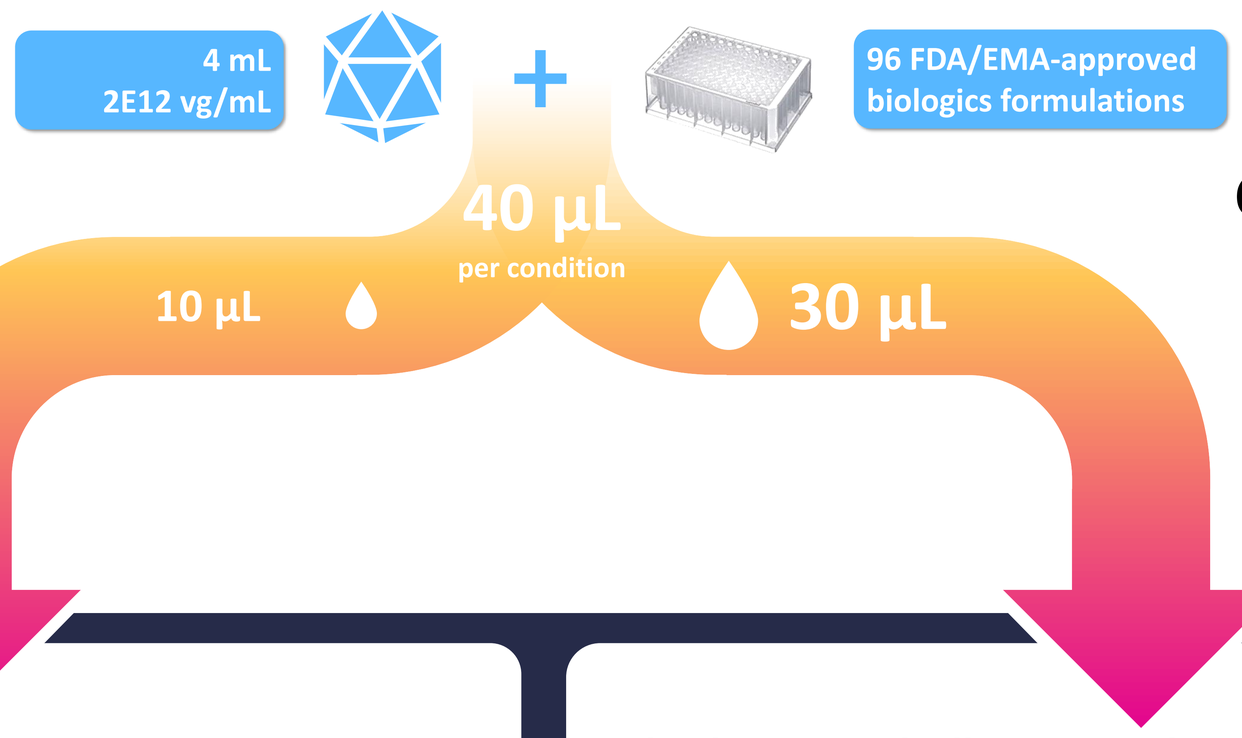
Rapid AAV pre-formulation screening using a commercially available buffer kit & minimal sample requirement

Philipp Beck, Astrid Stockinger, Sonya Schermann

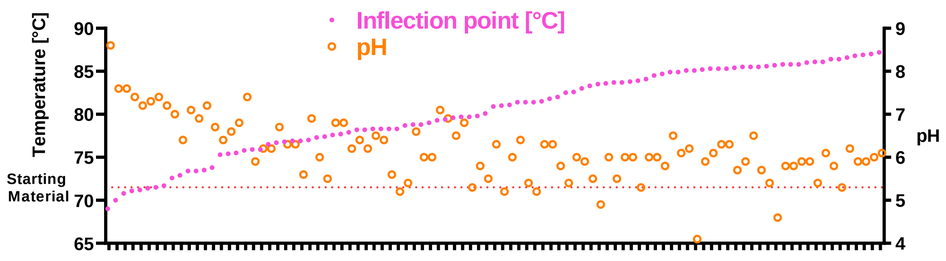
P095

Material constraints are a limiting factor in early stage AAV gene therapy development programs. This is especially true for initial, broad formulation studies, that consume substantial amounts of material and time. We established a **low-volume, high-throughput pre-formulation screening** approach that requires only very low sample amounts and provides results within two days.

Starting with only **4 mL** of an AAV vector at **2-4E12 vg/mL**, we used a commercially available buffer kit containing 96 FDA/EMA-approved biologics formulations to generate thermal capsid (**nanoDSF**) and inter-particle (**DLS**) stability data.
Only **40 µL of sample** per condition are sufficient to generate a starting point for focused buffer screenings.

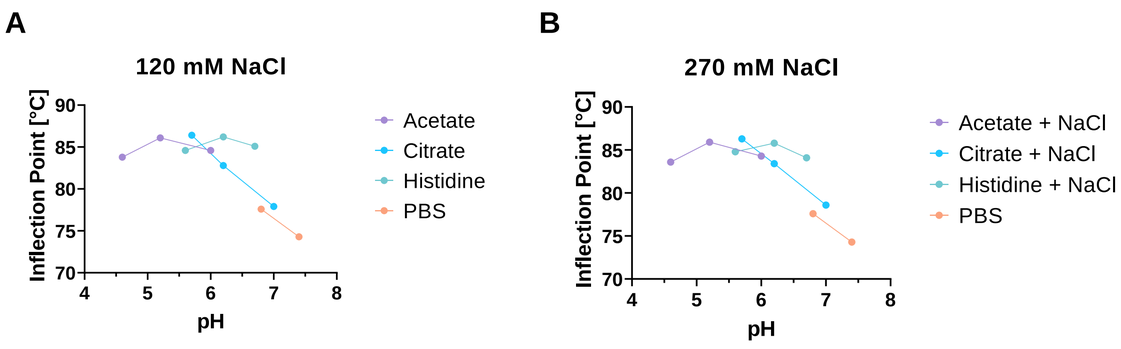


Inversed relationship: lower pH ⊖ higher melting point



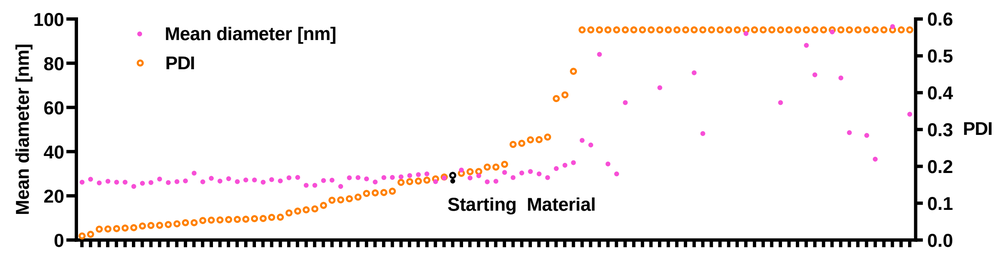
Capsid stability of the serotype used in the study was found to be **strongly influenced by pH**, whereas other **formulation parameters & excipients had negligible effects** on AAV melting temperature (T_m). Thermal **stability increased with decreasing pH**, with up to +15 °C improvement in T_m compared to the basic formulation of the starting material.

Adjustment of ionic strength has no influence on melting temperature

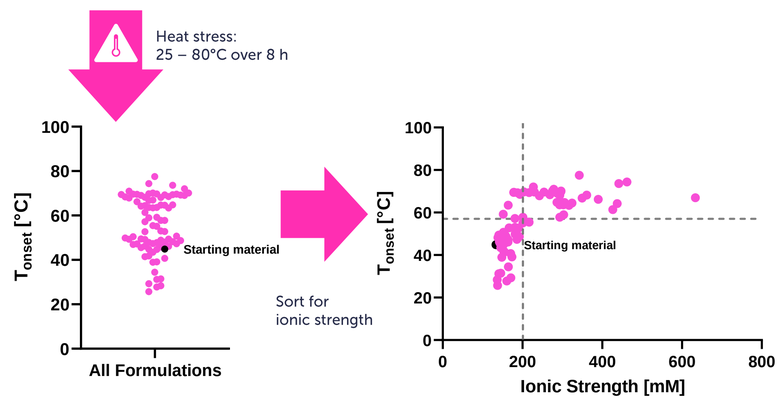


The pH range of 4.5 – 6.8 was more closely evaluated in a focused screening using acetate, histidine, citrate (all 40 mM) and PBS: **at pH < 6.7, unfolding temperatures are > 80 °C**, while buffers at neutral pH levels resulted in AAV unfolding < 80 °C (A/B). This pattern occurs **independent of the buffer substance** used as observed for citrate and PBS in B. Importantly, additional NaCl did not influence the unfolding temperatures (A/B) and thus the thermal stability of the capsid is **not affected** by this change in **ionic strength**.

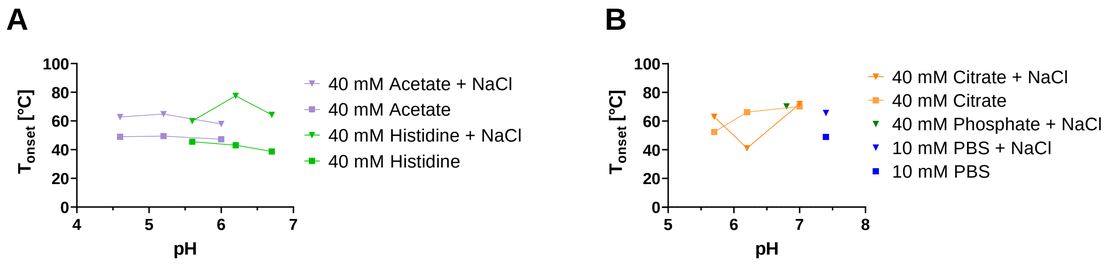
Ionic strength dictates resistance towards aggregation upon heat stress



- Colloidal status of formulations was determined directly after mixing AAV with the buffer kit (above). This already demonstrated that some of the formulations are not suitable (high polydispersity index, PDI).
- Monitoring temperature-induced onset of AAV aggregation (T_{onset}) showed that **ionic strength is a main contributor to colloidal stability upon heat stress** (right), confirming published data[1] and validating our platform.



Adjustment of ionic strength leads to higher aggregation temperature with some buffers



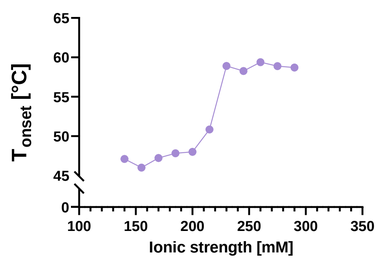
- As shown in the box above & left, pH < 7 and ionic strength > 200 mM confers colloidal and thermal stability.
- Next, the pH range of 4.5 – 6.8 was more closely evaluated in a focused screening using acetate, histidine, citrate and PBS. Addition of 150 mM NaCl to buffers lead to an increase in T_{onset} (A/B). Citrate is a special case since the ionic strength of the 40 mM citrate buffer is already high enough to result in a T_{onset} >60°C (except for one likely outlier at pH at pH 6.2 (B).

Summary

- Using nanoDSF, we showed that conformational stability is strongly influenced by pH, whereas other formulation parameters & excipients had negligible effects on AAV melting temperature. Specifically, **thermal stability increased with decreasing pH**, with up to **+15 °C improvement** in T_m compared to the basic formulation of the starting material.
- We demonstrated that T_{onset} could be **increased by up to +25 °C** compared to the starting material and that a **minimum ionic strength-level** (around 200 mM) is required for colloidal stabilization which is in line with published data.[1] **Ionic strength** was found to be the **only major contributor** to colloidal stability.
- The data provides a solid basis to inform further rational formulation development. The **approach is capsid agnostic**: it can be applied to **any AAV serotype** or other novel modalities based on **protein nanoparticles.**

Ionic strength threshold: NaCl-titration

A closer examination of the effect of ionic strength by addition of NaCl to 40 mM Acetate at pH 5.5 was performed: A threshold exists in the **200-225 mM** range above which **further increase** of ionic strength **does not increase** the **colloidal stability** of AAV in the formulation during heat stress.



Poster downloads

[1] JF Wright et al. *"Identification of Factors that Contribute to Recombinant AAV2 Particle Aggregation and Methods to Prevent Its Occurrence during Vector Purification and Formulation"* Molecular Therapy, Vol. 12, No. 1, 2005

Aim higher

Ascend | Fraunhoferstraße 9b | 82152 Planegg | Germany

www.ascend-gctx.com
philipp.beck@ascend-gctx.com