Poster 900

Next-generation automated AAV engineering platform for rapid identification of efficient and specific capsids in non-human primates

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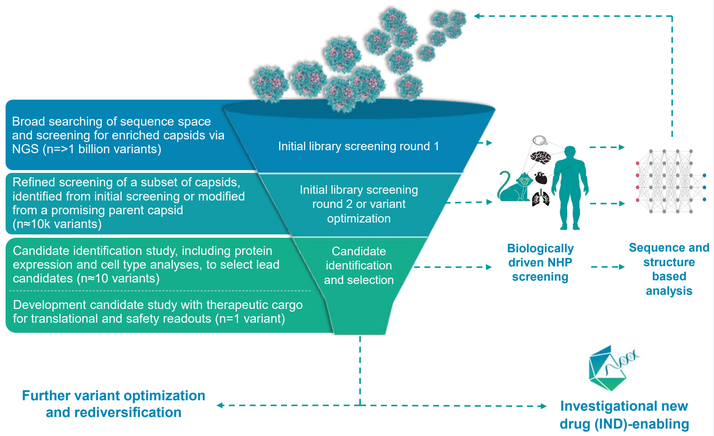
Capsida Biotherapeutics' novel, NHP-driven, AAV engineering platform



Capsida's platform for directed evolution and screening in primates generates novel AAV capsids capable of reaching target tissues throughout the body following intravenous (IV) delivery

Scalable generation of high-complexity and high-fidelity AAV libraries

- Engineering across multiple surface-exposed loops enables the generation of high-complexity libraries capable of targeting multiple organs and cell types
- Novel DNA assembly methodology with onboard error correction enables large-scale assembly of high-fidelity, transfection grade library DNA in an automated, scalable format

Fully automated screening platform enables rapid selection of improved capsids

- Automated tissue and sample processing capabilities drive biweekly screening campaigns in NHPs
- Automated capabilities allow for interrogation of multiple therapeutic areas in parallel
- Increased volume of processed tissue has led to dramatic improvements in data quality feeding into the analysis pipeline

Rich biological datasets are driving predictive analytical capabilities

- Large-format next generation sequencing (NGS) platform feeds data into a bioinformatics pipeline for analysis and data mining
- As capsids progress through engineering, candidate identification and lead candidate selection, biological data enables machine learning to generate predictive analysis

Abstract

The rapid development of gene therapies, in recent years, has highlighted the critical need for more efficient and specific delivery vectors for targets throughout the human body. Previous engineering platforms, which focused on high-throughput screening and directed evolution in lower-order animal models led to dramatically improved capsids for a variety of targets, including organ-level and cell type-level specificity.[1,2] However, the translatability of these engineered capsids has proven challenging.[3,4]

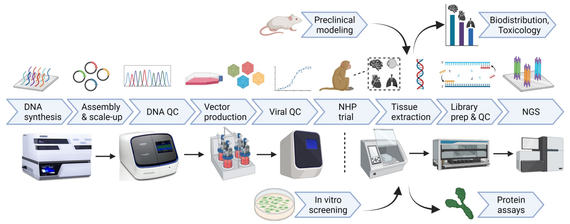
To bypass the challenges of translating capsids engineered *in vitro* or in lower-order species, while still leveraging the success of directed evolution in a high-throughput and high-capacity manner, Capsida Biotherapeutics has developed an automated adeno-associated virus (AAV) engineering platform capable of interrogating targets through the body, directly in non-human primates (NHPs) and human cell lines at a rapid pace. This platform has generated several iterations of improved capsids for targets in the central nervous system (CNS) and is now being leveraged for targets throughout the body. In addition to increased capacity and throughput, the automated engineering platform has simultaneously improved data quality by increasing the volume of interrogated primate tissues, enabling greater predictive analysis in the bioinformatics platform and more rapid identification of improved capsids.

References

1. Deverman BE *et al. Nat Biotechnol* 2016;34:204–9
2. Kumar SR *et al. Nat Methods* 2020;17:541–50
3. Hordeaux J *et al. Mol Ther Methods Clin Dev* 2018;10:79–88
4. Liguore WA *et al. Mol Ther* 2019;27:2018–37

1. Automated solutions to standard wet lab protocols enable greatly increased capacity

Version 1 automation platform (2021) – Islands of automation



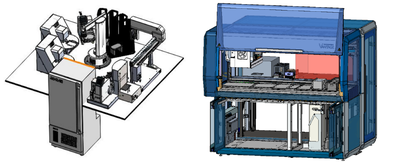
A highly complex workflow underlies any viral engineering efforts – a high-throughput platform capitalizes on industrialization with an assembly line of specialized robotics

The complexity of activities necessary to support high-throughput screening of biomolecules directly in primates, and transition them into the clinic, necessitates automated facilitation. The process of low-throughput screening in lower-order animal models was redesigned from the ground up to leverage robotic capabilities

Key automation advantages:

- Data quality
  - Consistent process for every run, no accidental cross-sample mixing
- Throughput
  - Process more samples at once, re-assign employees to human-critical work, run samples during off-hours

Version 2 automation platform (2022) – Integrated work cells

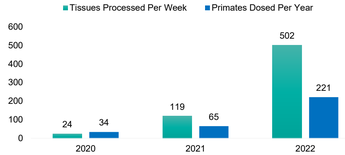


Video

Integrated work cells that consolidate multiple devices into single systems allow for >5x scale-up in processing capacity. This ability to screen larger numbers and volumes of tissues is critical to support the rapid generation of potentially life-saving capsid variants

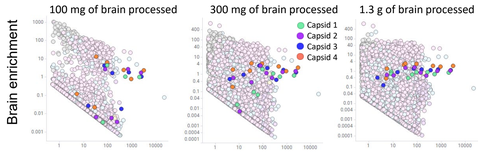
- The scale of Capsida's activities has exponentially increased since inception, driven by Version 1 automation in 2021 and Version 2 in 2022
- The flexible nature of our directed evolution platform means greater capacity enables expansion into novel therapeutic areas beyond the CNS

Platform scalability



2. Process scale-up significantly improves data quality

$$cpm = \frac{reads\ of\ variant}{total\ reads} * 1E6 \qquad enrichment = \frac{cpm\ (measured\ output\ in\ tissue)}{cpm\ (input\ virus)}$$



Control capsid dose-titration curves highlight improved variant resolution upon brain region aggregation

In a library of 20,000 novel variants injected into NHPs, four controls were intentionally dosed in replicate across a range of input concentrations. Limits of detection with traditional processing cause low quality data for any variants not injected at a high dose. By processing greater tissue volumes, control curve linearity is extended further, reflecting the ability to quantify CNS enrichment of low-prevalence variants. This effectively increases the number of variants from which data is captured in each engineering study, minimizing engineering rounds needed to achieve a desired capsid.

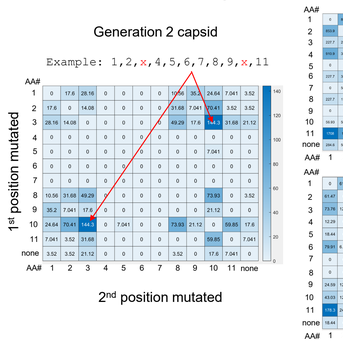
Poster PDF

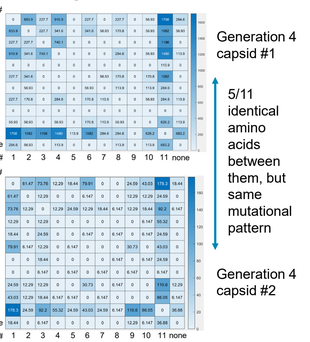
Video

Please scan this quick response (QR) code with your smartphone camera or app to obtain a copy of these materials. Alternatively, please click here to download the video.

3. Refined analyses enabled by increased sampling capacity

Mutational hotspot analysis highlights differences between capsid families and paths for further mutagenesis

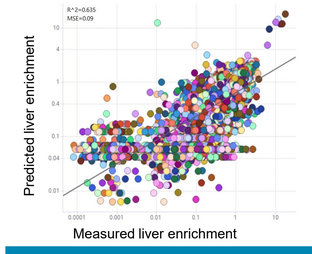




- A multi-site mutational substitution strategy was used on generation 2 and generation 4 capsids (see poster 905), and this library of >10k variants was screened across multiple NHPs. Capsids identified with CNS enrichment greater than the parent were analyzed for mutational pattern
- Positional crossplots sum brain enrichment across top variants and reveal dark 'mutation pair hotspot' points, indicating regions for additional mutagenesis
- Shared patterns between certain capsids solidify the assignment of capsid motifs and point to different *in vivo* mechanisms of action that can be further investigated
- These same analyses are being actively applied to additional capsid properties, such as vector productivity and liver detargeting

4. Machine learning generates accurate capsid phenotype predictions

Machine learning models trained on our rich biological datasets generate accurate predictions of capsid performance, demonstrating the power of machine learning to expedite the search for ideal capsids. A random forest ensemble model trained on data from two primate studies testing 40,000 capsid variants *in vivo* yielded accurate predictions of liver detargeted variants never seen by the model. Model-based feature selection algorithms enable an efficient search of capsid sequence space by finding highly predictive amino acid properties at key positions



$$MSE = \frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2$$

Mean squared error

$$R^2 = 1 - \frac{RSS}{TSS}$$

RSS = sum of squares of residuals
TSS = total sum of squares

Model exhibits low mean squared error of prediction and strong correlation between predicted and measured capsid variant performance in a given tissue following IV delivery to a primate. Predicting capsid liver enrichment values allows us to carry forward only those vectors with a minimized chance of causing liver toxicity in the clinic

Conclusions

- Capsida Biotherapeutics' fully automated, high-throughput platform screens billions of novel capsids to find those that are improved in primate CNS transduction following IV delivery
- The capacity of the platform is being leveraged to optimize capsid efficacy and specificity, with the ability to expand into multiple additional therapeutic areas
- The wealth of biological data generated by our platform is a fertile substrate for structural and bioinformatic analysis, guiding future engineering rounds