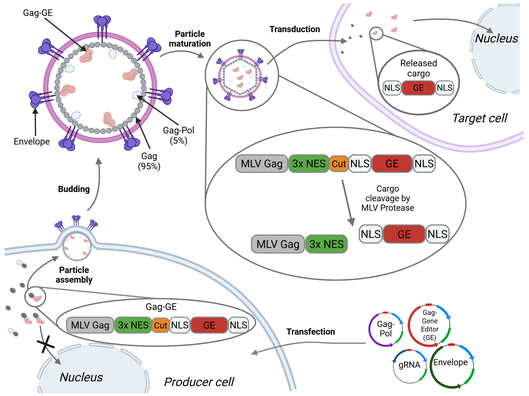
# Engineered Virus-Like Particles with Minimal Viral Components for Improved Base Editing Efficiency *In Vivo*

Karol Budzik, Dylan Dautel, Liana Behmoiras, Chen Wang, Geoffrey Burns, Stephen Pyon, Melissa Leitner, Matteo Gentili, Anne Richard, Angela Dao, Mahmudul Hasan, Claudia Pereira, Luca Biasco, Melissa Bonner, Yi Yu

nChromaBio, Boston, MA

## 1. DLVR-X for Gene Editor Delivery and the Need for Cargo Release Improvement

### The Importance of Properly Timed Cargo Cleavage

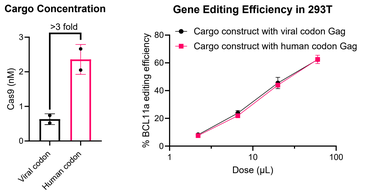



DLVR-X are engineered virus-like particles (eVLPs) based on Murine Leukemia Virus (MLV)[1]. Viral protease-mediated cleavage of Gag-GE (gene editor) and Gag needs to be tightly controlled for optimal particle assembly and potency.

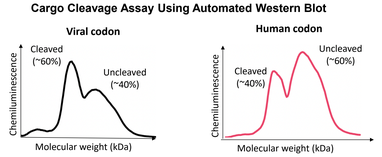
[1]Banskota, Samagya, et al. "Engineered virus-like particles for efficient in vivo delivery of therapeutic proteins." Cell 185.2 (2022)

### ~60% of Cargo is Cleaved in DLVR-X

Codon optimization of Gag-Cargo construct improved cargo loading but not the potency.

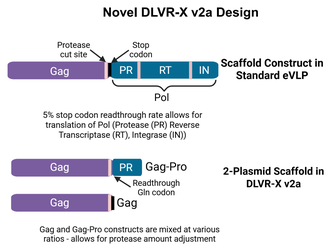



The cargo cleavage assay reveals that ~60% of the cargo is cleaved in standard DLVR-X particles. When cargo expression increases, the cleavage efficiency is decreased to ~40%.

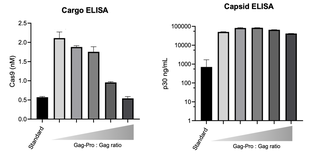



## 2. RT- and IN- Free DLVR-X (DLVR-X v2a) Improves Both Cargo Cleavage and Particle Assembly

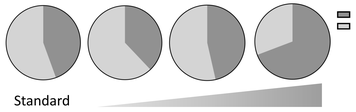
### DLVR-X v2a Improves Particle Titers but Leads to Premature Cargo Cleavage




DLVR-X v2a design results in higher Cas9 content and substantially improves particle assembly. Increasing the Gag-Pro:Gag ratio decreases editing and Cas9 content.



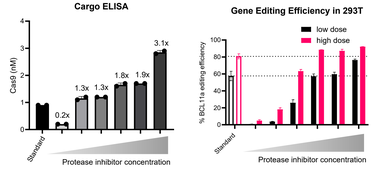

Premature Cas9 cleavage in *producer cells* increases with higher Gag-Pro:Gag ratio



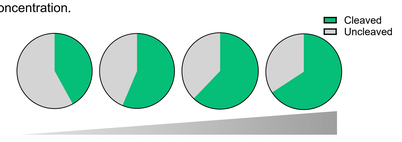

* Uncleaved/cleaved ratio calculated using peak height in cleavage assay


### Adding Protease Inhibitor Prevents Premature Cargo Cleavage and Improves DLVR-X v2a Particle Assembly

Increasing concentrations of the protease inhibitor improve the potency of DLVR-X v2a and cargo loading.

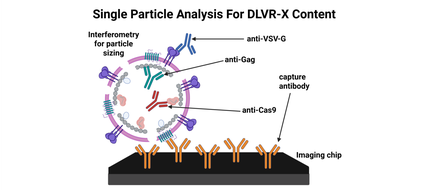



Cargo release in DLVR-X v2a particles improves with protease inhibitor concentration.

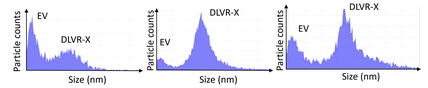



* Uncleaved/cleaved ratio calculated using peak height in cleavage assay

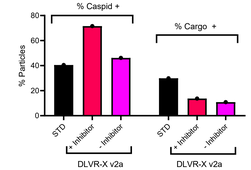
DLVR-X v2a with protease inhibitor leads to improved particle formation.




Size distribution indicates fewer EVs in DLVR-X v2a

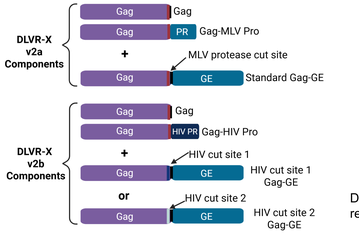



DLVR-X v2a increased capsid loading but not per particle cargo loading.

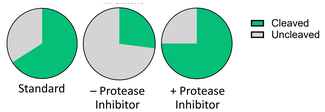



## 3. Chimeric DLVR-X v2b with HIV Protease Works at Lower Protease Inhibitor Concentration

HIV protease is more sensitive to protease inhibitors than MLV protease, as a result, requires less inhibitor.

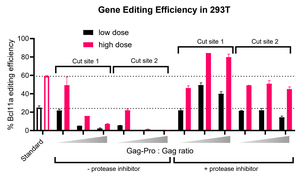



**Cargo release in DLVR-X v2b**

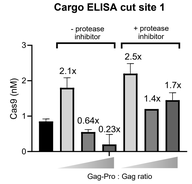



* Uncleaved/cleaved ratio calculated using peak height in cleavage assay

DLVR-X v2b with HIV protease and cargo with cut site 1 leads to better editing than standard DLVR-X.



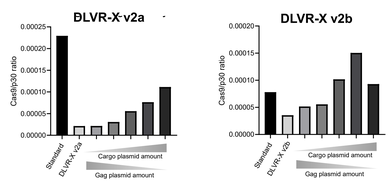

DLVR-X v2b resulted in improved cargo loading and release at lower protease inhibitor concentrations.



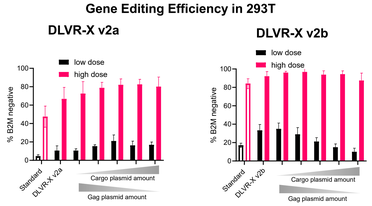

## 4. Optimized DLVR-X v2 Designs Resulted in > 10x Higher Particle Titers and ~2-fold Increase Potency in Mouse Livers at Normalized Doses

### Further Optimization Improves Cargo Packaging

Plasmid ratio optimizations lead to higher cargo/capsid ratios based on ELISA analysis.

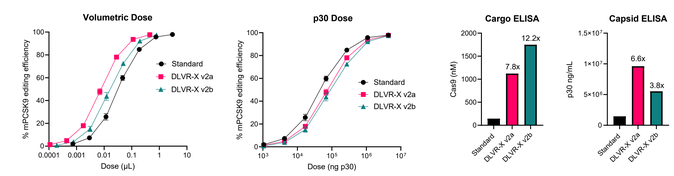



**Gene Editing Efficiency in 293T**



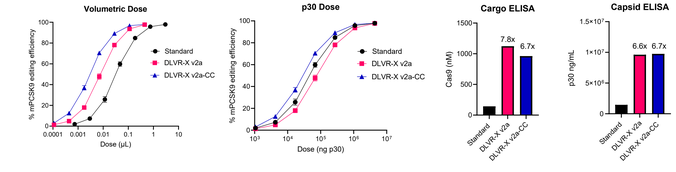

### DLVR-X v2 Particles have Increased Productivity and Potency in Mouse Livers

Optimized DLVR-X v2 particles show up to 12-fold increase in productivity. A slight decrease in editing in N2A cells was observed with capsid protein (p30) dose normalization.

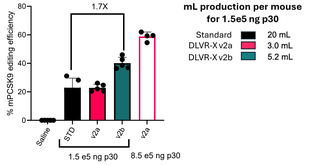



DLVR-X v2a with further cargo loading improvements resulted in higher editing in N2A cells with p30 normalization While maintaining ~ 7-fold increase in productivity.

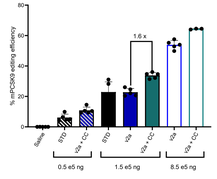
**For details on the loading strategy, see poster 1693.**




At the same p30 dose, equal (DLVR-X v2a) or higher (DLVR-X v2b) potency was observed in mouse liver.




Additional cargo loading improvements increase the *in vivo* potency of DLVR-X v2 .




## Conclusions and Next Steps

- DLVR-X enables efficient macromolecule delivery, including gene editors and their RNPs.
- Here, we demonstrate the development of DLVR-X v2, the first RT- and IN-free MLV eVLPs for gene editor delivery. A two-plasmid scaffold was used to allow the tuning of viral protease expression, and a protease inhibitor can be used during the particle production process to inhibit premature cargo release.
- DLVR-X v2 showed ~10-fold higher p30 titers than standard DLVR-X in scaled-up productions, and after p30 normalization, equal (DLVR-X v2a) or higher (DLVR-X v2b) potency was observed in mouse liver. DLVR-X v2 designs dramatically decrease the scale needed for particle production and future manufacturing costs.
- Alternative cargo loading strategy further improved the *in vitro* and *in vivo* potency of DLVR-X v2a.
- Future work includes further DLVR-X v2a and v2b optimization with the alternative cargo loading strategy for the application in different therapeutic programs.