Conditional Trimerization and Lytic Activity of HIV-1 gp41 Variants Containing the Membrane-Associated Segments

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Supporting Information

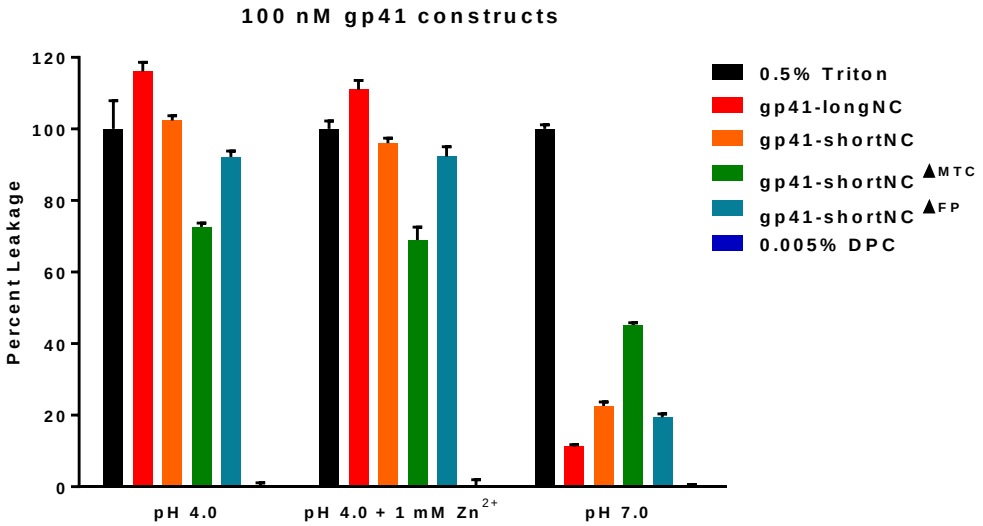

Figure S1. Liposomal release assay of various gp41 constructs (100 nM) at pH 4.0, pH 4.0 with 1 mM Zn^{2+}, and pH 7.0. Evidently, addition of 1 mM Zn^{2+} (10,000 times that of the protein concentration) does not change the content leakage induced by gp41 constructs, thus confirming that the fusion activity is not affected by the hexahistidine tags. In addition, for all tested gp41 constructs, the fusion activities are attenuated when changing from pH 4.0 to pH 7.0.

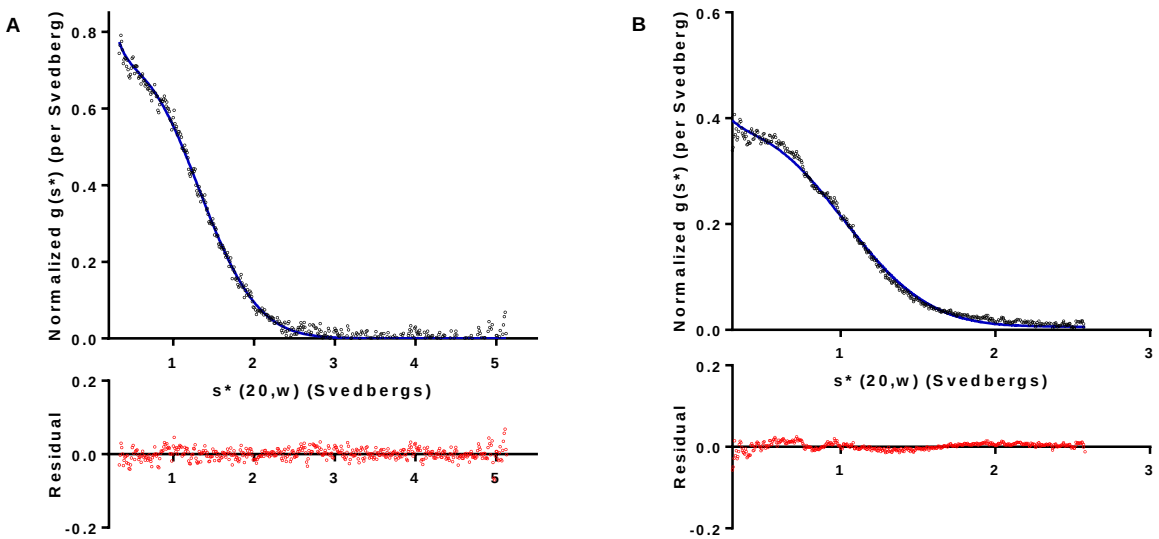

Figure S2. AUC of gp41-shortNC $^{\Delta FP}$ (panel A) and gp41-MTC (panel B) at pH 7. The s* distribution of both proteins were fit to a single component model. For gp41-shortNC $^{\Delta FP}$, $S_{20,w}$ and apparent M_w (S/D) values are 0.904 (0.884, 0.924) *S* and 13.3 (12.7, 14.3) kDa respectively, and the calculated M_w is 13.2 kDa. For gp41-MTC, $S_{20,w}$ and apparent M_w (S/D) values are 0.693 (0.685, 0.698) *S* and 9.4 (9.0, 9.7) kDa respectively, and the calculated M_w is 9.3 kDa.

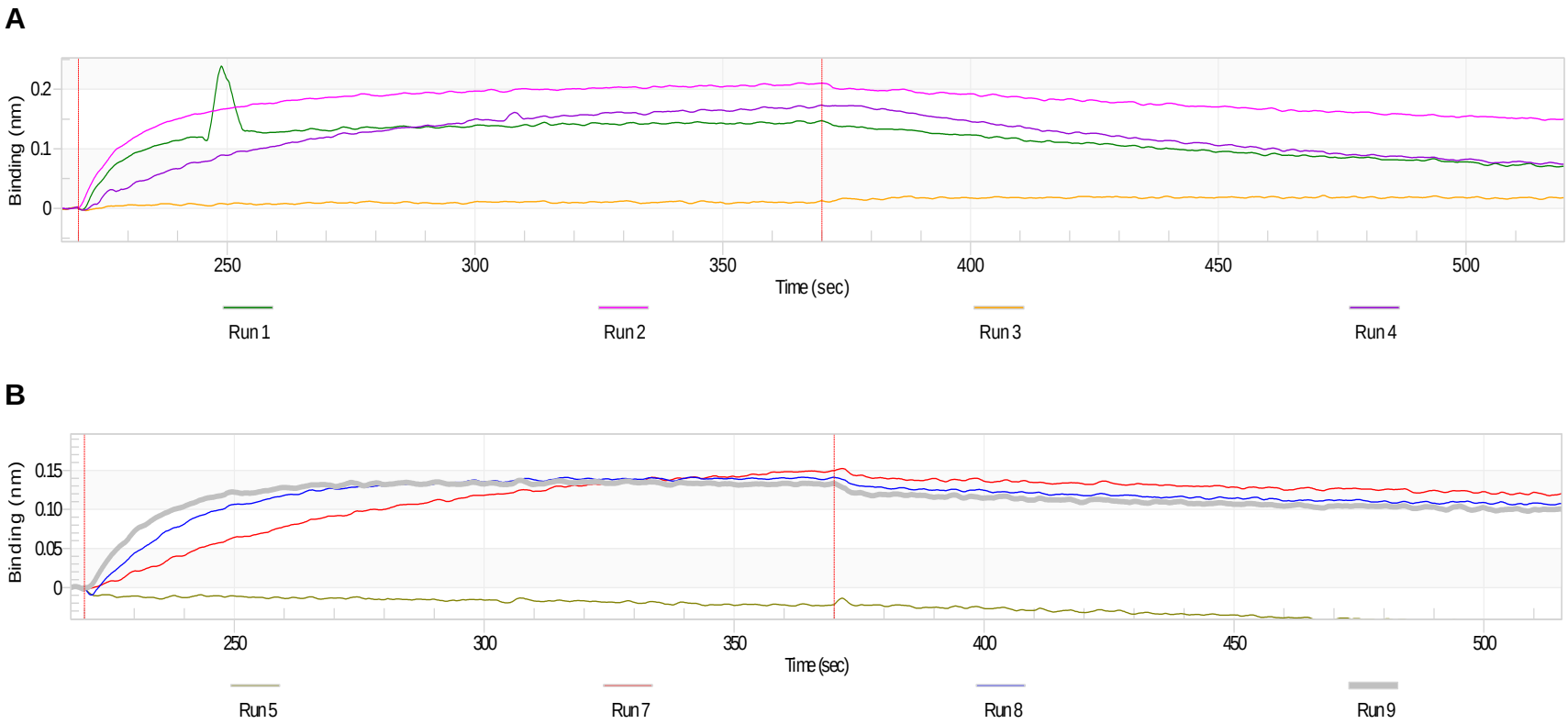

Figure S3. Representative binding data of 2F5 (A) and 4E10 (B) with gp41 constructs measured by biolayer interferometry at pH 4. For antibody 2F5 (panel A), gp41-longNC (purple), gp41-shortNC (pink) and gp41-shortNC $^{\Delta FP}$ (green) show clear binding activities, while gp41-shortNC $^{\Delta MTC}$ (orange) shows no binding. Similarly, for antibody 4E10 (panel B), gp41-longNC (red), gp41-shortNC (blue) and gp41-shortNC $^{\Delta FP}$ (gray) show clear binding activities, while gp41-shortNC $^{\Delta MTC}$ (brown) shows no binding.

Table S1. Effect of GdnHCl on DPC micelles. DPC micelle diameters were determined by dynamic light scattering (DLS).

[GdnHCl] (M)	DPC micelle diameter (nm)
0	4.4
1	3.5
2	3.4
3	3.7
4	5.0
5	4.0
6	63[a]
7	24[a]

[a] Note that the DLS data quality was significantly affected at high concentrations of GdnHCl, thus the values may not be accurate.