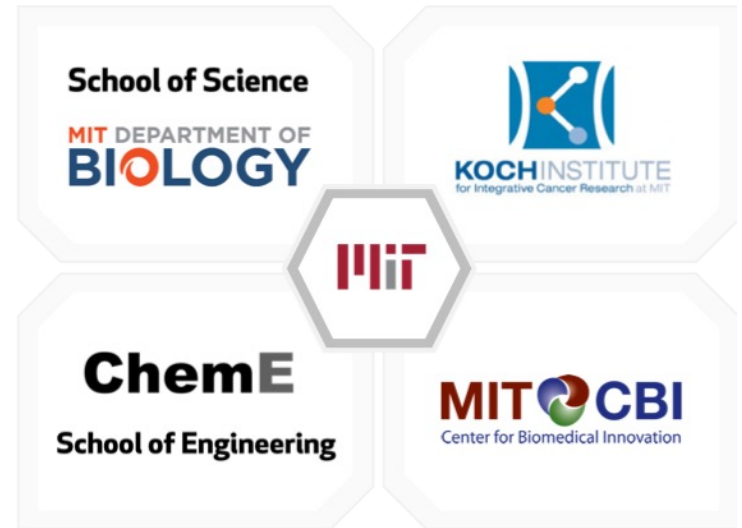


Rational Design of rAAV Production via Mechanistic Modeling

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Project team: Continuous Viral Vector Manufacturing Based on Mechanistic Modeling and Novel Process Analytics



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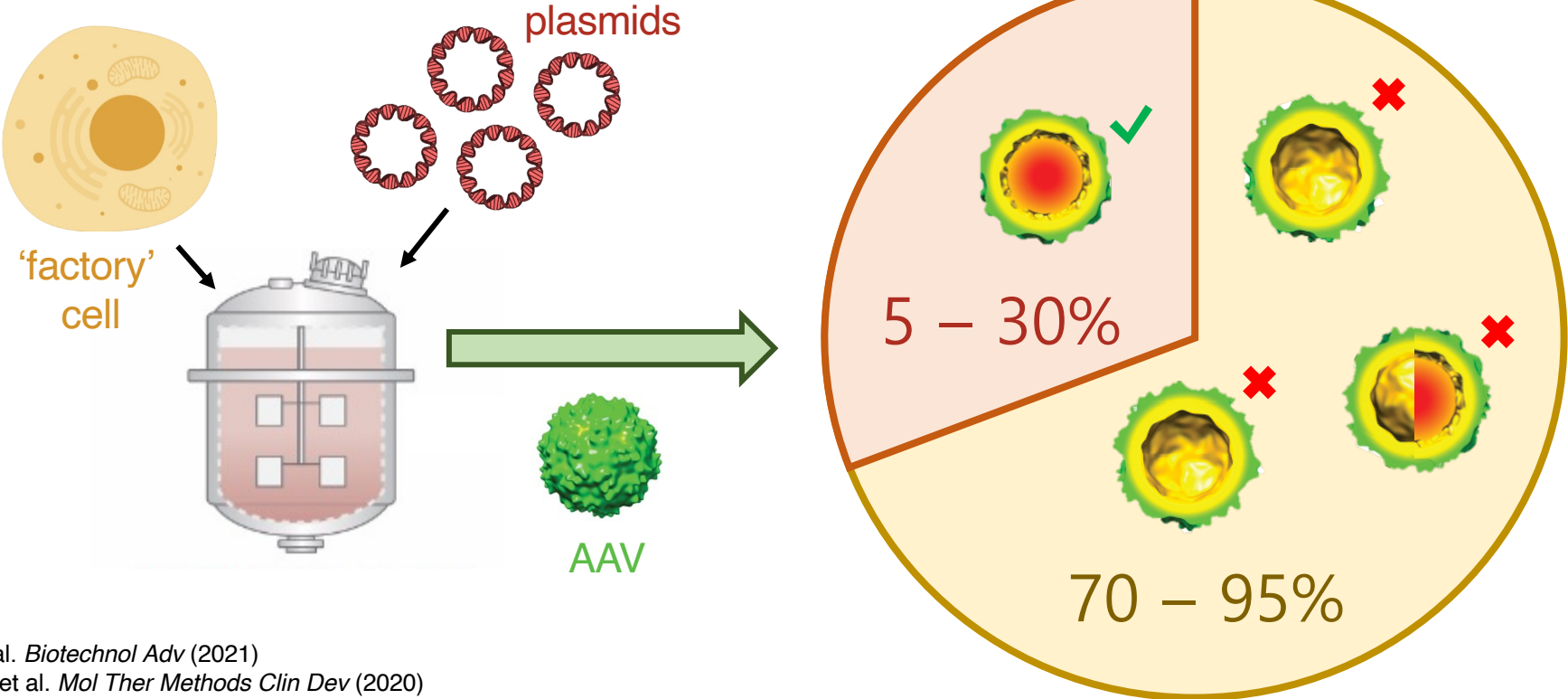


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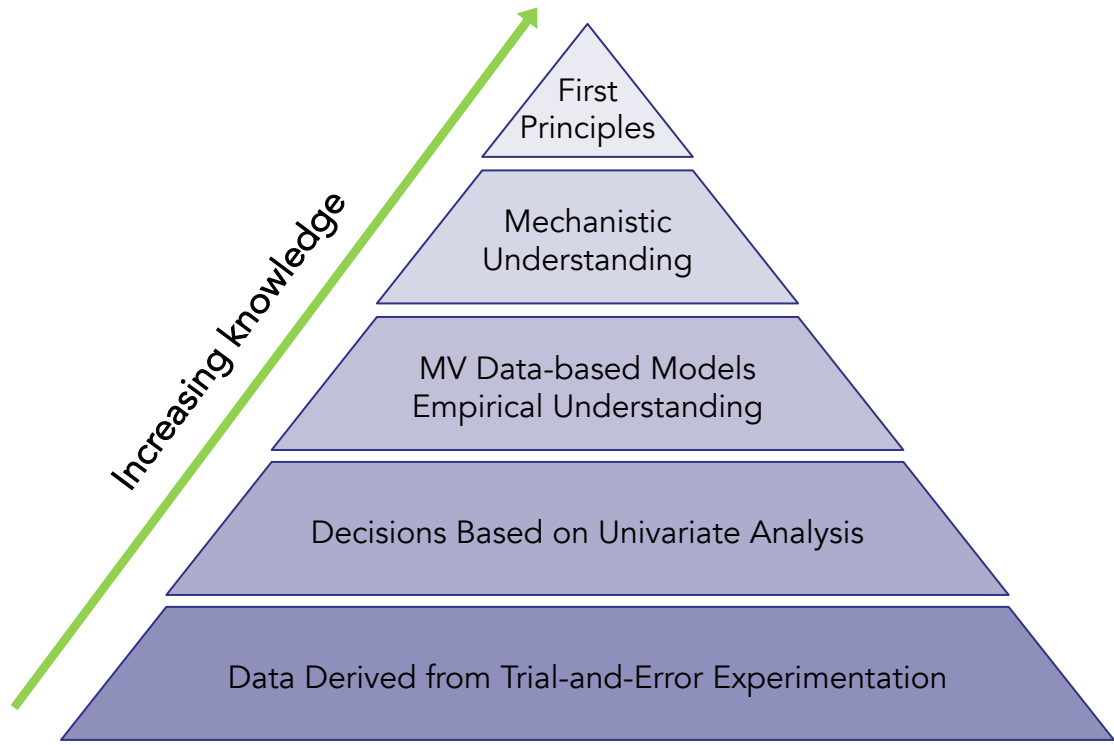
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Conventional rAAV production workflow results in high ratio of empty capsid in crude harvest



Sha et al. *Biotechnol Adv* (2021)
Gimpel et al. *Mol Ther Methods Clin Dev* (2020)
Joshi et al. *Mol Ther Methods Clin Dev* (2019)
Wang et al. *Mol Ther Methods Clin Dev* (2017)

Mechanistic models leverage increased knowledge and understanding



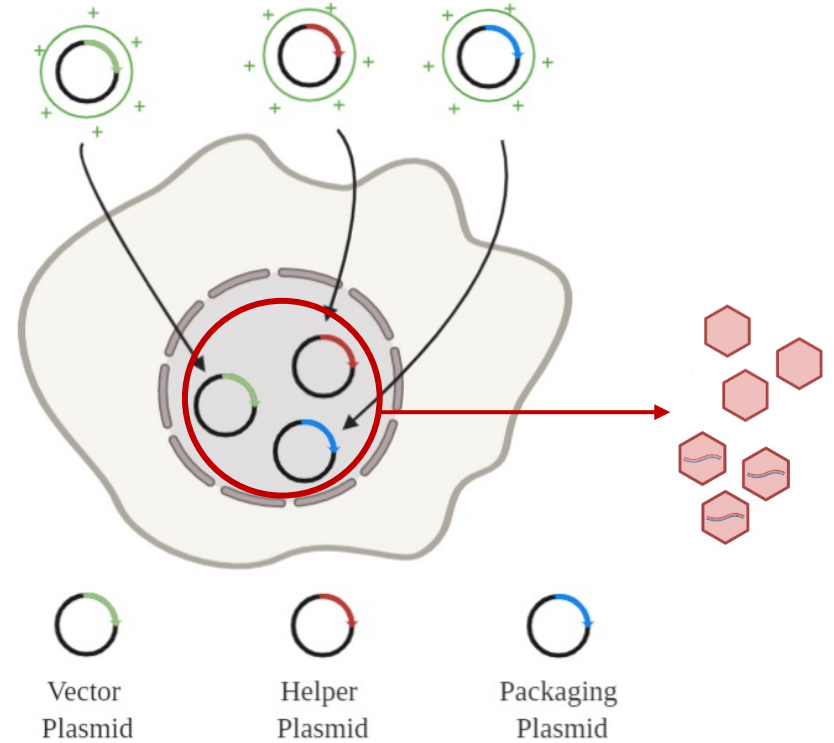
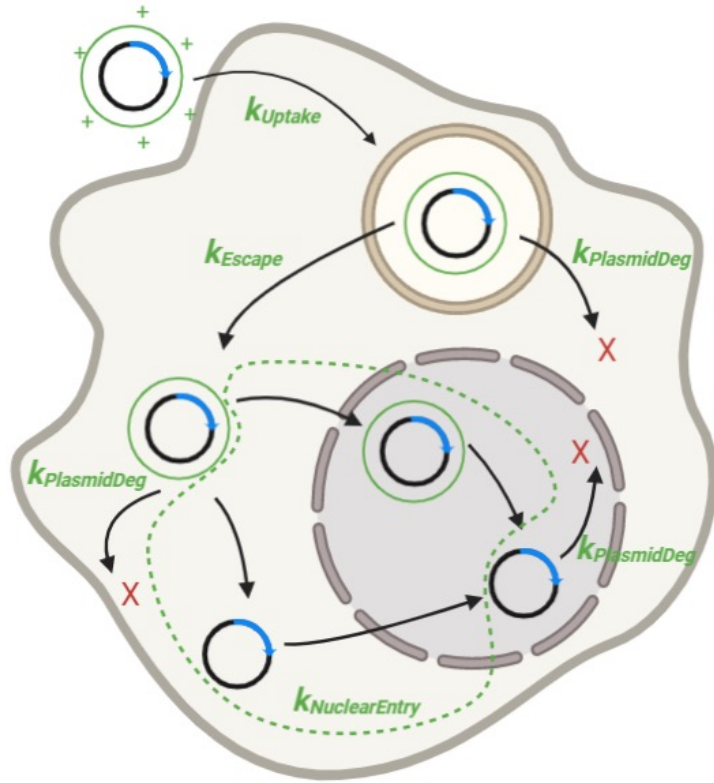
Adapted from WHO (2008)

Develop a mechanistic model for rAAV production via triple transfection

1. Combine virology, cell biology, and process dynamics
2. Utilize experimental data to estimate model parameters and to iteratively improve upon the developed model

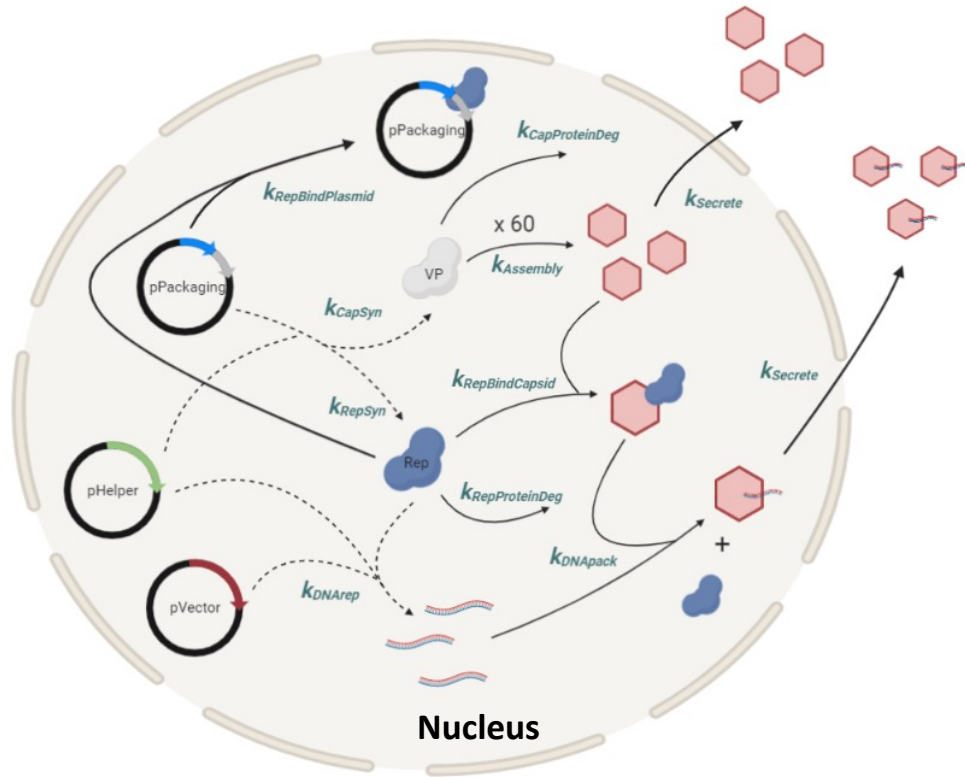
Expected outcome: the analysis using a mechanistic model will reveal the trajectory and bottlenecks in rAAV production, therefore improving our capability of process designs

Mechanistic model for PEI-mediated delivery



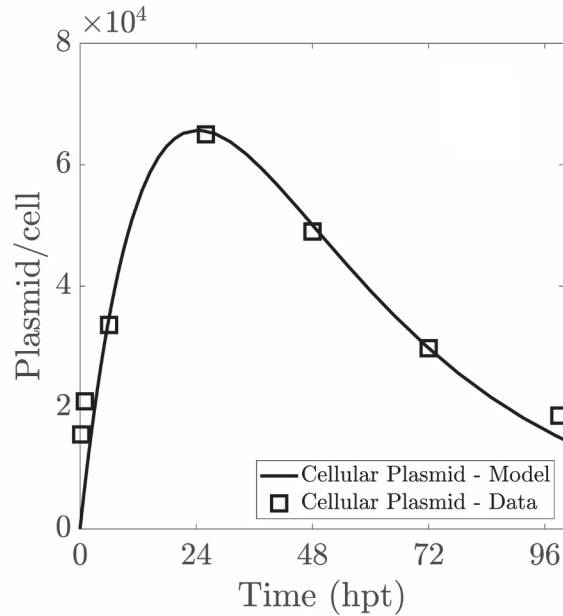
Adapted from Varga et al. Gene Therapy (2005)

Mechanistic model for viral production

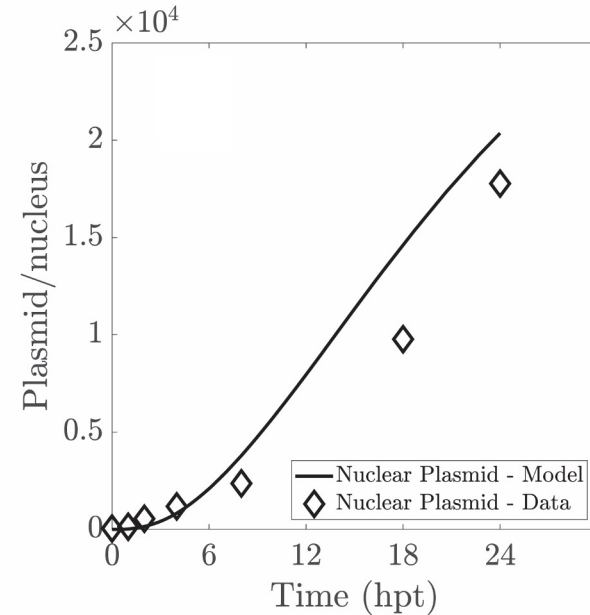


- Adapted from wild-type AAV synthesis
- Includes
 - Rep protein and viral protein (VP) synthesis
 - Rep protein regulatory functions
 - Capsid assembly
 - Viral DNA replication
 - DNA packaging into capsid
 - Capsid secretion from the nucleus to the cytosol
- Empty/full capsid has the same secretion kinetics

Model vs. data for dynamics of plasmid content in cell and nucleus

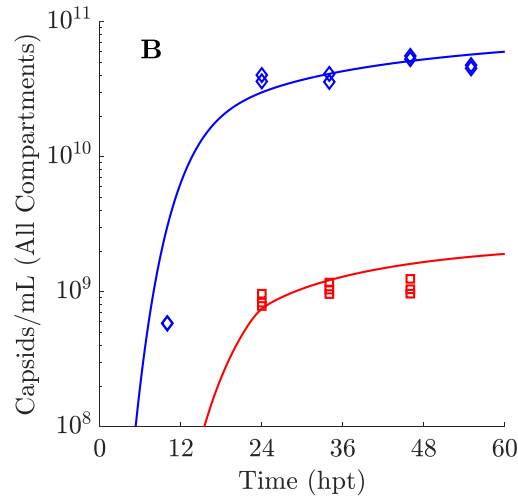
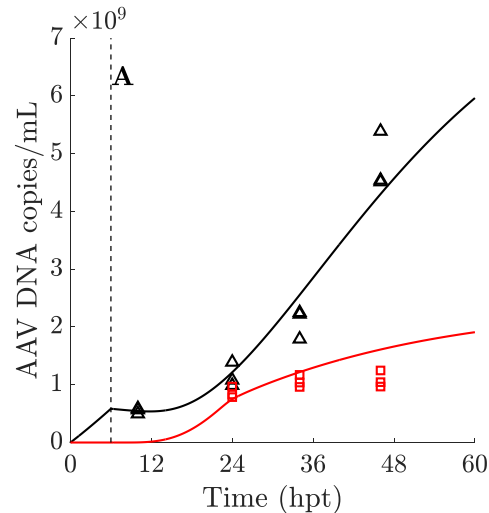


Plasmids in cells – fitted.
Data from Carpentier et al. (2006)



Plasmids in nucleus – predicted.
Data from Glover et al. (2010)

Model vs. data for the dynamics of viral production

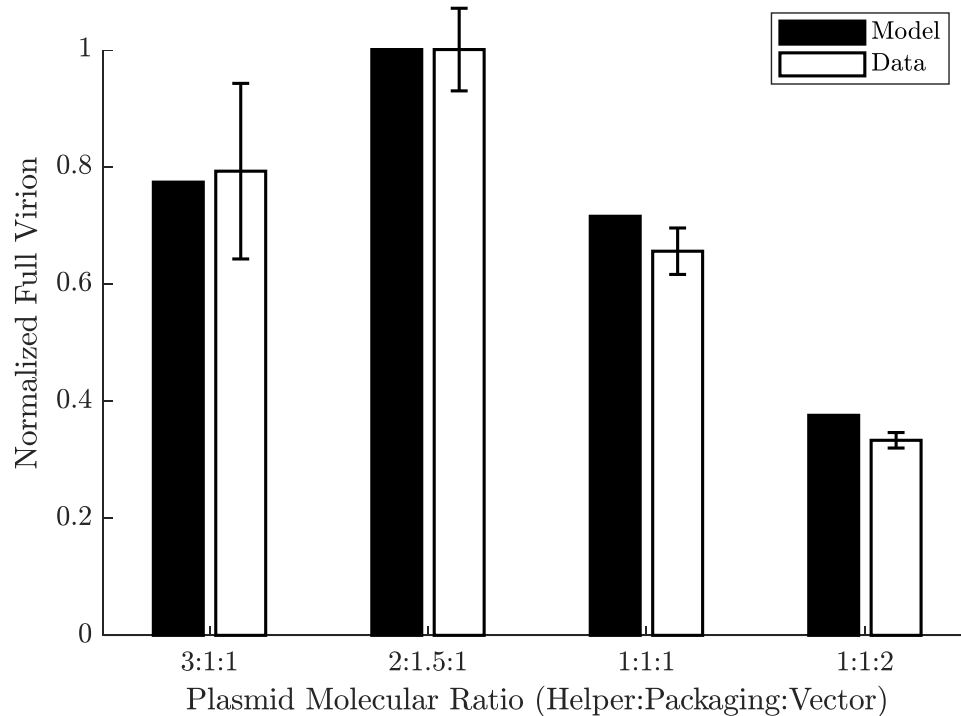


△ Replicated viral DNA - Data □ Full virion - Data ◇ Total capsid - Data
— Replicated viral DNA - Model — Full virion - Model — Total capsid - Model

Data (in-house experiment) for parameter estimation:

- Quantity of capsids over time
- Quantity of full capsids over time
- Quantity of all AAV DNA species over time (vector plasmid, replicated AAV, encapsidated AAV)

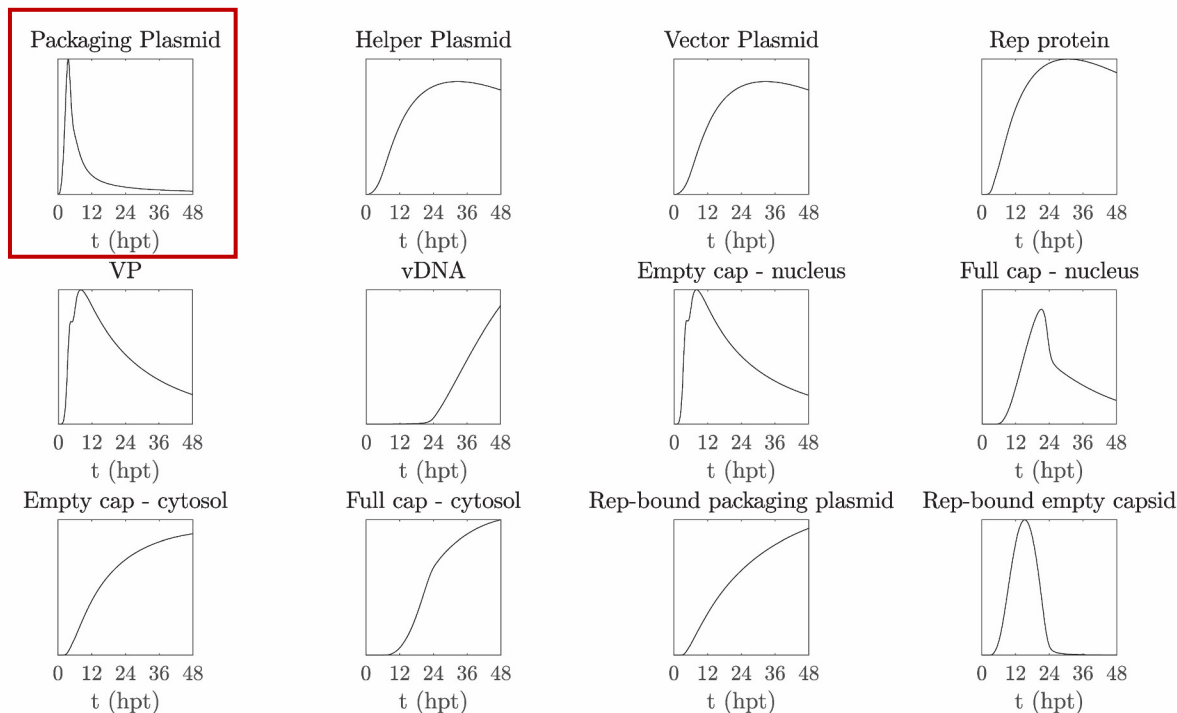
Model capable of predicting relative changes in productivity at varied plasmid ratios



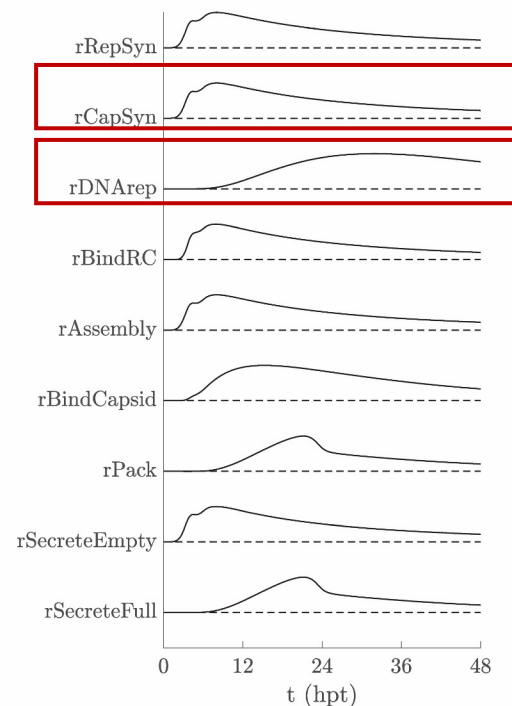
Data from
Grieger et al. (2016)

Majority of empty capsids in harvest can be explained by discoordination of capsid formation and DNA synthesis

A. Species concentrations



B. Viral production reaction rates



Extending the capsid production timeline could improve full:empty capsid ratio

Novel multi-stage transfection experimental design

Hypothesis:

Feeding plasmid over time can extend capsid production timeline and reduce empty particles

Measurements:

Rep protein

pAAV-GFP/pHelper/pRC5

Replicated viral DNA

Total capsid

Full capsid

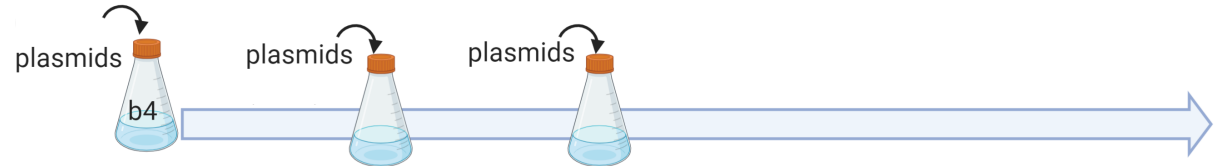
One-stage transfection



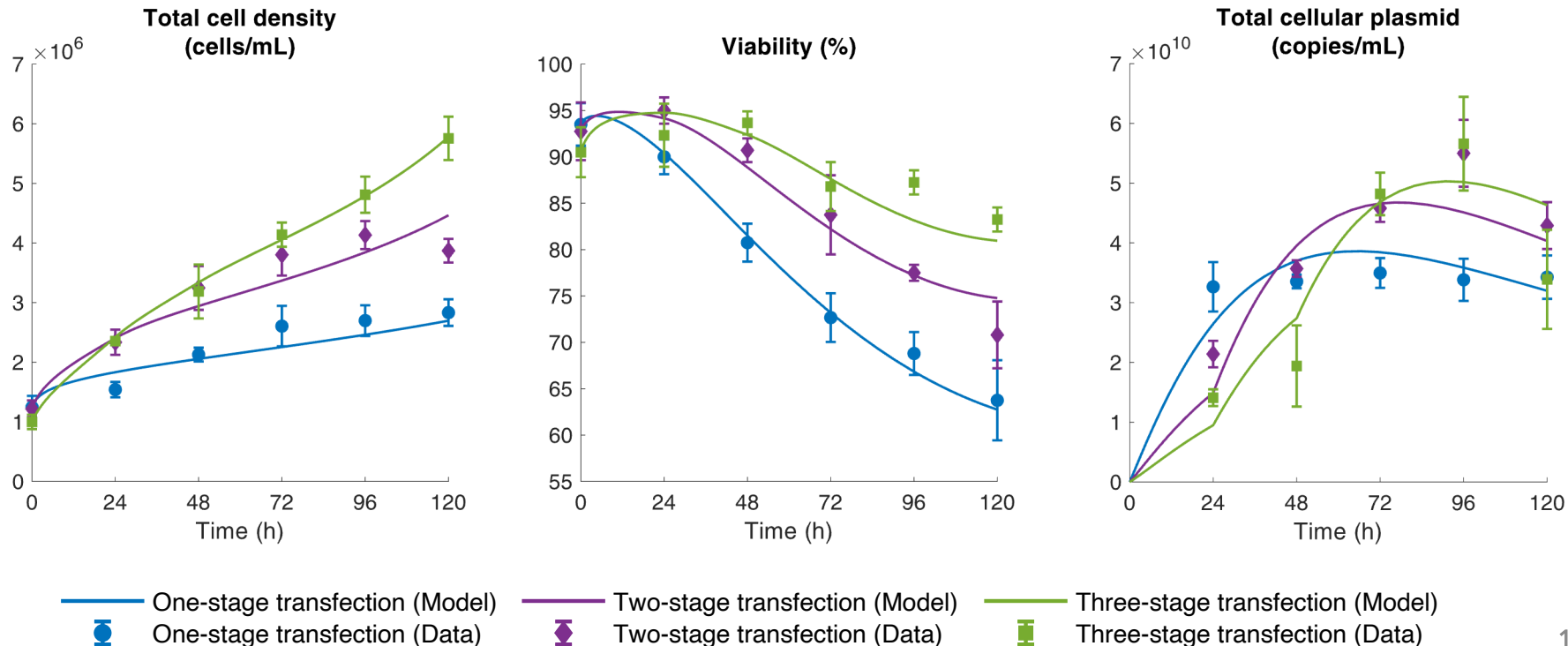
Two-stage transfection



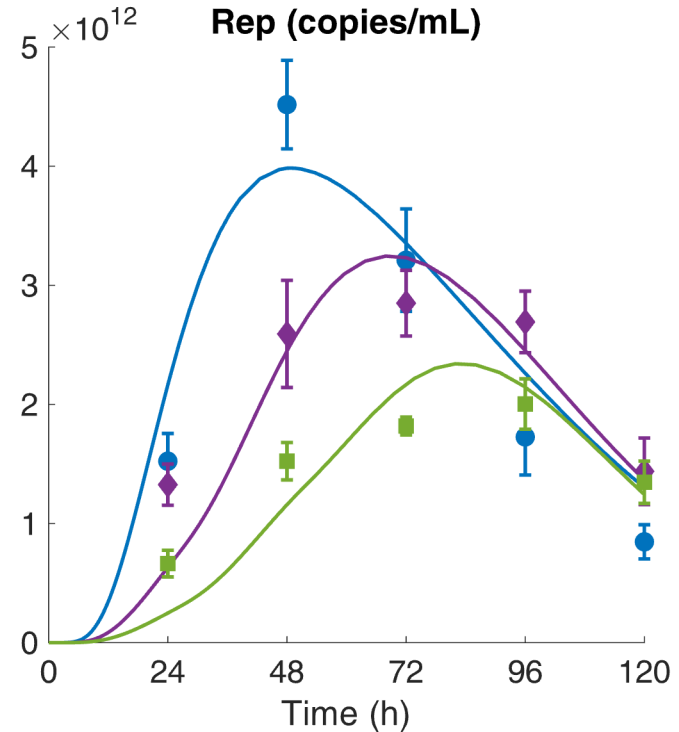
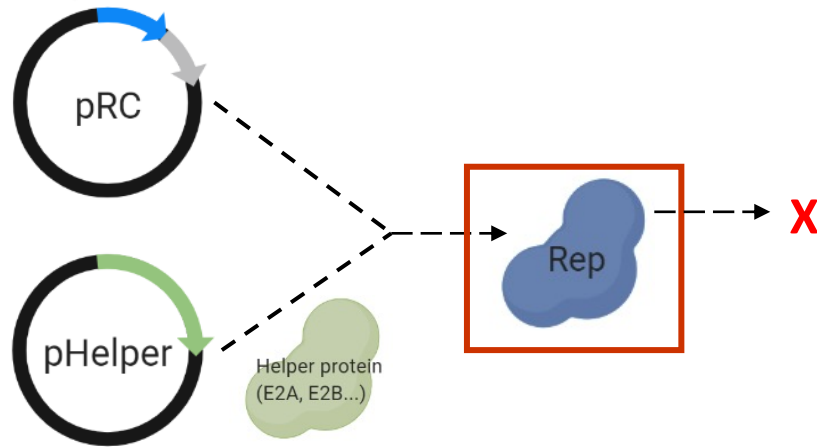
Three-stage transfection



Multi-stage transfections result in better growth and more plasmid uptake at later phases



Multi-stage transfection delays the peak of Rep protein production

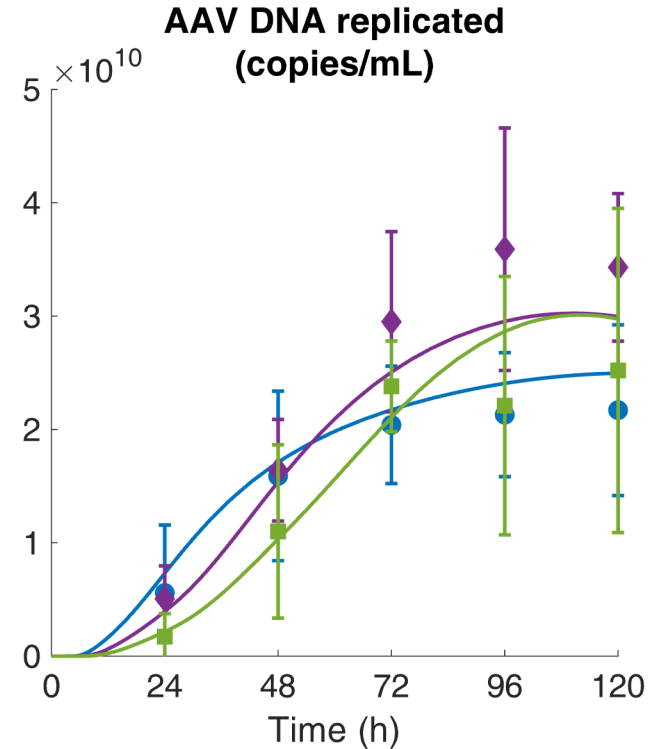
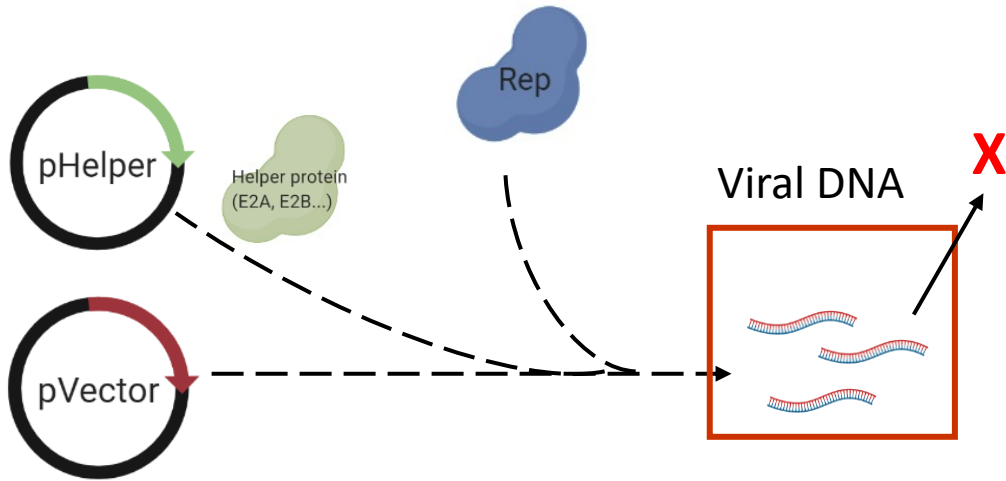


— One-stage transfection (Model)
● One-stage transfection (Data)

— Two-stage transfection (Model)
◆ Two-stage transfection (Data)

— Three-stage transfection (Model)
■ Three-stage transfection (Data)

Viral DNA (vDNA) replication dynamics are similar across transfection methods

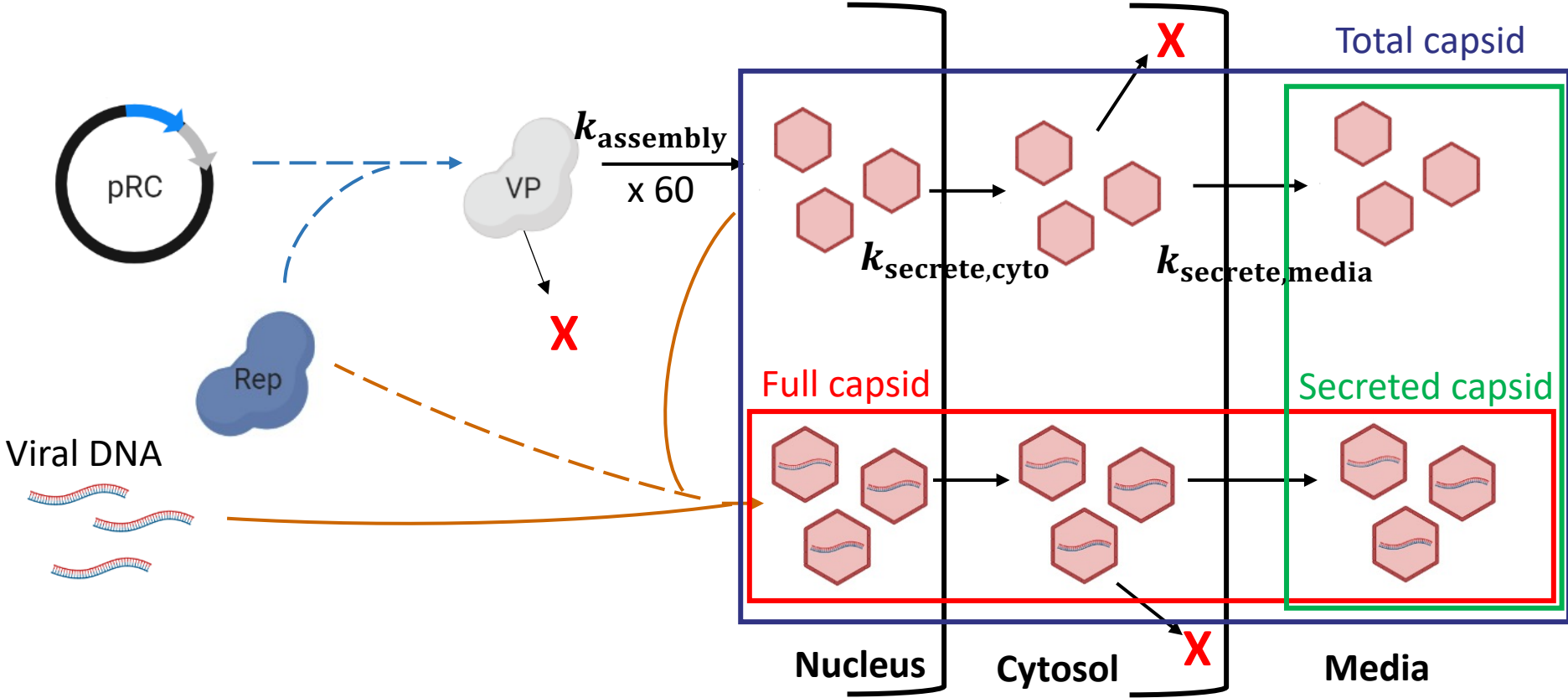


— One-stage transfection (Model)
● One-stage transfection (Data)

— Two-stage transfection (Model)
◆ Two-stage transfection (Data)

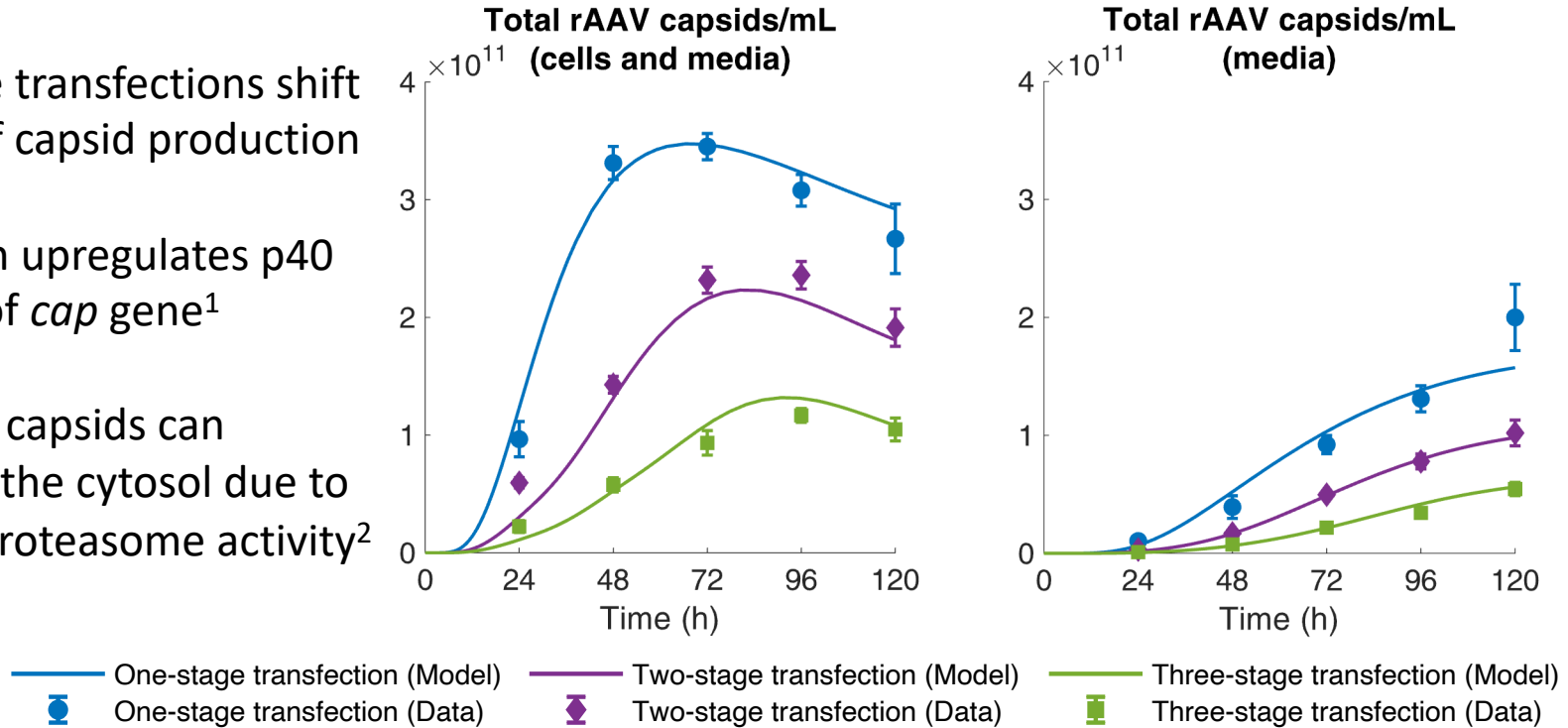
— Three-stage transfection (Model)
■ Three-stage transfection (Data)

Capsid assembly and packaging modeling includes more details



Spreading out transfection reduces total capsid production

- Multi-stage transfections shift the peak of capsid production
- Rep protein upregulates p40 promoter of *cap* gene¹
- Assembled capsids can degrade in the cytosol due to ubiquitin-proteasome activity²

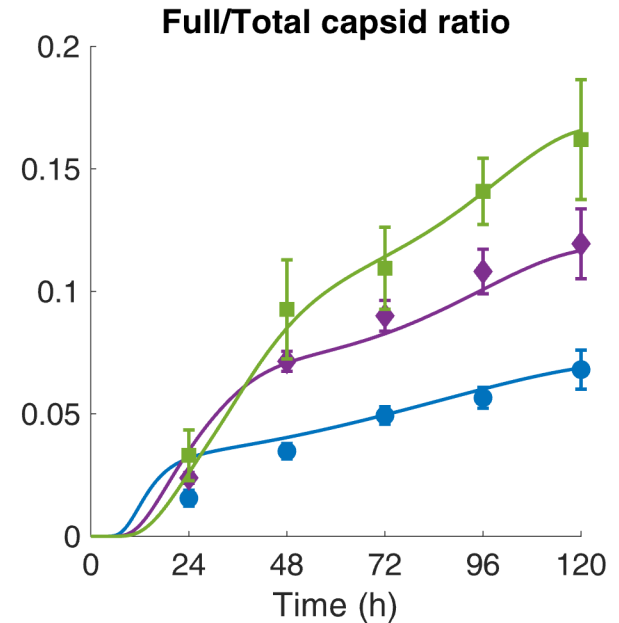
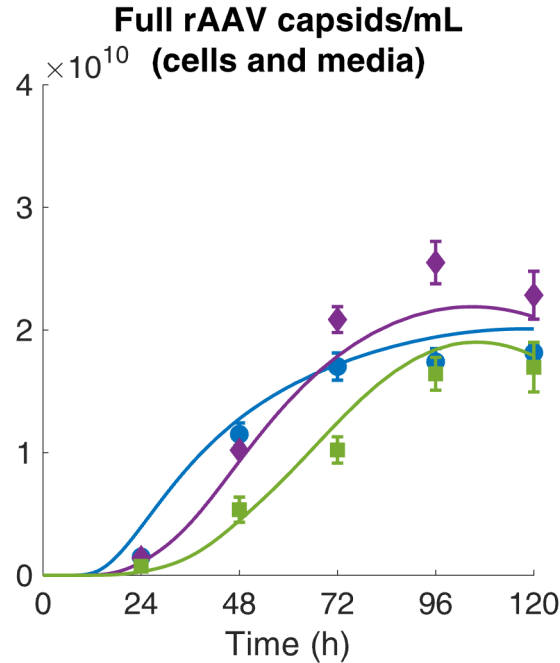


1. Pereira et al. *J Virol.* (1997)

2. Yan et al. *J Virol.* (2002)

Multi-stage transfection methods increase the full capsid ratio in harvest

- Viral titers are comparable across one-stage and multi-stage transfection methods
- Full capsid ratio is significantly improved by multi-stage transfection
- Model can capture the differences across transfection methods



— One-stage transfection (Model)
● One-stage transfection (Data)

— Two-stage transfection (Model)
◆ Two-stage transfection (Data)

— Three-stage transfection (Model)
■ Three-stage transfection (Data)

Conclusion

- Developed a mechanistic model that captures rAAV production dynamic changes across one-stage transfection and multi-stage transfection methods
- Designed and demonstrated a multi-stage transfection experiment to improve full capsid ratio in viral harvest via triple transfection based on analysis from mechanistic modeling
- Novel transfection technique paves way for improvement of viral vector product quality

Thank You!

