peaks (Peak 1, Peak 2 and Peak 3 in (A)) were combined, concentrated and characterized by SDS-PAGE and western blotting. The SUMO-Vpu C-terminal protein was found in Peak 2, eluting at ca. 18 ml, which corresponds to a protein monomer of ca. 21 kDa molecular weight, based on elution of standard protein mixture (Figure 4 in the Main Text).



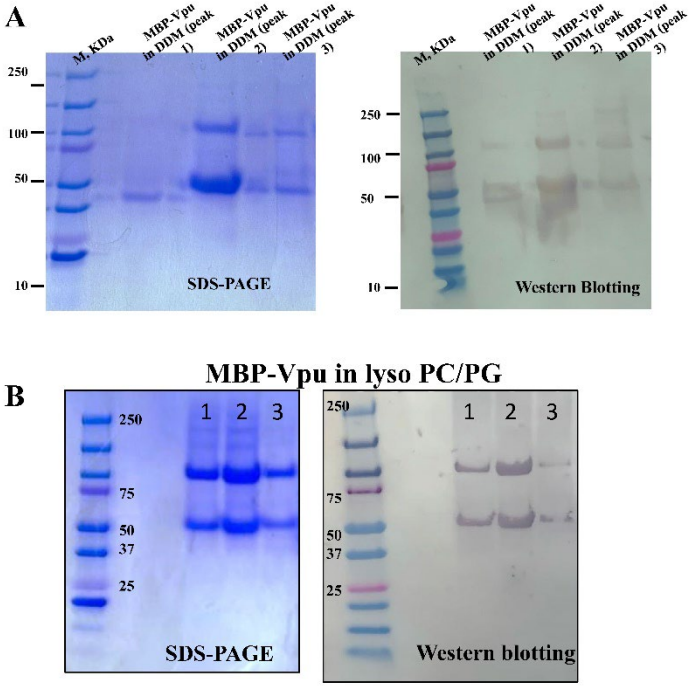
Supporting Figure 3. Representative images of nsEM on 10 µM MBP-Vpu in buffer. The 100 nm bar is in black. Well-formed uniform size MBP-Vpu oligomers are visible.



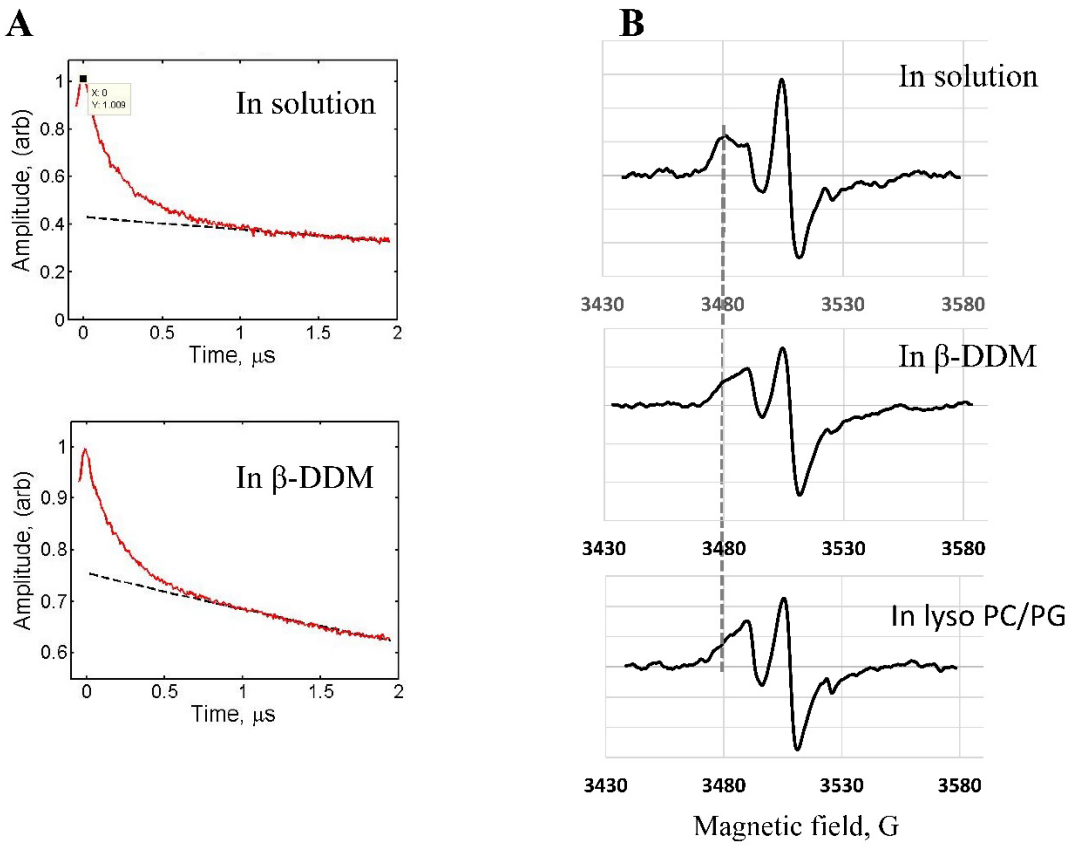
Supporting Figure 4. Particle size distribution for MBP-Vpu oligomers in solution. Analysis of the protein particles' size distribution within representative area of nsEM image (left) for sample of 5 µM MBP-Vpu was conducted using the ImageJ software. A set of more than thirty particles with visually similar shape was selected manually and their areas were calculated. The particle area distribution (right) is relatively narrow (except few outliers) with average particle area of 298.8 nm^2. Ideally, this corresponds to a circle with a 9.7-9.8 nm radius and ca. 20 nm diameter.



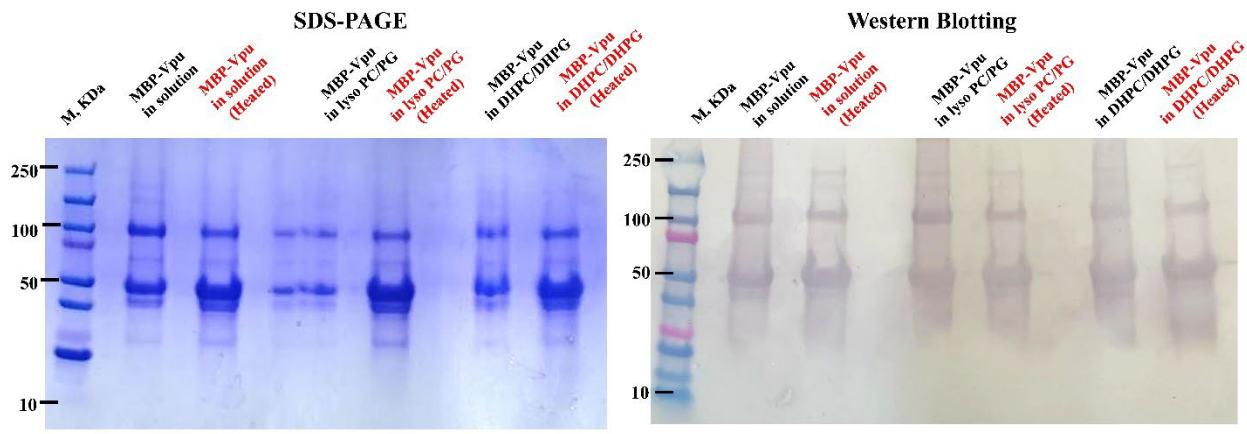
Supporting Figure 5. A coarse model of the MBP-Vpu pentamer: (A) The crystal structure of MBP without substrate (PDB ID 1PEB); the larger size of MBP monomer is about 6.8 nm. (B) A possible arrangement of MBP-VPU monomers in the pentameric structure, as viewed from top. MBP moieties rendered based on 1PEB crystal structure and colored in magenta surround the central pentameric domain shown as a cartoon. The MBP- Vpu linkers are depicted in blue; the TM helices of Vpu monomers are drawn as orange circles. (C) The nsEM data for 5 μM MBP-Vpu in buffer (the same as in Figure 5).



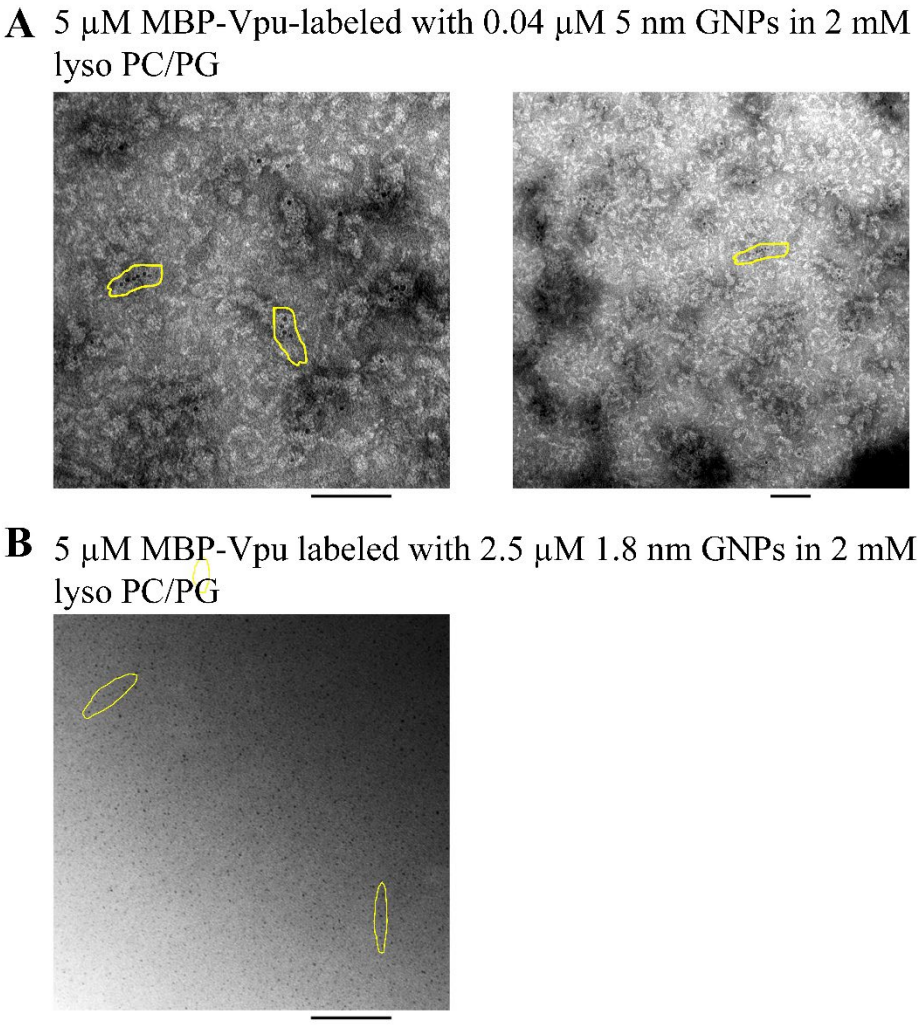
Supporting Figure 6. SDS-PAGE and WB analysis of SEC fractions corresponding to Peaks (shoulder) 1, 2 and 3 of MBP-Vpu in DDM and lyso PC/PG (Figure 6). Each of the three fractions contains MBP-Vpu, corresponding to different oligomeric state of the protein.



Supporting Figure 7. EPR data for the spin-labeled at residue Q36C MBP-Vpu. (A) Raw DEER signals (red) and baseline (black) for the protein in solution (top) and in β-DDM (bottom). (B) CW EPR spectra for the protein in solution, β-DDM and lyso PC/PG. Upon transition from buffer to hydrophobic environment, the low magnetic field CW EPR spectral line shifts toward higher magnetic fields indicating increased spin label mobility and consequently protein region restructuring.



Supporting Figure 8. SDS-PAGE and WB of MBP-Vpu in buffer, lyso PC/PG and DHPC/DHPG with and without heating the samples before loading them onto the gel. For the heat treatment, samples were prepared using standard procedure and then heated at 97 °C for 10 min.



Supporting Figure 9. Representative EM images of 5 μM MBP-Vpu labeled with GNPs. (A) nsEM on MBP-Vpu labeled with 0.04 μM 5 nm GNPs in 2 mM lyso PC/PG. Some of the arrays of GNP-labeled protein are outlined in yellow. Note, not each MBP-Vpu monomer has a GNP attached, as the protein concentration was significantly higher than the concentration of GNPs. (B) EM images of non-stained MBP-Vpu labeled with 2.5 μM 1.8 nm GNPs. In this case the protein is not visible, but arrays of GNPs attached to the protein can be traces as well (outlined in yellow). In both cases, the protein was first labeled with GNPs to its His_8-tag, and then reconstituted in lyso PC/PG. The 100 nm bars are below the micrographs.