Dyno-86m: Optimizing Intravitreal Delivery to the Non-Human Primate Retina with Machine-Guided AAV Capsid Design

Shireen Abesteh, Ian Brenckle, Zac Cargill, Ina Chen, Sam Chen, Daniela Deny, Barbara Diaz-Rohrer, Jumana Fathima, Jeff Gerold, Arpan Ghosh, Jacob Grush, Sarah Hilton, Rishi Jajoo, Eric Kelsic, Helene Kuchwara, Jamie Kwasnieski, Kathy Lin, Erik Lykken, Eryney Marrogi, Katie Maryak, Patrick McDonel, Hanna Mendes Levitin, Amanda Miles, Nishith Nagabhushana, Daniela Pignatta, Brandon Satinsky, Bhavica Saxena, Cem Sengel, Abigail Sheridan, Timothy Tarbell, Meghan Thommes, **Heikki Turunen**, Adrian Veres, Nick White, Sam Wolock
Dyno Therapeutics, Inc., Watertown, Massachusetts, USA

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Abstract

There is an unmet need for safer and more efficient payload delivery methods to enable gene therapies with improved clinical outcomes in the eye. Dyno's platform enables machine-guided design of optimized capsid variants, *in vivo* characterization of capsid libraries, and *in vivo* validation of capsid transduction properties. Through this approach we designed and validated an AAV capsid variant, dyno-86m, with remarkably improved efficiency in transducing the neural retina following intravitreal (IVT) delivery into cynomolgus macaque non-human primate (NHP) eyes. Single-cell sequencing and histological analyses confirmed that dyno-86m dramatically outperforms AAV2 and is consistently better than external engineered IVT capsid variants in transducing photoreceptors, at rates with clinically meaningful potential.

Summary

- **Single-nuclei RNA sequencing demonstrates transduction of all retinal cell types by dyno-86m in cynomolgus macaque**
- **Quantification of transduction by histology confirmed snRNAseq and NGS based measurements**
- **As shown by histology and snRNAseq, dyno-86m transduced NHP retina cells up to 3x more efficiently than an external engineered capsid, and by NGS 80x more efficiently than AAV2, with no reduction in AAV production efficiency**
- **The dyno-86m capsid is available for immediate licensing. Contact bd@dynotx.com to learn more about licensing and emerging validated capsids from more recent NHP studies**

Platform Overview

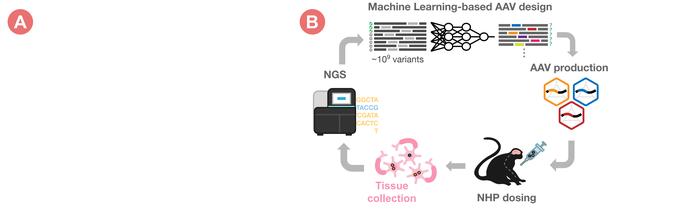

Figure 1. Dyno's capsid discovery platform. **(A)** Dyno utilizes machine guided design and high throughput DNA synthesis to engineer variants with improved functional properties in large, high quality libraries. **(B)** Optimization of variants is iterative and builds on previous data. Variants are designed *in silico* and characterized *in vivo* by NGS of variant-associated barcodes from NHP tissues. The data are used to train machine learning models to enable design of next-generation libraries.

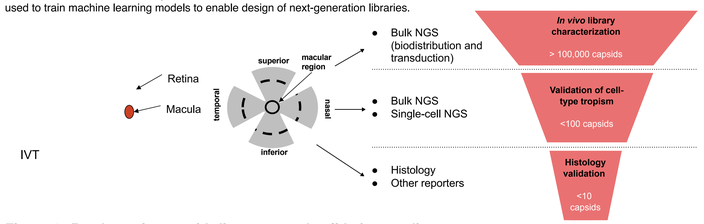

Figure 2. Readouts for capsid discovery and validation studies. Initial characterization of variants employs complex (>1e5 variants) capsid libraries and utilizes NGS readouts to measure biodistribution and transduction in ocular and non-ocular tissues. *In vivo* validation is performed in smaller scale studies and provides bulk and single-cell NGS (<100 variant/experiment scale) as well as histology based readouts (<10 variant/experiment scale).

NGS Validation

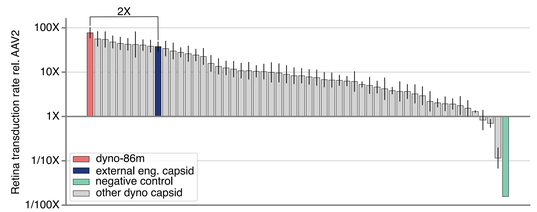

Figure 3. The dyno-86m capsid outperforms an external engineered capsid and dramatically outperforms AAV2 in transducing the NHP retina following IVT delivery. Transduction of capsid variants in NHP retina relative to AAV2 and normalized to variant abundance (measured by NGS of expressed variant-associated barcodes) in a library of <100 variants. The dyno-86m capsid originates from an *in vivo* library characterization study. An external capsid is highlighted as a reference based on its consensus status as an oft-compared-to engineered capsid which has been reported to exhibit significantly improved retinal delivery over AAV2. Error bars show 95% confidence intervals based on SEM across replicates.

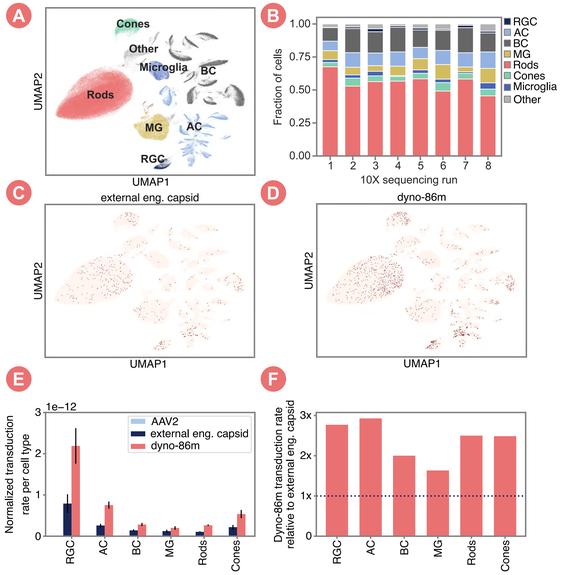

Figure 4. Single-nucleus RNA sequencing shows dyno-86m consistently transduces all NHP retinal cell types more efficiently than the external engineered capsid. **(A)** UMAP projection of ~320,000 profiled retinal cells and **(B)** relative ratios of retinal cell types in 8 separate 10X runs. **(C-D)** UMAP projection shaded by transduction events by the external engineered capsid and dyno-86m, respectively. **(E)** Transduction rates (fraction of transduced nuclei normalized by dosage of test articles) in retinal cell types. **(F)** Fold improvement of dyno-86m transduction rate as compared to the external engineered capsid. RGC=retinal ganglion cells; AC=amacrine cells; BC=bipolar cells; MG=Müller glia.

Histology Validation

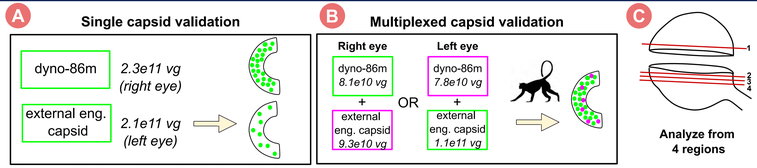

Figure 5. Design of histological validation study. Dyno-86m transduction efficiency was compared to the external engineered capsid by immunofluorescent (IF) staining of eGFP and mCherry reporter proteins in cynomolgus macaque. **(A)** Vectors were injected into separate eyes, or **(B)** mixed together at roughly equal ratios (as measured by NGS) and co-injected into the same eyes. **(C)** Collection strategy of retinal sections for IF staining.

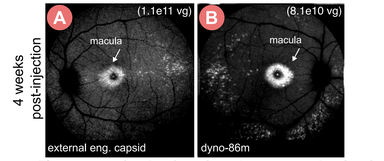

Figure 6. Fluorescent fundus imaging shows transduction in the NHP macula and peripheral retina. Representative fluorescent fundus images show eGFP expression resulting from transduction at 4 weeks post-dosing by **(A)** the external engineered capsid and **(B)** dyno-86m.

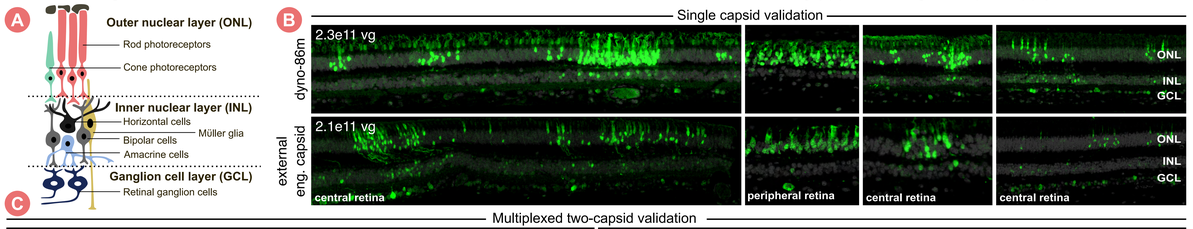

Figure 7. The dyno-86m capsid transduces cells in all retinal layers in NHP non-macular retina. **(A)** Schematic overview of retinal cell layers. **(B)** IF staining shows strong transduction in the ONL by dyno-86m (top) and by the external engineered capsid (bottom). **(C)** IF staining of two eyes injected with two reporter combinations shows stronger transduction by dyno-86m as compared to the external engineered capsid.

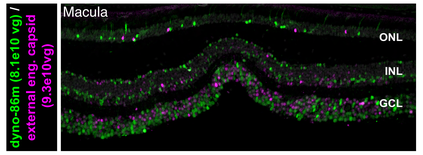

Figure 8. The dyno-86m capsid transduces cells in all retinal layers in the NHP macula. Transduction by dyno-86m shown in green and by the external engineered capsid in magenta.

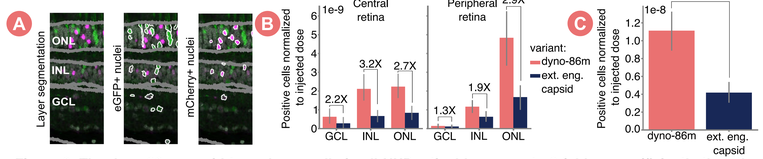

Figure 9. The dyno-86m capsid transduces cells in all NHP retinal layers up to 3-fold more efficiently than the external engineered capsid. **(A)** Example images showing automated quantification of eGFP and mCherry expressing cells. **(B)** Quantification of dyno-86m and the external engineered capsid transduction in different retinal layers and regions or **(C)** across the whole retinal cross-section normalized to the dose per vector (n=4 sections/eye averaged across 2 injected eyes).