Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.


# Supporting Information (SI) for:

## Highly modular hepatitis B virus-like nanocarriers for therapeutic protein encapsulation and targeted delivery to triple negative breast cancer cells

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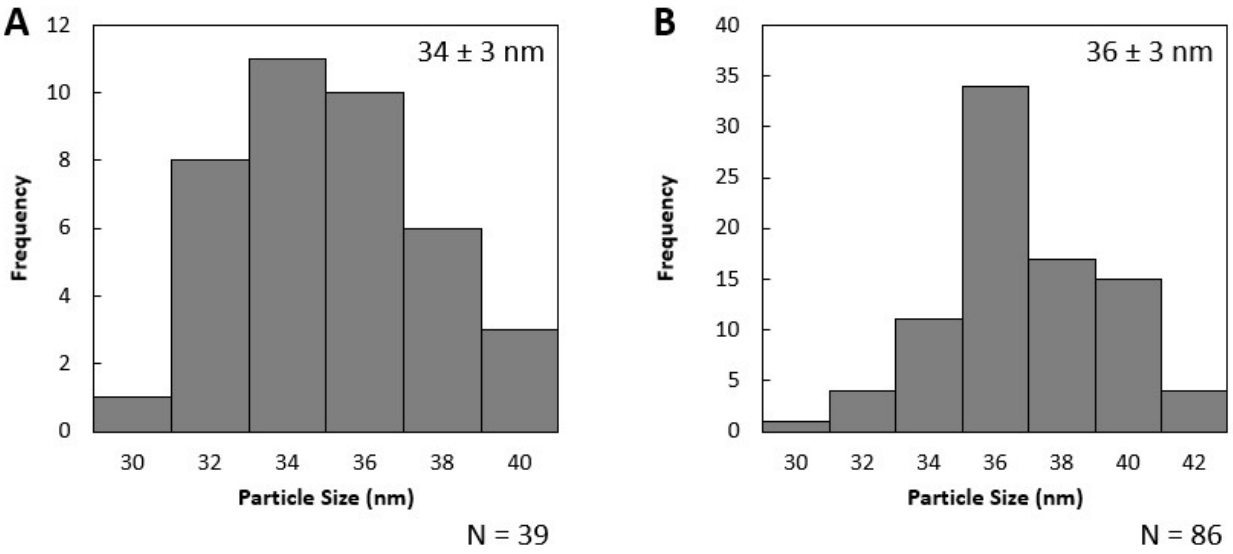


**Figure S1:** Histogram of $HBV_{SpyTag}$ particle sizes. Measurements of particle sizes from TEM images via Image J were taken of $HBV_{SpyTag}$ from (A) pooled sucrose gradient ultracentrifugation fractions 7-10 and (B) ELP[KV8F-40]-SpyCatcher ($ELP_{40}$-SpyCatcher) purification samples. Error in average particle diameter is the standard deviation of the respective measured diameters.

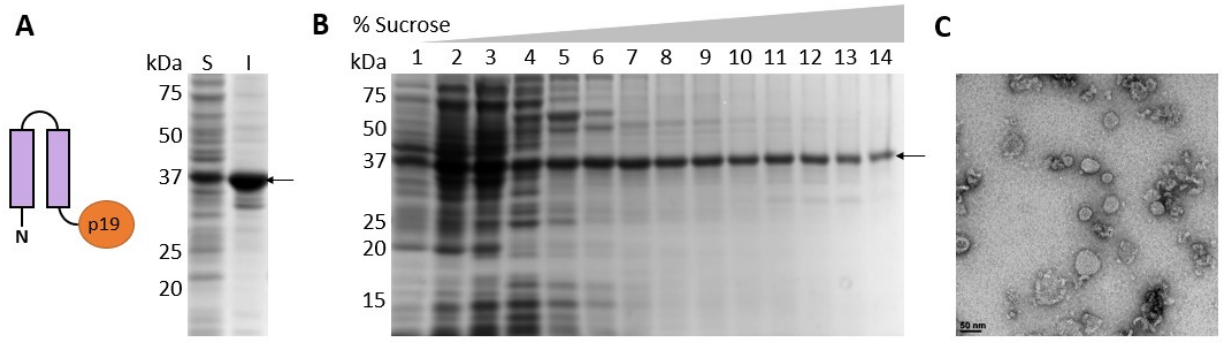


**Figure S2:** Analysis of HBV-p19 protein self-assembly. **(A)** HBV-p19 was expressed in BL21 and the soluble and insoluble lysates were assessed with SDS-PAGE. Arrow shows the location of the HBV-p19. **(B)** Sucrose gradient ultracentrifugation separated HBV-p19 proteins that formed capsid-sized nanostructures (fractions 7-10) from proteins that were not assembled (fractions 1-6) and proteins that formed larger aggregates (fractions 11-14). Arrow shows the HBV-p19 protein across the different fractions. **(C)** TEM demonstrated the protein structures that were formed with pooled fractions (8 and 9) from the sucrose gradient analysis.

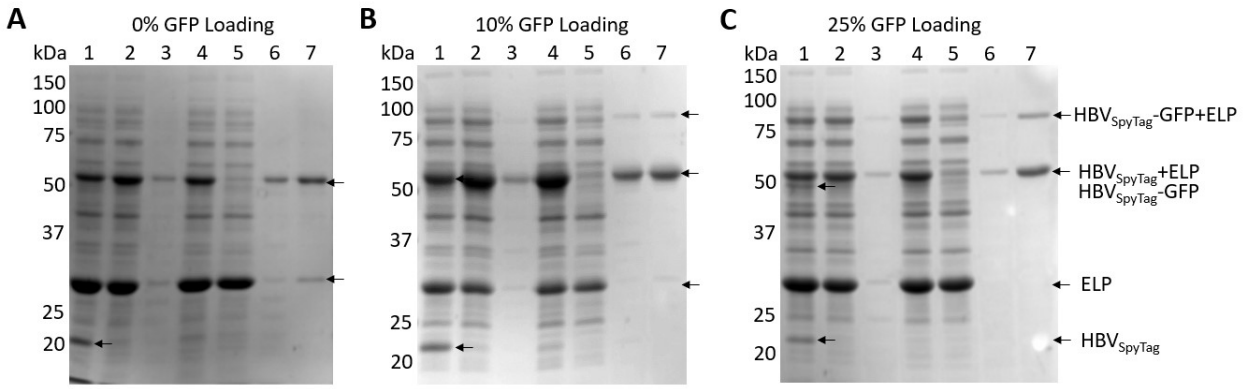


**Figure S3:** $ELP_{40}$-SpyCatcher reaction with $HBV_{SpyTag}$(GFP) lysates and subsequent inverse transition cycling. SDS-PAGE analysis was performed to assess the ligation of $ELP_{40}$ purification tags to $HBV_{SpyTag}$ VLPs with 0% **(A)**, 10% **(B)**, or 25% **(C)** GFP loading and their subsequent purification through ITC: (1) ELP-SpyCatcher mixed with $HBV_{SpyTag}$(GFP) lysate at a 2:1 molar ratio, (2) Protein sample after overnight reaction, (3) Insoluble proteins post-reaction, (4) Soluble proteins post-reaction, (5) ITC: soluble contaminants, (6) ITC: Insoluble contaminants, (7) ITC: purified VLPs. The percentages are the average per VLP.

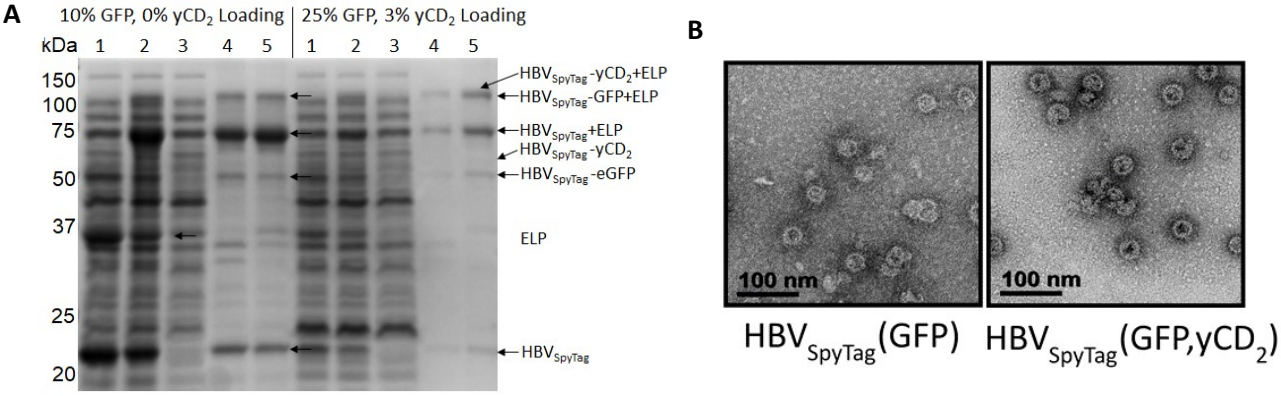


**Figure S4:** Purification of tri-expressed VLPs: $HBV_{SpyTag}(GFP,yCD_2)$ **(A)** $ELP_{40}$-SpyCatcher reaction with tri-expressed $HBV_{SpyTag}(GFP,yCD_2)$ VLPs and subsequent cycle of ITC: (1) ELP-SpyCatcher mixed with $HBV_{SpyTag}(GFP,yCD_2)$ respective sample at a 2:1 molar ratio, (2) Post-reaction protein sample, (3) ITC: soluble contaminants, (4) ITC: Insoluble contaminants, (5) ITC: purified VLPs. Arrows point to labeled corresponding protein species. The percentages are the average per VLP. (B) Purified HBVSpyTag(GFP,yCD2) remained intact as confirmed by TEM.

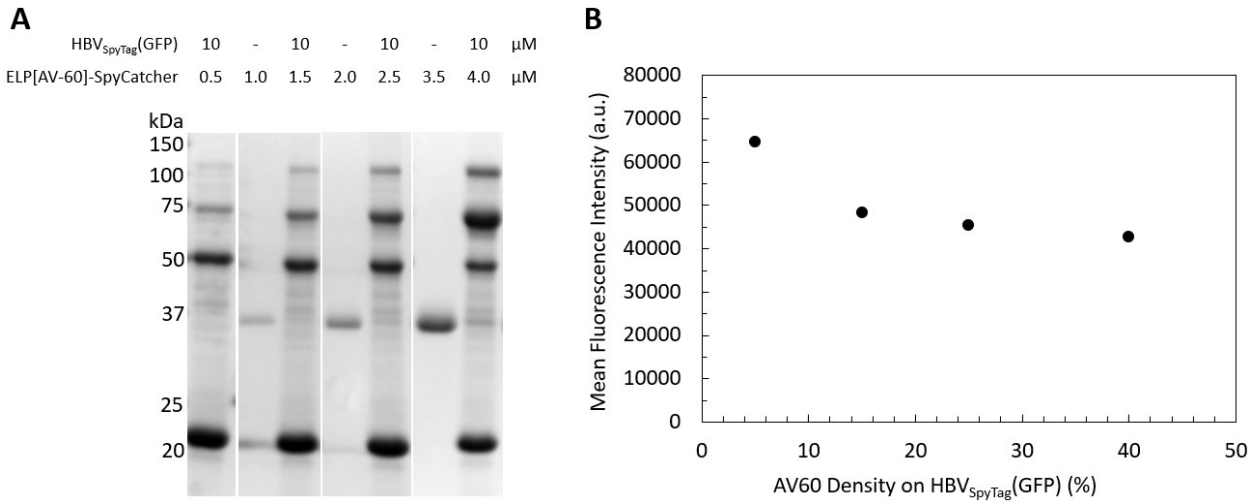


**Figure S5:** Assessing how ligation density of ELPs on exterior of HBV$_{SpyTag}$(GFP) VLPs affect non-specific uptake in SUM149 cells. **(A)** SDS-PAGE demonstrated modification of HBV$_{SpyTag}$(GFP) the exterior of the VLP with a 15%, 25% or 40% ligation density of ELP[AV-60]-SpyCatcher (ELP$_{60}$-SpyCatcher). **(B)** Mean fluorescence intensity of HBV$_{SpyTag}$(GFP) modified with 5-40% ELP$_{60}$-SpyCatcher was delivered to SUM149 cells and assessed by flow cytometry. The percentages are the average per VLP.

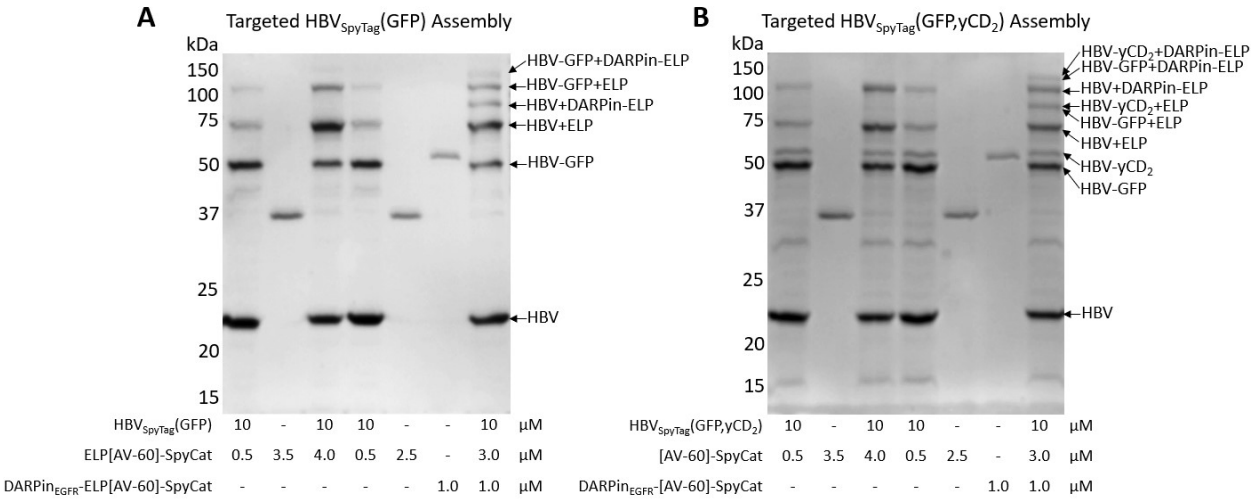


**Figure S6:** Assembly of targeted HBV$_{SpyTag}$(GFP) and HBV$_{SpyTag}$(GFP,yCD$_2$) for cell-specific delivery. SDS-PAGE demonstrated modification of dual-expressed HBV$_{SpyTag}$(GFP) **(A)** and tri-expressed HBV$_{SpyTag}$(GFP,yCD$_2$) VLPs **(B)** with a total 40% ligation density of ELP$_{60}$-SpyCatcher, with targeted VLPs having a 10% density of DARPin$_{EGFR}$ added at the N-terminus of 25% of the ligated ELP$_{60}$-SpyCatcher proteins. The percentages are the average per VLP.

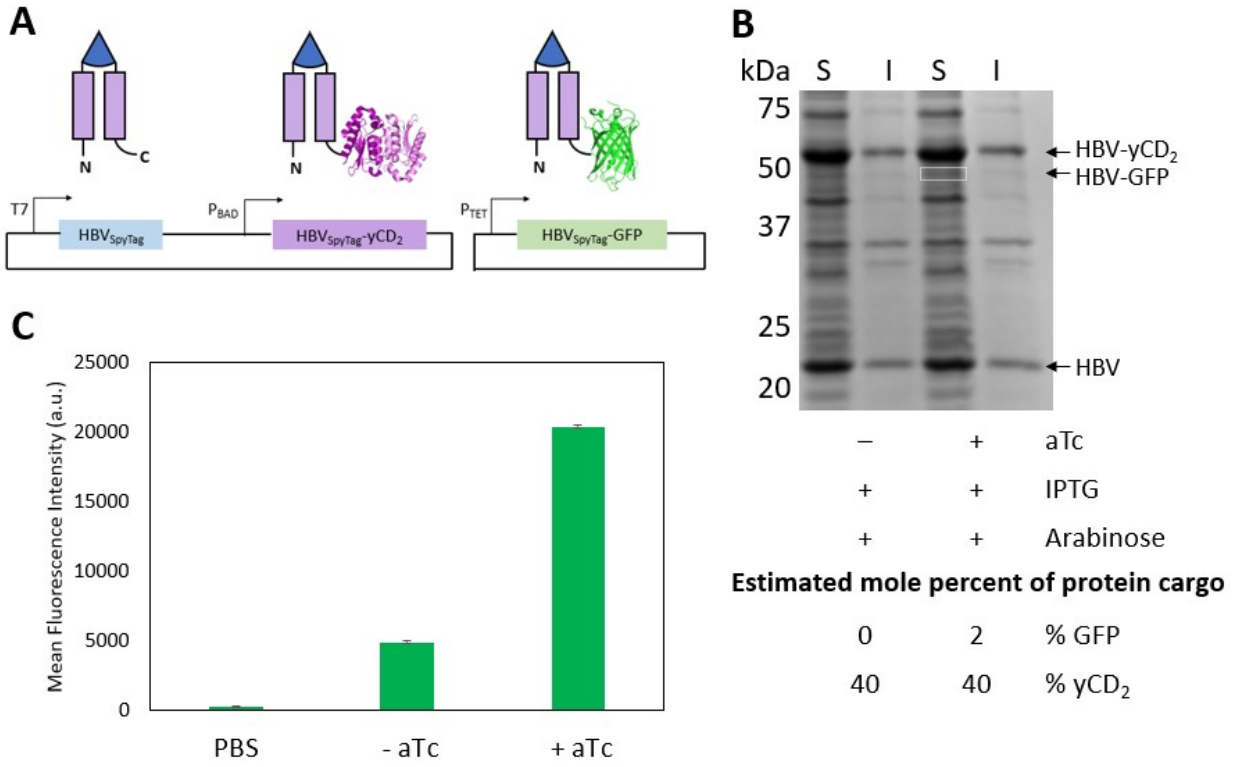


**Figure S7:** Alternative tri-expression system for high $yCD_2$ loading in VLP. **(A)** The original tri-expression system was altered by switching the *E. coli* promoter controlling the $HBV_{SpyTag}$-$yCD_2$ and $HBV_{SpyTag}$-GFP genes. **(B)** SDS-PAGE analysis of soluble and insoluble lysates resulting from inducing expression with IPTG and arabinose or aTc, IPTG, and arabinose using the modified tri-expression system. Estimated mole percent of protein cargoes was assessed by using densitometry. Boxed protein band on SDS-PAGE corresponds to HBV-GFP protein. The percentages are the average per VLP. **(C)** Fluorescence of the soluble lysate samples with or without aTc was measured to confirm GFP production (Ex: 470 nm, Em: 520 nm). Error bars are standard deviation from technical replicates (N = 3).

*Section 2: Supplementary Information*

**Tables S1:** DNA primers used in restriction enzyme cloning

| **Name:** | **Sequence:** |
|---|---|
| KpNI-GGGSGGG-SpyTag-GG-BamHI(+) | CGGAGGGGGAAGCGGTGGAGGTGCACAC ATAGTAATGGTAGACGCCTACAAGCCGAC GAAGGGTGGAG |
| KpNI-GGGSGGG-SpyTag-GG-BamHI(–) | GATCCTCCACCCTTCGTCGGCTTGTAGGCG TCTACCATTACTATGTGTGCACCTCCACCG CTTCCCCCTCCGGTAC |
| NcoI-DARPin_EGFR_Fw | GATGATCCATGGATCTGGGCAAAAAATTG TTAGAAG |
| NdeI-S-$(G_4S)_2$-GGSA-DARPin_EGFR_Rev | GATGATCATATGGCTTCCACCACCTCCTG AGCCGCCACCACCAGAGCCGCCGCTAGC ATTCAGTTTCTGCAGAATTTCCG |
| SacI-$HBV_{SpyTag}$_Fw | GATGATGAGCTCGATCCGGCTGCTAAC |
| XhoI-$HBV_{SpyTag}$_Rev | GATGATCTCGAGCCAACAACAGTAGTCTC CGGAAGTGTTG |
| XhoI-A-$(G_3S)_3$-$G_4$T-AgeI (+) | TCGAGTGCAGGTGGCGGTAGTGGTGGCGG GTCTGGAGGTGGCTCAGGCGGTGGAGGGA CTA |
| XhoI-A-$(G_3S)_3$-$G_4$T-AgeI (–) | CCGGTAGTCCCTCCACCGCCTGAGCCACC TCCAGACCCGCCACCACTACCGCCACCTG CAC |
| XhoI-GFP_Fw | GATGATACCGGTATGGTGAGCAAGGGCG |
| SacI-His6-GFP_Rev | GATGATGAGCTCTTAGTGATGGTGATGGT GATGCTTGTACAGCTCGTCCATG |
| AgeI-yCD_Fw | GATGATACCGGTGTGACCGGCGGAATG |
| BamHI-$G_3$-yCD_Rev | GATGATGGATCCACCGCCACCTTCTCCAA TATCCTCAAACCAGTC |
| BamHI-$G_4$S-yCD_Fw | GATGATGGATCCGGTGGCGGGGGTTCGGT GACCGGCGGAATG |

| | |
|---|---|
| AvrII-His6-yCD_Rev | GATGATCCTAGGTTAGTGATGGTGATGGT GATGTTCTCCAATATCCTCAAACCAGTC |
| SphI-HBV$_{SpyTag}$-GFP-h6 Fw | GATGATGCATGCATGGACATCGACCCTTA TAAAG |
| HBV$_{SpyTag}$-GFP-h6-AvrII Rev | GATGATCCTAGGTCATTAATGGTGATGGT GGTGATGCTTGTACAGCTC |

**Tables S2:** DNA primers used in Gibson assembly cloning

| **Name:** | **Sequence:** |
|---|---|
| pET24a-HBV$_{SpyTag}$ vector_Fw | GGGTCCTCAACGACAGGAG |
| pET24a-HBV$_{SpyTag}$ vector_Rev | CTGGTTAGCAGAATGAATCACCGATAC |
| araC-pBad promoter_Fw | ATCGGTGATTCATTCTGCTAACCAGTAA GGCAGTTATTGGTGCCC |
| araC-pBad promoter_Rev | CAAATTCTTTATAAGGGTCGATGTCCAT GACTAGTCTCCTTCTTAAAGTTAAAC |
| HBV$_{SpyTag}$-GFP_Fw | GACATCGACCCTTATAAAGAATTTGGAG |
| HBV$_{SpyTag}$-GFP_Rev | AACAGATCTGATATCGGCGCTGGCGCCT AGGGCTTTGTTAGCAGCCGGATC |
| rrn1-rrn2 term_Fw | CGCCAGCGCCGATATC |
| rrn1-rrn2 term_Rev | ATCGTGCTCCTGTCGTTGAGGACCCAAG CTTAAGCTTGCGTTCTTCG |