DGE-AUC: Adapting the Power of Density Gradient Separations for Gene Therapy Analytics

Ross VerHeul[1], Shawn Sternisha[2], Emilie Bouda[1], Shaofeng Wang[1], Akash Bhattacharya[3]

Beckman Coulter Life Sciences, [1]Indianapolis, Indiana; [2]Miami, Florida; [3]Loveland, Colorado USA

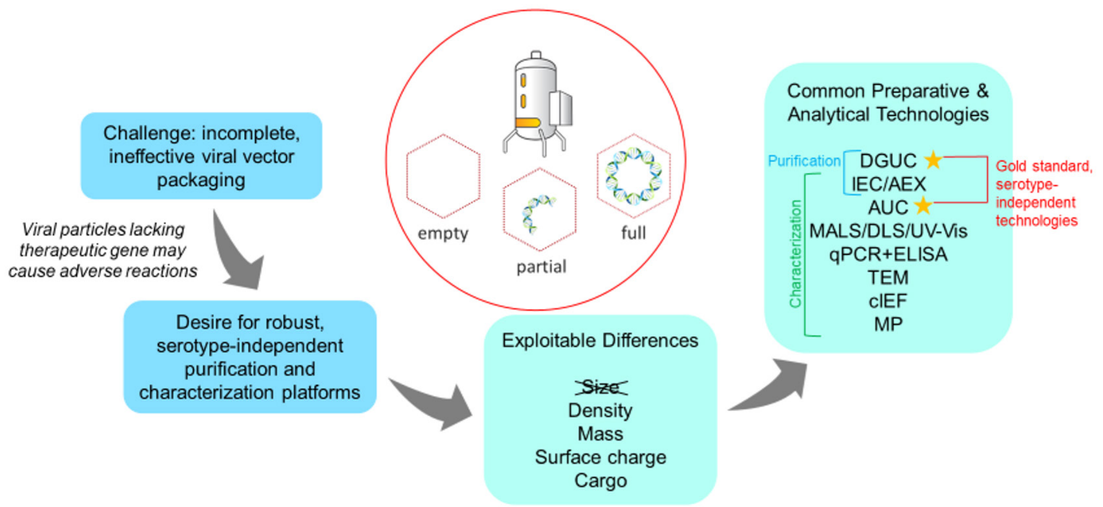
Abstract

Density gradient ultracentrifugation (DGUC) has long been considered a staple technology providing high-resolution purification of numerous materials in gene therapy, including AAV, adenovirus, and plasmids, from bench to manufacturing scales. DGUC separates particles via buoyant density and is well-known to efficiently separate otherwise challenging materials such as empty, intermediate, and full viral capsids. While traditionally used for purification, this approach has recently been adapted for analytical purposes supporting gene therapy by using an analytical ultracentrifuge (AUC), such as the Optima AUC.

Density gradient equilibrium AUC (DGE-AUC) is a highly simplified analytical method that provides the same high-resolution benefits as its prep-scale counterpart, along with numerous advantages over the current gold standard sedimentation velocity (SV) methods. DGE-AUC with CsCl gradients is amenable to AAV, adenovirus, and other large viral particles, providing high-resolution data with significantly less sample (>30X sensitivity) than SV-AUC, and readily enables the use of multiwavelength analysis without compromising data quality. Furthermore, DGE-AUC is serotype-agnostic with intuitive interpretation and analysis (not requiring specialized AUC software), and thus is poised to benefit a wide array of gene therapy & viral vector users where traditional analytical methods fall short.

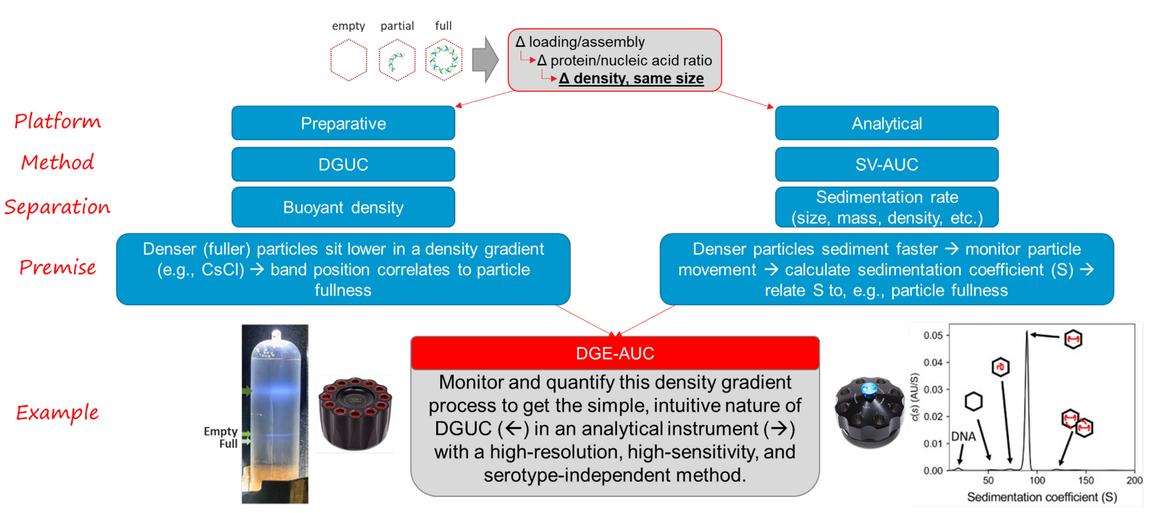
Addressing a common challenge in gene therapy

Many technologies exist to quantify the loading efficiency of viral vectors



Gold standard: exploiting differences particle density

Centrifugation offers unique, high-resolution methods for viral vectors & more



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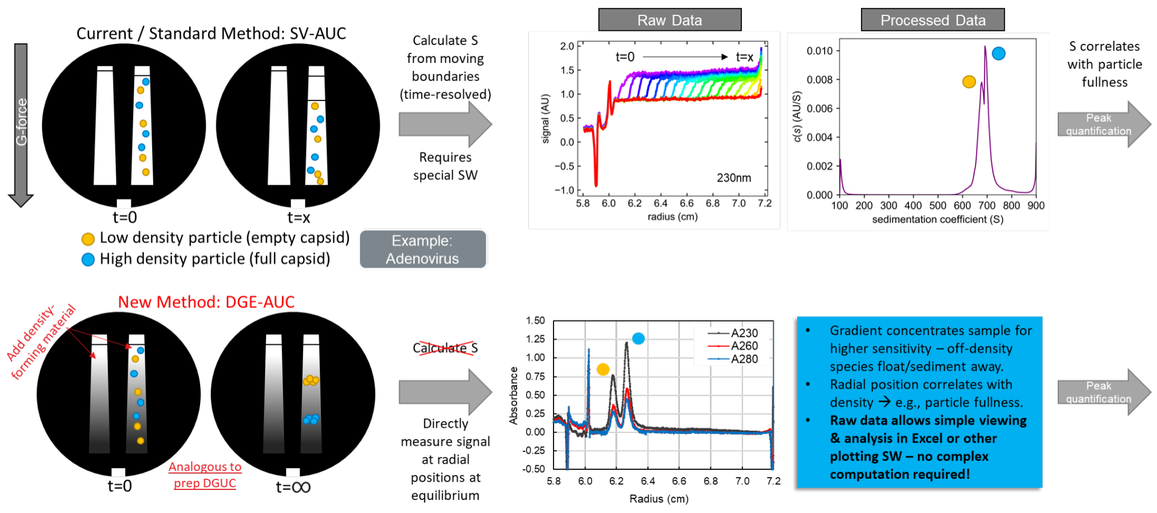
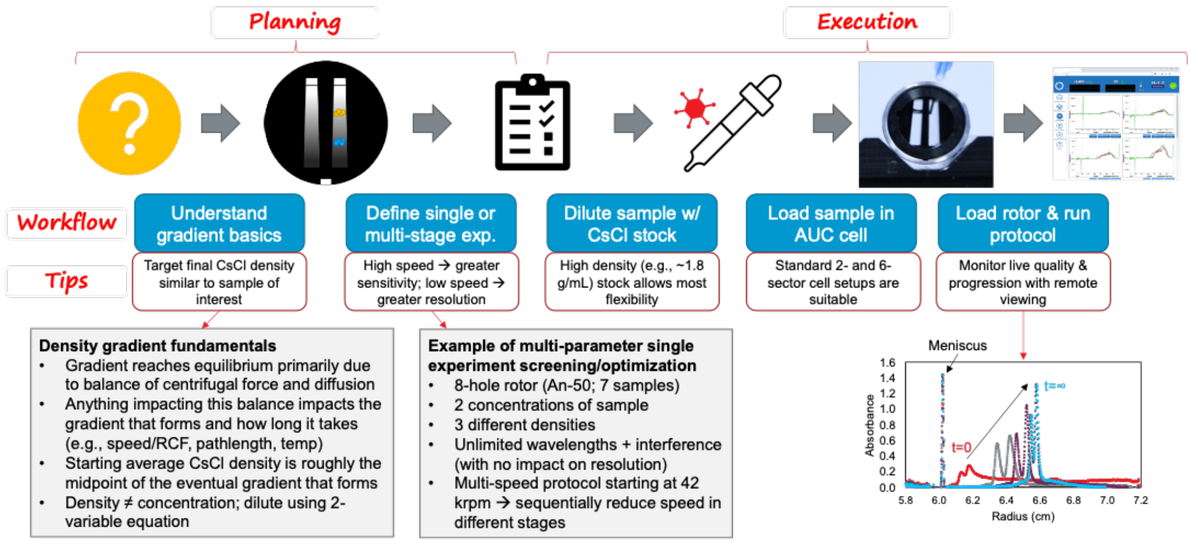
Introduction to DGE-AUC

DGE-AUC offers rapid, intuitive data interpretation without complex calculations



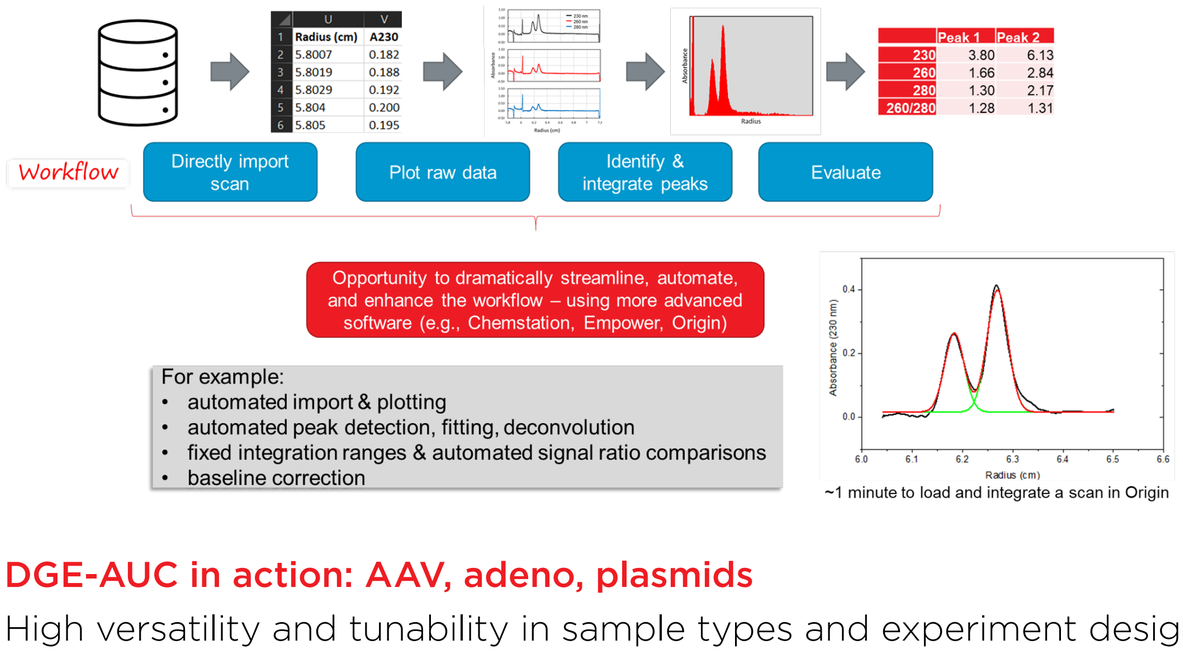
Simple implementation & optimization of DGE-AUC

Leverage the well-known fundamentals of DGUC to implement quickly



Simplified analysis with no complex computations

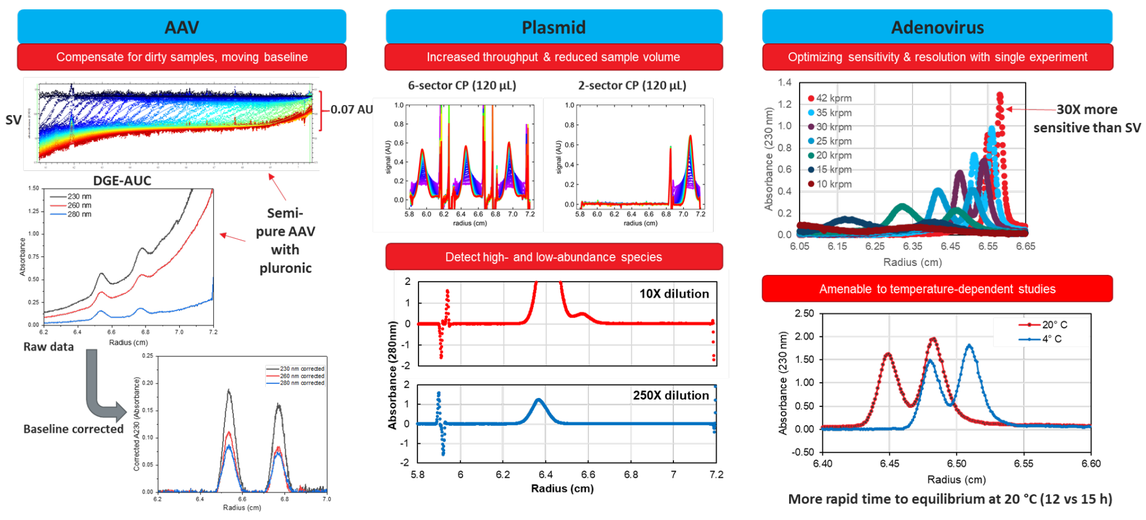
Example: Adenovirus analysis using only Excel



~1 minute to load and integrate a scan in Origin

DGE-AUC in action: AAV, adeno, plasmids

High versatility and tunability in sample types and experiment design



Summary of DGE-AUC advantages

- DGE-AUC is a versatile, modern day analytical method based on well-known foundational understanding of CsCl density gradients

- More data (DGE-AUC is orthogonal to well-established SV-AUC methods)
- More relatable (analogous to industry-standard CsCl prep gradients; intuitive interpretation)
- More straightforward (no complex black box math; no special AUC software; no supercomputers)
- More sample types (serotype agnostic; not size-limited; e.g., AAV, adeno, plasmids)
- Less sample (more than 30X greater sensitivity is achievable)
- Faster optimization (multi-parameter optimization screen achievable in a single experiment)
- Higher throughput (at least 3x samples; e.g., 6-sector CPs)
- Higher resolution (not time-resolved; no wavelength/resolution trade-off; scan averaging)
- More tunability (tune throughput, sensitivity, and resolution with CP, volume, rpm, density, temp, etc)
- More tolerant (of common buffer components, including stabilizers like pluronics, sucrose)

For more information:

Visit Beckman.com or contact

Ross VerHeul: rverheul@beckman.com

References

1. Maruno et al. (2021). DOI: 10.1016/j.xphs.2021.06.031

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