Supplementary Materials

Engineering the HK97 Virus-Like Particle as a nanoplatform for biotechnology applications.

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DNA Sequences

HK97 GP5 (wild type)

ATGTCTGAACTCGCTCTCATTCAAAAAGCTATCGAAGAATCCCAGCAGAAAATGACCCAGCTTTTCGATGCGCAGA
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ATAACTTCGTGAAAAACATGCTGACCATCCTGTGCGAAGAGCGTCTGGCGCTGGCGCATTATCGCCCGACAGCAA
TCATCAAGGGCACCTTCTCTTCTGGCTCATGA

*For the A302C variant the GCA codon has a change to the codon TGT

*S305C has a change at the highlighted position to the codon TGT

HK97 GP4

ATGGCACCTGAAATCGTAAAAACGCTGTCCTTCGACGAGACAGAAATCAAATTCACCGGTGACGGGAAGCAGGG
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ATAA

GFP-targeting peptide fusion

ATGGGCGGAGGAGGCAAGGGGGTGAAGGAAGTAATGAAGATCAGTCTGGAGATGGACTGCACTGTTAACGGCG
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HK97 GP5-LPETG

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HK97 GP5-RGDS S305C

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CTAA

Protein Sequences

GP5

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TKAQAAGTFTVGGFDMASQVWDRMDATVEVSREDRDNFVKNMLTILCEERLALAHYRPTAIIKGTFSSGS

GP4

MAPEIVKTLSFDETEIKFTGDGKQGIFEGYASVFNNTDSDGDIILPGAFKNALANQTRKVAMFFNHKTWELPVGKWDSL
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GFP-targeting peptide fusion

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GDGIETIRDVENWLRDSVGLTKSQAVGLIARFKSAIRSESEGDGNEAQINALLQSIKSFPSNLGK

GP5-LPETG

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ADVVAEKALKPESDITFSKQTANVKTIAHWVQASRQVMDDAPMLQSYINNRLMYGLALKEEGQLLNGDGTGDNLEGL
NKVATAYDTSLNATGDTRADIIAHAIYQVTESEFSASGIVLNPRDWHNIALLKDNEGRYIFGGPQAFTSNIMWGLPVVP
TKAQAAGTFTVGGFDMASQVWDRMDATVEVSREDRDNFVKNMLTILCEERLALAHYRPTAIIKGTFSSGSRSAGGGG
AGGGTLPETGGS

GP5-RGD S305C

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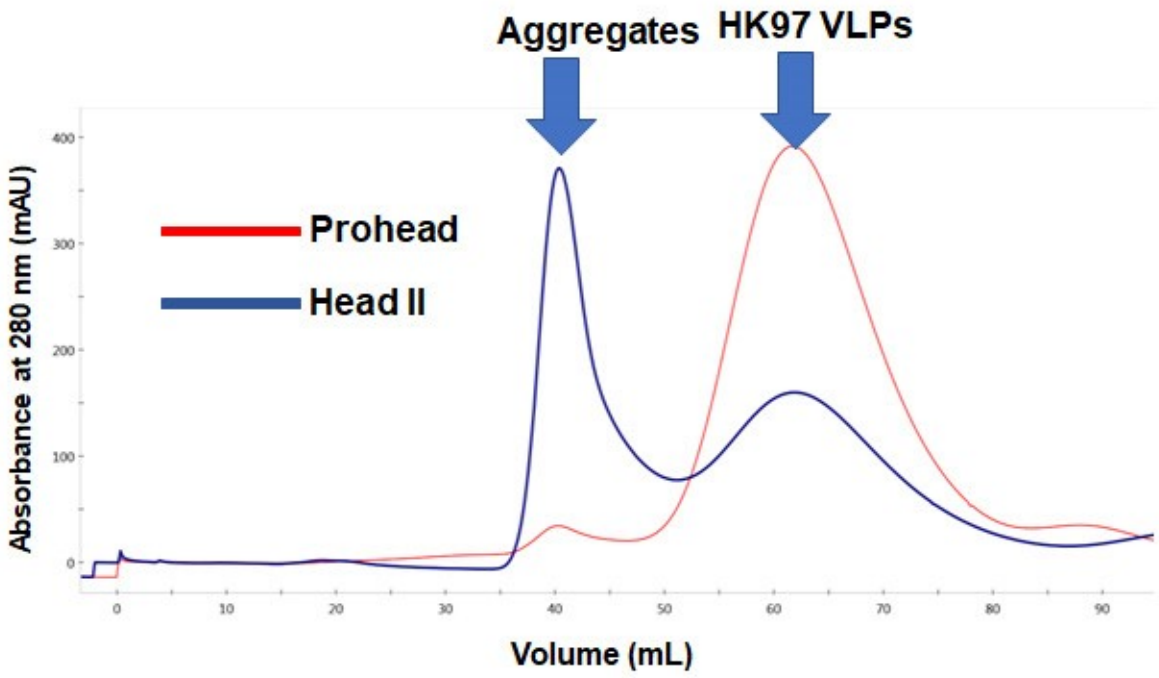


Figure S1. Comparison of HK97 Prohead size exclusion chromatography purification to mature Head II size exclusion chromatography. The size exclusion chromatography HK97 Head II elution profile shows an increased intensity for the peak corresponding to aggregates near the void volume relative to the elution profile for a Prohead HK97 VLP sample.

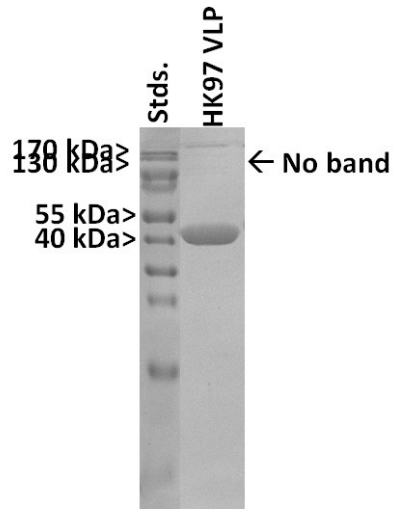


Figure S2. SDS-PAGE analysis of the HK97 VLP derived from wild type GP5 prepared using TCA precipitation. Preparation by TCA precipitation removed a band associated with polymerization of GP5 subunits that is normally observed by common SDS-PAGE preparation methods utilized in our lab.

			Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm):	52.33	Peak 1:	54.47	100.0	12.10
PdI:	0.009	Peak 2:	0.000	0.0	0.000
Intercept:	0.960	Peak 3:	0.000	0.0	0.000
Result quality :	Good				

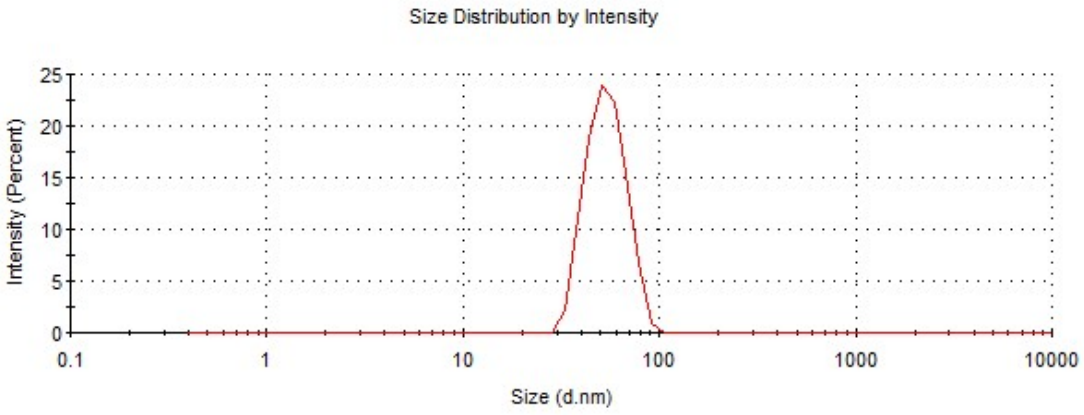


Figure S3. Representative dynamic light scattering intensity spectra and distribution values for the Prohead I HK97 VLP. Values indicate homogeneous nanoparticles and sizes consistent with the expected HK97 Prohead VLPs.

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 52.85	Peak 1:	55.02	100.0	12.17
PdI: 0.010	Peak 2:	0.000	0.0	0.000
Intercept: 0.971	Peak 3:	0.000	0.0	0.000
Result quality : Good				

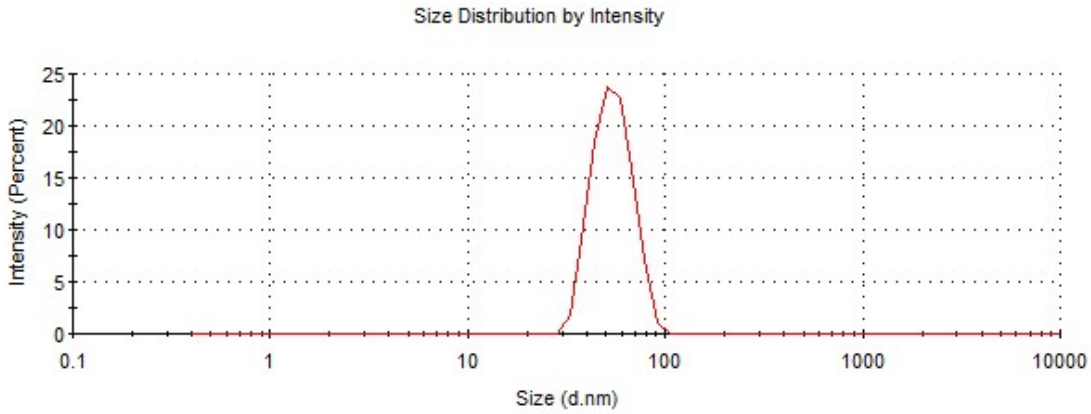


Figure S4. Representative dynamic light scattering intensity spectra and distribution values for the Prohead II HK97 VLP. Values indicate homogeneous nanoparticles and sizes consistent with the expected HK97 Prohead VLPs.

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 67.00	Peak 1:	74.19	100.0	25.94
PdI: 0.130	Peak 2:	0.000	0.0	0.000
Intercept: 0.953	Peak 3:	0.000	0.0	0.000
Result quality : Good				

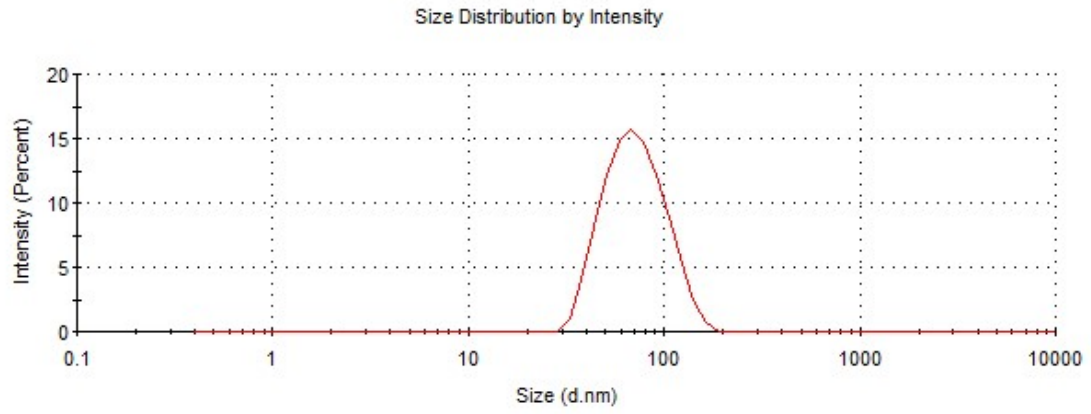
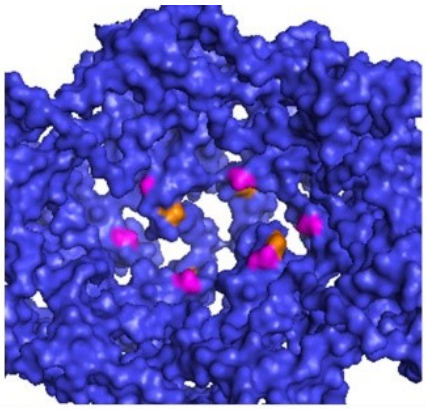


Figure S5. Representative dynamic light scattering intensity spectra and distribution values for the Head II HK97 VLP. Values indicate homogeneous nanoparticles and sizes consistent with the expected HK97 Head VLPs.



Wild Type GP5	1-MSEL…PQAFTSN…SSGS-385
GP5 A302C	1-MSEL…PQCFTSN…SSGS-385
GP5 S305C	1-MSEL…PQAFTCN…SSGS-385

Figure S6. Close up view of the Prohead HK97 VLP internal surface and sequence comparison of wild type to GP5 mutant variants. Close up view of the interior surface of a GP5 hexamer comprising the Prohead HK97 VLP. The A302 position is colored orange and the S305 position is colored magenta. An amino acid sequence overlay of the different modifications made from the wild type GP5 is shown below the structural model.

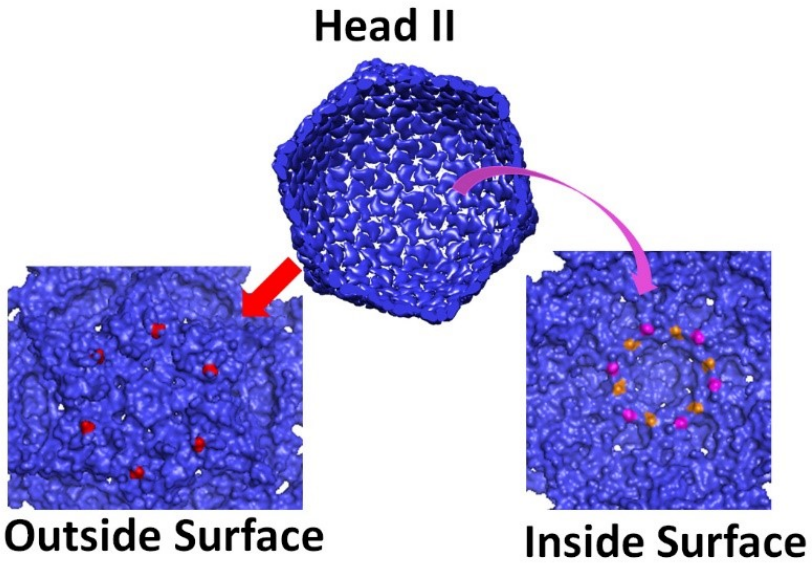


Figure S7. Molecular model of the Head II HK97 structure showing the internal and external surfaces with highlighted amino acid residues. The structural model of the Head II HK97 VLP shows that the A302 (orange) and S305 (magenta) residues are still exposed on the interior surface upon transformation from the Prohead form. The outer surface also has the C-terminal residue (red) visible in the model, indicating that transformation to the Head II does not drastically alter the positioning of the C-terminus.

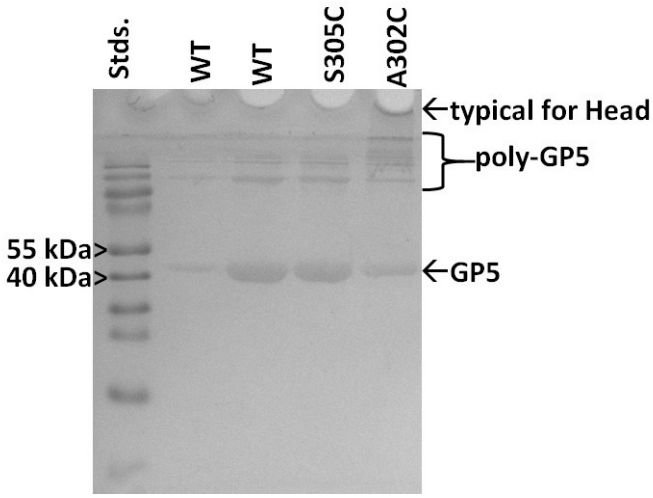


Figure S8. SDS-PAGE comparison of HK97 GP5 variants. The GP5 A302C variant (A302C) showed noticeably more polymers of GP5 (poly-GP5) than wild type (WT) and the GP5 S305C variant (S305C), especially increased staining in the well area, similar to what is observed for Head II transformed HK97 VLPs.

			Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm):	64.11	Peak 1:	69.28	100.0	19.84
PdI:	0.069	Peak 2:	0.000	0.0	0.000
Intercept:	0.967	Peak 3:	0.000	0.0	0.000
Result quality :	Good				

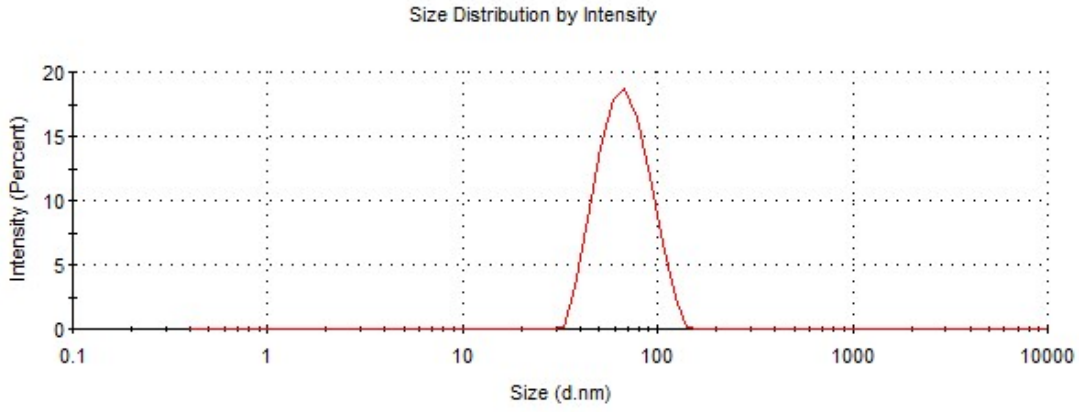


Figure S9. Representative DLS data for the HK97 A302C VLP (prohead I) after purification. The diameter was found to be higher than expected for the Prohead I form (56 nm), but more similar in diameter to the Head morphology (66 nm).

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 52.69	Peak 1:	55.20	100.0	12.68
PdI: 0.026	Peak 2:	0.000	0.0	0.000
Intercept: 0.970	Peak 3:	0.000	0.0	0.000
Result quality : Good				

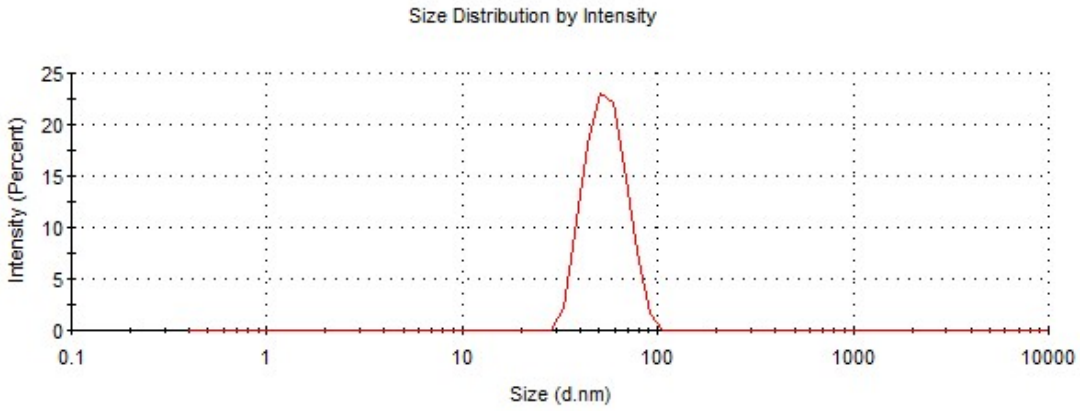


Figure S10. Representative dynamic light scattering intensity spectra and distribution values for the Prohead I HK97 S305C VLP prior to fluorescent labeling. Values indicate homogeneous nanoparticles and sizes consistent with the expected HK97 Prohead VLPs.

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 51.71	Peak 1:	54.84	100.0	13.98
PdI: 0.041	Peak 2:	0.000	0.0	0.000
Intercept: 0.959	Peak 3:	0.000	0.0	0.000
Result quality : Good				

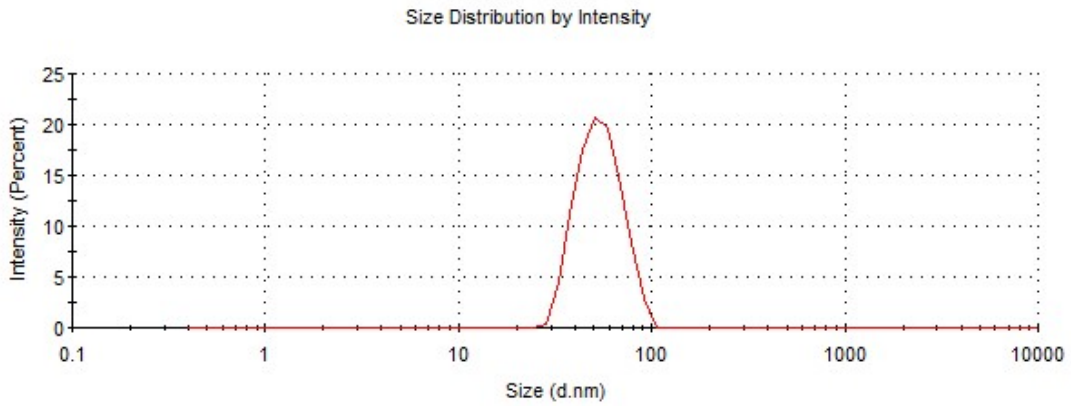


Figure S11. Representative dynamic light scattering intensity spectra and distribution values for the Prohead I HK97 S305C VLP after labeling with 5-IAF. Values indicate homogeneous nanoparticles with sizes unchanged after labeling and purification.

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 54.49	Peak 1:	55.70	100.0	8.312
PdI: 0.019	Peak 2:	0.000	0.0	0.000
Intercept: 0.967	Peak 3:	0.000	0.0	0.000
Result quality : Good				

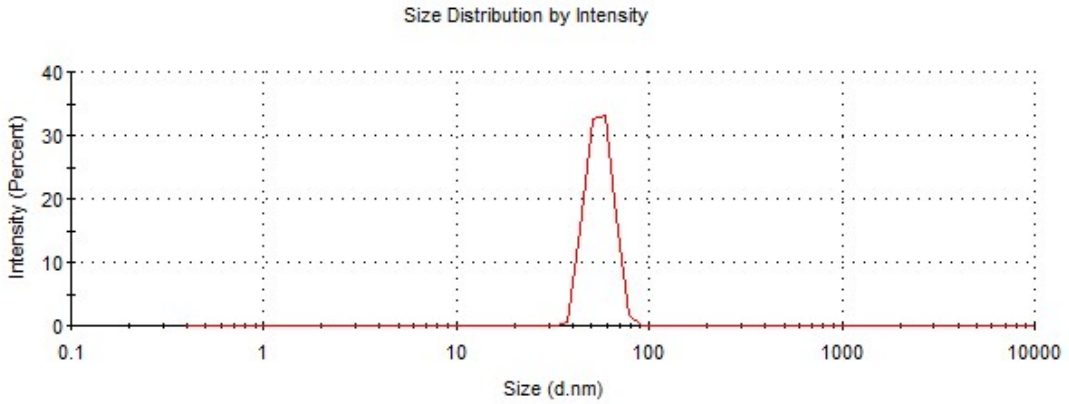
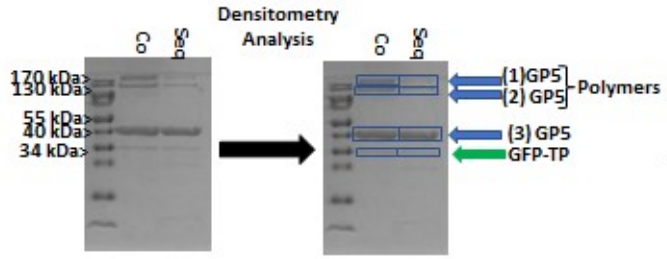


Figure S12. Representative dynamic light scattering intensity spectra and distribution values for the Prohead I HK97 VLP/GFP-TP through co-expression strategy. Values indicate homogeneous nanoparticles and sizes consistent with the expected HK97 Prohead VLPs, essential unchanged upon encapsulation of GFP-TP.



Band	Co-Expression BC Sum	Seq.- Expression BC Sum
(1) GP5	3,475,381	1,489,471
(2) GP5	4,158,848	2,007,873
(3) GP5	13,042,066	11,350,896
GFP TP	2,595,264	2,052,497
	Analysis	
Total Sum GP5 Intensity	20,676,295	14,848,240
Ratio GP5/GFP TP	7.97	7.23
GFP TP/VLP	53	58

Figure S13. Representative SDS-PAGE and densitometry analysis for determination of GFP-TP packaged per HK97 VLP by co-expression and sequential expression methods.

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 56.96	Peak 1:	58.62	100.0	10.03
PdI: 0.027	Peak 2:	0.000	0.0	0.000
Intercept: 0.961	Peak 3:	0.000	0.0	0.000
Result quality : Good				

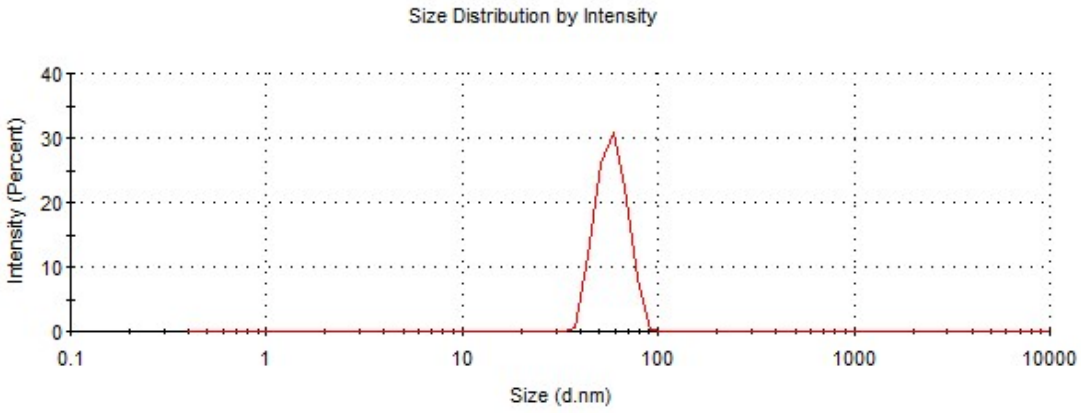


Figure S14. Representative dynamic light scattering intensity spectra and distribution values for the Prohead I HK97 VLP/GFP-TP through sequential 2-plasmid strategy. Values indicate homogeneous nanoparticles and sizes consistent with the expected HK97 Prohead VLPs, essential unchanged upon encapsulation of GFP-TP.

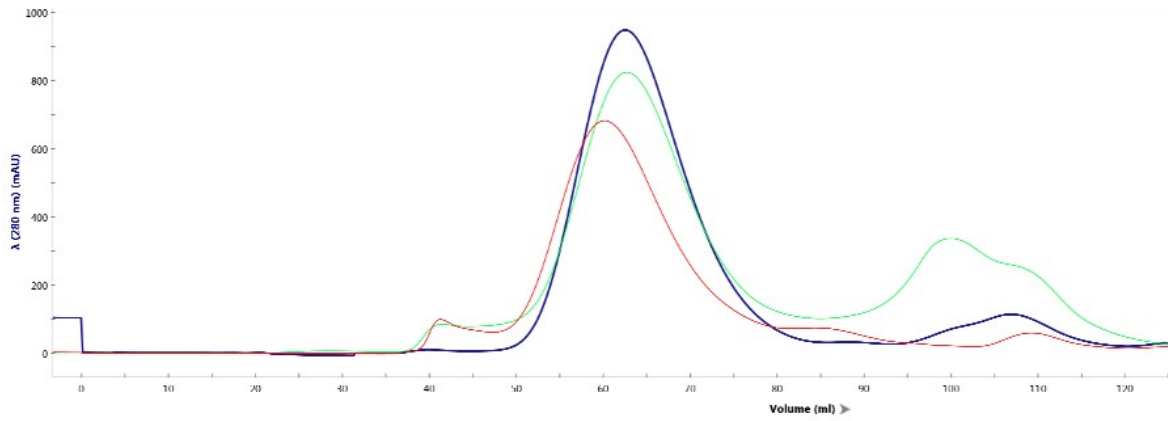


Figure S15. Size exclusion chromatography traces comparing the HK97 VLP/GFP-TP constructs to Prohead I HK97 VLPs. SEC chromatograms for the purification of HK97 VLP/GFP-TP produced by co-expression (blue trace) or sequential expression (green trace) of the GFP-TP and CP show indistinguishable elution profiles for the HK97 VLP in comparison with Prohead I HK97 VLP sample (red trace) aside from minor shifts due to times the runs were performed.

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 54.24	Peak 1:	57.61	100.0	14.60
PdI: 0.049	Peak 2:	0.000	0.0	0.000
Intercept: 0.955	Peak 3:	0.000	0.0	0.000
Result quality : Good				

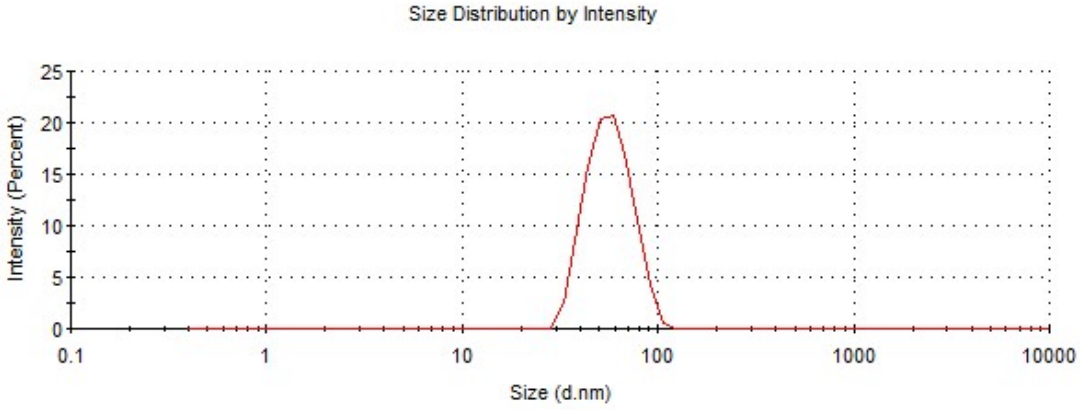


Figure S16. Representative dynamic light scattering intensity spectra and distribution values for the Prohead I LPETG-HK97 VLP. Values indicate homogeneous nanoparticles and sizes consistent with the expected HK97 Prohead VLPs, indicating that the addition of a short flexible peptide sequence does not add significant diameter to the VLP, as expected.

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 98.27	Peak 1:	155.7	90.7	73.96
PdI: 0.328	Peak 2:	20.21	9.3	7.562
Intercept: 0.965	Peak 3:	0.000	0.0	0.000
Result quality : Refer to quality report				

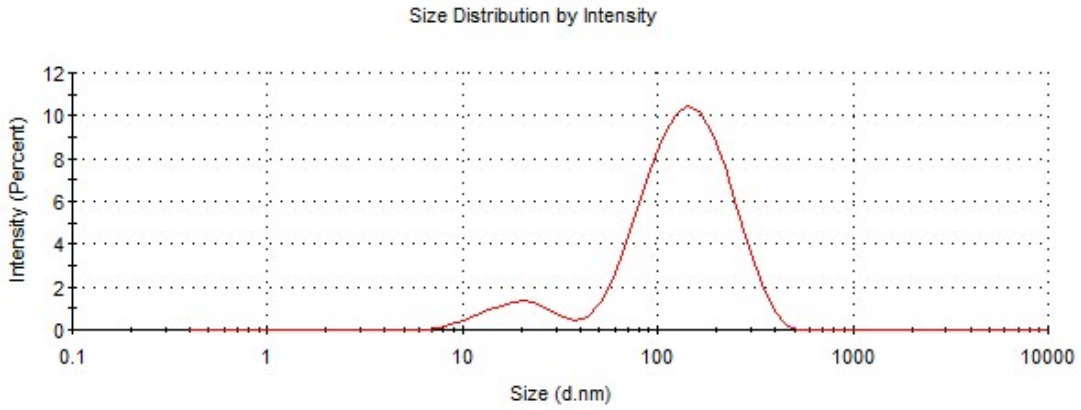


Figure S17. DLS analysis of sortase ligation reaction of polyG-GFP with LPETG-HK97 VLPs. The results show a heterogeneous mixture distributed between small particles (sortase and polyG-GFP) and larger particles, consistent with attachment of polyG-GFP to the exterior of the HK97 VLP.

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 55.42	Peak 1:	58.12	100.0	13.56
PdI: 0.025	Peak 2:	0.000	0.0	0.000
Intercept: 0.970	Peak 3:	0.000	0.0	0.000
Result quality : Good				

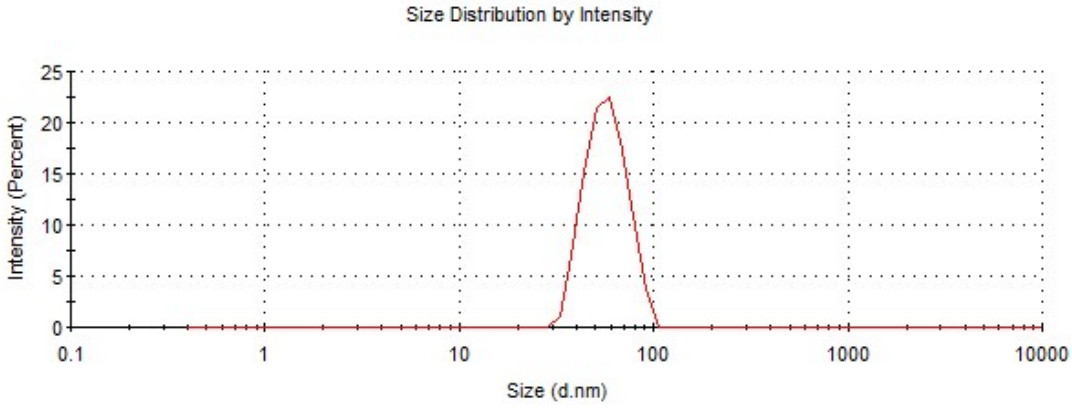


Figure S18. Representative dynamic light scattering intensity spectra and distribution values for the Prohead I RGDS-HK97 S305C VLP unlabeled. Values indicate homogeneous nanoparticles and sizes consistent with the expected HK97 Prohead VLPs, indicating that the addition of a short RGDS peptide sequence does not add significant diameter to the VLP, as expected.

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 55.59	Peak 1:	60.29	100.0	17.75
PdI: 0.069	Peak 2:	0.000	0.0	0.000
Intercept: 0.957	Peak 3:	0.000	0.0	0.000
Result quality : Good				

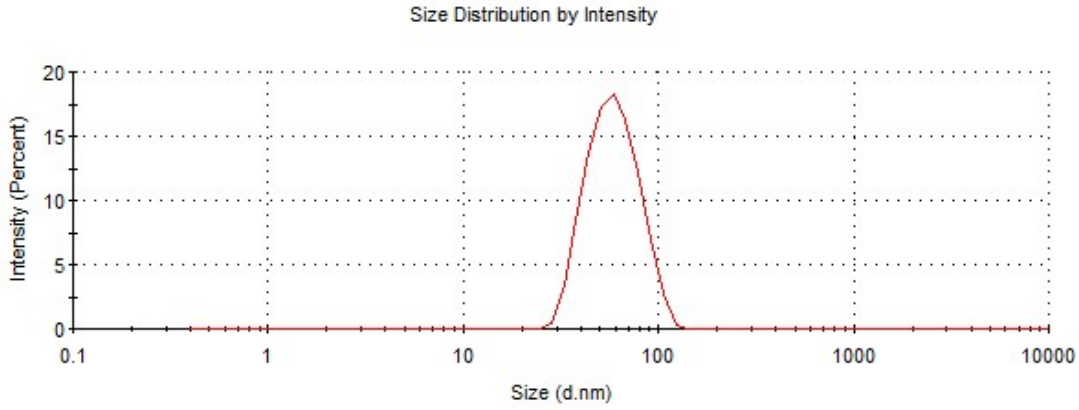


Figure S19. Representative dynamic light scattering intensity spectra and distribution values for the Prohead I RGDS-HK97 S305C VLP after labeling with 5-IAF. Values indicate homogeneous nanoparticles with sizes unchanged after labeling and purification.

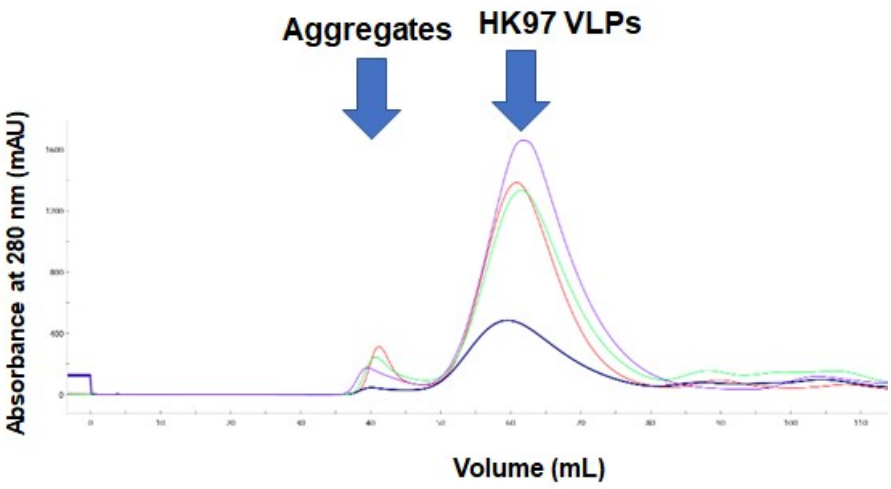


Figure S20. Size exclusion chromatography traces comparing several variants of Prohead I HK97 VLPs examined in this study. SEC chromatograms comparing the elution profiles of Prohead II (purple trace), HK97 S305C VLP (green), LPETG HK97 VLP (red trace), and RGDS-HK97 VLP (blue trace) show very little shift in the elution of the VLP particles, suggesting no change upon modification of the HK97 VLP.