APPLICATIONS OF ULTRACENTRIFUGATION IN PURIFICATION AND CHARACTERIZATION OF BIOMOLECULES

Akash Bhattacharya,[1] *Ross VerHeul*,[2] Eric Von Seggern,[1] Stephen Otts,[2] Beckman Coulter, Inc., [1]Loveland, CO & [2]Indianapolis, IN, USA

INTRODUCTION

Ultracentrifuges spin samples with centrifugal forces typically spanning 100,000 – 1,000,000 x g. At these high forces, the constituent molecules in the sample separate based on their physical properties (e.g., size, mass, density, anisotropy). Accordingly, ultracentrifugation is commonly used to purify, as well as characterize, low-molecular weight polymers up to multi-megaDalton protein complexes and organelles.
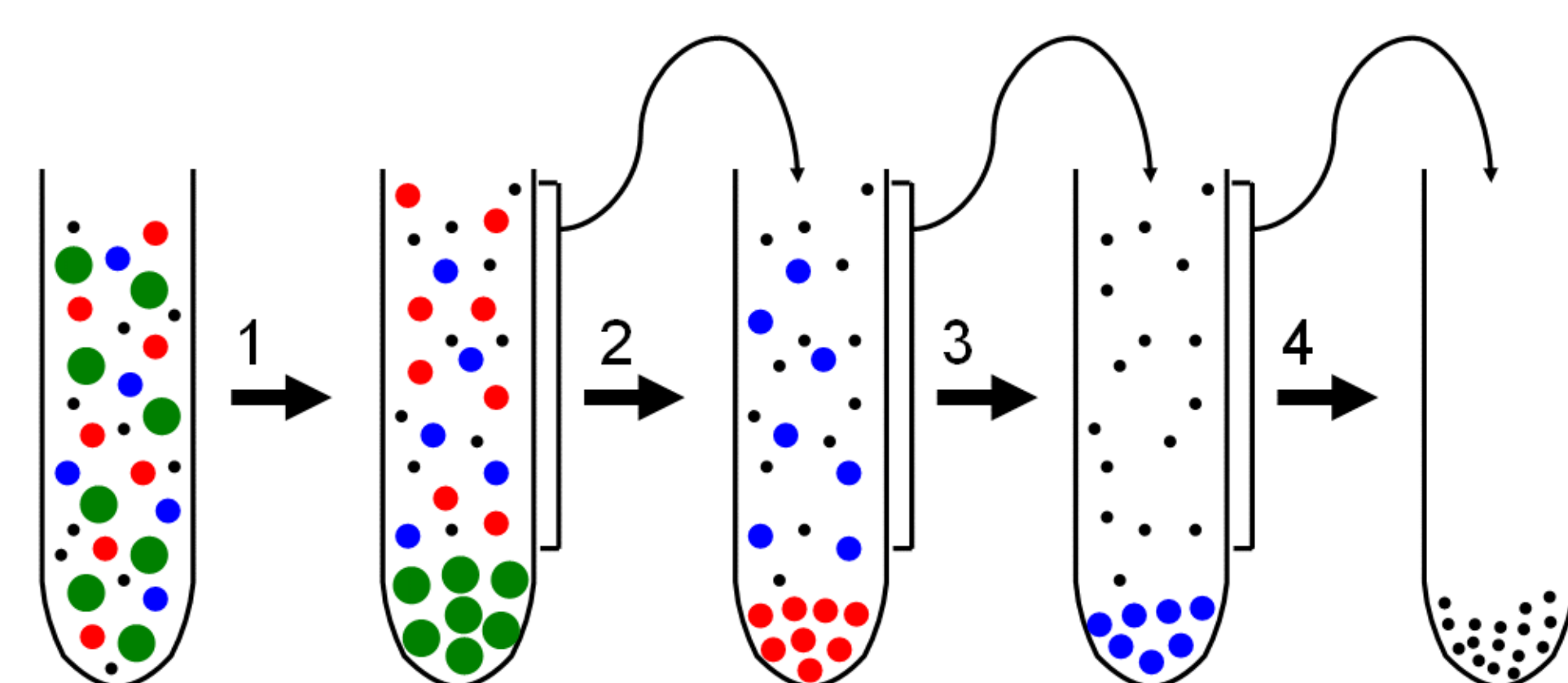
PREPARATIVE ULTRACENTRIFUGATION

Differential Ultracentrifugation

» Particles are separated on the basis of their size and mass (sedimentation coefficient, S).

» Multiple pelleting steps may be used for iterative enrichment.

» Ideal for separating particle groups of very different sizes.

Density Gradient Ultracentrifugation (DGUC)

» Soluble particles are separated in a liquid column of varying density (density gradient).

» In rate zonal experiments, particles migrate at varying rates, dictated by their S-values, and are time-dependent.

» Isopycnic separations are time-insensitive, where particles migrate to their apparent buoyant density in the gradient.

» Ideal for high-resolution separation of small materials with similar physical properties.
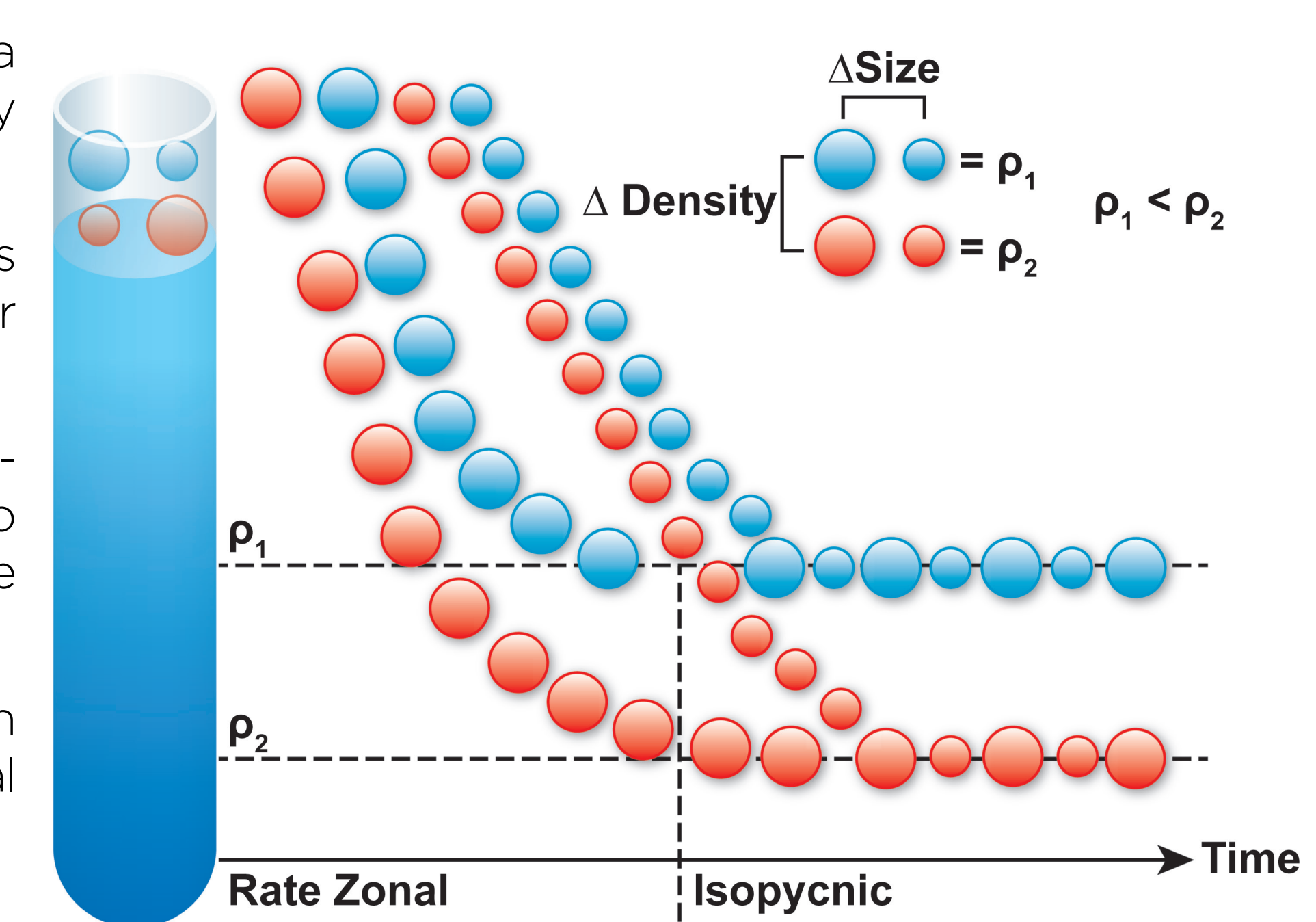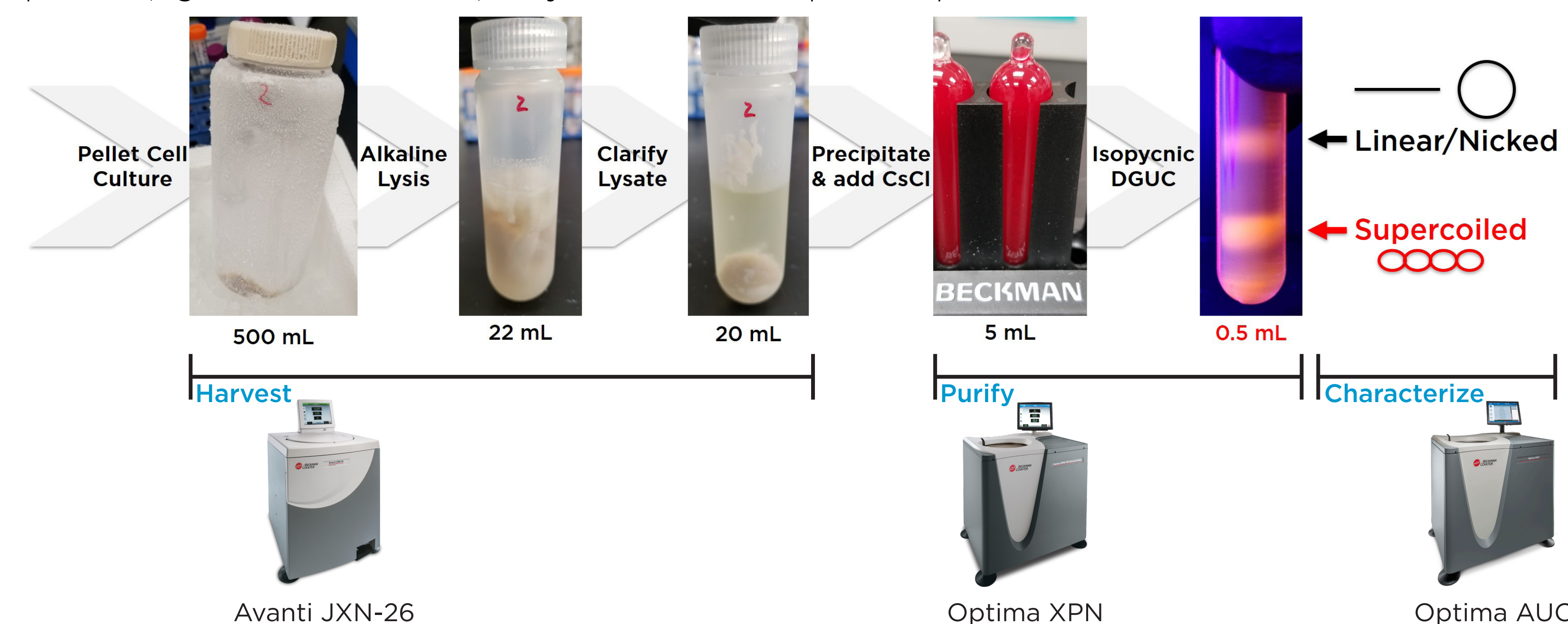
Example Workflow for DGUC Purification of Plasmid DNA

» Plasmid DNA may be extracted from bacteria using a variety of methods.

» The workflow below depicts a common alkaline lysis extraction and purification via a cesium chloride (CsCl) density gradient method with ethidium bromide.

» Newer density gradient materials (i.e., iodixanol/OptiPrep) and DNA-interacting probes (e.g., DAPI & GelGreen) may also be used in plasmid purification.

Pellet Cell Culture — 500 mL — Alkaline Lysis — 22 mL — Clarify Lysate — 20 mL — Precipitate & add CsCl — 5 mL — Isopycnic DGUC — 0.5 mL — Linear/Nicked, Supercoiled

Harvest — Avanti JXN-26
Purify — Optima XPN
Characterize — Optima AUC

AUC HISTORY

- Theodor Svedberg invents AUC, Chemistry Nobel Prize 1926
- Jean Perrin describes Sedimentation Equilibrium, Physics Nobel Prize 1926
- Ole Lamm describes the sedimentation and diffusion of samples in a sector-cell, 1930s
- Edward Pickels starts Spinco and builds the first commercial AUC – the Model E, 1947
- Beckman acquires Spinco, 1954
- Meselson & Stahl experiment, 1958
- Schachman develops Rayleigh Interference detection, 1958
- Van-Holde Weischet graphical analysis is developed, 1978
- Beckman introduces the Proteomelab XLA/XLI AUC instrument, 1990s
- Demeler develops UltraScan, 1998, Shuck develops Sedfit, 2000
- Beckman releases the new generation Optima AUC with multi-wavelength capability
- New frontier: Viral vectors, nanoparticles, liposomes

AUC FUNDAMENTALS

Forces on Particles
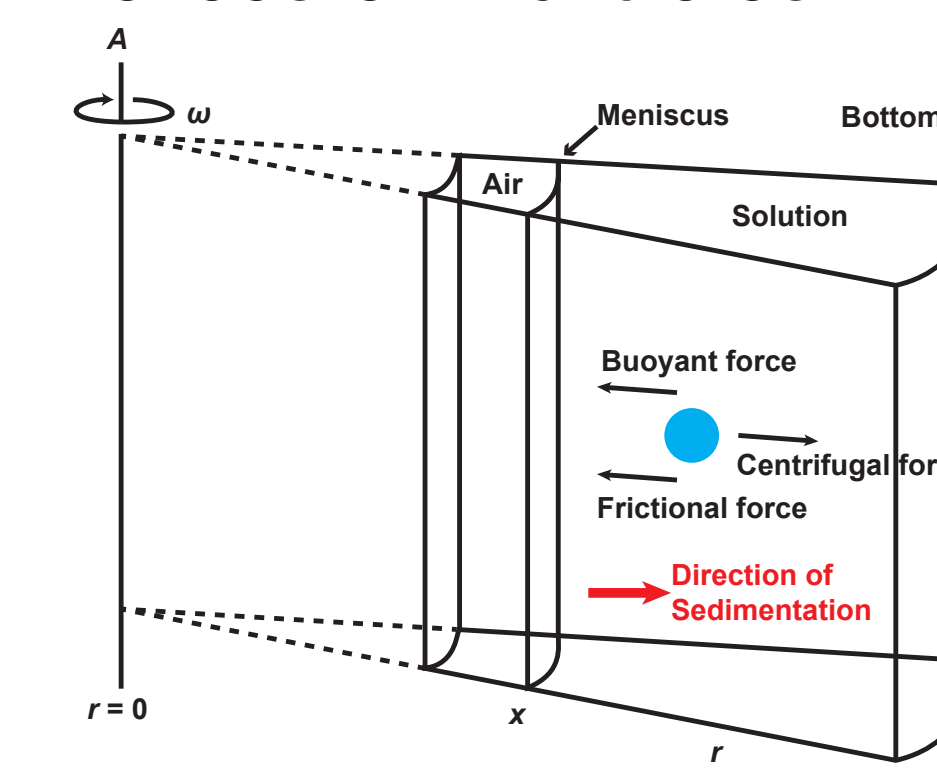
AUC Optics Schematic
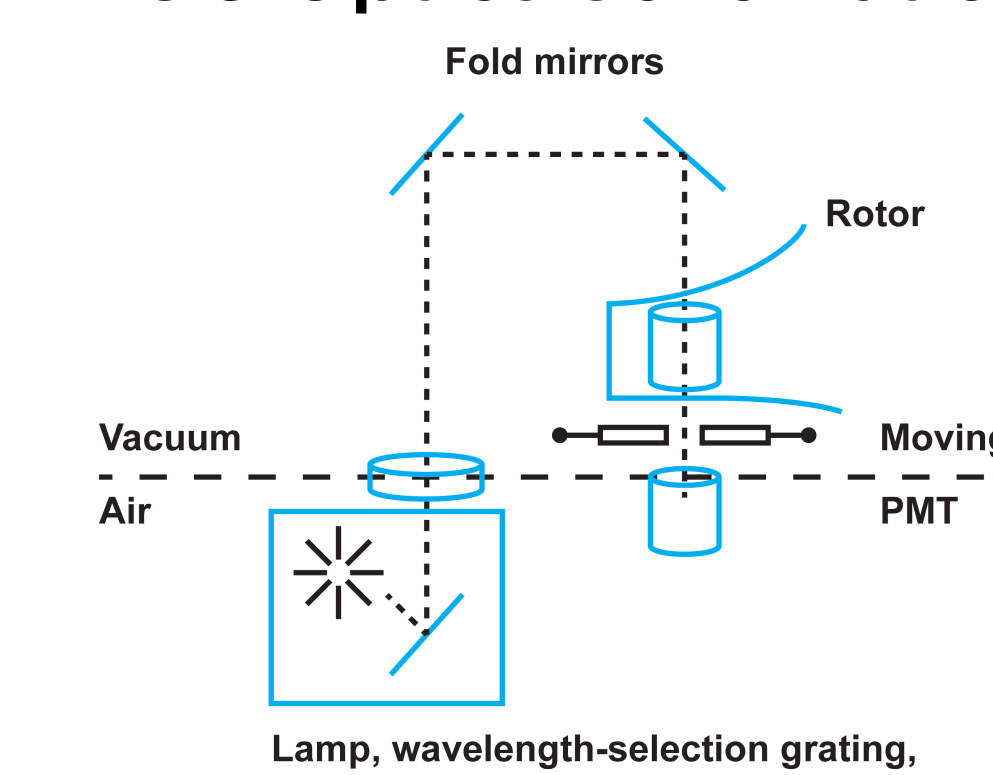
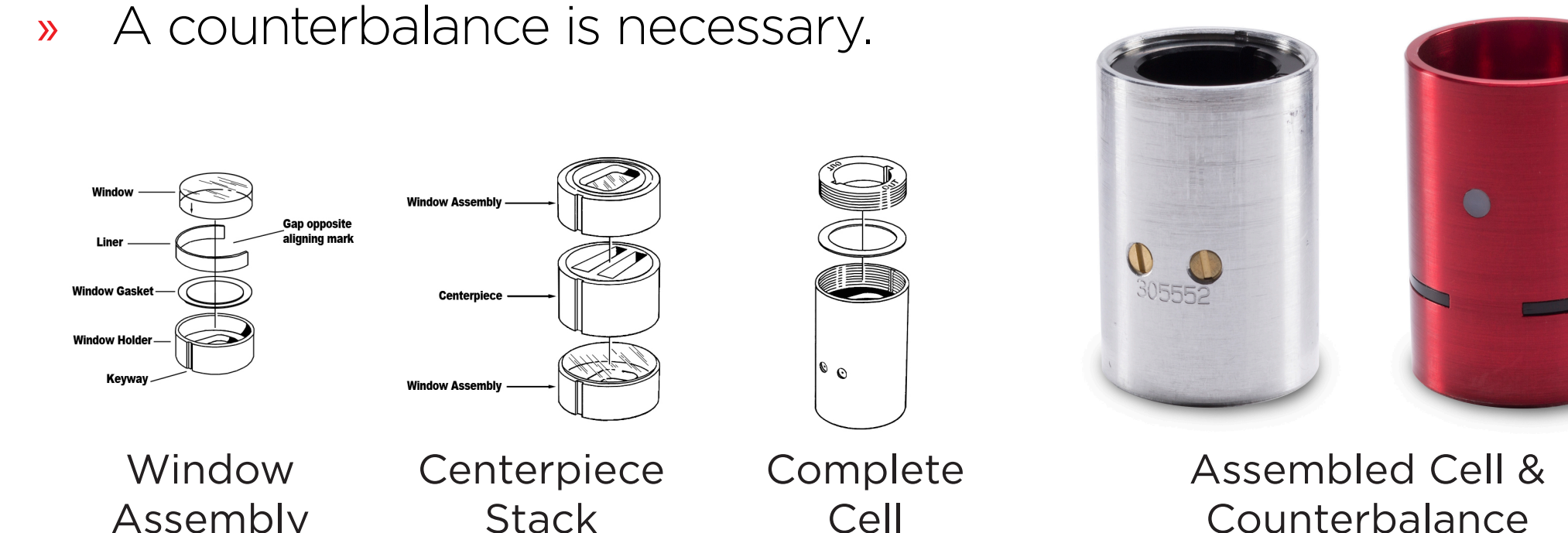### Lamm Equation:

$$\frac{\partial C}{\partial t} = \frac{-1}{r}\left(\frac{\partial}{\partial r}\left(s\omega^2 r^2 C - Dr\frac{\partial C}{\partial r}\right)\right)$$

Components of an AUC Cell

» Two-sector centerpiece sandwiched between two quartz or sapphire windows & assembled into a cylindrical housing.

» A screw ring is torqued to seal the cell.

» A counterbalance is necessary.

Window Assembly — Centerpiece Stack — Complete Cell — Assembled Cell & Counterbalance
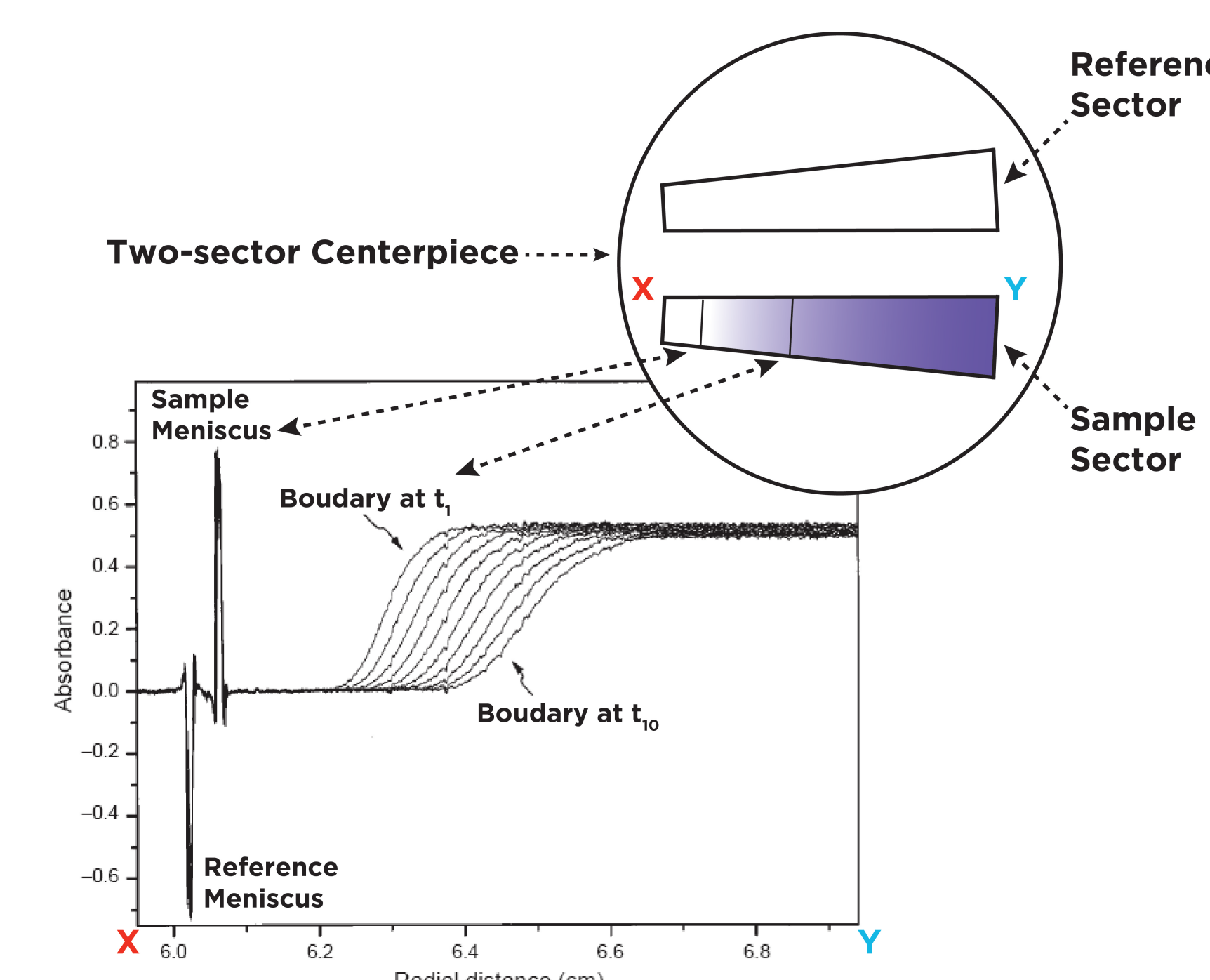
AUC Rotor & Optics

» 4- and 8-hole rotors are rated to 60,000 and 50,000 rpm, respectively.

» Interference and multiwavelength optics can be used in tandem.

An-60 — An-50 — Optics in Position

Introduction to Sedimentation Velocity Data

» Sample migration produces moving boundaries which contain sedimentation and diffusion information.

Two-sector Centerpiece
Reference Sector
Sample Sector
Boundary at t
Boundary at t₁₀
Sample Meniscus
Reference Meniscus

References: (1) By Thomasione, Public Domain, commons.wikimedia.org/w/index.php?curid=37665969. (2) Ultrascan: www.ultrascan3.aucsolutions.com. (3) Sedfit: www.analyticalultracentrifugation.com.
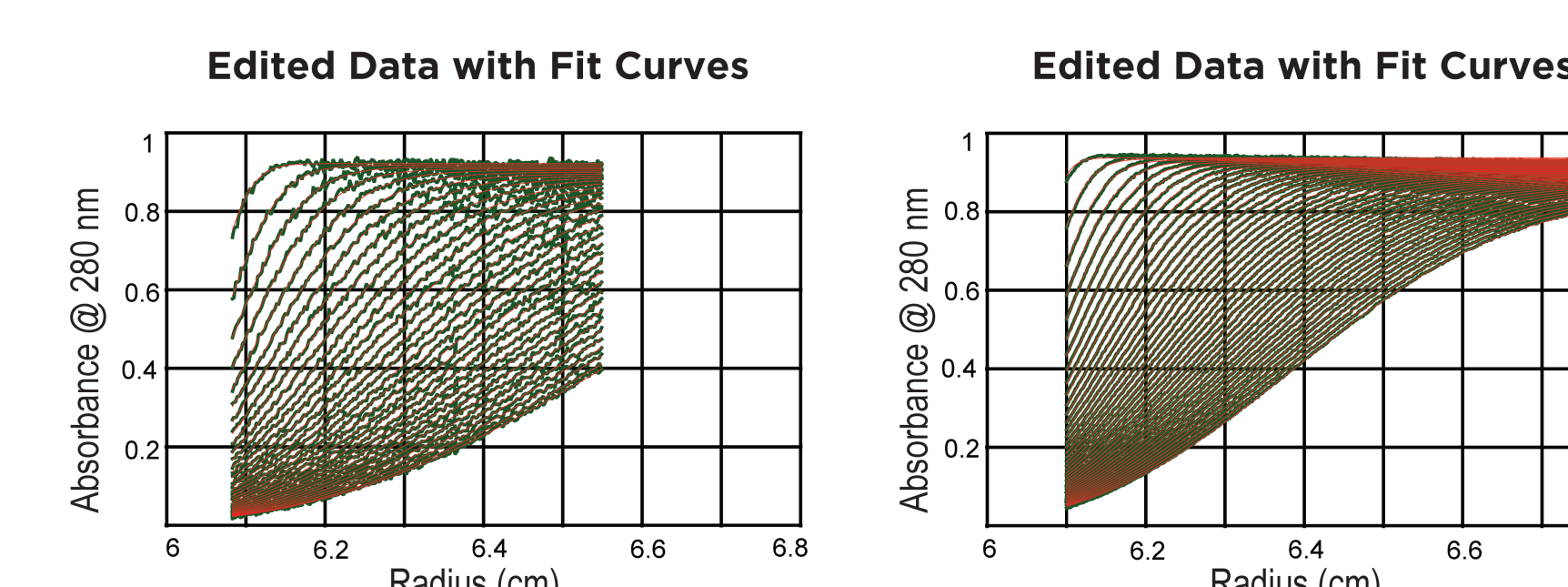
Experimental Setup

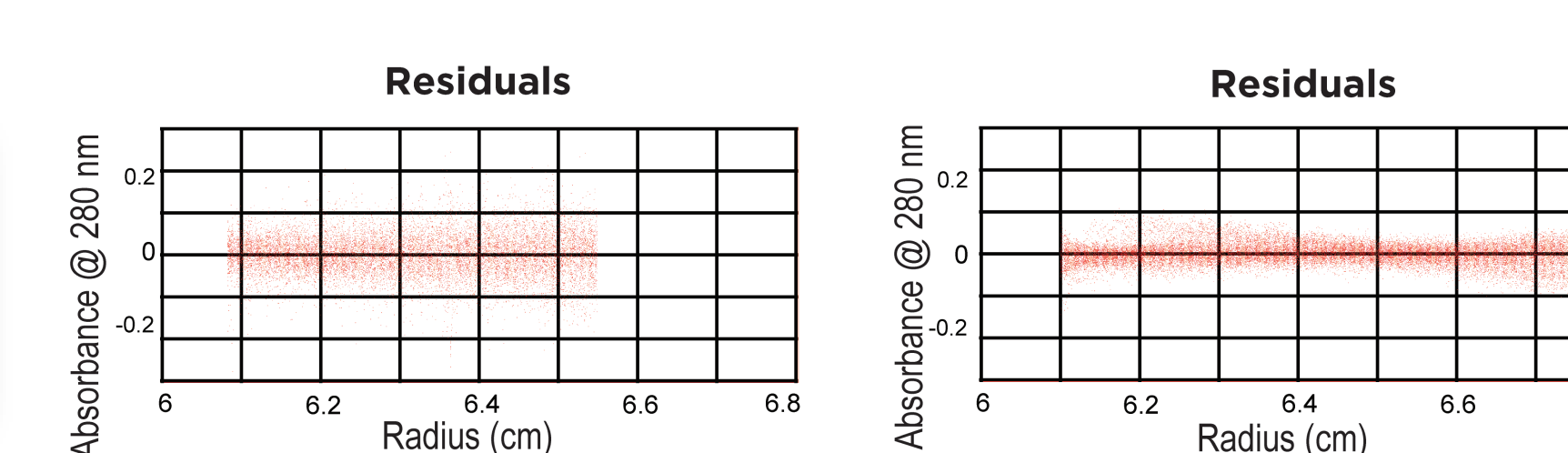40,000 rpm with 200+ absorbance scans (280 nm)
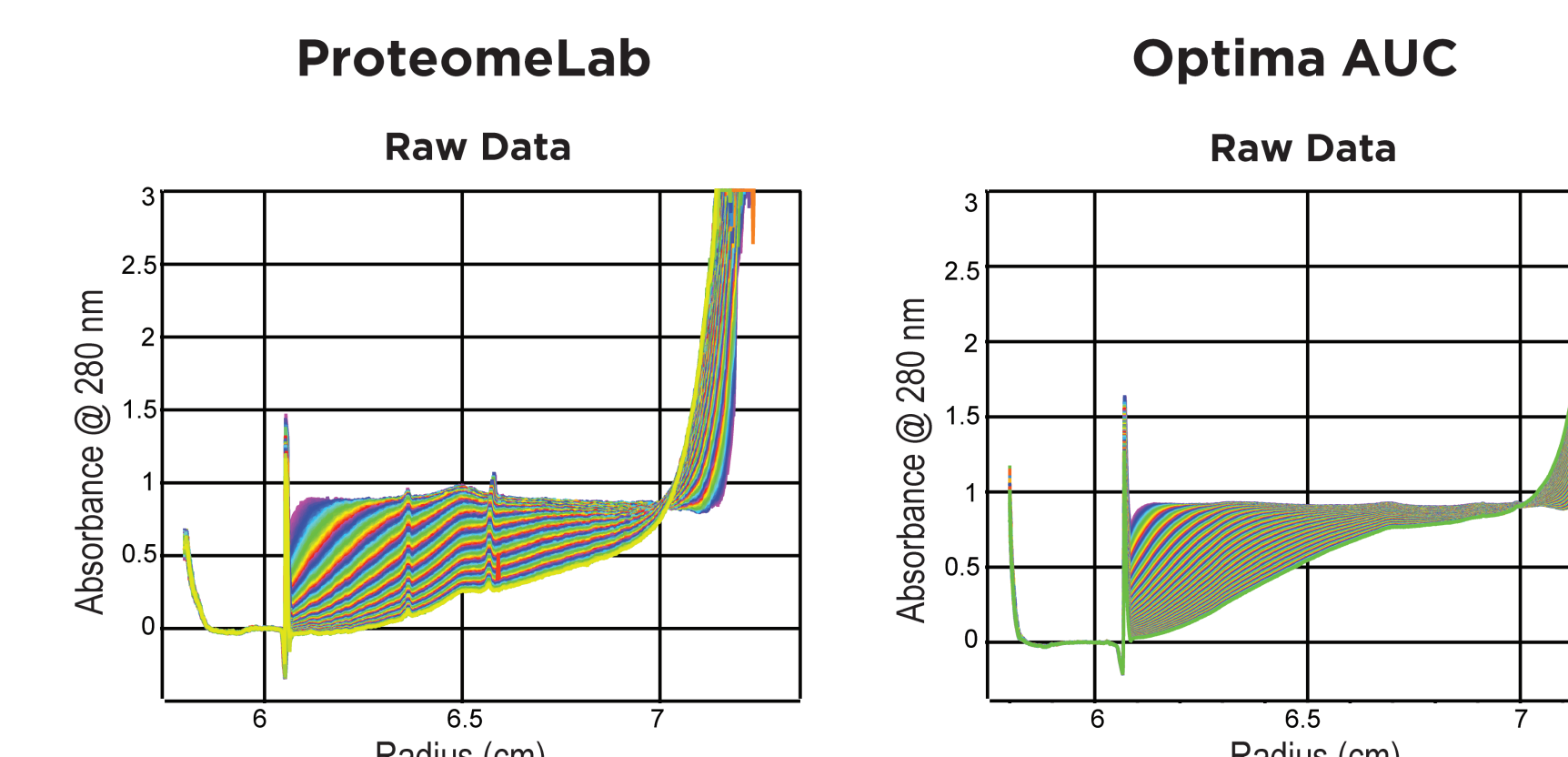
Data Edits

Eliminate spikes, define the meniscus, and limit radial range
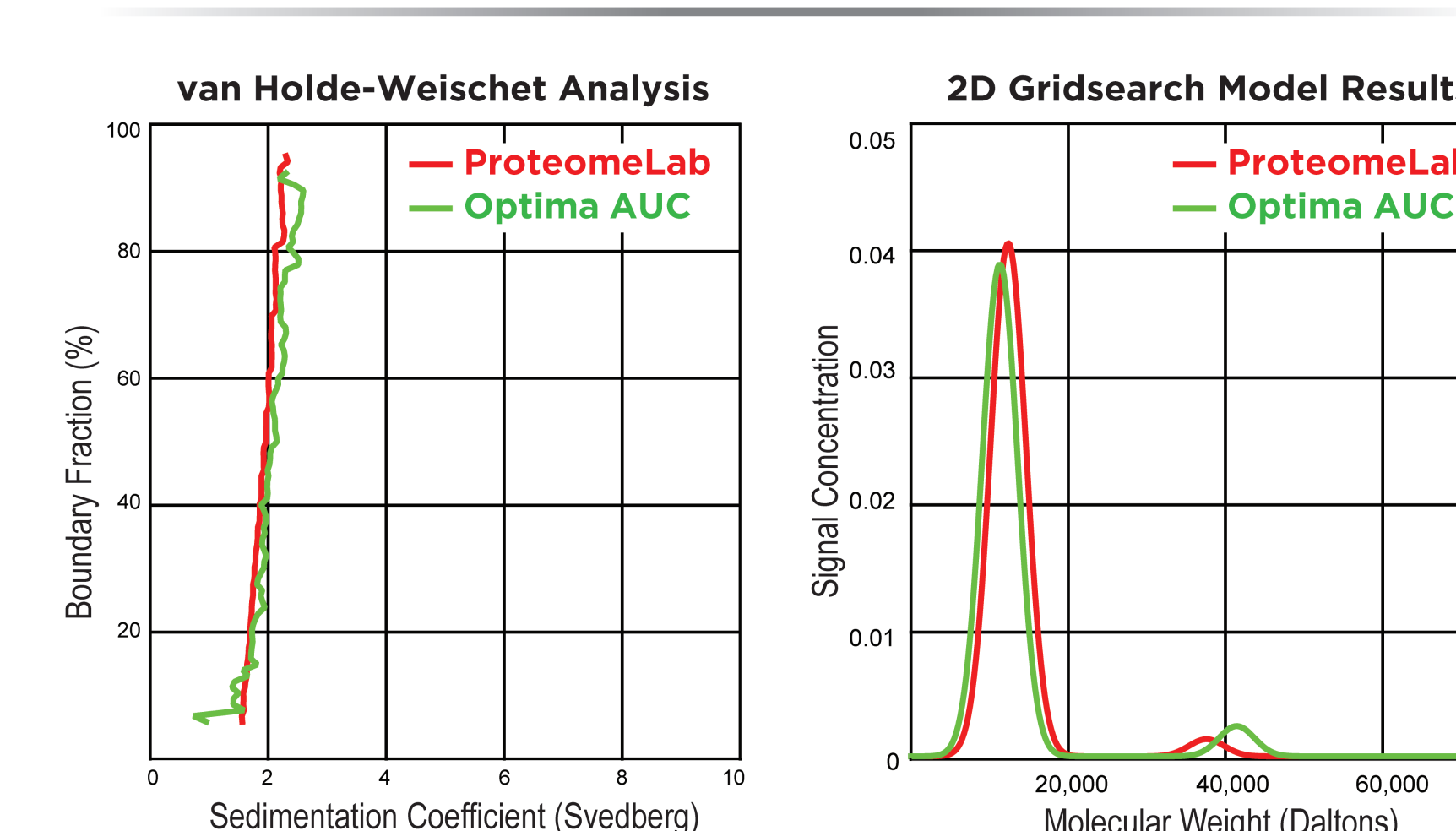
Data Processing

Process data via finite element methods and filter out noise
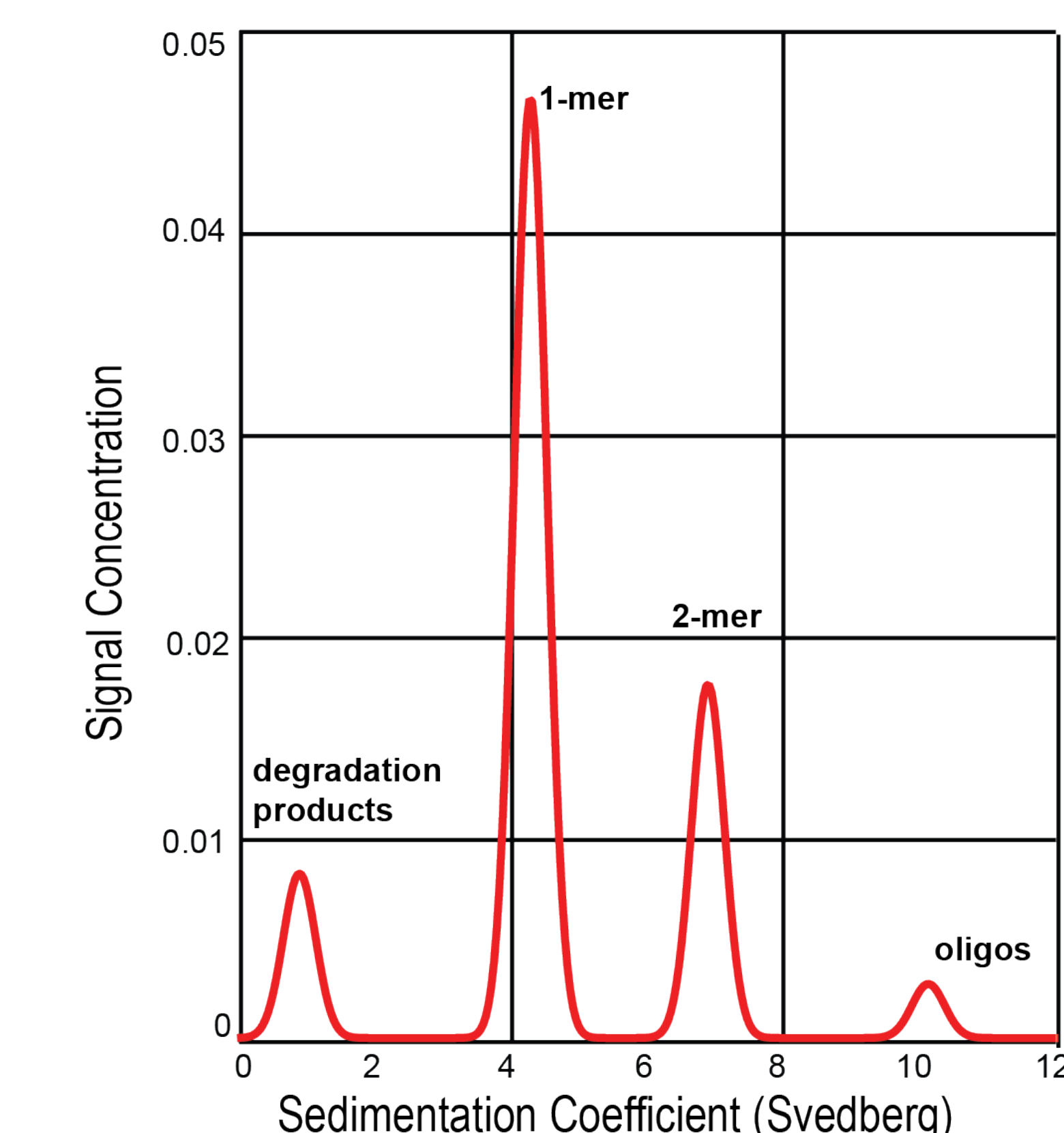
Plot & Interpret

Interpret results from population distributions vs MW or model-free graphical interpolation plots

ProteomeLab — Raw Data
Optima AUC — Raw Data
Edited Data with Fit Curves
Residuals
van Holde-Weischet Analysis
2D Gridsearch Model Results

QUALITY CONTROL & CHARACTERIZATION USING AUC

Quantification of Aggregation and Degradation of Biological Samples and Elucidation of Molecular Shape

BSA Concentration Distribution

1-mer, 2-mer, oligos, degradation products

AAV9 Empty vs Full Viral Packaging

Empty 70.94% SV=59.7
Full 25.25% SV=84.3
AAV9 in MgCl2, BTP and Glycine

danaher

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