# 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.

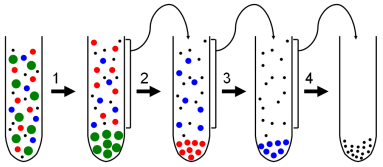
## PREPARATIVE ULTRACENTRIFUGATION

### 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.



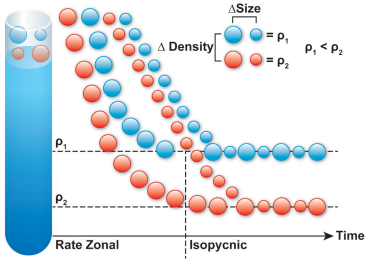

### Density Gradient Ultracentrifugation (DGUC)

» 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.

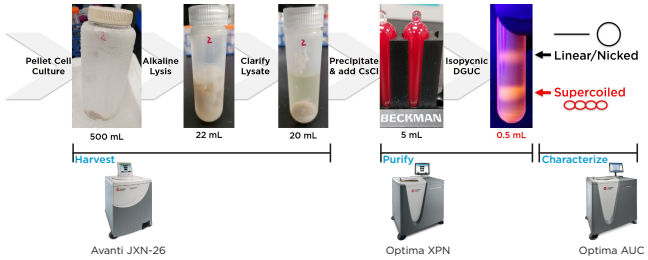



### Example Workflow for DGUC Purification of Plasmid DNA

» 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.




## AUC HISTORY

Theodor Svedberg invents AUC, Chemistry Nobel Prize 1926

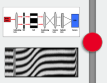
Jean Perrin describes Sedimentation Equilibrium, Physics Nobel Prize 1926

Ole Lamm describes the sedimentation and diffusion of samples in a sector-cell, 1930s

Edward Pickel starts Spinco and builds the first commercial AUC – the Model E, 1947

Beckman acquires Spinco, 1954

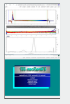
Meselson & Stahl experiment, 1958



Schachman develops Rayleigh Interference detection, 1958

Van-Holde Weischet graphical analysis is developed, 1978

Beckman introduces the Proteomelab XLA/XLI AUC instrument, 1990s



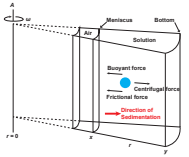
Demeler develops Ultrascan, 1998, Shuck develops Sedfit, 2000

Beckman releases the new generation Optima AUC with multi-wavelength capability

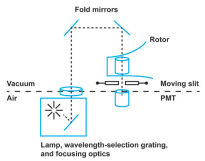
New frontier: Viral vectors, nanoparticles, liposomes

## AUC FUNDAMENTALS

### Forces on Particles




### AUC Optics Schematic




### Lamm Equation:

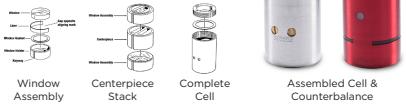
$$\frac{\partial C}{\partial t} = \frac{-1}{r}\left(\frac{\partial}{\partial r}\left(s\omega^2 r^2 C - D r \frac{\partial C}{\partial r}\right)\right)$$

### 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

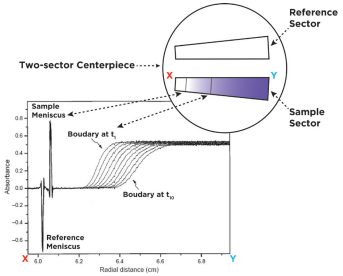
» 4- and 8-hole rotors are rated to 60,000 and 50,000 rpm, respectively.

» Interference and multiwavelength optics can be used in tandem.

An-60 | An-50 | Optics in Position

### Introduction to Sedimentation Velocity Data

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




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

## AUC WORKFLOW CASE STUDY WITH INSULIN

### Comparison of ProteomeLab vs Optima AUC

**Experimental Setup**

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

**Data Edits**

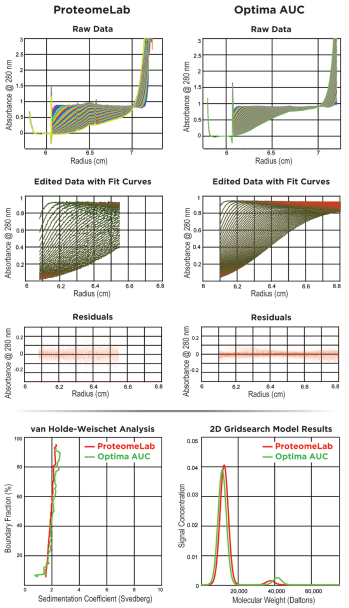
Eliminate spikes, define the meniscus, and limit radial range

**Data Processing**

Process data via finite element methods and filter out noise

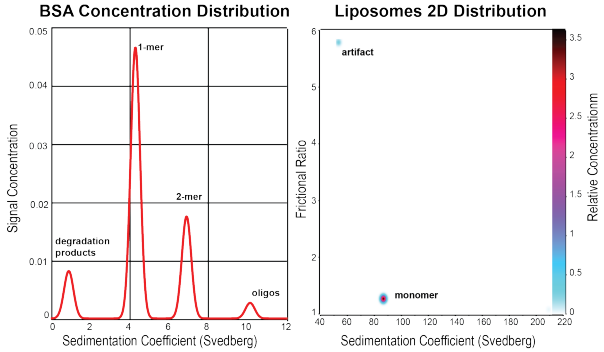
**Plot & Interpret**

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




## QUALITY CONTROL & CHARACTERIZATION USING AUC

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



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