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Proceedings of the 25th Analytical Ultracentrifugation Workshops and Symposium

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Abstract

The 25th International Analytical Ultracentrifugation (AUC) Workshops and Symposium (AUC2022) took place at the University of Lethbridge in Lethbridge, Canada, in July 2022. In total, 104 attendees (Attendance Profile: 104 attendees, 69 in-person, 35 remote. Brazil 1, Canada 24, China 1, Czech Republic 2, Finland 1, France 3, Germany 22, India 3, Italy 1, Japan 4, Spain 1, Switzerland 3, Taiwan 1, United Kingdom 5, United States 32) participated in the event and presented the latest advances in the field. While the primary focus of the conference was to showcase the applications of AUC in chemical, life sciences, and nanoparticle disciplines, several presentations also integrated complementary methods, such as isothermal titration calorimetry, microscale thermophoresis, light scattering (static and dynamic), small-angle X-ray scattering, X-ray crystallography, and cryo-electron microscopy. Additionally, the delegates gained valuable hands-on experience from 20 workshops covering a broad range of applications, experimental designs and systems, and the latest software innovations in solution biophysics. The AUC2022 special volume highlights the sustained innovation, utility and relevance of AUC and related solution biophysical methods across various disciplines, including biochemistry, structural biology, synthetic polymer chemistry, carbohydrate chemistry, protein and nucleic acid characterization, nano-science, and macromolecular interactions.

Introduction and conference organization

During the 23rd International Analytical Ultracentrifugation Workshops and Symposium held in Glasgow, UK in 2017, it was proposed that after the 24th international event in Christchurch, New Zealand, the 25th Anniversary Workshops and Symposium be held at the University of Lethbridge, Lethbridge, Canada. Subsequently, Dr. Borries Demeler joined the University of Lethbridge in the fall of 2018 which boosted the morale to host this international event, for the first time, in Canada. The event lasted from July 10 to 15, 2022, and also included an excursion to the beautiful Waterton National Park, which was a highly memorable event for all participants (Fig. 1). The delegates also went on a boat

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tour and briefly visited Montana, USA from Waterton National Park, Canada. As chairs of the organizing committee, Borries Demeler and Trushar Patel were thrilled to host 104 delegates who attended the event, most of whom were from Canada, France, Germany, Japan, Switzerland, the UK, and the USA.

We would like to sincerely thank the scientific organizing committee which included Alexander Bepperling (Novartis, Germany), Olwyn Byron (University of Glasgow, UK), Helmut Cölfen (Universität Konstanz, Germany), Renwick Dobson (University of Canterbury, New Zealand), Karen Fleming (Johns Hopkins University, USA), Chris Horne (Walter and Eliza Hall Institute of Medical Research: Melbourne, VIC, AU), Liwen Qi (Tsinghua University, China), as well as David Scott (University of Nottingham, UK). Furthermore, we also recognize the efforts and dedication of the Local Organizing Committee that included Maulik Badmalia, Amy Henrickson, Tyler Mrozowitch, and Quadir Siddiqui from the University of Lethbridge.

Following the format of previous events, the first two days of the 25th anniversary event featured 20 workshops presented by experts in their fields, followed by a ‘Ask the Experts’ roundtable discussion moderated by Jeffrey Fagan, Natl. Institute of Standards and Technology. The workshops offered newcomers to AUC, an introduction to the basics of hydrodynamics and experimental designs, followed by detailed presentations on AUC of high-concentration solutes, AUC of membrane proteins, polymers, micelles, nanoparticles and nanocarriers, as well as strategies to analyze lipid nanoparticles, and gene therapies based on adeno-associated virus vectors (AAV). An important novel development in the field, multi-wavelength AUC, which adds an orthogonal characterization dimension to the traditional hydrodynamic dimension was discussed by several workshops. Innovations in hardware (the Beckman-Coulter Optima AUC™) were presented, and advances in software, which covered topics, such as Good Manufacturing Practices (GMP) and analysis software for AUC experiments, such as UltraScan, SedFit, SedAnal, DCDT + , Sednterp, as well as software for small-angle light scattering analysis and for hydrodynamic modeling with UltraScan-SOMO and Hullrad. The tradition of a strong emphasis on training was evident from the helpful willingness of all experts to share their knowledge and tricks of their trade with colleagues, and continually grow this important branch of solution biophysics. Encouraging was the trend for strong participation by the younger generation (Table 1). Students and postdoctoral fellows, as well as emerging scientists turned out in greater numbers for oral and poster presentations than in previous conferences. Also, participation by female scientists was up slightly from our previous conference, although there is still room for improvement. Collectively, the AUC community should make a concerted effort to attract new students into this exciting field to foster growth, new ideas and directions.

Svedberg award

The AUC community has traditionally awarded the Svedberg Award to individuals who have made significant contributions to the field of AUC and hydrodynamics. This year, the committee nominated and elected Drs. Jose Garcia de la Torre and Walter Stafford for their life-long support of the community.

Outline of articles covered in 25th international analytical ultracentrifuge workshops and symposium special volume

The AUC2022 Conference Proceedings contain 24 exciting AUC research and methods development articles. They cover a wide range of topics that can be loosely grouped in the following themes: Improvements in data analysis packages and approaches, adeno-associated viral (AAV) vectors and nanoparticles, protein characterization using AUC, and complementary approaches, and protein–ligand interactions.

Improvements of data analysis packages and approaches

In order to determine the sedimentation coefficient and molecular weight of biomolecules, it is essential to remove time-invariant noise from AUC data. Each AUC data analysis package utilizes specific approaches to remove such noise. Mortezazadeh and Demeler (2023a) developed a new method that averages repeated radial incident light measurements as a function of the photomultiplier response at different wavelengths. This method effectively removes the majority of time-invariant noise, specifically from intensity data, and integrated it into the UltraScan AUC data refinement pipeline. They demonstrated that this improved method of removing time-invariant noise is beneficial for processing sedimentation equilibrium data as well as for analytical buoyant density equilibrium experiments where correcting time-invariant noise is challenging.

Understanding the functions of biomolecules often requires studying their physicochemical properties and solution behavior. Several methods are available to calculate hydrodynamic parameters of biomolecules, such as proteins and nucleic acids, and these methods are continuously refined and improved by researchers. For example, Fleming et al. (2023) developed an improved version of HullRadSAS, enabling reliable calculation of various hydrodynamic parameters for proteins and nucleic acids based on their high-resolution structure. This enhanced utility allows for the calculation of translational diffusion coefficients, hydrodynamic radii, intrinsic viscosities, radius of gyration, and sedimentation coefficients. One unique feature of this utility is the ability to calculate surface shell and entrained water, which is critical for large protein and nucleic acid molecules. Additionally, Brookes and Rocco (2023) developed a web-based tool as part of UltraScan-SOMO that can rapidly and reliably calculate hydrodynamic properties from high-resolution or scattering-based bead models. It provides parameters, such as partial specific volume, hydration, translational diffusion coefficient, Stokes radius, sedimentation coefficient, intrinsic viscosity, radius of gyration, electron pair distance distribution function, and circular dichroism patterns.

The SEDNTERP program has been widely utilized by the AUC community for over two decades. Recently, Philo updated this package to incorporate diffusion and sedimentation velocity data, calculated the refractive index of buffers, refractive index increment values, viscosity and density values, and corrected sedimentation and diffusion coefficients to standard solvent conditions (Philo 2023). The article also provides background information on many of the hydrodynamic parameters. The biotech industry has a keen interest in investigating therapeutic proteins in high-concentration environments such as human serum.

Ranasinghe et al. (2023) examined the suitability of double-stranded DNA molecules for use as a molecular standard for AUC because molecular reference materials to validate AUC instruments are so far not available. A suitable reference material must satisfy multiple requirements, such as stability, homogeneity, solution ideality, variable size and anisotropy, and it must be low cost, abundant, and it must be usable at multiple speeds and temperatures. In this study, multiple topologies of supercoiled, relaxed, and linearized small DNA mini-circles were evaluated by AUC for this purpose. They found that open circular and linearized DNA satisfy the requirements nicely, with superior homogeneity and colloidal stability observed over multiple months and temperatures. Supercoiled DNA—This work will be of interest to biopharma companies looking to validate their AUC instruments for analytics in a Good Manufacturing Processes (GMP) environment.

Bishop and Correia (2023) developed simulation protocols based on Gilbert theory to aid in designing sedimentation velocity experiments for studying self-association. By simulating different concentrations of molecules, they identified specific patterns indicating the number of building blocks involved in the reaction. They also examined the effects of factors like cooperativity and thermodynamic non-ideality on the patterns. This information can be useful for studying high-concentration drug solutions. The researchers also described the software SEDANAL, which helps analyze experimental data and provides important parameters for understanding the interactions between molecules. Furthermore, this special issue includes another software package called SViMULATE, developed by Brautigam (2023). SViMULATE allows users to simulate sedimentation velocity data interactively, providing a graphical overview. It accommodates various user-provided parameters and generates simulated data in various formats for additional analysis. One of the benefits of these programs is their ability to calculate various hydrodynamic parameters for simulated molecules. Additionally, they can emulate data for various experimental modalities and data acquisition systems.

Multi-wavelength analytical ultracentrifugation (MW-AUC) has emerged as a powerful feature of AUC, with applications in various areas of life sciences. This method shows great promise to facilitate the processing of data involving mixtures of macromolecules and small molecules with different chromophores. To address this, Mortezazadeh and Demeler (2023b) developed a data analysis utility called the spectral decomposition residual visualization module, which they integrated with the UltraScan package. This module monitors the accuracy of spectral decomposition, resulting in robust and reliable data analysis for samples containing mixtures of macromolecules and small molecules. Additionally, Savelyev et al. (2023) developed another utility (integrated into UltraScan) to analyze and accurately quantify peaks from analytical buoyant density equilibrium (ABDE) experiments. This utility can calculate the concentration distribution of the density-forming gradient material at equilibrium. Notably, this method facilitates quantification of empty, partial and filled AAV capsids, and supports measurements for both ionic and non-ionic density-forming materials and can process data collected using UV and AVIV fluorescence optical systems.

Correia et al. (2023) demonstrated the application of the fluorescence detection system in studying macromolecular interactions in serum samples. They showed that the fluorescence detection system can be applied to study complexes such as the bilirubin–HSA complex,

whose sedimentation is influenced by non-ideality and the Johnston–Ogston effect due to the presence of high concentrations of IgG. Their work also highlighted the ability to study fluorescently labeled IgG in serum samples using the fluorescence detection system. They used the SEDANAL package to process AUC data in their study.

Finally, Winzor et al. (2023a) studied the relationship between diffusion coefficients and protein concentration using traditional boundary spreading measurements and synthetic boundary measurements in the analytical ultracentrifuge for two globular proteins and observed a minor negative concentration dependence of the diffusion coefficient, both in experimental observations and theoretical predictions. However, the concentration dependence fell within the limits of experimental uncertainty inherent in diffusion coefficient measurements. They also examined the ionic strength dependence of the concentration dependence coefficient using dynamic light scattering. Despite the thermodynamic constraints of constant temperature and pressure, good agreement was found between predicted and published experimental ionic strength dependencies for lysozyme and immunoglobulin. The same authors also discussed the discrepancy between experimental values of the light scattering second virial coefficient for proteins and those predicted based on excluded volume statistical mechanics (Winzor et al. 2023b). They suggested that a better theoretical description of published results for lysozyme can be achieved by considering an experimental parameter that measures the difference between the thermodynamic excluded volume term and its hydrodynamic counterpart. This parameter is a combination of factors that quantify the concentration dependence of the translational diffusion coefficient obtained from dynamic light scattering measurements.

Adeno-associated viral (AAV) vectors and nanoparticles

One of the challenges faced in the development of AAV-based drug delivery systems is the packaging of AAV with nucleic acid therapeutics. To avoid immuno-toxicity issues associated with empty AAV, accurate quantification of loaded therapeutics and empty AAVs is crucial. While chromatography and light-scattering-based methods are currently used, Yarawsky et al. (2023a) demonstrated the effectiveness of AUC in accurately determining the amounts of filled, partially filled and empty AAV capsids, ultimately reducing immuno-toxicity. Their article also highlights the application of integrated analyses that employ multiple sedimentation velocity data analysis models to reliably determine the hydrodynamic parameters of individual species within the mixture of empty, partially filled, and filled AAVs. Similarly, Saleun et al. (2023) contributed to this field by providing an overview of previously published information on AAVs and demonstrating that classical detection at 260 nm, as well as interference optics in AUC can be used to determine weight percentages of capsid populations and the size of the genome incorporated in rAAV particles.

This special volume also includes an article by Wawra et al. (2023), which demonstrates that AUC is an appropriate method for characterizing enveloped viruses that can also serve as drug delivery vehicles. The authors utilized a vesicular stomatitis virus-based oncolytic virus with glyco-proteins on the envelope. They performed density gradient and density contrast experiments, as well as nanoparticle tracking analysis, to determine the partial specific

volume and hydrodynamic diameter of the virus, respectively. Subsequently, they conducted AUC experiments and integrated the details of partial specific volume and hydrodynamic radius to reliably determine the solution behavior of the virus under investigation.

The next article by Sternisha et al. (2023) in this volume highlights the application of AUC to study the composition of Adenovirus. The authors demonstrate the efficiency gains when characterizing viral vectors by density gradient rather than sedimentation velocity methods. Lipid nanoparticles have emerged as reliable and robust tools for therapeutic delivery, offering benefits, such as low immunogenicity and targeted delivery. Recent work by Bepperling and Richter (2023) demonstrates that density contrast sedimentation velocity experiments can reliably determine molecular weight distributions. In combination with density obtained from density contrast sedimentation velocity experiments, it is also possible to determine the mRNA copy number of a degradable lipid nanoparticle formulation.

Finally, Bepperling and Best (2023) conducted an important study utilizing multiple AUC-based approaches, including boundary sedimentation velocity AUC, MWL–AUC, band sedimentation AUC, and CsCl density gradient sedimentation equilibrium AUC, to assess their benefits and disadvantages for characterizing AAVs. Their findings suggest that while all techniques provide reliable results for AAV characterization, specific sample requirements and various data analysis approaches should be considered.

Protein characterization using AUC and complementary approaches

Outer membrane proteins often contain unfolded regions that enable their interaction with chaperones to perform their functions. However, due to a lack of information regarding their possible conformations, further research is necessary in this area. In light of this, Devlin et al. (2023) developed a unique pipeline that integrates wet-lab and computational approaches. They first utilized available experimental data on unfolded outer membrane proteins and refined this information through sedimentation velocity experiments under denaturing conditions. Subsequently, they employed coarse-grained modeling and molecular dynamics simulations based on the sedimentation velocity data to obtain a comprehensive range of unfolded conformations. These approaches provide a foundation for studying the conformations, biogenesis, and interactions of outer membrane proteins.

Non-ideality effects in hydrodynamic measurements typically arise from the large size of molecules or the charge or elongated nature of biomolecules. A recent investigation by Yarawsky et al. (2023b) focused on the recombinant B-repeat superdomain of the biofilm-related accumulation-associated protein (Aap) from *Staphylococcus epidermidis*, which contains either five full B-repeats or one full B-repeat. The study revealed that the extended nature of these proteins leads to a phenomenon known as viscous fingering. Viscous fingering is typically observed at very high concentrations for globular proteins. However, in the case of the investigated proteins, viscous fingering occurred at a concentration of 5 mg/mL, demonstrating their non-ideal behavior and the presence of non-ideal effects at relatively low concentrations for extended proteins.

Abu Hammad et al. (2023) utilized sedimentation equilibrium and velocity experiments to study two different glycoforms of IgG1 antibody. Both glycoforms had variations in terms

of their glycosylation patterns and types of sialylation. Their work demonstrated that despite the differences in types of glycosylation, both glycoforms of antibodies have similar weight average molar masses as well as sedimentation coefficient distributions.

Sodium dodecyl sulfate (SDS) is commonly used in laboratories to denature proteins and disrupt large biological complexes, among other purposes. However, a recent study led by Henrickson et al. (2023) shed light on an important aspect of the effect of SDS on certain proteins. The authors discovered that in the absence of SDS, the myotoxin-II protein remains monomeric. However, upon the addition of small amounts of SDS below its critical micelle concentration, myotoxin-II forms a stable hexamer. The study also demonstrated that the presence of SDS, a phospholipid mimetic, leads to the hexamerization of myotoxin-II, which could explain the pathology of this snake venom.

Protein–ligand interactions

Biomolecular interactions play critical roles in various cellular processes and host–pathogen communications. Several methods, such as isothermal titration calorimetry, microscale thermophoresis, and analytical ultracentrifugation, are available to study biomolecular interactions. To further enhance the field of biomolecular interactions, Watkin et al. (2022) summarized the theory of diffusion, microfluidics principles, and outlined the application of microfluidic systems in studying protein–ligand interactions. They also compared this technique with other commonly used techniques for studying interactions and highlighted potential challenges.

Netrin family proteins, along with their receptors, are involved in various cellular functions and signaling pathways, such as axon migration. Unc5B is one of the key interacting partners of Netrin-1, and their binding leads to repulsion of axon growth cones. Using the MW-AUC technique, Gabir et al. (2023) demonstrated that Netrin-1 exists in a monomer–dimer equilibrium and that netrin-1 dimerization is pH dependent. Furthermore, their work also revealed that netrin-1 and Unc5B interact with each other in an equimolar ratio.

Conclusions

In conclusion, the 25th International Analytical Ultracentrifugation Workshop and Symposium held at the University of Lethbridge was a huge success. This event provided opportunities to trainees (students, postdoctoral fellows) and investigators from academia, industry and government to learn about hardware and software innovations, experimental designs to address cutting edge research challenges in gene therapy through viral vectors, and vaccine technology involving lipid nanoparticles, synthetic polymers and other nanoparticles. Participants disseminated research via oral and poster presentations, with plenty of opportunity to network and exchange ideas. Finally, we look forward to the 26th International Analytical Ultracentrifugation Workshops and Symposium on July 22–27, 2024, in Germany, organized by Johannes Walter and Alexander Bepperling. For additional information on AUC2023, please visit https://www.auc2024.fau.de/.

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References

Abu Hammad K, Dinu V, MacCalman TE et al. (2023) Comparative sedimentation equilibrium analysis of two IgG1 glycoforms: IgGCri and IgGWid. Eur Biophys J. 10.1007/s00249-023-01656-x

Bepperling A, Best J (2023) Comparison of three AUC techniques for the determination of the loading status and capsid titer of AAVs. Eur Biophys J. 10.1007/s00249-023-01661-0

Bepperling A, Richter G (2023) Determination of mRNA copy number in degradable lipid nanoparticles via density contrast analytical ultracentrifugation. Eur Biophys J. 10.1007/s00249-023-01663-y

Bishop GR, Correia JJ (2023) Simulation of Gilbert theory for self-association in sedimentation velocity experiments: a guide to evaluate best fitting models. Eur Biophys J. 10.1007/s00249-023-01634-3

Brautigam CA (2023) SViMULATE: a computer program facilitating interactive, multi-mode simulation of analytical ultracentrifugation data. Eur Biophys J. 10.1007/s00249-023-01637-0

Brookes EH, Rocco M (2023) Beyond the US-SOMO-AF database: a new website for hydrodynamic, structural, and circular dichroism calculations on user-supplied structures. Eur Biophys J. 10.1007/s00249-023-01636-1

Correia JJ, Bishop GR, Kyle PB et al. (2023) Sedimentation velocity FDS studies of antibodies in pooled human serum. Eur Biophys J. 10.1007/s00249-023-01652-1

Devlin T, Fleming PJ, Loza N et al. (2023) Generation of unfolded outer membrane protein ensembles defined by hydrodynamic properties. Eur Biophys J. 10.1007/s00249-023-01639-y

Fleming PJ, Correia JJ, Fleming KG (2023) Revisiting macromolecular hydration with HullRadSAS. Eur Biophys J. 10.1007/s00249-022-01627-8

Gabir H, Gupta M, Meier M et al. (2023) Investigation of dynamic solution interactions between NET-1 and UNC-5B by multi-wavelength analytical ultracentrifugation. Eur Biophys J. 10.1007/s00249-023-01644-1

Henrickson A, Montina T, Hazendonk P et al. (2023) SDS-induced hexameric oligomerization of myotoxin-II from *Bothrops asper* assessed by sedimentation velocity and nuclear magnetic resonance. Eur Biophys J. 10.1007/s00249-023-01658-9

Mortezazadeh S, Demeler B (2023a) Systematic noise removal from analytical ultracentrifugation data with UltraScan. Eur Biophys J. 10.1007/s00249-023-01631-6

Mortezazadeh S, Demeler B (2023b) A spectral decomposition quality assessment tool for multi-wavelength AUC experiments with UltraScan. Eur Biophys J. 10.1007/s00249-023-01640-5

Philo JS (2023) SEDNTERP: a calculation and database utility to aid interpretation of analytical ultracentrifugation and light scattering data. Eur Biophys J. 10.1007/s00249-023-01629-0

Ranasinghe M, Fogg JM, Catanese J et al. (2023) Suitability of double-stranded DNA as a molecular standard for the validation of analytical ultracentrifugation instruments. Eur Biophys J. 10.1007/s00249-023-01671-y

Saleun S, Mas C, Le Roy A et al. (2023) Analytical ultracentrifugation sedimentation velocity for the characterization of recombinant adeno-associated virus vectors sub-populations. Eur Biophys J. 10.1007/s00249-023-01650-3

Savelyev A, Brookes EH, Henrickson A et al. (2023) A new UltraScan module for the characterization and quantification of analytical buoyant density equilibrium experiments to determine AAV capsid loading. Eur Biophys J. 10.1007/s00249-023-01641-4

Sternisha SM, Wilson AD, Bouda E et al. (2023) Optimizing high-throughput viral vector characterization with density gradient equilibrium analytical ultracentrifugation. Eur Biophys J. 10.1007/s00249-023-01654-z

Watkin SAJ, Bennie RZ, Gilkes JM et al. (2022) On the utility of microfluidic systems to study protein interactions: advantages, challenges, and applications. Eur Biophys J. 10.1007/s00249-022-01626-9

Wawra S, Kessler S, Egel A et al. (2023) Hydrodynamic characterization of a vesicular stomatitis virus-based oncolytic virus using analytical ultracentrifugation. Eur Biophys J. 10.1007/s00249-023-01649-w

Winzor DJ, Dinu V, Scott DJ et al. (2023a) Retrospective rationalization of disparities between the concentration dependence of diffusion coefficients obtained by boundary spreading and dynamic light scattering. Eur Biophys J. 10.1007/s00249-023-01664-x

Winzor DJ, Dinu V, Scott DJ et al. (2023b) Experimental support for reclassification of the light scattering second virial coefficient from macromolecular solutions as a hydrodynamic parameter. Eur Biophys J. 10.1007/s00249-023-01665-w

Yarawsky AE, Zai-Rose V, Cunningham HM et al. (2023a) AAV analysis by sedimentation velocity analytical ultracentrifugation: beyond empty and full capsids. Eur Biophys J. 10.1007/s00249-023-01646-z

Yarawsky AE, Dinu V, Harding SE et al. (2023b) Strong non-ideality effects at low protein concentrations: considerations for elongated proteins. Eur Biophys J. 10.1007/s00249-023-01648-x

Fig. 1.
AUC 2023 delegates group picture at Cameron Falls, Waterton National Park

Table 1

Metrics for gender and career stage participation

	Gender		Career stage		
	Female	**Male**	**Student**	**Emerging**	**Established**
Registrations	27% (28)	73% (76)			
Session chairs	40% (4)	60% (6)	0	2	8
Talks	34% (10)	66% (19)	13	5	11