

Investigating the Aggregation, Genome Release, and Self-Interactions of AAV Formulations

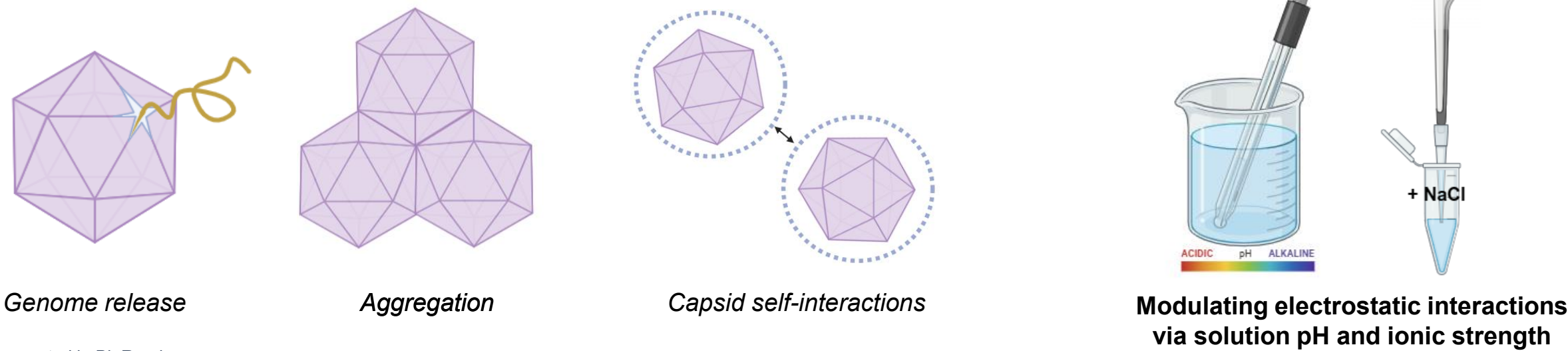
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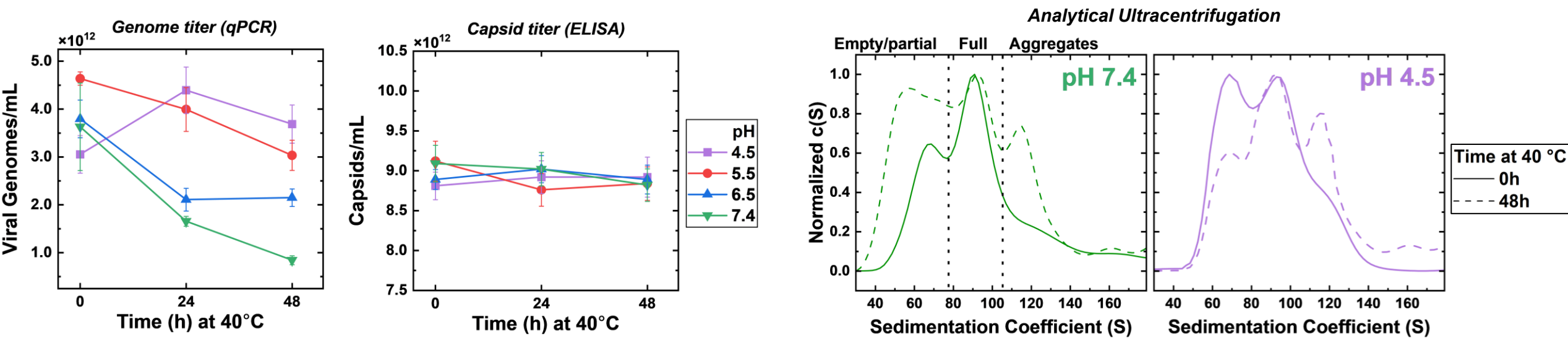
CASSS Cell and Gene Therapy Symposium

Goal is understanding how formulation conditions contribute to stability of AAVs



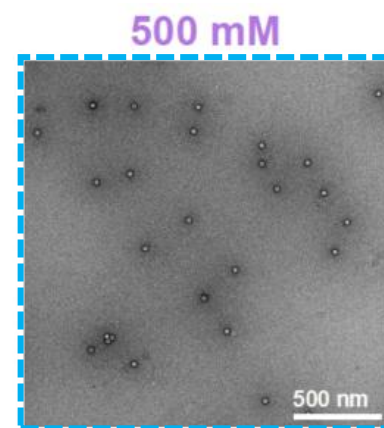
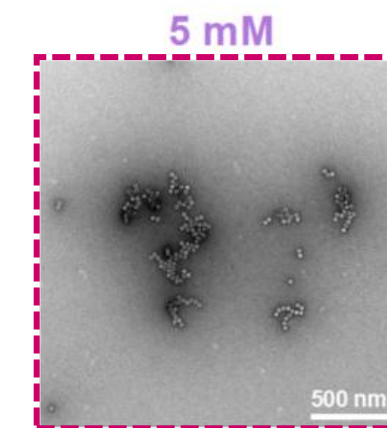
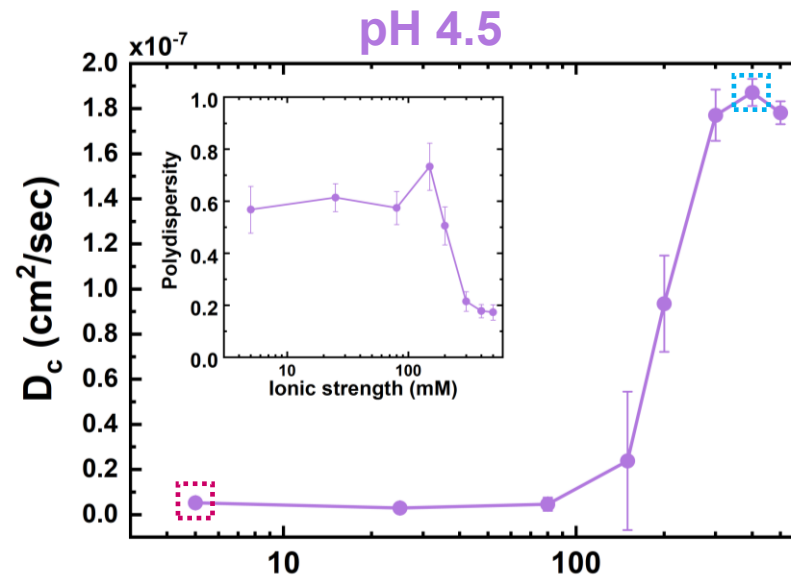
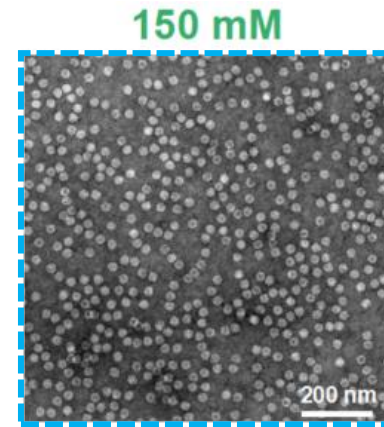
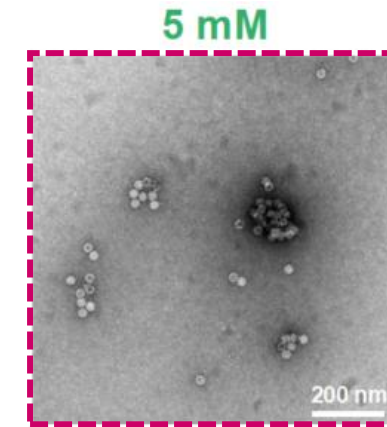
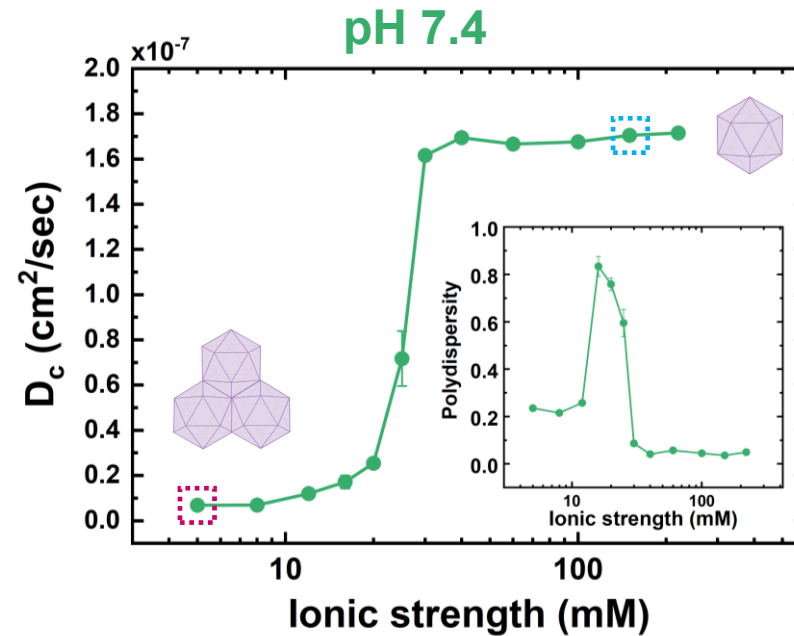
Cartoons created in BioRender

Thermal stress studies revealed pH-dependent differences in genome release



Capsids aggregate reversibly at low ionic strength

- Ionic strength increased by spiking in NaCl
- Similar trend for both full and empty capsids

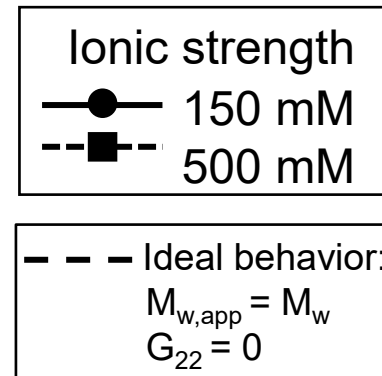
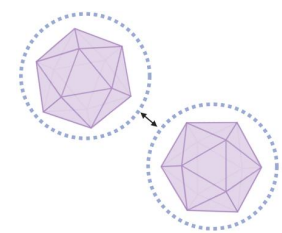
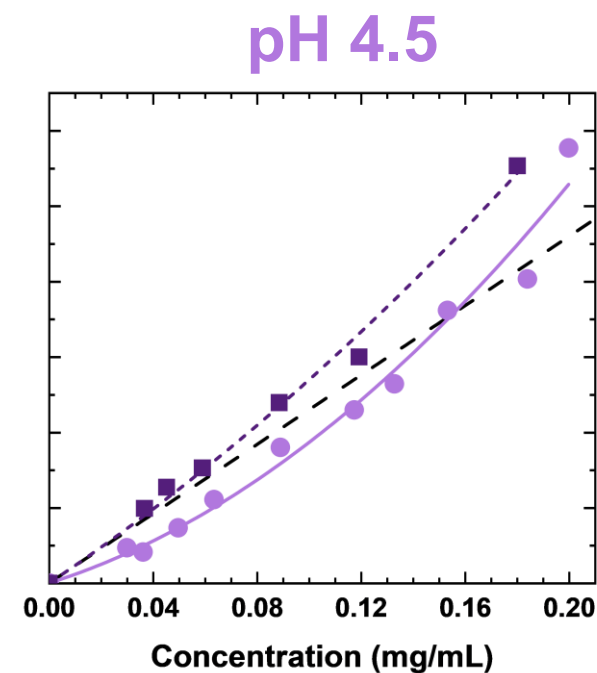
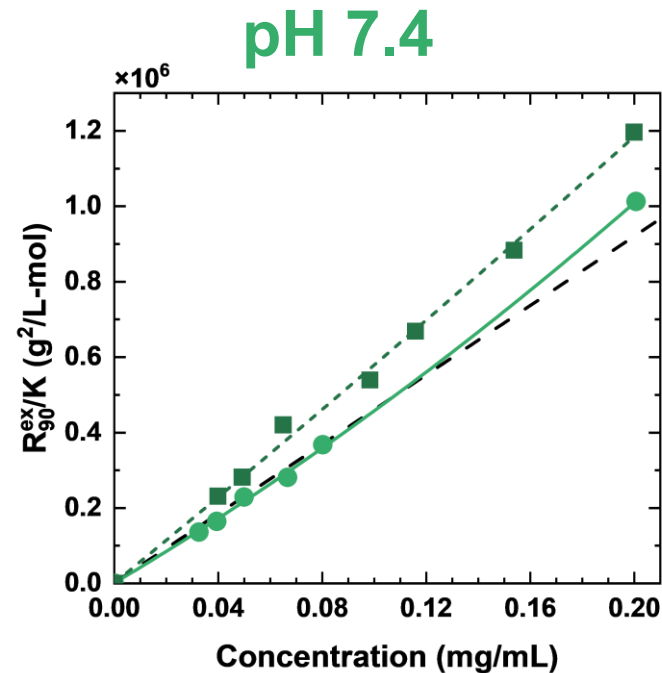


Static light scattering experiments reveal attractive capsid self-interactions

$$\frac{R_{90}^{ex}}{K} = M_{w,app}c_2 + M_w G_{22}c_2^2$$

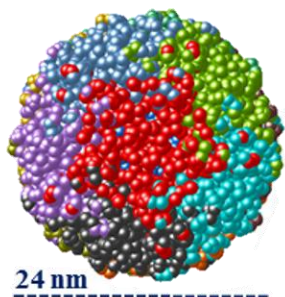
$G_{22} > 0$ (upward curvature) net attractions
 $G_{22} < 0$ (downward curvature) net repulsions

- Both pH 4.5 and 7.4 show strong electrostatic attractions – stronger at pH 4.5

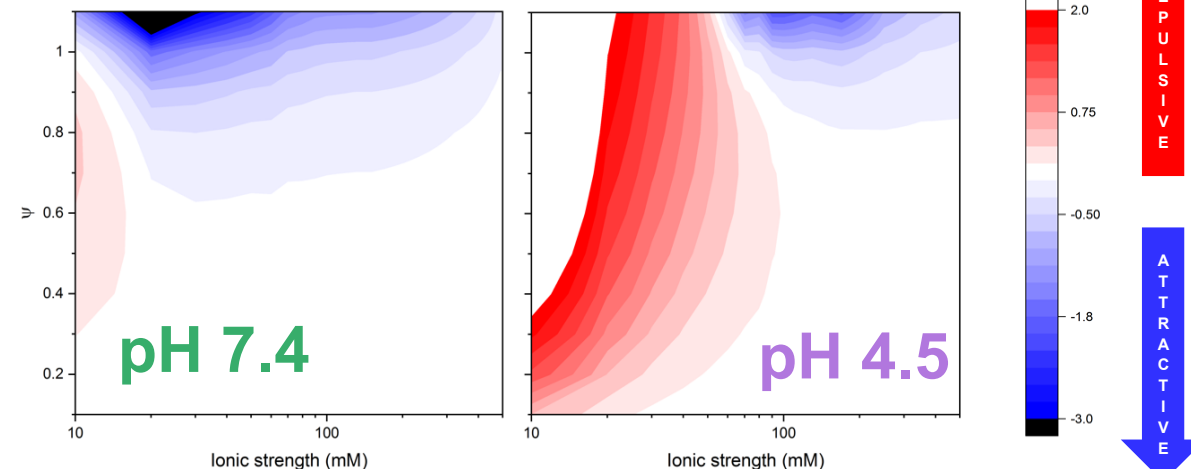


Coarse-grained model predictions of second osmotic virial coefficient (B_{22})

1 bead per charge site and per viral protein (1bC/VP) Model



- Currently limited to two-body interactions between intact empty capsids
- Predicts repulsions at low ionic strength, and higher sensitivity to ionic strength at low pH



Electrostatic interactions play a significant role in stability of AAV capsids

- The insights from this work can aid in the selection of solution conditions during the development of AAV-based pharmaceuticals
- The experimental techniques and computational model contribute to the available toolbox of methods used to analyze AAV product quality and stability
- Future work: exploring other serotypes, transgenes, empty capsids, various buffer compositions and salts

