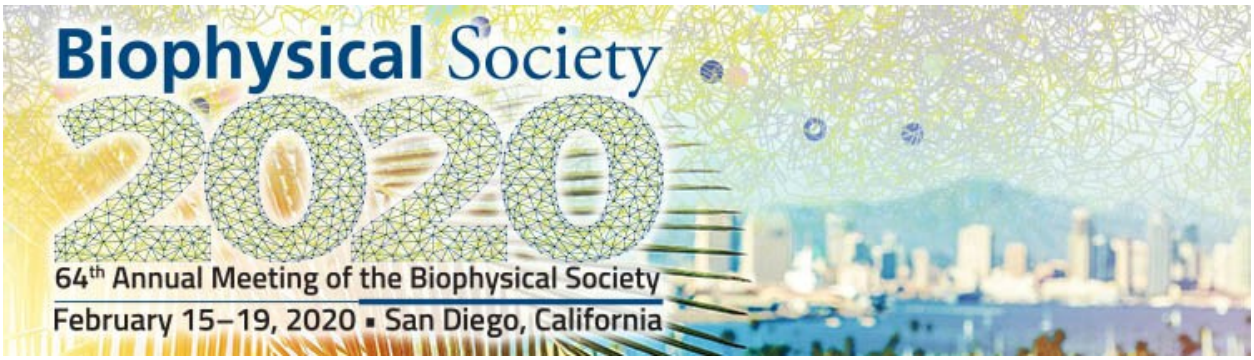

Monday, February 17
8:30 AM – 10:00 AM
Room 33C
Beckman Coulter Life Sciences

Get the High-Resolution Separation That You Have Been Searching for with Preparative and Analytical Ultracentrifugation

Introduction: Purification of biological products, including biotherapeutics, involves the separation of cells from the culture media, followed by extensive processing to isolate the target of interest. Relatively simple separations are often achieved via differential centrifugation (pelleting), though high-resolution separations often utilize density gradient ultracentrifugation to yield high purity. In this presentation, we will discuss the full gamut of preparative (ultra)centrifugation, which permits the isolation and purification of biological components ranging from small peptides and nanoparticles to large nucleic acids, viruses, and organelles. We will then discuss the analytical/characterization aspects of ultracentrifugation, which allow quantitation of size, mass, shape, and density of the biological components that have been purified, along with exploration of their thermodynamic properties and binding interactions. Modern examples will be discussed for both preparative and analytical ultracentrifugation.

Purification: Modern centrifuges reach incredibly high speeds (with centrifugal acceleration sometimes exceeding 1,000,000 x *g*) to aid the high-resolution separation of particles, typically in the micro- or nanometer range, by size and/or density. Today's gene therapy products, such as viral vectors, require high-quality purification to ensure the consistent production of safe, efficacious therapeutics of the highest quality to further advance this rapidly growing field and deliver solutions to patients in need. Density gradient ultracentrifugation (DGUC) is a centrifuge-based technique for providing superior purification of viral vectors (e.g., isolating full AAV particles from partial and empty capsids), along with other materials (such as plasmid DNA) in gene therapy production workflows. Though a well-established and mature method, DGUC is sometimes viewed as dated, challenging to design and conduct, or only suited for small-scale research applications. In this workshop, we'll address these perceptions and discuss the premise of DGUC as a modern, high-resolution purification technique for AAVs and plasmid DNA. We'll also provide guidance on how to get started with DGUC and optimize this technique for gene therapy workflows.

Characterization: Analytical ultracentrifugation (AUC) is one of the most versatile biophysical tools used today for the characterization of biological samples ranging from small drug molecules to intact viruses, vesicles and microparticles. AUC works with biological samples in the native state and does not depend on a reporter species or custom-coated substrates. AUC separates biomolecules based upon both molecular mass and anisotropy and can also be used to quantify interactions between different species. In this talk, we will start with the principles of AUC and take a tour through the technology behind modern AUC, including detection methods. We then look at advancements of the latest gen Optima AUC. Next, we go through experiment design – including the use of simulation tools. Following, we will

address the different types of AUC experiments (equilibrium and velocity), compare and contrast their merits with sample data, and touch upon the principles of data processing. Finally, we will explore a variety of applications with a focus on the unique advantages that AUC brings to the study of various biotherapeutics, polymers, nanoparticles, and others – and how AUC compares to and complements other analytical techniques.

Speakers

Ross VerHeul, Senior Applications Scientist, Beckman Coulter Life Sciences
Akash Bhattacharya, Senior Applications Engineer, Beckman Coulter Life Sciences