

August 24-30, 2025

Tsinghua University, Beijing, China

AUC 2025

the 27th International
Analytical Ultracentrifugation
Workshop and Symposium

BOOK OF ABSTRACTS



Welcome!

We cordially invite you to AUC 2025, the 27th International Analytical Ultracentrifugation Workshop and Symposium, to be held in Beijing, China, on August 24-30, 2025, on campus of the Tsinghua University. Tsinghua University, with over 113 years of academic heritage, embodies a legacy of innovation and unwavering excellence. At Tsinghua, our assembly of innovative minds and visionaries is striving to fulfill the significant needs of the country and actively building a community with a shared future for mankind.

The focus of AUC 2025 is on studies of biological macromolecules, interacting systems, synthetic polymers, nanoparticles, and colloids. Techniques featured include analytical ultracentrifugation (AUC), dynamic and static light scattering (DLS, MALS), small angle X-ray and neutron scattering (SAXS, SANS), microscale thermophoresis (MST), nuclear magnetic resonance (NMR), surface plasma resonance (SPR), field flow fractionation (FFF), mass photometry (MP) and calorimetry (ITC, DSC), and electron microscopy (SEM, TEM, CT) are featured.

AUC 2025 will open with several workshops, which begin on August 25th, and end on Tuesday, August 26th. These workshops will be given by leading experts in their respective fields. On Wednesday, August 27th there will be an intercultural exchange. The symposium will start on the morning of Thursday, August 28th, with our keynote lecture and continue until the afternoon of Friday, August 29th. The symposium will offer two poster sessions, an exhibitor fair, and roundtable discussions. On Saturday, August 30th, a visit to the National Protein Science Facility will be arranged.

We warmly welcome the AUC community to Beijing and to Tsinghua University. We hope you will find this week's program both inspiring and rewarding.

Wenqi Li, Xiaodong Ye, Shaowei Li, Mengdi Chen
Co-Chairs, AUC 2025

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Organization

Scientific Organizing Committee (SOC):

- Byron, Olwyn (University of Glasgow, UK)
- Chen, Mengdi (East China Normal University, China)
- Demeler, Borries (University of Lethbridge, Canada)
- Dobson, Renwick (University of Canterbury, New Zealand)
- Ebel, Christine (University Grenoble Alpes, France)
- Fleming, Karen (Johns Hopkins University, USA)
- Li, Shaowei (Xiamen University, China)
- Li, Wenqi (Tsinghua University, China)
- Nischang, Ivo (Friedrich Schiller University Jena, Germany)
- Patel, Trushar (University of Lethbridge, Canada)
- Uchiyama, Susumu (Osaka University, Japan)
- Walter, Johannes (FAU, Germany)
- Ye, Xiaodong (University of Science and Technology of China, China)

Local Organizing Committee (LOC):

- Li, Wenqi (Tsinghua University, China)
 - Ye, Xiaodong (University of Science and Technology of China, China)
 - Li, Shaowei (Xiamen University, China)
 - Chen, Mengdi (East China Normal University, China)
 - Chang, Qing (Tsinghua University, China)
 - Chu, Wendan (Tsinghua University, China)
-
- Li, Yuxin (Tsinghua University, China)
 - Wang, Zhaoxing (Tsinghua University, China)



sponsor





GUSI: Basics and Advanced GUSling

- **Chad Brautigam** Biomedicine Hall E302

GUSI is a software program that is designed to ease the generation of figures from biophysical data formats. It is particularly useful in combination with SEDFIT and SEDPHAT, as these latter programs send data directly to GUSI. Covered in this workshop will be the basics of GUSI's layout and operation, including working with multiple data sets, manipulating colors and markers, and generating legends. Advanced GUSI concepts, including rudimentary line fitting, formatting files for input to GUSI, and exploring the wide array of biophysical data types pre-formatted in the program will also be discussed.

AUC GMP analysis with UltraScan

- **Borries Demeler** Biomedicine Hall E303

In this workshop I will present how to process AUC experiments in a GMP environment with UltraScan. UltraScan's GMP module is fully compliant with 21-CFR-part11 requirements and largely automates the entire workflow from data acquisition to GMP reporting, electronic signatures and audit trails. UltraScan is the only software to offer a GMP compliant data acquisition module for the Optima AUC instrument and integrates directly with the Optima over encrypted ethernet connections without modifying the data as is done in the Beckman data acquisition software. Automated data acquisition, live data monitoring, LIMS data import, editing, analysis and reporting is processed on a high performance computing platform which parallelizes the data analysis process for up to 16 samples and up to 100 wavelength for each sample simultaneously, and drastically reduces the time required for a complete experimental analysis. A PDF report is automatically generated that includes integrations of s , D , f/f_0 , MW or radial metrics. Here I will demonstrate how to analyze generic sedimentation velocity experiments in automated GMP mode, and I will demonstrate AAV capsids in analytical buoyant density equilibrium (ABDE) mode. Finally, I will demonstrate how to switch the data source from Optima AUC instrument to disk to permit automated GMP analysis of datasets acquired on older Proteomelab XLA/XLI instruments, or through data import using the UltraScan tar.gz extraction/conversion routine for OpenAUC data formats.



Analysis of Reversible Self-Associations by AUC using sedfit/sedphat

- **Alexander Bepperling** Biomedicine Hall E402

The workshop will be a hands-on training using sedfit, sedphat and Gussi. Please download the respective software from: <https://sedfitsedphat.nibib.nih.gov/software> and <https://www.utsouthwestern.edu/research/core-facilities/mbr/software/>. Unzip and place all three software packages under C:\sedfit on your computer. We will analyse a series of sedimentation velocity experiments of a self-associating protein, which I have recorded beforehand, using sedfit. Afterwards we will construct a isotherm in Gussi and export it to sedphat. There, we will setup a model and fit the data to obtain an association constant. The repeat session will be similar, but we will analyze a hetero-association and I will focus more on the sedphat part.

SV-AUC of Membrane Proteins

- **Christine Ebel** Biomedicine Hall E403

The AUC challenge of solubilized membrane protein samples is principally that detergent-solubilized membrane proteins represent a multicomponent system, in which different components (protein, detergent, lipid, water) interact to form the different types of sedimenting particles (species). The samples are therefore necessarily polydisperse, principally because of the presence of free detergent micelles in solution. Depending on the specific conditions, this complexity can be disentangled by taking advantage of several key distinctions between protein and detergent properties. For example, detergents and proteins have distinct optical properties (different extinction coefficients and increments of refractive index), and different buoyant properties (partial specific volumes). Moreover, membrane protein-protein interactions (as protein stability) can significantly depend on the type and concentration of detergent. In addition to a presentation of the theoretical considerations and mathematical formalism, we will discuss practical implementation and analysis of sedimentation velocity experiments and analysis of membrane protein/detergent solutions. Topics to be covered include the following: How to determine the parameters needed for data analysis (partial specific volumes and increment of refractive indexes); Strategies for the design of the cells and experiments; How to analyze the data using sedfit/sedphat/gussi programs; How to evaluate size distribution and non-interacting species, sample homogeneity, protein association state, amounts of bound detergent, and the limits of these analysis.



Multisignal Sedimentation Velocity

- **Chad Brautigam** Biomedicine Hall E302

Knowing the stoichiometry of interacting macromolecules is vital to a comprehensive understanding of the interaction's thermodynamics. An excellent means to discover stoichiometry is multisignal sedimentation velocity (MSSV). This method leverages the ability of modern ultracentrifuges to probe the sedimentation of macromolecules using different wavelengths of light and different detection modalities. This ability, coupled with knowledge of the components' signal increments, allows a global analysis of MSSV experiments to be conducted, decomposing the resulting solute distributions into component distributions, allowing the molar ratio of co-sedimenting solutes to be determined. When this datum is coupled with the hydrodynamic properties of the complex, an accurate value for stoichiometry can be derived. This workshop will cover experimental strategies and hands-on data analysis of MSSV experiments in SEDPHAT.

AUC and orthogonal methods for the estimation of size distribution of AAV vectors

- **Susumu Uchiyama** Biomedicine Hall E303

AUC and orthogonal methods for the estimation of size distribution of AAV vectors including full and empty ratio will be explained. Methods includes, three AUC methods (SV-AUC, BS-AUC, and DGE-AUC), mass photometry, charge detection mass spectrometry, chromatography, and cryo-electro microscopy. Parameters necessary for the quantitative analysis in each method will be introduced.



Cooperative SLS and DLS measurements: from principles to advanced characterizations on macromolecular, colloidal and self-assembly systems

● **Tianbo Liu** Biomedicine Hall E402

Static and dynamic laser light scattering techniques (SLS and DLS) are two different techniques with different principles. They often share a good fraction of instruments; therefore it is convenient to perform SLS and DLS on the same system to obtain much more valuable information.

SLS represents the traditional scattering technique, which measures the total scattered intensity from a solution at different scattering angles to get a scattering curve. SLS assumes that the incident and scattering lasers have the identical wavelength – referred as elastic scattering. SLS can be used to determine the weight-average molecular weight (MW), radius of gyration (R_g) of particles, as well as the nature and magnitude of intermolecular interactions via the second virial coefficient (A_2).

DLS measures the scattered light fluctuations within very short time range, raised by the random Brownian movement of particles. The Brownian movements lead to the Doppler's effect, resulting in tiny changes in the wavelength of the scattered light from that of the incident light. By means of a digital correlator, such tiny changes can be determined and amplified by the time correlation function and consequently be used to determine the Brownian move speed of particles – in diffusion coefficient and then the hydrodynamic radius (R_h).

The combination of SLS and DLS can be very powerful in determining the various structures and processes in solutions, such as the shape, size and mass of particles, biological complexes and supramolecular assemblies. In the workshop, examples on different areas will be given, such as the self-assembly of surfactants into micelles and vesicles, the polyelectrolyte behavior of biological macromolecules, accurate determination of the shapes of particles, as well as the time-resolved online measurements to obtain the kinetics of assembly/formation processes.



Basic Principles of Analytical Ultracentrifugation and Its Applications in Macromolecular Characterization

● **Xiaodong Ye** Biomedicine Hall E403

This workshop provides an introduction to Analytical Ultracentrifugation (AUC), a powerful and versatile technique for investigating macromolecules and their interactions in solution. We will begin with a brief overview of the historical development of AUC, followed by an explanation of key theoretical foundations and parameters, including the Svedberg equation, the Lamm equation, sedimentation and diffusion coefficient, partial specific volume, and frictional ratio.

The core focus will be on the Sedimentation Velocity (SV) method—the most widely used AUC technique—accompanied by brief discussions of Sedimentation Equilibrium (SE) and Band Sedimentation. We will address how to optimize experimental conditions for various types of samples and analytical goals, with particular attention to rotor speed, sample concentration, and detector selection. As part of this, we will also introduce the use of fixed-position optical detection to monitor time-resolved transmission intensity, a method that can be applied to determine nanoparticle size distributions using commercial AUC instruments. The workshop will also cover data acquisition and analysis strategies. Throughout the session, examples from our own research and the literature will be used to illustrate the capabilities of AUC in analyzing complex systems, including:

- Polymers with different topologies
- DNA conformational transitions
- Protein-polyelectrolyte interactions



Tools for multidimensional particle characterization via AUC

● **Johannes Walter** Biomedicine Hall E302

Analytical Ultracentrifugation is perfectly suited for the analysis of particle size distributions (PSDs). However, the characterization of polydisperse PSDs is making high demands to any characterization technique. For sedimentation analysis, it has to be taken into account that the sedimentation rate scales with the particle size squared. To tackle such challenging systems, multi-wavelength (MW) gravitational sweep (GS) experiments at a fixed radial position were developed which are based on a continuously increasing rotor speed. GS experiments are of particular importance when studying polydisperse PSDs due to the much larger dynamic range compared to traditional sedimentation velocity (SV) experiments.

HDR-MULTIFIT can be used to analyze GS data and provides the ability to determine the optical properties of individual components in polydisperse mixtures and to relate this data to the hydrodynamic properties. For spherical NPs having well-defined refractive indices, high dynamic range (HDR) particle size analysis is possible. The analysis benefits from the direct fractionation of different particle sizes in the measurement cell during sedimentation, while the optimum signal to noise ratio for the whole PSD is achieved by automatically tuning the wavelength used for data evaluation.

Besides GS experiments, it is demonstrated that HDR-MULTIFIT can also be used for the post-processing of sedimentation coefficient distributions from sedimentation velocity experiments (derived by e.g., SEDFIT, SEDANAL, UltraScan, DCDT+). Concentration coupling and the simultaneous determination of size and density are further capabilities offered by HDR-MULTIFIT.

In addition, HDR-SVFIT, a tool for MW analysis of SV data will be presented which provides the possibility to conduct analysis of hundreds of wavelengths simultaneously on a standard office computer. Such results can be processed to derive sedimentation coefficient dependent extinction and emission spectra.

The workshop will tackle the following topics:

1. Determination of particle sizes and their distributions
2. The influence of scattering and the role of Mie's theory
3. Correction of size distributions and their weighting
4. Multi-wavelength analysis of GS experiments
5. Concentration coupling experiments for polydisperse distributions
6. Simultaneous analysis of size, density and refractive index increment using density variation
7. Multi-wavelength analysis of SV experiments including global regularization schemes
8. Post-processing of MW analysis results

Most importantly, this workshop will include a hands on training on HDR-MULTIFIT, HDR-SVFIT and related tools.



US-SOMO Hydrodynamics-1

● **Emre Brookes, Mattia Rocco, Olwyn Byron** Biomedicine Hall E303

UltraScan SOLution MOdeler (US-SOMO)[1,2] processes atomic and lower-resolution bead model representations of biological and other macromolecules to compute various hydrodynamic parameters, such as the sedimentation and diffusion coefficients, relaxation times and intrinsic viscosity. This allows the researcher to validate structural models against experimental data. The tools available in US-SOMO have been shown to provide the best known computations of hydrodynamic parameters from experiment [3]. The US-SOMO hydrodynamics workshop will consist of two sessions. In the first session, attendees will be given an introduction to computational hydrodynamics and techniques. In the second session, users will utilize US-SOMO to compute hydrodynamics parameters from structures and to use automated batch processing to compute over multiple structures. A brief introduction to the US-SOMO web version <https://somoweb.genapp.rocks> will be presented. It is suggested that attendees attend both sessions, as earlier material will not be covered again during the two session course. Registered attendees will receive detailed software installation instructions in the week prior to the workshop. References:

[1] Rocco M, Byron O., 2015, "Hydrodynamic Modeling and Its Application in AUC." , Methods Enzymol. 2015;562:81-108. doi: 10.1016/bs.mie.2015.04.010.

[2] Rocco, M., Brookes, E. & Byron, O. US-SOMO: Methods for Construction and Hydration of Macromolecular Hydrodynamic Models. In: Encyclopedia of Biophysics, Roberts, G. & Watts, A., European Biophysical Societies (eds). Springer, Berlin, Heidelberg (2021). doi: 10.1007/978-3-642-35943-9_292-1.

[3] Brookes, E. and Rocco, M., 2018. Recent advances in the UltraScan SOLution MOdeller (US-SOMO) hydrodynamic and small-angle scattering data analysis and simulation suite. European Biophysics Journal, 47(7), pp.855-864. doi:10.1007/s00249-018-1296-0.



Analysis and Fractionation of Nanoparticles by Analytical Ultracentrifugation

● **Mengdi Chen** Biomedicine Hall E402

Analytical ultracentrifugation (AUC) has proven to be a valuable tool for the study of colloids since its invention by Svedberg, who initially used it for the particle size characterization of gold colloids. The character of colloids raises analytical questions, which are often different from those which are commonly addressed for biopolymers; therefore, AUC analysis of colloidal systems differs from that of well-defined proteins. Colloids are often extremely polydisperse owing to an inherent tendency to aggregate and thus must be treated as interacting polydisperse systems. Furthermore, colloids are often hybrid systems (e.g. inorganic-organic) and have a particle size and density distribution. A further complicating feature is that many colloids, especially inorganic species, are extremely small, so diffusion broadening of boundaries can make AUC analysis of such systems difficult.

This talk highlights which answers AUC can give for colloidal systems despite the previously mentioned obstacles, which restrict the use of many common AUC evaluation techniques for proteins. Some examples will be presented and discussed

- (1) Pt and ZrO₂ colloids as examples for the determination of particle size distributions of extremely small colloids in the nanometer range;
- (2) an interacting binary mixture of reactive lattices with a protein, which leads to aggregation as an example for the quantitative assessment of colloid aggregation phenomena;
- (3) iron oxides in a carrageenan microgel, coated metal particles and a growing inorganic mineral inside an organic aggregate as organic-inorganic hybrid particles, as well as ferritin, an organic-inorganic hybrid colloid which has a particle size and density distribution as an example for the combination of AUC with other size-dependent but density-independent methods like flow-field-flow fractionation to yield further insight into the nature of the different oligomers; and
- (4) synthetic boundary crystallization ultracentrifugation as an example of how fast processes like crystal growth can be transferred to a spatial resolution of species which can be analyzed by the much slower AUC technique. These examples illustrate how common problems in colloid chemistry can be solved by AUC and which pitfalls and future perspectives exist.



AUCAgent, a new software aimed for automatically analysis of AUC

● **Wenqi Li** Biomedicine Hall E403

AUCAgent is an innovative AUC data analysis software. It incorporates automated meniscus detection capabilities, enabling direct processing of the raw intensity data in experiments conducted without reference solutions, substantially boosting experimental data acquisition throughput. Its comprehensive experimental recording module ensures complete data traceability while providing robust support for researchers' data processing and experimental reporting needs. Moreover, the integrated data quality control module facilitates the identification of scans with optimal signal-to-noise ratios, thereby enhancing the reliability of analytical outcomes. This workshop is designed to provide a comprehensive introduction to some of the current functions and usage methods of AUCAgent.



Analytical Ultracentrifugation of Colloidal Systems Featuring Dispersity

● **Ivo Nischang** Biomedicine Hall E302

1. Basic relations

(intrinsic) diffusion coefficient, (intrinsic) sedimentation coefficient, intrinsic viscosity, partial specific volume, translational friction properties, molar mass, hydrodynamic invariant, hydration

2. Sedimentation velocity experiments

Sedimentation-diffusion analysis

absolute molar mass, alternative methods (size-exclusion chromatography / field-flow fractionation—multi-angle light scattering (SEC / FFF—MALS))

(Self-)assemblies in solution

hydration, degree of aggregation, sizing

Nanoscale drug delivery systems

solution complexity, colocalization studies, serum experiments

3. Sizing aspects (AUC, DLS, SEC / FFF—MALS)

hydrodynamic size estimates based on sedimentation analysis (AUC), sedimentation-diffusion analysis (AUC), diffusion measurements (DLS), and radii of gyration (MALS)



US-SOMO Hydrodynamics-2

● **Emre Brookes, Mattia Rocco, Olwyn Byron** Biomedicine Hall E303

UltraScan SOLUTION MOdeler (US-SOMO)[1,2] processes atomic and lower-resolution bead model representations of biological and other macromolecules to compute various hydrodynamic parameters, such as the sedimentation and diffusion coefficients, relaxation times and intrinsic viscosity. This allows the researcher to validate structural models against experimental data. The tools available in US-SOMO have been shown to provide the best known computations of hydrodynamic parameters from experiment [3]. The US-SOMO hydrodynamics workshop will consist of two sessions. In the first session, attendees will be given an introduction to computational hydrodynamics and techniques. In the second session, users will utilize US-SOMO to compute hydrodynamics parameters from structures and to use automated batch processing to compute over multiple structures. A brief introduction to the US-SOMO web version <https://somoweb.genapp.rocks> will be presented. It is suggested that attendees attend both sessions, as earlier material will not be covered again during the two session course. Registered attendees will receive detailed software installation instructions in the week prior to the workshop. References:

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[2] Rocco, M., Brookes, E. & Byron, O. US-SOMO: Methods for Construction and Hydration of Macromolecular Hydrodynamic Models. In: Encyclopedia of Biophysics, Roberts, G. & Watts, A., European Biophysical Societies (eds). Springer, Berlin, Heidelberg (2021). doi: 10.1007/978-3-642-35943-9_292-1.

[3] Brookes, E. and Rocco, M., 2018. Recent advances in the UltraScan SOLUTION MOdeller (US-SOMO) hydrodynamic and small-angle scattering data analysis and simulation suite. European Biophysics Journal, 47(7), pp.855-864. doi:10.1007/s00249-018-1296-0.



Comprehensive characterization of protein-based vaccine antigens by analytical ultracentrifugation and complementary techniques

● **Shaowei Li** Biomedicine Hall E402

Since its introduction by Dr. Theodor Svedberg, analytical ultracentrifugation (AUC) has evolved into a pivotal biophysical tool for analyzing biological macromolecules, their complexes, and their interactions with other molecules. It is now widely applied across biology, medicine, chemistry, physics, and related disciplines. In this workshop, I will explore the technical features of AUC, with particular emphasis on the complementarity between sedimentation velocity (SV) and sedimentation equilibrium (SE) methods. Drawing on more than two decades of research experience in recombinant vaccine development and structural vaccinology, I will discuss experimental considerations, data analysis strategies, and applications of AUC in characterizing diverse protein-based vaccine antigens. Selected published AUC results will be presented, along with suggestions for best practices, and will be complemented by discussions on integrating AUC with other analytical techniques such as surface plasmon resonance (SPR), size-exclusion chromatography (SEC), and dynamic light scattering (DLS). These examples will illustrate how AUC and complementary methods can be applied synergistically throughout the vaccine development process. The aim is to provide practical, experience-based guidance for the use of AUC in research, quality control, and regulatory evaluation in vaccine-related fields. This workshop will also serve as a prelude to my upcoming symposium presentation, "Versatility of Analytical Ultracentrifugation in Vaccine R&D," thereby offering a practice-to-theory knowledge continuum for AUC in vaccine research and development.



Optima AUC cGMP Suite Analysis Management Software Introduction

● **Qikun Yu** Biomedicine Hall E403

Ensuring that analytical ultracentrifugation (AUC) data fully complies with cGMP requirements has always been a significant challenge for quality management and regulatory compliance in the biopharmaceutical industry. Data integrity, traceability and complete audit trails are not only mandatory regulatory requirements but also core safeguards for ensuring product quality and patient safety.

Beckman Coulter has introduced Optima cGMP Suite analysis management software specifically designed for AUC, aiming to completely solve the AUC cGMP dilemma and provide enterprises with powerful, user - friendly, and validated compliance tools.

The core advantages of this innovative cGMP software are as follows:

- Seamless integration into your cGMP workflow: Designed to meet stringent regulatory requirements such as 21 CFR Part 11.
- Robust data integrity and security: Ensuring tamper - proof and traceable processes from raw data acquisition to final report generation.
- Simplified compliance management: Automated audit trails, electronic signatures (optional), and user - rights management significantly reduce compliance workload and risks.
- Enhanced efficiency and confidence: Helping you generate reliable reports that meet cGMP standards more quickly, accelerating the R & D process and product launch.

Want to gain a deeper understanding and hands - on experience with this transformative software? Join our upcoming Workshop!



Analysis of Reversible Hetero-Associations by AUC using sedfit/sedphat

- **Alexander Bepperling** Biomedicine Hall E302

The workshop will be a hands-on training using sedfit, sedphat and Gussi. Please download the respective software from: <https://sedfitsedphat.nibib.nih.gov/software> and <https://www.utsouthwestern.edu/research/core-facilities/mbr/software/>. Unzip and place all three software packages under C:\sedfit on your computer. We will analyse a series of sedimentation velocity experiments of a self-associating protein, which I have recorded beforehand, using sedfit. Afterwards we will construct an isotherm in Gussi and export it to sedphat. There, we will setup a model and fit the data to obtain an association constant. The repeat session will be similar, but we will analyze a hetero-association and I will focus more on the sedphat part.

SV-AUC of Membrane Proteins Repeat Session1

- **Christine Ebel** Biomedicine Hall E303

The AUC challenge of solubilized membrane protein samples is principally that detergent-solubilized membrane proteins represent a multicomponent system, in which different components (protein, detergent, lipid, water) interact to form the different types of sedimenting particles (species). The samples are therefore necessarily polydisperse, principally because of the presence of free detergent micelles in solution. Depending on the specific conditions, this complexity can be disentangled by taking advantage of several key distinctions between protein and detergent properties. For example, detergents and proteins have distinct optical properties (different extinction coefficients and increments of refractive index), and different buoyant properties (partial specific volumes). Moreover, membrane protein-protein interactions (as protein stability) can significantly depend on the type and concentration of detergent. In addition to a presentation of the theoretical considerations and mathematical formalism, we will discuss practical implementation and analysis of sedimentation velocity experiments and analysis of membrane protein/detergent solutions. Topics to be covered include the following: How to determine the parameters needed for data analysis (partial specific volumes and increment of refractive indexes); Strategies for the design of the cells and experiments; How to analyze the data using sedfit/sedphat/gussi programs; How to evaluate size distribution and non-interacting species, sample homogeneity, protein association state, amounts of bound detergent, and the limits of these analysis.



Analytical Ultracentrifugation of Colloidal Systems Featuring Dispersity Repeat Session4

● **Ivo Nischang** Biomedicine Hall E402

1. Basic relations

(intrinsic) diffusion coefficient, (intrinsic) sedimentation coefficient, intrinsic viscosity, partial specific volume, translational friction properties, molar mass, hydrodynamic invariant, hydration

2. Sedimentation velocity experiments

Sedimentation-diffusion analysis

absolute molar mass, alternative methods (size-exclusion chromatography / field-flow fractionation—multi-angle light scattering (SEC / FFF—MALS))

(Self-)assemblies in solution

hydration, degree of aggregation, sizing

Nanoscale drug delivery systems

solution complexity, colocalization studies, serum experiments

3. Sizing aspects (AUC, DLS, SEC / FFF—MALS)

hydrodynamic size estimates based on sedimentation analysis (AUC), sedimentation-diffusion analysis (AUC), diffusion measurements (DLS), and radii of gyration (MALS)



Bring SV-AUC into the cGMP environment: Considerations and Expectations

● **Lake Paul** Biomedicine Hall E403

Analytical Ultracentrifugation (AUC) has long been considered the gold standard in biophysical characterization, offering unmatched capabilities in size distribution analysis and particle quantitation. Historically confined to research and development, AUC is now gaining traction in regulated environments—driven in part by the rise of Cell and Gene Therapy products that demand high-resolution, orthogonal methods.

This workshop will explore the transition of AUC from R&D to cGMP and Quality Control applications. We will highlight BioAnalysis' successful implementation of AUC in compliance with ICHQ2(R1/R2) guidelines, demonstrating its utility not only in early-phase development but also in late-phase and commercial release testing. Key topics will include method validation aligned with regulatory expectations, rigorous computer software validation specific to AUC workflows, and practical strategies for troubleshooting common implementation challenges.

Participants will leave with a framework for integrating AUC into their own quality systems.



Simulating AUC Data

- **Chad Brautigam** Biomedicine Hall E302

The ability to simulate a sedimentation velocity (SV) experiment before actually conducting it is a critical aid to experimental planning. Among the many questions that can be addressed are: How long will the experiment take? How many scans will I get? How will the downstream analyses behave with a given set of parameters? In this workshop, I will cover the theoretical and mathematical underpinnings of SV simulations and compare the ways simulation is handled in several freeware programs. In depth instruction on the use of SViMULATE will be offered, including advanced noise-generation algorithms, using PDB files as the basis for simulation, interacting systems, and non-ideal sedimentation.

AUC and orthogonal methods for the estimation of size distribution of AAV vectors

Repeat Session 2

- **Susumu Uchiyama** Biomedicine Hall E303

AUC and orthogonal methods for the estimation of size distribution of AAV vectors including full and empty ratio will be explained. Methods includes, three AUC methods (SV-AUC, BS-AUC, and DGE-AUC), mass photometry, charge detection mass spectrometry, chromatography, and cryo-electro microscopy. Parameters necessary for the quantitative analysis in each method will be introduced.



Cooperative SLS and DLS measurements: from principles to advanced characterizations on macromolecular, colloidal and self-assembly systems Repeat Session 2

● **Tianbo Liu** Biomedicine Hall E402

Static and dynamic laser light scattering techniques (SLS and DLS) are two different techniques with different principles. They often share a good fraction of instruments; therefore it is convenient to perform SLS and DLS on the same system to obtain much more valuable information.

SLS represents the traditional scattering technique, which measures the total scattered intensity from a solution at different scattering angles to get a scattering curve. SLS assumes that the incident and scattering lasers have the identical wavelength – referred as elastic scattering. SLS can be used to determine the weight-average molecular weight (MW), radius of gyration (R_g) of particles, as well as the nature and magnitude of intermolecular interactions via the second virial coefficient (A_2).

DLS measures the scattered light fluctuations within very short time range, raised by the random Brownian movement of particles. The Brownian movements lead to the Doppler's effect, resulting in tiny changes in the wavelength of the scattered light from that of the incident light. By means of a digital correlator, such tiny changes can be determined and amplified by the time correlation function and consequently be used to determine the Brownian move speed of particles – in diffusion coefficient and then the hydrodynamic radius (R_h).

The combination of SLS and DLS can be very powerful in determining the various structures and processes in solutions, such as the shape, size and mass of particles, biological complexes and supramolecular assemblies. In the workshop, examples on different areas will be given, such as the self-assembly of surfactants into micelles and vesicles, the polyelectrolyte behavior of biological macromolecules, accurate determination of the shapes of particles, as well as the time-resolved online measurements to obtain the kinetics of assembly/formation processes.



Basic Principles of Analytical Ultracentrifugation and Its Applications in Macromolecular Characterization

Repeat Session 2

● **Xiaodong Ye** Biomedicine Hall E403

This workshop provides an introduction to Analytical Ultracentrifugation (AUC), a powerful and versatile technique for investigating macromolecules and their interactions in solution. We will begin with a brief overview of the historical development of AUC, followed by an explanation of key theoretical foundations and parameters, including the Svedberg equation, the Lamm equation, sedimentation and diffusion coefficient, partial specific volume, and frictional ratio.

The core focus will be on the Sedimentation Velocity (SV) method—the most widely used AUC technique—accompanied by brief discussions of Sedimentation Equilibrium (SE) and Band Sedimentation. We will address how to optimize experimental conditions for various types of samples and analytical goals, with particular attention to rotor speed, sample concentration, and detector selection. As part of this, we will also introduce the use of fixed-position optical detection to monitor time-resolved transmission intensity, a method that can be applied to determine nanoparticle size distributions using commercial AUC instruments. The workshop will also cover data acquisition and analysis strategies. Throughout the session, examples from our own research and the literature will be used to illustrate the capabilities of AUC in analyzing complex systems, including:

- Polymers with different topologies
- DNA conformational transitions
- Protein-polyelectrolyte interactions



Tools for multidimensional particle characterization via AUC Repeat Session 3

● **Johannes Walter** Biomedicine Hall E302

Analytical Ultracentrifugation is perfectly suited for the analysis of particle size distributions (PSDs). However, the characterization of polydisperse PSDs is making high demands to any characterization technique. For sedimentation analysis, it has to be taken into account that the sedimentation rate scales with the particle size squared. To tackle such challenging systems, multi-wavelength (MW) gravitational sweep (GS) experiments at a fixed radial position were developed which are based on a continuously increasing rotor speed. GS experiments are of particular importance when studying polydisperse PSDs due to the much larger dynamic range compared to traditional sedimentation velocity (SV) experiments.

HDR-MULTIFIT can be used to analyze GS data and provides the ability to determine the optical properties of individual components in polydisperse mixtures and to relate this data to the hydrodynamic properties. For spherical NPs having well-defined refractive indices, high dynamic range (HDR) particle size analysis is possible. The analysis benefits from the direct fractionation of different particle sizes in the measurement cell during sedimentation, while the optimum signal to noise ratio for the whole PSD is achieved by automatically tuning the wavelength used for data evaluation.

Besides GS experiments, it is demonstrated that HDR-MULTIFIT can also be used for the post-processing of sedimentation coefficient distributions from sedimentation velocity experiments (derived by e.g., SEDFIT, SEDANAL, UltraScan, DCDT+). Concentration coupling and the simultaneous determination of size and density are further capabilities offered by HDR-MULTIFIT.

In addition, HDR-SVFIT, a tool for MW analysis of SV data will be presented which provides the possibility to conduct analysis of hundreds of wavelengths simultaneously on a standard office computer. Such results can be processed to derive sedimentation coefficient dependent extinction and emission spectra.

The workshop will tackle the following topics:

1. Determination of particle sizes and their distributions
2. The influence of scattering and the role of Mie's theory
3. Correction of size distributions and their weighting
4. Multi-wavelength analysis of GS experiments
5. Concentration coupling experiments for polydisperse distributions
6. Simultaneous analysis of size, density and refractive index increment using density variation
7. Multi-wavelength analysis of SV experiments including global regularization schemes
8. Post-processing of MW analysis results

Most importantly, this workshop will include a hands on training on HDR-MULTIFIT, HDR-SVFIT and related tools.



US-SOMO Hydrodynamics-1 Repeat Session 3

● **Emre Brookes, Mattia Rocco, Olwyn Byron** Biomedicine Hall E303

UltraScan SOLUTION MOdeler (US-SOMO)[1,2] processes atomic and lower-resolution bead model representations of biological and other macromolecules to compute various hydrodynamic parameters, such as the sedimentation and diffusion coefficients, relaxation times and intrinsic viscosity. This allows the researcher to validate structural models against experimental data. The tools available in US-SOMO have been shown to provide the best known computations of hydrodynamic parameters from experiment [3]. The US-SOMO hydrodynamics workshop will consist of two sessions. In the first session, attendees will be given an introduction to computational hydrodynamics and techniques. In the second session, users will utilize US-SOMO to compute hydrodynamics parameters from structures and to use automated batch processing to compute over multiple structures. A brief introduction to the US-SOMO web version <https://somoweb.genapp.rocks> will be presented. It is suggested that attendees attend both sessions, as earlier material will not be covered again during the two session course. Registered attendees will receive detailed software installation instructions in the week prior to the workshop. References:

[1] Rocco M, Byron O., 2015, "Hydrodynamic Modeling and Its Application in AUC.", *Methods Enzymol.* 2015;562:81-108. doi: 10.1016/bs.mie.2015.04.010.

[2] Rocco, M., Brookes, E. & Byron, O. US-SOMO: Methods for Construction and Hydration of Macromolecular Hydrodynamic Models. In: *Encyclopedia of Biophysics*, Roberts, G. & Watts, A., European Biophysical Societies (eds). Springer, Berlin, Heidelberg (2021). doi: 10.1007/978-3-642-35943-9_292-1.

[3] Brookes, E. and Rocco, M., 2018. Recent advances in the UltraScan SOLUTION MOdeller (US-SOMO) hydrodynamic and small-angle scattering data analysis and simulation suite. *European Biophysics Journal*, 47(7), pp.855-864. doi:10.1007/s00249-018-1296-0.



Analysis and Fractionation of Nanoparticles by Analytical Ultracentrifugation Repeat Session 3

● **Mengdi Chen** Biomedicine Hall E402

Analytical ultracentrifugation (AUC) has proven to be a valuable tool for the study of colloids since its invention by Svedberg, who initially used it for the particle size characterization of gold colloids. The character of colloids raises analytical questions, which are often different from those which are commonly addressed for biopolymers; therefore, AUC analysis of colloidal systems differs from that of well-defined proteins. Colloids are often extremely polydisperse owing to an inherent tendency to aggregate and thus must be treated as interacting polydisperse systems. Furthermore, colloids are often hybrid systems (e.g. inorganic-organic) and have a particle size and density distribution. A further complicating feature is that many colloids, especially inorganic species, are extremely small, so diffusion broadening of boundaries can make AUC analysis of such systems difficult.

This talk highlights which answers AUC can give for colloidal systems despite the previously mentioned obstacles, which restrict the use of many common AUC evaluation techniques for proteins. Some examples will be presented and discussed

- (1) Pt and ZrO₂ colloids as examples for the determination of particle size distributions of extremely small colloids in the nanometer range;
- (2) an interacting binary mixture of reactive lattices with a protein, which leads to aggregation as an example for the quantitative assessment of colloid aggregation phenomena;
- (3) iron oxides in a carrageenan microgel, coated metal particles and a growing inorganic mineral inside an organic aggregate as organic-inorganic hybrid particles, as well as ferritin, an organic-inorganic hybrid colloid which has a particle size and density distribution as an example for the combination of AUC with other size-dependent but density-independent methods like flow-field-flow fractionation to yield further insight into the nature of the different oligomers; and
- (4) synthetic boundary crystallization ultracentrifugation as an example of how fast processes like crystal growth can be transferred to a spatial resolution of species which can be analyzed by the much slower AUC technique. These examples illustrate how common problems in colloid chemistry can be solved by AUC and which pitfalls and future perspectives exist.



AUCAgent, a new software aimed for automatically analysis of AUC Repeat Session 3

● **Wenqi Li** Biomedicine Hall E403

AUCAgent is an innovative AUC data analysis software. It incorporates automated meniscus detection capabilities, enabling direct processing of the raw intensity data in experiments conducted without reference solutions, substantially boosting experimental data acquisition throughput. Its comprehensive experimental recording module ensures complete data traceability while providing robust support for researchers' data processing and experimental reporting needs. Moreover, the integrated data quality control module facilitates the identification of scans with optimal signal-to-noise ratios, thereby enhancing the reliability of analytical outcomes. This workshop is designed to provide a comprehensive introduction to some of the current functions and usage methods of AUCAgent.



US-SOMO Hydrodynamics-2 Repeat Session 4

● **Emre Brookes, Mattia Rocco, Olwyn Byron** Biomedicine Hall E302

UltraScan SOLution MOdeler (US-SOMO)[1,2] processes atomic and lower-resolution bead model representations of biological and other macromolecules to compute various hydrodynamic parameters, such as the sedimentation and diffusion coefficients, relaxation times and intrinsic viscosity. This allows the researcher to validate structural models against experimental data. The tools available in US-SOMO have been shown to provide the best known computations of hydrodynamic parameters from experiment [3]. The US-SOMO hydrodynamics workshop will consist of two sessions. In the first session, attendees will be given an introduction to computational hydrodynamics and techniques. In the second session, users will utilize US-SOMO to compute hydrodynamics parameters from structures and to use automated batch processing to compute over multiple structures. A brief introduction to the US-SOMO web version <https://somoweb.genapp.rocks> will be presented. It is suggested that attendees attend both sessions, as earlier material will not be covered again during the two session course. Registered attendees will receive detailed software installation instructions in the week prior to the workshop. References:

[1] Rocco M, Byron O., 2015, "Hydrodynamic Modeling and Its Application in AUC." , Methods Enzymol. 2015;562:81-108. doi: 10.1016/bs.mie.2015.04.010.

[2] Rocco, M., Brookes, E. & Byron, O. US-SOMO: Methods for Construction and Hydration of Macromolecular Hydrodynamic Models. In: Encyclopedia of Biophysics, Roberts, G. & Watts, A., European Biophysical Societies (eds). Springer, Berlin, Heidelberg (2021). doi: 10.1007/978-3-642-35943-9_292-1.

[3] Brookes, E. and Rocco, M., 2018. Recent advances in the UltraScan SOLution MOdeller (US-SOMO) hydrodynamic and small-angle scattering data analysis and simulation suite. European Biophysics Journal, 47(7), pp.855-864. doi:10.1007/s00249-018-1296-0.



Comprehensive characterization of protein-based vaccine antigens by analytical ultracentrifugation and complementary techniques

Repeat Session 4

● **Shaowei Li** Biomedicine Hall E303

Since its introduction by Dr. Theodor Svedberg, analytical ultracentrifugation (AUC) has evolved into a pivotal biophysical tool for analyzing biological macromolecules, their complexes, and their interactions with other molecules. It is now widely applied across biology, medicine, chemistry, physics, and related disciplines. In this workshop, I will explore the technical features of AUC, with particular emphasis on the complementarity between sedimentation velocity (SV) and sedimentation equilibrium (SE) methods. Drawing on more than two decades of research experience in recombinant vaccine development and structural vaccinology, I will discuss experimental considerations, data analysis strategies, and applications of AUC in characterizing diverse protein-based vaccine antigens. Selected published AUC results will be presented, along with suggestions for best practices, and will be complemented by discussions on integrating AUC with other analytical techniques such as surface plasmon resonance (SPR), size-exclusion chromatography (SEC), and dynamic light scattering (DLS). These examples will illustrate how AUC and complementary methods can be applied synergistically throughout the vaccine development process. The aim is to provide practical, experience-based guidance for the use of AUC in research, quality control, and regulatory evaluation in vaccine-related fields. This workshop will also serve as a prelude to my upcoming symposium presentation, "Versatility of Analytical Ultracentrifugation in Vaccine R&D," thereby offering a practice-to-theory knowledge continuum for AUC in vaccine research and development.



Optima AUC cGMP Suite Analysis Management Software Introduction Repeat Session 4

● Qikun Yu Biomedicine Hall E402

Ensuring that analytical ultracentrifugation (AUC) data fully complies with cGMP requirements has always been a significant challenge for quality management and regulatory compliance in the biopharmaceutical industry. Data integrity, traceability and complete audit trails are not only mandatory regulatory requirements but also core safeguards for ensuring product quality and patient safety.

Beckman Coulter has introduced Optima cGMP Suite analysis management software specifically designed for AUC, aiming to completely solve the AUC cGMP dilemma and provide enterprises with powerful, user - friendly, and validated compliance tools.

The core advantages of this innovative cGMP software are as follows:

- Seamless integration into your cGMP workflow: Designed to meet stringent regulatory requirements such as 21 CFR Part 11.
- Robust data integrity and security: Ensuring tamper - proof and traceable processes from raw data acquisition to final report generation.
- Simplified compliance management: Automated audit trails, electronic signatures (optional), and user - rights management significantly reduce compliance workload and risks.
- Enhanced efficiency and confidence: Helping you generate reliable reports that meet cGMP standards more quickly, accelerating the R & D process and product launch.

Want to gain a deeper understanding and hands - on experience with this transformative software? Join our upcoming Workshop!



AUC LNP analysis with UltraScan's Custom Grid analysis - ZOOM

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Lipid nanoparticles (LNPs) are an important drug delivery vehicle for vaccines, cancer drugs and others. Their composition is a critical quality attribute that requires characterization of particle size, molar mass, cargo load and density distributions. Using UltraScan's Custom Grid analysis, it is possible to design grids that permit the simultaneous analysis of both sedimenting and floating particles mixed in a single sample. A density matching approach customized in our laboratory for the analysis of LNP formulations can be combined with the custom grid analysis to comprehensively characterize the composition on LNPs using D2O density matching. In this talk, I will describe the elements and approaches needed to implement D2O density matching and custom grid analysis for LNPs.



SAXS-A-FOLD: a website for fast ensemble modeling optimizing the fit of AlphaFold or user-supplied protein structures with flexible regions to SAXS data

Mattia Rocco, J. E. Curtis, and Emre Brookes

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The public-domain website SAXS-A-FOLD (<https://saxsafold.genapp.rocks>) implements an approach using protein modules/domains linked by unstructured regions and generates ensembles of models optimized against Small-Angle X-ray Scattering (SAXS) data [Brookes et al. J. Appl. Cryst., in press]. SAXS $I(q)$ and $P(r)$ data are uploaded, together with a starting structure either taken from the AlphaFold2 database or user-provided. A Monte Carlo method that adjusts backbone dihedral allowed angles along chosen segments of potential flexibility is used to generate a pool of tens of thousand sequential conformations. $I(q)$ and $P(r)$ are calculated with fast established methods on a reduced pool subset, and combinations of best-fitting models are found by non-negative least squares (NNLS) against the experimental data. A final set of models is then chosen by re-applying NNLS on the $I(q)$ calculated on these pre-selected models by a local implementation of the more physically advanced WAXSiS method.



Fractionation of Colloidal Particles: From AUC to UC

Mengdi Chen

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Slight non-uniformity in nanoparticles size or shape can lead to deviations in the observed properties from predictions based on ideal monodisperse nanoparticles. For reducing nanoparticle polydispersity, post-synthesis treatment serves as a complementary approach to synthesis optimization, yet it is often overlooked. Centrifugation is theoretically a versatile method for fractionating all types of polydisperse nanoparticles. However, due to the challenges of precisely controlling fractionation in the centrifugal field and the complex interactions between nanoparticles and ligands, centrifugation is largely confined to a washing step following nanoparticle synthesis. In this talk, we will demonstrate how the quantitative results from analytical ultracentrifuge (AUC) can be used to establish suitable centrifugal conditions in a preparative ultracentrifuge (UC), to form gradient structures and achieve highly effective fractionation of nanoparticles.



MINI TALK

Inner Filter Effect Correction and Engineering Optimization for Fluorescence Detection in AUC

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The inner filter effect limits the applicability of fluorescence detection in Analytical Ultracentrifuge (AUC) for high-concentration analyte measurements. We developed a correction method specifically optimized for confocal optical structure, replacing uncorrected data with a concentration-proportional, difference-invariant 1-f. Leveraging the multi-sample detection capability of AUC, we designed a real-time parameter calculation method to achieve self-calibration, which ensure that the difference-invariant is not affected by systematic noise and variations in environmental factors. Experimental results show that our method enhances the linear correlation coefficient of fluorescein solutions (0.86-109.9 μM) from 0.9166 to 0.9965, demonstrating significantly improved concentration-response linearity. In addition, we have optimized the mechanical configuration of the fluorescence detection system, where the enhanced structural stability avoids the adjustment of the reflector and contributes to improved signal quality.



Characterization methods of rAAV at different development stages

Susumu Uchiyama

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AUC is now considered as gold standard for the estimation of full and empty ratio of AAV vectors but generally sample amount required for the analysis is considered large. This could be correct in some cases but not always true. In this talk I will introduce AUC methods and other "orthogonal methods" for the quantitative AAV vector size characterization. These methods can be applied depending on the development stages of therapeutic AAV vectors.



Unlocking the Power of AUC in PROTAC and Molecular Glue Discovery: The VATC Assay

Lake Paul

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Analytical Ultracentrifugation (AUC) is a highly informative yet underutilized technique in the PROTAC and Molecular Glue field. While researchers often rely on multiple orthogonal assays to gather insights into complex formation, binding affinities, and size distributions, a single AUC rotor experiment can deliver all this—and more.

At BioAnalysis, we've redefined AUC's role in Targeted Protein Degradation (TPD) with the development of the Velocity Assay for Ternary Complexes (VATC). This powerful, multi-attribute assay provides simultaneous quantification of binary and ternary complexes, estimation of complex shape (BCF and TCF), binding affinity (Kd), confirmation of the Hook Effect, and a ranking system for PROTAC and Glue candidates—all in one run.

VATC streamlines data acquisition, reduces reliance on multiple techniques, and accelerates compound selection while cutting both time and cost. It enables early, in-depth characterization of mechanism and interaction, guiding project teams before they commit to resource-intensive downstream assays.

This talk will showcase the VATC platform across various PROTAC and Molecular Glue systems and highlight recent and upcoming publications supporting its broad applicability and impact.



Advancing Analytical Ultracentrifugation: Beckman Coulter's Research and Future Horizons

Lutz Ehrhardt

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In the talk I will give a quick overview over the current AUC instrumentation, then AUC related research work done at Beckman Coulter will be presented in examples of Multiwavelength-AUC, LNP characterization using density variation. An additional application example will show the link between preparative Rate Zonal Density Gradient Ultracentrifugation and the characterization of extracellular vesicles by AUC.



The Application of AUC Technology in the Quality Control of Biopharmaceuticals

Wenqi Li

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Analytical ultracentrifugation (AUC) is a widely used method for characterizing macromolecular solutions. It employs spectroscopic detection (based on light absorption, interference, or fluorescence) to monitor the sedimentation behavior of samples within a centrifuge cell. This technique is critical for biopharmaceutical quality control, enabling purity analysis, aggregation state assessment, molecular weight determination, and structural studies – essential for ensuring product quality and stability. Biopharmaceuticals, including antibody drugs, cell therapies, vaccines, and nucleic acid-based drugs, face increasingly stringent and standardized quality control requirements throughout their lifecycle, driven by optimized production processes, advancing analytical technologies, and evolving regulatory standards from bodies like China's National Medical Products Administration (NMPA). Our platform's project, "Application of Analytical Ultracentrifugation Technology in the Quality Control of Protein-Based Drugs," has been successfully approved as a 2024 National Pharmaceutical Standards Improvement Project by the National Pharmacopoeia Commission. This report integrates insights from the R&D process and fundamental properties of biopharmaceuticals to systematically propose tailored quality control analytical methods aligned with their preparation and process development.



MINI TALK

Determination of extinction coefficient for full and empty recombinant adeno-associated virus particles by sedimentation velocity analytical ultracentrifugation

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Recombinant adeno-associated virus (rAAV) is a non-enveloped virus that encapsidates single-stranded DNA (ssDNA) within an icosahedral capsid. rAAV has recently emerged as a popular gene delivery vector. Here, we determined the extinction coefficients (ϵ) of full and empty particles of rAAV serotype 2, 5, 6, 8, and 9, using sedimentation velocity analytical ultracentrifugation (SV-AUC) with multi-wavelength detection. At 260 nm, the absorbance maximum of nucleic acids, the ϵ of full particles was approximately five-fold higher than that of empty particles. At 280 nm, near the absorbance maximum of proteins, the ϵ difference decreased to about two-fold. At 230 nm, the difference was smaller, yet the ϵ of full particles remained approximately 1.2-fold higher than that of empty particles. Overall, this study provides practical guidance for accurate quantification of rAAV using SV-AUC.



Mechanisms of transcriptional regulation by the RpiR family of proteins

Renwick Dobson

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In this talk I will present our efforts to define how bacteria regulate the uptake of amino sugars through the modulation of transcriptional regulation. Sialic acids are a large group of nine-carbon amino sugars that are widely distributed among mammals and higher metazoans. They are usually found conjugated to exterior cell surfaces. Commensal and pathogenic bacteria that colonise heavily sialylated niches (e.g., the mammalian respiratory tract and gut) can scavenge sialic acid from their surrounding environment and use it as a carbon, nitrogen and energy source—that is, they eat your glycoconjugates for breakfast. Sequestration and degradation of sialic acid involves specific amino sugar transporters responsible for the import into the bacterial cell and five catabolic enzymes that successively degrade sialic acid. Sialic acid utilization is essential for a range of human pathogenic bacteria.



The application of analytical ultracentrifugation for structural and mechanistic studies of autophagy receptors

Lifeng Pan

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Autophagy is a well-regulated and critical intracellular catabolic process involving lysosome-dependent degradation pathway, whose functional abnormalities are closely related to a large number of human diseases, such as inflammation, cancer, and neurodegenerative diseases. As a unique type of adaptor proteins, autophagy receptors can selectively recognize and target relevant substrates, including endogenous protein aggregates, dysfunctional organelles, and glycogen, for autophagic degradations, and play pivotal roles in numerous physiological processes in mammalian cells. However, until now, many of the detailed working mechanisms of autophagy receptors during selective autophagy are still poorly understood. Analytical ultracentrifugation is a powerful technique for characterizing the homogeneity and the aggregation status of protein samples. In this talk, I will present several examples to show how we combine analytical ultracentrifugation method with traditional structure biology approaches to facilitate the structural and mechanistic studies of autophagy receptors.



AUC Analysis of the Membrane Protein γ -Secretase

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γ -secretase is an intramembrane protease complex consisting of nicastrin, presenilin-1/2, A ϕ H-1a/b, and Pen-2. Hydrolysis of the 99-residue transmembrane fragment of amyloid precursor protein (APP-C99) by γ -secretase produces β -amyloid (A β) peptides. Pathogenic mutations in PSEN1 and PSEN2, which encode the catalytic subunit presenilin-1/2 of γ -secretase, lead to familial Alzheimer's disease in an autosomal dominant manner. However, the underlying mechanism of how the mutant PSEN gene may affect the function of the WT allele remains to be elucidated. Here we report that each of the loss-of-function γ -secretase variants that carries a PSEN1 mutation suppresses the protease activity of the WT γ -secretase on A β production. Each of these γ -secretase variants forms a stable oligomer with the WT γ -secretase in vitro in the presence of the detergent CHAPSO, but not digitonin. This observation is supported by AUC analysis of γ -secretase. Importantly, robust protease activity of γ -secretase is detectable in the presence of CHAPSO, but not digitonin. These experimental observations suggest a dominant negative effect of the γ -secretase, in which the protease activity of WT γ -secretase is suppressed by the loss-of-function γ -secretase variants through hetero-oligomerization.



Enterohaemorrhagic Escherichia coli AdhE spiroosome length correlates with enzymatic directionality and is perturbed by salicylidene acylhydrazides

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Enterohaemorrhagic Escherichia coli causes sporadic, and sometimes large-scale, food poisoning outbreaks, for which antibiotic treatment in humans is contraindicated. As an alternative form of therapy, previous studies developed the family of salicylidene acylhydrazide (SA) anti-virulence compounds. One target of the SA compounds is AdhE, an enzyme that converts acetyl-CoA to ethanol and vice versa. AdhE oligomerises, forming helicoidal filaments, heterogeneous in length, called spiroosomes. We show it is possible to only partially fractionate AdhE spiroosomes because in vitro they oligomerise in the absence of stimuli and that spiroosome formation is necessary to regulate the direction of AdhE enzymatic reactions. We also show that the SA compound ME0054 binds and perturbs AdhE spiroosomes, enhancing the conversion of ethanol to acetyl-CoA. This mechanistic understanding of how ME0054 impacts AdhE function will help in the development of SA compounds as novel anti-virulence inhibitors.



MINI TALK

Defining the biological interactions of the transcriptional regulator HiNanR

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Protein-DNA interactions are essential for cellular functions, including transcriptional regulation. The RpiR family transcriptional regulators are understudied. These proteins regulate gene expression in response to the presence of various sugar metabolites, such as sialic acids. Sialic acids are scavenged by pathogenic bacteria, e.g. *Haemophilus influenzae*, as an energy source and to disguise themselves from the host's immune system by attaching sialic acids to their own external surfaces. Most protein genes involved in the sialic acid pathways are in the *nan* and *siaPQM* operons. These operons are regulated by two transcription regulators: HiNanR, an RpiR transcriptional regulator, and the cAMP receptor protein (HiCRP). CRPs are well-known, but there is limited knowledge about HiNanR. My research aims to determine the molecular mechanisms used by HiNanR to regulate the operons in response to sialic acids.



Analytical Ultracentrifugation and the Saving the Oseberg Viking Artefacts project

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Both sedimentation velocity and sedimentation equilibrium in the analytical ultracentrifuge (AUC), used in conjunction with other hydrodynamic, spectroscopic and imaging techniques, are playing important roles in the design of new bioinspired polymer consolidants for saving valuable maritime archaeological objects – such as the Oseberg Viking ship artefacts in Norway.

This presentation will focus on how the AUC – and some important advances in molecular hydrodynamics – is helping the production of consolidants based on natural products like chitosans (and the related aminocelluloses), lignins, terpenes and siloxanes and providing the Conservator with a choice or “toolbox” of materials to choose from depending on the nature and state of disrepair of a particular artefact.

Tribute will also be paid to Dr GM Pavlov for his significant contribution to the development of important methodology which has been used.

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Versatility of Analytical Ultracentrifugation in Vaccine R&D

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Since its debut, analytical ultracentrifugation (AUC) has gradually become an important biophysical technique for analyzing and studying biomacromolecules, their complexes, and their interactions with other molecules. It has been widely applied across multiple disciplines, including biology, medicine, chemistry, and physics. This presentation will briefly introduce the technical features of AUC and, drawing on two decades of experience applying AUC in vaccine research and development, will illustrate its unique capabilities in characterizing recombinant vaccine antigens in terms of protein molecular weight, particle size, and aggregation state. The talk will further discuss applications of AUC in epitope mapping, process development, and quality control of vaccines; highlight its role in the development of emerging vaccine modalities such as mRNA vaccines and viral vector vaccines; and offer brief perspectives on the future directions of AUC in biopharmaceutical research and development.



Small differences can have a huge impact: Support of clone selection of a glycoprotein by AUC

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Glycoproteins can pose considerable analytical challenges for routine biopharma development due to the heterogeneity of the glycan moiety. They are often difficult to analyze by work horse methods like mass spectrometry rendering their true molar mass in solution unclear. This is particularly true for early process development where the glycosylation is not as well controlled as during stages like manufacturing. Here we present an example of a glycoprotein, which showed small differences in the sedimentation coefficient (<0.2 S) of individual clones, which turned out to be quite substantial differences in molecular weight. Further evidence by a fluorescence-based thiol assay revealed also differences in disulfide bridging. This underlines the importance of biophysical characterization being involved early in project development.



MINI TALK

Determination of Broad Size-Density Distributions of Polymeric Nanoparticles by Analytical Ultracentrifugation Using Solvent Density Variation

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Accurate determination of the size, density, and composition of nanoparticles (NPs) is crucial for their applications, as density often reflects composition. Analytical ultracentrifugation (AUC) is one of the most precise techniques for nanoparticle characterization. In typical experiments, sedimentation velocity analytical ultracentrifugation (SV-AUC) directly yields sedimentation coefficient distributions. For simple systems with narrow size and density distributions—such as monodisperse proteins, nucleic acids, or polymeric nanoparticles—the solvent density variation can be applied to determine density (or partial specific volume) and subsequently infer size and molecular weight. However, for systems with broad distributions in both size and density, the conventional boundary fraction approach may become less reliable. This is because sedimentation coefficients are simultaneously influenced by both parameters, and changing solvent density can cause different particle populations to shift their contributions to the boundary fraction, making component matching during extrapolation problematic.

In this study, we first used Brownian dynamics simulations to model (i) monodisperse nanoparticles, (ii) binary systems differing in both size and density (including cases where smaller particles had either lower or higher densities), and (iii) systems with broad distributions in both size and density. These simulations illustrate the inherent difficulty—especially in complex and polydisperse systems—of extracting size and

density distributions solely from sedimentation coefficient distributions using the conventional boundary fraction approach. We then propose a new analytical approach in which the sedimentation coefficient distribution obtained for each solvent condition is mapped onto a set of possible size–density combinations. By taking the intersection of these solution sets across all solvent densities, the feasible parameter space is substantially constrained. This enables the reconstruction of broad size and density distributions. Tests on simulated systems with broad distributions confirm the accuracy and robustness of the method.



Strategies for the characterization of functional particle systems by analytical ultracentrifugation

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The dispersion stability and functional properties of particles are significantly influenced by their surface properties. Core-shell particles are promising materials for designing absorption and scattering properties of colloidal crystals, and surface ligands and their hydrophilic and hydrophobic interactions can be exploited to guide the adsorption behavior of particles at liquid-gas or liquid-solid interfaces. Analytical ultracentrifugation (AUC) has great potential for multidimensional characterization of particles. Since it provides access to the sedimentation properties [1] and densities of nanoparticles [2], it can also assess particle functionalization.

For our first example, we will focus on polystyrene-polydopamine (PS@PDA) core-shell particles, which are used as building blocks for novel colloidal photonic crystals [3]. Understanding the particles' properties in solution, especially their morphology and composition, is crucial to advancing the hierarchical complexity of this system. We conducted analytical buoyant density equilibrium (ABDE) experiments with AUC, supplemented by H₂O/D₂O density contrast AUC methods to examine the density and hydration of the PDA shell. ABDE-AUC experiments involve forming a density gradient in situ within the AUC cell using a gradient-forming material. The particles then

migrate to a position where their density matches that of the surrounding medium. Furthermore, density contrast AUC experiments in various H₂O/D₂O solvent mixtures allow us to determine the particles' anhydrous density. In our methodology, we first designed the gradients using the Hermans-Ende model. Then, we determined the anhydrous and buoyant density of bare PS particles. We validated our findings through a comprehensive approach that included scanning electron microscopy and sedimentation velocity measurements. Next, we moved on to PS@PDA particles synthesized under varying reaction parameters, specifically the concentrations of the core particles and the dopamine monomer. We obtained the buoyant and anhydrous densities and extracted the optical spectra of the particles in the formed gradient. This combination of AUC methods allows us to assess critical quality attributes of the functional particles. For example, we can determine the shell porosity, cross-linking density, and water adsorption of the dopamine shell, as well as its effect on the particles' optical properties

Our second example considers silica-PNIPAM core-shell particles with varying degrees of softness, which are controlled by the concentration of the crosslinker. Transferring particle monolayers from liquid interfaces to solid substrates is important for structuring surfaces with colloidal lithography [4]. Recent research has shown that particle assembly on a substrate after drying can differ significantly from its arrangement at the air-water interface, especially when soft particles such as microgels or core-shell particles are used [5–7]. In these cases, dangling polymer chains can adsorb to the liquid interface and stretch out to produce a pronounced, compressible corona surrounding the core. The final structure after drying depends on the interplay between interfacial particle morphology, immersion capillary forces, and the adhesion of the polymer corona to the substrate. Controlling these individual contributions is therefore key to understanding and ultimately predicting the resulting particle structure on the solid substrate. Studying the influence of particle properties on monolayer compression at the interface and its rearrangement upon drying requires in-depth knowledge of shell properties (e.g., grafting density, shell thickness, and degree of solvation) depending on the synthesis parameters (e.g., ligand concentration and degree of cross-linking). To that end, we prepared a set of silica-PNIPAM core-shell particles with varying degrees of softness, which we controlled via crosslinker concentration. We compared these core-shell microgel particles to a non-crosslinked hairy particle system

composed of grafted PNIPAM. In this case, we varied the grafting density to modify the interfacial corona. We will demonstrate that combining sedimentation and thermogravimetric analyses provides detailed information on the shell morphology, in particular, the particle's hydrodynamic diameter, the thickness of the PNIPAM shell as well as the total water uptake of the shell, which can be linked to a degree of swelling.

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Advances in the van der Waals (vdW) bead modeling method in US-SOMO

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US-SOMO has a prominent section for bead modeling based hydrodynamic calculations. For atomic-level structures, the van der Waals (vdW) one bead per non-hydrogen atom modeling method, including in each bead the theoretically "bound" hydration waters, was developed. We present its latest version, comparing the calculated translational diffusion coefficient D_t , intrinsic viscosity $[\eta]$ and rotational correlation time τ for a set of proteins structures with their corresponding carefully evaluated literature experimental values. The GRPY method excluding buried beads was used. By moving outwardly by roughly half of a water radius the centers of hydrated beads, we could match D_t within -0.8 ± 2.1 % (range $-3.6 - +3.7$ %, 22 structures), $[\eta]$ within -0.2 ± 5.2 % (range $-8.7 - +8.1$ %, 12 structures), and τ within -1.2 ± 4.5 % (range $-7.5 - +4.8$ %, 7 structures). Given the uncertainties in the experimental values especially for $[\eta]$ and τ , the vdW method appears to be very reliable.



An Integrative Method for 3D Structure Determination of Large RNAs

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RNA's diverse functions and its therapeutic potentials are dictated by its structure and conformational dynamics. 3D structure determination of large RNAs by conventional structural techniques including X-ray crystallography, NMR and cryo-EM remains challenging due to large RNA's inherent flexibility and increased dynamics. As of July 20, 2025, high-resolution RNA-only structures (2,008) only account for 0.9% of a total number of 239,267 structures deposited in Protein Databank, and the majority of them are less than 100 nucleotides in length. There is a strong impetus to develop alternative approaches. A growing trend in the field is to comprehensively analyze RNA structure by using multiple complementary experimental and computational techniques, in other words, hybrid methods. Recently, we develop efficient methods for posttranscriptional site-directed spin labeling (SDSL) of large RNAs using the TPT3-NaM unnatural base pair system^{1, 2}, which opens new possibility for application of electron paramagnetic resonance (EPR) spectroscopy of pulsed electron-electron double resonance (PELDOR) in investigating the structures of large RNAs. Enabled by the SDSL method and RNA perdeuteration using deuterated nucleotides during enzymatic synthesis, we demonstrate long-range distance measurement on a large RNA up to 140 Å by PELDOR spectroscopy³. By combined use of the SDSL method, PELDOR spectroscopy, small angle X-ray scattering and computational modeling, we develop an integrative method for 3D structure determination of large RNAs from the RNA genomes of Zika virus and SARS-CoV-2.



MINI TALK

AUCAgent: A new software for AUC data analysis

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Analytical ultracentrifugation (AUC) is a pivotal technique for the accurate analysis of macromolecules, extensively employed in both academic research and industrial settings. However, current data-analysis workflows face significant challenges. Existing AUC software, though indispensable, suffers from functional constraints that impede advanced research, relies on simplistic single-model processing, and delivers a suboptimal user experience. Developing a next-generation AUC data-analysis platform is therefore imperative. This modern solution will:

- Provide customizable analytical models to tackle complex research questions.
- Enable flexible data processing through advanced computational methods.
- Offer an optimized user interface to minimize operational barriers.

By bridging these critical gaps, the platform will deliver comprehensive and robust analytical support for macromolecular research.



Human DDX1 interacts and unwinds NEAT1 RNA G-quadruplexes and modulates paraspeckle formation

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DDX1 is a key human protein involved in a number of biological activities. On the other hand, NEAT1 is a human long non-coding RNA that is also emerging as a key regulator of cellular processes. Our biophysical and biochemical work demonstrates a strong link between these two human components. We discovered that DDX1 forms a stable complex with NEAT1 RNA and resolves G-quadruplex structures to facilitate proper RNA folding. The small-angle X-ray scattering work revealed multiple binding configurations between DDX1 and NEAT1, suggesting dynamic structural remodeling during complex formation. We also found that DDX1 depletion disrupts paraspeckle formation despite compensatory upregulation of NEAT1 and core paraspeckle proteins, establishing DDX1 as an essential factor in nuclear body assembly.



Molecular macroions studied by the AUC - What new information can we obtain?

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Between traditional simple ions and large colloidal particles, we found that there exists a transitional stage – macroionic solutions. In this regime, the charged solutes with 1-6-nm-size possess fundamentally different behaviors from the above two categories. Examples of macroions include well-defined molecular clusters, metal-organic cages, small nanoparticles and biomacromolecules such as globule proteins. Macroions can be very soluble in water; however, they also tend to strongly attract with each other in dilute solutions, leading to the complex self-assembly and phase transitions, regulated by the electrostatic interactions.

A simple macroionic solution contains merely the macroions and their counterions, both are hydrated. Counterions are found to be loosely associated with the macroions. Depending on their valance and hydrated sizes, the number of associated counterions and their distances to the macroions vary. When the macroions are moderately charged, the counterions can further mediate and attract macroions together to form extended 2-D sheets. The sheets can either enclose and self-assemble into hollow, spherical, single layered blackberry type supramolecular structures or remain as tough sheets in dilute solutions – leading to the gelation and phase transitions.

While my group has applied many techniques to study macroions in the past

two decades – SLS, DLS, ITC, Zeta potential analyzer, TEM and AFM, our newly purchased AUC provides us new aspects of these fascinating systems. AUC has been used to determine important parameters, such as the thickness of the hydration shells of nanoscale molecular clusters with well-defined size, shape and molecular weight, for the first time; the distribution and locations of metal counterions around a spherical, porous macroion; the intermolecular distance between a dimer of macroions formed by counterion-mediated attraction. In addition, we are trying multiwavelength AUC technique to explore the host-guest interactions involving cage-shaped macromolecules.



ICE2 is Essential for the Little Elongation Complex (LEC)-Mediated Small Nuclear RNA Transcription by RNA Polymerase II

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The evolutionarily conserved little elongation complex (LEC), which is composed of four core subunits: ICE1, ICE2, ELL and ZC3H8, regulates RNA polymerase II (RNAPII)-mediated snRNA transcription. However, the assembly and mechanisms of the LEC complex are not well understood. Here, we report the crystal structure of ICE1/ICE2 binary complex of human LEC, which reveals the interaction interfaces between the ICE1 and ICE2. The structure demonstrates that the N-terminal of ICE2 bind the C-terminal domain of ICE1. Based on the structure, we also identified key amino acids that influence the interactions between the two subunits. Moreover, our results indicate that ICE2 is required for the occupancy of RNAPII on snRNA gene. Collectively, our results revealed the structural insight into the ICE1/ICE2 binary complex and the function of ICE2 in LEC-mediated snRNA transcription.



MINI TALK

The Applications of Analytical Ultracentrifugation Sedimentation Velocity in the Characterization of HPV Virus-Like Particles and Vaccine Development

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Analytical Ultracentrifugation Sedimentation Velocity (AUC-SV) is a fundamental technique for characterizing the assembly and stability of virus-like particles (VLPs). By employing the high-resolution $c(s)$ distribution model, AUC-SV precisely determines the sedimentation coefficient (S value) and diffusion coefficient, thereby elucidating the homogeneity, oligomeric state, and assembly mechanisms of the particles. In the study of HPV VLPs, AUC-SV offers distinct advantages by quantitatively analyzing the molecular weight of unassembled components, which facilitates the evaluation of VLP assembly efficiency, homogeneity, and stability. For instance, in the investigation of HPV59 L1 VLP, AUC-SV demonstrated that the homogeneity of mature VLP (mVLP, 140S) significantly surpasses that of immature VLP (imVLP, 118S/139S), and confirmed that the C175S mutant exclusively formed homogeneous pentamers (9.3S), providing direct evidence for the VLP assembly mechanism. Furthermore, in the design of chimeric VLP (chVLP), AUC-SV, in conjunction with HPSEC, indicated that the C175A and C428A mutants co-assembled at a 1:1 molar ratio to form homogeneous particles (123.4S), with physicochemical properties closely resembling those of wild-type VLP. Compared to dynamic light scattering (DLS) and transmission electron microscopy (TEM), AUC-SV can detect low-abundance aggregates (<1%) and directly assess the long-term stability of VLPs in their native solution environment, thereby providing a reliable basis for batch-to-batch consistency and quality control of multivalent vaccines. These studies underscore the indispensable role of AUC in the development of HPV vaccines, establishing a technical foundation for the rational design and rapid translation of next-generation multivalent vaccines.



Analytical Ultracentrifugation as a Tool for Exploring COSAN Assemblie

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COSAN anion ($[\text{Co}(\text{C}_2\text{B}_9\text{H}_{11})_2]^-$) is composed of two bulky dicarbollide semicages, each of them bearing two negative charges that “sandwich” a metal cation Co^{3+} . Its low charge density confers to COSAN the possibility to bind with an unexpectedly strong affinity to hydrophilic neutral interfaces, a water-mediated effect we called the superchaotropic effect, and to apolar surfaces (hydrophobic interactions). These and possibility to make intermolecular (B-H...H-C) bonds, and electrostatic interactions, provide multiple ways for intermolecular interactions. We used sedimentation velocity analytical ultracentrifugation (SV-AUC) to revisit (1) the aggregation behavior of COSAN into micelles, a topic still under debate, and (2) the nature of the protein assemblies induced by COSAN. This study highlights the complementary role of SV-AUC in understanding supramolecular assemblies, offering useful insights into the behavior of COSAN nano-ions and their interactions with biomacromolecules.



The function of C9ORF72

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Massive GGGGCC (G4C2) repeat expansion in C9orf72 and the resulting loss of C9orf72 function are the key features of ~50% of inherited amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) cases. However, the biological function of C9orf72 remains unclear. We report the cryo-EM structure of the human C9ORF72-SMCR8-WDR41 complex at a resolution of 3.2 Å. The structure reveals the dimeric assembly of a heterotrimer of C9ORF72-SMCR8-WDR41. In the protomer, C9ORF72 and WDR41 are joined by SMCR8 without direct interaction. WDR41 binds to the DENN domain of SMCR8 by the C-terminal helix. Structural and biochemical analysis reveals the GAP activity of the C9ORF72-SMCR8 complex for Rab8a and Rab11a. Of note, the C9orf72-SMCR8 complex is a major negative regulator of primary ciliogenesis. Mechanistically, the C9orf72-SMCR8 complex suppresses the primary cilium as a RAB8 GAP. These results reveal the unexpected impact of C9orf72 on primary ciliogenesis and shed light on the pathogenesis of diseases caused by the loss of function of C9orf72.



Structural insights into the complete high-affinity IgE receptor (FcεRI) and implications for allergic responses

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Allergic diseases affect over 25% of individuals in industrialized nations and constitute a significant public health challenge. The high-affinity IgE receptor (FcεRI), predominantly expressed on mast cells and basophils, is important for allergic responses. Previous structural work has focused mainly on soluble extracellular domains, overlooking transmembrane segments and their interactions with coreceptors—a gap that limits our understanding of receptor signaling. Using cryo-electron microscopy single-particle analysis, we determined the first near-atomic resolution structure of the full-length FcεRI complex, including its transmembrane regions. Our findings reveal an IgE-induced transition of FcεRI from a dimeric to a monomeric state and support a dissociation-dependent activation mechanism. These insights provide a structural basis for developing targeted therapies against allergic diseases.



MINI TALK

Analytical Ultracentrifugation Reveals the Process and Mechanism of HEV Virus-Like Particle Disassembly Triggered by Neutralizing Antibody 8C11

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Neutralizing antibodies (nAbs) are critical immune effectors that eliminate viruses, typically by blocking receptor engagement or interfering with viral conformational changes. In this study, using hepatitis E virus (HEV) as a model, we elucidated a novel neutralization mechanism in which a potent nAb, 8C11, induces physical collision-mediated disassembly of viral particles. Analytical ultracentrifugation (AUC), in combination with high-resolution structural biology, was employed to quantitatively analyze the dynamic structural changes of HEV virus-like particles (VLPs) upon binding to 8C11. Sedimentation velocity (SV-AUC) measurements provided the first direct evidence that 8C11 binding converts intact VLPs (~51.3S) into p495 dimer-antibody complexes (~7.5S) within 2 hours, accompanied by a marked reduction in sedimentation coefficient, indicative of large-scale structural disruption. AUC not only enabled precise determination of the molecular mass and composition of the disassembly products, but also revealed the time dependence and antibody dose effect of the dissociation process, offering a robust means to differentiate particle stability under distinct binding modes. Together with cryo-electron microscopy (cryo-EM), we confirmed that physical collision leads to loss of capsid symmetry and exposure of the viral genome. This work not only uncovers a new mode of virus neutralization mediated by 8C11, but also highlights the unique power of AUC in probing the kinetics and mechanisms of virus-antibody interactions, providing valuable technical support for the design of broad-spectrum neutralizing antibodies based on structural disruption.



Multidimensional Colloids Characterization

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An important trend in colloid science is the requirement to understand and design systems of increasing complexity. Available techniques for particle characterization are predominantly focused on size, even simultaneous size and shape characterization is a major challenge, in particular for nanoparticles (NPs). Property functions as comprehensive description of such particle systems define the target in product design and are understood along the five dimensions of particle size and shape, surface, structure and composition and their respective distributions. While the characterization of 1D size distributions is well established, the independent description and measurement of two or more distributed properties is a highly challenging task. Recent progress in the description and characterization of multivariate particle property distributions will be presented and discussed. Analytical ultracentrifugation (AUC) combines outstanding resolution with high reproducibility. Novel sensors based on absorption and emission optics provide access to the optical properties of size-selected colloids. This allows accurate size measurement of multimodal samples due to the excellent separation power. In principle, all particle systems with size, shape or composition dependency of optical properties can be analyzed avoiding tedious counting by TEM or AFM. Recent approaches based on size- and shape-dependent optical properties of plasmonic NPs allow to measure full number-weighted 2D size and length distributions of gold nanorods or of 5-sided gold double pyramids in one single experiment. In a

similar approach, size and compositional distributions of noble metal alloy nanoparticles can be obtained. Further examples include the core-shell analysis of quantum dots, their size-dependent band gap determination or the analysis of 2D materials such as graphene or MoS₂. Analytical ultracentrifugation is considered now as gold standard in NP characterization due to the unprecedented resolution, accuracy and reproducibility. While AUC is a relatively expensive technique, recent progress in nanoparticle chromatography presents a rather cheap, highly promising and scalable alternative. The characterization of even multimodal colloids with sizes from below 1 nm up to 100 nm is demonstrated for several materials. Examples include the separation of highly luminescent carbon nanodots, polymers, semiconducting gold clusters and plasmonic gold, silver NPs and their alloys. The most recent example is the full 2D characterization of plasmonic nanorods by transferring techniques from AUC, namely the optical back-coupling method, to nanoparticle chromatography.

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Absolute properties of (synthetic) polymers

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The molecular understanding of (synthetic) polymers and macromolecular systems is key to their technological advancement in modern areas of science. With the inception of the “polymer age”, an experimental framework to study polymers has grown and matured. Next to (synthetic) polymer solutions being disperse systems, the polymers by themselves show dispersity in their properties, particularly the molar mass. This makes them inherently difficult to study when compared to well-defined biomacromolecules such as proteins.

Our approach to reveal their fundamental properties on a molecular scale makes use of the methods available by an analytical ultracentrifuge (AUC). Those methods are complemented by viscometric and light scattering approaches. Throughout all polymer systems, we found that the translational friction properties of synthetic polymers are impacted much more significantly by large values of hydration, i.e., solvent being present within their occupied volume. Polymers also show much lower values of sedimentation coefficients and higher nonideality than more dense, less hydrated globular proteins.

In this contribution, we address the very core of the fundamental problem to determine accurate molar masses of (synthetic) polymers. This is because at unknown dispersity the accuracy of molar mass determinations by sedimentation-diffusion analysis may fail tremendously. The highly diffuse

sedimentation boundaries and often observed apparent existence of several species can be an artefact from numerical analysis. With examples varying from low to medium to very high dispersity, and the framework of hydrodynamics, we demonstrate ways to enable high accuracy of molar mass estimates.



Probing Soybean Protein-Polyelectrolyte Interactions and Structural Transitions in Soybean Proteins Using Analytical Ultracentrifugation

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Analytical ultracentrifugation (AUC) provides a powerful, first-principle approach for probing the interactions, stoichiometry, and structural transitions of macromolecules in solution. In this presentation, we focus on the complexation behavior between soybean glycinin (11S) and synthetic diblock polyelectrolytes (PEG-b-PSS) under varying ionic strengths. Despite possessing similar net charges, 11S and PEG-b-PSS form stable complexes depending on the ionic strength of the solutions and the degree of polymerization of the PSS block. We demonstrate that binding strength predominates at moderate ionic strength, enabling complex formation. At very low ionic strength (0.025 M), PEG-b-PSS induces the dissociation of 11S into subunits and peptides, revealing a unique pathway for structural modulation. To provide a broader understanding of soybean protein assembly and stability, we also briefly discuss the structural formation mechanisms of 15S and 21S globulins. In particular, we highlight how disulfide bond formation—governed by surface sulfhydryl content—drives the transformation of 11S into higher-order assemblies such as 15S and 21S. These findings collectively underscore the versatility of AUC in characterizing complex, multicomponent protein systems and reveal new insights into the mechanisms of protein-polyelectrolyte association.



MINI TALK

Application of Multi-Speed Sedimentation Velocity Method to Characterize Human-Serum-Albumin-based Nanoparticle Drug Carriers

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Human-serum-albumin (HSA)-based nanoparticles have emerged as attractive carrier systems for the targeted transport of diverse therapeutic agents and diagnostic probes because of its multifaceted advantages. For nanoparticle-based medicines, the particle size and size distribution is one of the most widely accepted defining characteristic. Here we report a multi-speed sedimentation-velocity analytical ultracentrifugation (SV-AUC) strategy for interrogating the microparticle-related critical quality attributes (CQAs) of generic HSA-bound paclitaxel injectables. By performing staged low- and high-speed AUC runs, we determined nanoparticle size and size distribution, quantified unbound albumin aggregates, and validated the analytical methodology. Complementary techniques—negative-stain transmission electron microscopy, field-flow fractionation coupled with static light scattering, size-exclusion chromatography, and mass photometry—were employed to benchmark the strengths and limitations of each approach for assessing nanoparticle size distribution.



MINI TALK

AUC a powerful Biopharma comparability assessment tool for studying peptide and protein aggregation - case study peptides, mAb and ADC

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Analytical Ultracentrifugation (AUC) is a gold-standard and a niche biophysical technique used in biopharma for characterizing proteins. Protein aggregation is a Critical Quality Attribute- CQA as it impacts product safety, stability & efficacy. Early stage determination of CQA using HOS analytical tools like AUC helps in understanding and establishing biosimilarity of the inhouse and reference products. Peptide formulations and Drug products of Biologics pose a significant challenge in SV-AUC analysis. We would like to provide an insight into the challenges and ways to mitigate these challenges the assessment studies of drug product based peptide and other biologics therapeutics. We have analysed multiple peptide and therapeutic proteins including monoclonal antibodies using SV-AUC and other orthogonal techniques like 1D & 2D NMR.

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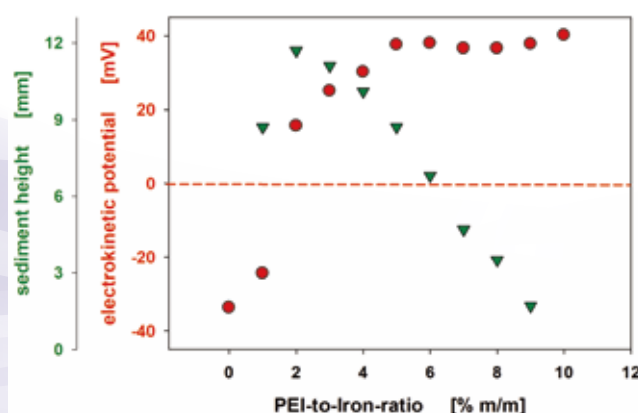
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Analytical centrifugation (AC): Quantification of separation behaviour due to electrostatic particle-particle-interaction, and suspension stability

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Many innovative dispersion-based products are featured by submicroscopic and nanoparticles with sophisticated functionality often based on highly complex surface structure. It is obvious that manifold interactions will take place between such dispersed particles and the continuous phase. A pragmatic answer gives the approach successfully developed by Hansen 50 years ago in case of organic liquids. On the other hand, DLVO theory was introduced to describe phenomena like agglomeration and flocculation by electrostatic and v. d. Waals forces about 75 years ago. Later steric interactions are accounted. This contribution aims to focus on structural and associated electrostatic aspects of surfaces of soft particles. A promising experimental approach consists in fingerprints obtained by analytical centrifugation (LUMiSizer). Extinction changes in dependence on centrifugation time are space resolved recorded from the bottom up to the top (25 mm) of up to 12 samples simultaneously. These extinction changes allow classifying the state of a suspension regarding particle-particle interaction. The traditional Zetapotential is also obtained. Changing the pH, the ionic strength or adding macromolecules answer questions upon the electrostatic interaction. Results clearly demonstrated that in-situ visualization of separation behaviour, like the extent of swarm or zone sedimentation as well as the determination of particle velocity distribution, proved to be a comprehensive and sensitive tool to classify stable, non-interacting dispersed particles versus unstable particles exhibiting flocculation/agglomeration. We like to raise the discussion and awareness regarding relationship between experimentally sound determined electrophoretic mobility and, on the other hand, employment of formal calculation of a Zeta-potential and questionable stability prediction of functionalized nanoparticles especially brushed or macromolecule coated ones. The presented approach is well applicable to mixtures of different types of particles as well as organic and ionic continuous phases.





A Complementary Tool to Analytical Ultracentrifugation (AUC) in Molecular Interaction Studies – Head-to-Head Data Comparison of Domestic SPR Instruments

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Precise characterization of biomolecular interactions is critical for life science research and drug development, with Surface Plasmon Resonance (SPR) and Analytical Ultracentrifugation (AUC) serving as complementary label-free technologies. SPR enables real-time surface-based kinetic analysis (k_a , k_d , KD) and high-throughput screening, while AUC provides solution-phase insights (molecular weight, aggregation state) under near-physiological conditions without surface interference. In line with national "domestic substitution" initiatives, this study compares a fully independent domestic SPR system (Polariton Life) with leading international platforms. The Polariton SPR features proprietary innovations: a high-sensitivity optical unit (maximum photon count 108, refractive index range 1.33–1.40), 16-flow cell microfluidics with precise delivery, and chemical-resistant PDMS components. Key metrics—RMS noise (<0.03 RU), baseline drift (<0.03 RU/min), and inter-channel consistency—match international standards.

Experimental validation across antigen-antibody binding, antibody-receptor interactions, peptide-antibody and LMW compound-protein systems shows comparable accuracy, reproducibility, and kinetic parameter determination between the domestic system and international counterparts. Independently developed consumables achieve $>90\%$ user satisfaction, ensuring supply security and cost control.

These findings confirm domestic high-end SPR instruments like Polariton's provide reliable, cost-effective alternatives. Integrating SPR and AUC enables comprehensive molecular interaction characterization, supporting advancements in life science and drug development.



AUCAgent: A new software for AUC data analysis

● **Chengshi Zeng** Tsinghua University, China

AUCAgent is an innovative AUC data analysis software. It incorporates automated meniscus detection capabilities, enabling direct processing of the raw intensity data in experiments conducted without reference solutions, substantially boosting experimental data acquisition throughput. Its comprehensive experimental recording module ensures complete data traceability while providing robust support for researchers' data processing and experimental reporting needs. Moreover, the integrated data quality control module facilitates the identification of scans with optimal signal-to-noise ratios, thereby enhancing the reliability of analytical outcomes.



Analytical Ultracentrifugation Reveals the Process and Mechanism of HEV Virus-Like Particle Disassembly Triggered by Neutralizing Antibody 8C11

● **Wenhui Xue** Xiamen University, China

Neutralizing antibodies (nAbs) are critical immune effectors that eliminate viruses, typically by blocking receptor engagement or interfering with viral conformational changes. In this study, using hepatitis E virus (HEV) as a model, we elucidated a novel neutralization mechanism in which a potent nAb, 8C11, induces physical collision-mediated disassembly of viral particles. Analytical ultracentrifugation (AUC), in combination with high-resolution structural biology, was employed to quantitatively analyze the dynamic structural changes of HEV virus-like particles (VLPs) upon binding to 8C11. Sedimentation velocity (SV-AUC) measurements provided the first direct evidence that 8C11 binding converts intact VLPs (~51.3S) into p495 dimer-antibody complexes (~7.5S) within 2 hours, accompanied by a marked reduction in sedimentation coefficient, indicative of large-scale structural disruption. AUC not only enabled precise determination of the molecular mass and composition of the disassembly products, but also revealed the time dependence and antibody dose effect of the dissociation process, offering a robust means to differentiate particle stability under distinct binding modes. Together with cryo-electron microscopy (cryo-EM), we confirmed that physical collision leads to loss of capsid symmetry and exposure of the viral genome. This work not only uncovers a new mode of virus neutralization mediated by 8C11, but also highlights the unique power of AUC in probing the kinetics and mechanisms of virus-antibody interactions, providing valuable technical support for the design of broad-spectrum neutralizing antibodies based on structural disruption.



Application of Multi-Speed Sedimentation Velocity Method to Characterize Human-Serum-Albumin-based Nanoparticle Drug Carriers

● **Qing Chang** Tsinghua University, China

Human-serum-albumin (HSA)-based nanoparticles have emerged as attractive carrier systems for the targeted transport of diverse therapeutic agents and diagnostic probes because of its multifaceted advantages. For nanoparticle-based medicines, the particle size and size distribution is one of the most widely accepted defining characteristic. Here we report a multi-speed sedimentation-velocity analytical ultracentrifugation (SV-AUC) strategy for interrogating the microparticle-related critical quality attributes (CQAs) of generic HSA-bound paclitaxel injectables. By performing staged low- and high-speed AUC runs, we determined nanoparticle size and size distribution, quantified unbound albumin aggregates, and validated the analytical methodology. Complementary techniques—negative-stain transmission electron microscopy, field-flow fractionation coupled with static light scattering, size-exclusion chromatography, and mass photometry—were employed to benchmark the strengths and limitations of each approach for assessing nanoparticle size distribution.



Characterizing the Self-Association of Tirzepatide by Analytical Ultracentrifugation (AUC)

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Tirzepatide, a dual GIP/GLP-1 receptor agonist, is a key therapy for type 2 diabetes and obesity. Understanding the solution behavior of tirzepatide and related therapeutic polypeptides, particularly their self-association, is critical. While static light scattering (SLS), often combined with dynamic light scattering (DLS), is commonly used, its limited size resolution typically provides only weight-average molecular weights and struggles to resolve monomeric, dimeric and higher-order oligomers. In contrast, analytical ultracentrifugation (AUC) operates under native conditions and effectively resolves and quantifies distinct oligomeric forms potentially relevant to biological activity. It provides precise determination of self-assembly state, offering orthogonal validation and complementary insights beyond traditional methods. This study employs AUC to investigate the impact of key factors (concentration, ionic strength, pH, and surfactants) on the self-assembly behavior of tirzepatide under formulation-relevant conditions.

Reference: ALLEN, D. P.; DOYLE, B. L.; QIAN, K. K.; HETRICK, E. M. PRESERVED GIP/GLP AGONIST COMPOSITIONS. WO 2024/086601 A2, 2024.



The Applications of Analytical Ultracentrifugation Sedimentation Velocity in the Characterization of HPV Virus-Like Particles and Vaccine Development

● **Shuyue Zhang** Xiamen University, China

Analytical Ultracentrifugation Sedimentation Velocity (AUC-SV) is a fundamental technique for characterizing the assembly and stability of virus-like particles (VLPs). By employing the high-resolution $c(s)$ distribution model, AUC-SV precisely determines the sedimentation coefficient (S value) and diffusion coefficient, thereby elucidating the homogeneity, oligomeric state, and assembly mechanisms of the particles. In the study of HPV VLPs, AUC-SV offers distinct advantages by quantitatively analyzing the molecular weight of unassembled components, which facilitates the evaluation of VLP assembly efficiency, homogeneity, and stability. For instance, in the investigation of HPV59 L1 VLP, AUC-SV demonstrated that the homogeneity of mature VLP (mVLP, 140S) significantly surpasses that of immature VLP (imVLP, 118S/139S), and confirmed that the C175S mutant exclusively formed homogeneous pentamers (9.3S), providing direct evidence for the VLP assembly mechanism. Furthermore, in the design of chimeric VLP (chVLP), AUC-SV, in conjunction with HPSEC, indicated that the C175A and C428A mutants co-assembled at a 1:1 molar ratio to form homogeneous particles (123.4S), with physicochemical properties closely resembling those of wild-type VLP. Compared to dynamic light scattering (DLS) and transmission electron microscopy (TEM), AUC-SV can detect low-abundance aggregates (<1%) and directly assess the long-term stability of VLPs in their native solution environment, thereby providing a reliable basis for batch-to-batch consistency and quality control of multivalent vaccines. These studies underscore the indispensable role of AUC in the development of HPV vaccines, establishing a technical foundation for the rational design and rapid translation of next-generation multivalent vaccines.

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