FAU

Friedrich-Alexander-Universität
Erlangen-Nürnberg

AUC2024

26th International Conference on Analytical Ultracentrifugation

BOOK OF ABSTRACTS

July 22 –27, 2024 · Banz Abbey/Germany

AUC2024.fau.de

Welcome

It is our pleasure to announce AUC 2024, the 26th International Analytical Ultracentrifugation Workshop and Symposium, to be held at Kloster Banz, Bad Staffelstein, Germany, July 22-27, 2024. Banz is a former Benedictine monastery and provides a stimulating environment for scientific exchange.

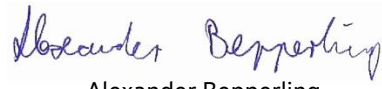
After more than 15 years, this conference is returning to Germany and we are honored to organize this prestigious meeting. AUC 2024 will focus on solution studies of biological macromolecules, interacting systems, synthetic polymers, and nanoparticles. While analytical ultracentrifugation is the main focus, dynamic and static light scattering, small angle X-ray and neutron scattering, microscale thermophoresis, surface plasmon resonance, field flow fractionation, calorimetry, and electron microscopy will also be presented.

AUC 2024 will open with a series of workshops beginning on Monday morning, July 22, and concluding on Tuesday, July 23. These workshops will be presented by leading experts in the field. On Wednesday, July 24, we will discover the beautiful landscape of Franconian Switzerland and German beer culture.

The workshop will be followed by a symposium, starting on the evening of Wednesday, July 24, with our Welcome Dinner and Svedberg Award Ceremony, and continuing through the afternoon of Saturday, July 27. The symposium will include poster sessions, an exhibition, and discussions.

We are looking forward to a scientific program full of expertise from around the world.

Johannes Walter



Alexander Bepperling

Conference Chairs of AUC 2024

Table of Contents

General Information

Conference Venue

Banz Abbey

Kloster-Banz-Straβe 1
96231 Bad Staffelstein
Germany

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Registration & Help Desk

The registration and help desk is located in the lower foyer. Registration is possible on these times and upon request (please contact the local organizing team, which can be identified by their green name tag):

- Sunday, 21 July, 17:00 – 18:00
- Monday, 22 July, 08:00 – 08:45
- Wednesday, 24 July, 17:30 – 18:15
- Thursday, 25 July, 08:00 – 08:45

The conference fee includes entrance to all sessions, the exhibition, coffee breaks, lunches and dinners, as well as the conference material. Please note that lunch during the social event on Wednesday is not included. Every participant will be provided with coupons for beverages served during lunches and dinners (2 per meal + 2 each for the get-together at 24 and 26 July evening). Snack and drinks are further sponsored for the trade exhibition and first poster session on Thursday.

Oral Presentations

All oral presentations take place in the lecture hall. They are limited to a total of 20 minutes for short talks and 25 minutes for full talks. This time includes a short discussion of about 5 minutes on the presented topic.

Speakers are expected to respect the schedule, to keep the time of their presentation and to present themselves to their session chair in advance to the session.

Speakers are requested to provide their presentation at least 15 minutes before the beginning of the session at the technical desk in the lecture hall. If necessary, speakers can use their own laptops for their presentation. Please inform the technical desk accordingly.

Conference Publications

After the conference, the authors of all conference contributions are invited to publish a journal article within a special conference issue in the **European Biophysics Journal**, which is gratefully offered once more for the 26th International Analytical Ultracentrifugation Workshop and Symposium 2024. Our timeline envisions 30 November 2024 as deadline for submission. More information on the submission process will be shared during the conference and will be also published on the conference website www.auc2024.fau.de.

Poster Presentations

Posters are displayed in the lecture hall surrounding the presentation area. Please mount your poster on Thursday either before the beginning of the symposium or during the lunch break. The poster boards are marked by the presenting author and title. The poster sessions are scheduled for Thursday and Friday, 25 & 26 July, 19:30 – 22:00. All posters will be part of both sessions and should be displayed until the end of the conference.

Poster Awards

The scientific committee will vote for the best poster presentations. The three posters with the most votes will be awarded with a poster prize during the concluding remarks on Saturday, 27 July, 12:30. All poster prizes are gratefully sponsored by AUC Solutions.

Exhibition

The exhibition is located in front of the main lecture. The exhibition area is accessible during the whole conference. The main exhibition time is parallel to the first poster session and starts on Thursday, 25 July, 19:30.

Welcome Dinner

The welcome dinner will take place at the restaurant of Banz Abbey and starts on Wednesday, 24 July, 18:30. The Svedberg Prize will be awarded during the welcome dinner.

Exhibitors & Sponsors

The exhibitor show will take place on the evening of Thursday, 25 July 2024.

Organization

Scientific Advisory Board

Alexander Bepperling	Sandoz, Germany
Johannes Walter	FAU, Germany
Olwyn Byron	University of Glasgow, United Kingdom
Borries Demeler	University of Lethbridge, Canada
Karen Fleming	Johns Hopkins University, USA
Trushar Patel	University of Lethbridge, Canada
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FAU

DESIGN OF PARTICULATE PRODUCTS CRC 1411

ENGINEERING OF ADVANCED MATERIALS
FAU COMPETENCE CENTER

FUNCTIONAL PARTICLE SYSTEMS

Program

Sun, 21 July Arrival

17:00 – 18:00 Registration & Help Desk

18:30 – 20:00 Dinner

Mon, 22 July Workshop

07:30 – 08:45 Breakfast

08:00 – 08:45 Registration & Help Desk

09:00 – 11:00 **Session 1**

11:00 – 11:30 Coffee Break

11:30 – 13:30 **Session 2**

13:30 – 14:30 Lunch

14:30 – 16:30 **Session 3**

16:30 – 17:00 Coffee break

17:00 – 19:00 **Session 4**

19:00 – 20:00 Dinner

from 20:00 Get-together

Tue, 23 July Workshops

07:30 – 08:45 Breakfast

09:00 – 11:00 **Session 5**

11:00 – 11:30 Coffee Break

11:30 – 13:30 **Session 6**

13:30 – 14:30 Lunch

14:30 – 16:30 **Session 7**

16:30 – 17:00 Coffee Break

17:00 – 19:00 **Session 8**

19:00 – 20:00 Dinner

from 20:00 Get-together

Wed, 24 July Social Event

07:30 – 08:45 Breakfast

during the day Social event

17:30 – 18:15 Registration & Help Desk

18:30 – 20:00 Welcome Dinner

from 20:00 Get-together

Thu, 25 July Symposium

07:30 – 08:45 Breakfast

08:00 – 08:45 Registration & Help Desk

08:45 – 09:00 Opening remarks by Johannes Walter | FAU Erlangen-Nürnberg & Alexander Bepperling | Sandoz, Germany

09:00 – 10:35 **Session 1: New methods and methods development – Part 1**
Chair: Johannes Walter | FAU Erlangen-Nürnberg, Germany

09:00 – 09:25 The involvement of Beckman Coulter Life Sciences in analytical ultracentrifugation from 1947 and beyond – A personal perspective

09:25 – 09:50 What you can learn from multiwavelength AUC

15:55 – 16:15 Biophysical analysis of PlzA-nucleotide interactions with multi-wavelength analytical ultracentrifugation
Sophia Bird | University of Lethbridge, Canada 55

16:15 – 16:45 Biophysical characterization of GFP-nanobody complex formation
Aysha Kinjo Demeler | University of Lethbridge, Canada 56

16:45 – 17:45 Technical Workshop by LUM GmbH

18:00 – 19:30 Dinner

19:30 – 22:00 Mixers, trade fair and poster session

Fri, 26 July Symposium

07:30 – 08:45 Breakfast

08:45 – 10:45 **Session 5: Biopharma applications**
Chair: Alexander Bepperling | Sandoz, Germany

08:45 – 09:10 Analytical ultracentrifugation: A platform technology for Proteolysis Targeting Chimeras (PROTACs)
Alexander E. Yarawsky | Bioanalysis, USA 57

09:10 – 09:35 Ionic strength and pH dependence of antibody reversible self-association probed by SV-AUC
Eric S. Day | Genentech, USA 58

09:35 – 10:00 A new twist on drug design: modifying metabolism to inhibit virulence
Ester Serrano | University of Glasgow, UK 59

10:00 – 10:20 Development of molecular standards to validate analytical ultracentrifugation instruments for GMP qualification
Reece Martin | University of Lethbridge, Canada 60

10:20 – 10:45 SV-AUC during the development of LNPs and ATMPs
Klaus Richter | Coriolis Pharma Research GmbH, Germany 61

10:45 – 11:05 Coffee Break

11:05 – 12:30 **Session 6: Protein applications - Biological interactions**
Chair: Olwyn Byron | University of Glasgow, UK

11:05 – 11:30 TRAPped in an elevator: amino-sugar uptake and utilisation by bacteria
Renwick Dobson | University of Canterbury, New Zealand 62

11:30 – 11:50 The impact of von Willebrand factor on immune complexes in hemophilia A
Ute Curth | Hannover Medical School, Germany 63

11:50 – 12:10 Characterization of Rabies virus phosphoprotein thermoresponsive liquid-liquid phase separation
Caroline Mas | Université Grenoble Alpes, France 64

12:10 – 12:30 Investigation of the complexation between glycinin and block copolymers through analytical ultracentrifugation
Xiaodong Ye | University of Science and Technology, China 65

12:30 – 14:00 Lunch

14:00 – 14:25 **Session 7: Nanoparticles and polymers - Part 1**
Chair: Jeffrey Fagan | NIST, USA

14:00 – 14:25 Leveraging analytical ultracentrifugation for comprehensive characterization and quantification of lipid nanoparticles
Amy Henrickson | Beckman Coulter Life Sciences, Canada 66

14:25 – 14:50 Hydrodynamic tools for assessing the molecular integrity of archaeological consolidants. The Saving Oseberg project
Stephen E. Harding | University of Nottingham, UK 67

14:50 – 15:10 Characterization of gold nanoparticles by cryogenic electron tomography and analytical ultracentrifugation
Quy Ong | Ecole polytechnique Federale de Lausanne, Switzerland 68

15:10 – 15:35 Quantifying the number of total and accessible functional groups on nanomaterials
Ute Resch-Genger | BAM, Germany 69

15:35 – 16:00 Coffee Break

16:00 – 17:35 **Session 8: Protein applications - Protein hydrodynamics**
Chair: Christine Ebel | Université Grenoble Alpes, France

16:00 – 16:25 The molecular basis for hydrodynamic properties of PEGylated proteins
Patrick Fleming | Johns Hopkins University, USA 70

16:25 – 16:50 Hydrodynamic and thermodynamic analysis of PEGylated human serum albumin
John Correia | University of Mississippi Medical Center, USA 71

16:50 – 17:15 Hydrodynamic comparison of the structure and interactions of the last line of defence antibiotics vancomycin and teicoplanin
Stephen E. Harding | University of Nottingham, UK 72

17:15 – 17:35 Poly acidic amino acids sequence drives variation of protein in hydrodynamic properties
Wengi Li | Tsinghua University, China 73

18:00 – 19:30 Dinner

19:30 – 21:00 Poster session and get-together

Sat, 27 July Symposium

07:30 – 08:45 Breakfast

08:45 – 09:55 **Session 9: Nanoparticles and polymers – Part 2**
Chair: Amy Henrickson | Beckman Coulter Life Sciences, Canada

09:55 – 10:45 **Session 10: Beadmodeling and hydrodynamics simulations**
Chair: Karen Fleming | Johns Hopkins University, USA

10:45 – 11:00 Coffee Break

11:00 – 12:30 **Session 11: Cross-disciplinary research**
Chair: Klaus Richter | Coriolis Pharma, Germany

12:30 – 12:45	Closing ceremony
12:45 – 14:00	Lunch
14:00 – 14:30	Business Meeting
15:00 – 17:00	Social event
18:00 – 19:30	Dinner for remaining attendees
from 20:00	Get-together

Sun, 28 July	Departure
07:30 – 09:00	Breakfast

Monday, 22 July | Session 1: 09:00 – 11:00

GUSSI: Basics and Advanced GUSSling (Chad Brautigam)

Seminar Room 3

GUSSI 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 GUSSI. Covered in this workshop will be the basics of GUSSI's layout and operation, including working with multiple data sets, manipulating colors and markers, and generating legends. Advanced GUSSI concepts, including rudimentary line fitting, formatting files for input to GUSSI, and exploring the wide array of biophysical data types pre-formatted in the program will also be discussed.

SAXS / SANS and conflicting X-ray / NMR structures (Alexey Savelyev)

Seminar Room 4

SAXS/SANS measurements provide extremely useful low-resolution scattering profiles meant to describe 3D structures. Moving toward higher resolution profiles often requires the use of MD simulation to extract relevant 3D geometrical information. Since MD simulations are based on the use of the underlying molecular force-fields (inter-atomic interaction parameters), SAXS /SANS data can help with refining simulation models. Additionally, SAXS/SANS is capable of resolving biomolecular structures from NMR and X-ray crystallography, the most frequently used structure determination techniques which, however, may or may not faithfully represent structure in solution.

LNP Density Matching (Borries Demeler)

Seminar Room 5

In this workshop we will use UltraScan to measure nucleic acid load in lipid nanoparticles (LNPs). There are two orthogonal methods presented, both of which are based on sedimentation velocity experiments (SVEs) that can be used to characterize drug loading:

1. Measuring density distributions based on D2O density matching
2. Multi-wavelength AUC to characterize differential absorbances of the liposome shell versus the nucleic acid absorbance spectrum.

Determining the amount of drug loaded into a LNP is of utmost importance for many biopharma applications. Determining the precise loading with nucleic acids is critical for achieving clinically relevant formulations, and to avoid antigenic materials in vaccines or gene therapy formulations. This can be challenging for many techniques

since overall LNP size and shape may not proportionally change with the cargo load. However, the density, or partical specific volume of LNPs is a sensitive predictor of loading state, regardless of particle size, and the absorbance profiles of liposomes and nucleic acids is a unique characteristic that can be followed by AUC.

SVEs can be used to determine the sedimentation and diffusion coefficients, and partial concentrations of all solutes present in a sample with high resolution. In this workshop we will demonstrate how multiple SVEs performed in different D2O:H2O ratios can be used to globally fit a partial specific volume (PSV) distribution for samples that are heterogeneous in density, and to combine this information with MW-AUC information to uniquely identify the LNP loading state. Furthermore, we can combine the PSV distributions with the corresponding sedimentation and diffusion coefficient distributions to derive accurate molar mass, particle size and anisotropy distributions. Software modules implemented in UltraScan specifically addressing MW-AUC and PSV distribution measurements by SVEs will be discussed.

Optima AUC Toolbox (Amy Henrickson, Lutz Erhardt, Shawn Sternisha)

Seminar Room 6

This workshop will provide a brief introduction to AUC and will highlight the different methods that are in the AUC toolbox. The methods that will be investigated will be:

1. Sedimentation velocity (SV)
2. Density matching
3. Density Gradient Equilibrium (DGE), also known as Analytical Buoyant Density Equilibrium (ABDE)
4. Band sedimentation (BS)
5. Sedimentation equilibrium (SE)
6. Multiwavelength (MW) as a tool for SV and DGE

For each method, we will provide an overview of its principles, the type of information it can provide, and a couple of examples where the technique has been used.

New Features in UltraScan for Multi-wavelength Spectral Deconvolution and Analysis (Saeed Mortezazadeh)

Franz-Josef-Strauβ-Raum

In this workshop we will present important new features in UltraScan that support the analysis of multi-wavelength AUC data, both in SV and in ABDE mode. The features will address the following topics:

1. Correction/elimination of time-invariant noise in primary intensity data by subtracting an averaged intensity profile
2. Correction/elimination of time-invariant noise in multi-wavelength ABDE experiments (which are time-invariant data to start with) without using finite element modeling, by using multi-wavelength reference scans, allowing up to 15 equilibrium samples to be measured in a single experiment
3. Creation of analyte intrinsic molar extinction profiles by globally fitting wavelength scans of a dilution series
4. A new CSV file format converter/loader/exporter
5. Quality assessment of multi-wavelength spectral deconvolutions using a new 3D viewer
6. A new multi-wavelength ABDE peak integration routine for AAV capsid species quantification

The workshop will be a demonstration of these features. Participants can bring their own (suitable) data and follow along on their own computers.

Monday, 22 July | Session 2: 11:30 – 13:30

Multisignal Sedimentation Velocity (Chad Brautigam)

Seminar Room 3

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 ration 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.

Accurate computation of biomolecular partial specific volume (Alexey Savelyev)

Seminar Room 4

The PSV is one of the primary hydrodynamic parameters which quantitatively characterizes solute–solvent interactions and is required for computational analysis and interpretation of the data from hydrodynamic experimental techniques such as AUC, SAXS/SANS, and FS spectroscopy. Particularly, in the downstream analysis of the AUC data (e.g. in the UltraScan suite of programs), the PSV is an input parameter required for reliable transformation of the sedimentation and diffusion distributions measured in AUC to molar mass distributions. While there is strong evidence that PSV depends on the solvation buffer conditions (most notably, on the ionic strength and pH), accurate buffer-dependent PSV values for different classes of biomolecules are not available. Our research, in which we addressed numerous approximations and shortfalls in existing methods to assess biomolecular PSV, lays solid foundation for further increase in our method's resolution which would enable us to study more subtle aspects of the interplay between quantitative changes in the solvation buffer conditions and the macroscopic quantities like PSV. Our findings (computed buffer-specific PSV values) are of immediate use to the high-resolution analysis of AUC data.

AAV Multi-wavelength analysis (Borries Demeler)

Seminar Room 5

Gene therapies deliver genetic material to host cells to silence or repress a mutated gene, replace it with a healthy gene, or introduce a new gene to help fight diseases. Multiple approaches, drug delivery vectors, and potential drug targets make this field versatile and successful. One of the most widely used vectors is adeno-associated viruses (AAVs), as they are not known to be pathogenic and result in a low immune response. AAV formulations consist of both loaded (containing genetic material) and empty (lacking genetic material) vectors, in varying ratios. Their production can contain contaminants such as partially loaded or aggregated vectors or unpackaged nucleic acids. This rapidly evolving field requires continuous development to improve the safety and efficiency of treatments, which requires objective characterization methods capable of identifying all components present in a formulation.

Analytical ultracentrifuge (AUC) offers exciting innovations that can revolutionize studies focusing on the molecular basis of diseases and their cures. Recent advances in AUC hardware and software have allowed for the development of two new methods that can overcome these quality control issues. The first is sedimentation velocity (SV) multi-wavelength AUC (MW-AUC) which allows for the accurate separation of spectrally different macromolecules (proteins, lipids, and nucleic acids), resulting in reliable identification of loaded vs. partial or empty AAVs and other contaminants, providing significantly improved quality control of gene therapies. The second is analytical buoyant density equilibrium (ABDE). This method is analogous to the techniques used during AAV purification, where density gradient forming solvents separate loaded AAVs, empty AAVs, and contaminants based on their densities and results in baseline separated peaks for each analyte (Figure 2). Combining ABDE with MW-AUC results in the accurate identification of each solute peak. Furthermore, ABDE experiments significantly improve throughput and sensitivity, significantly reducing the amount of sample volume and concentration compared to SV MW-AUC. These methods are innovative biophysical approaches that can be used to characterize many macromolecular assemblies in their physiological solution environment. They are universally applicable to other vector systems and other viral therapeutics, as well as including lipid nanoparticles, bacteriophage formulations, and other gene therapy systems.

In this workshop, we will discuss experimental design, and the tools implemented in UltraScan to process AAV samples with multi-wavelength SV and ABDE experiments, and demonstrate the superior resolution, improved throughput, and reduced sample requirements.

Basics of studying interacting systems by AUC (Alexander Yarawsky)

Seminar Room 6

This workshop is targeted to AUC users at all levels of expertise. This workshop will take the form of a presentation. A brief overview of AUC will be provided, with a specific focus on interacting systems and how they differ from non-interacting systems.

Topics will include:

1. Experimental design (SV-AUC vs EQ-AUC, choice of optical system, etc.)
2. Examining the raw data
3. Local analysis (g(s*)/WDA vs c(s) vs van Holde-Weischet)
4. Global analysis (Sw isotherms vs direct boundary fitting)
5. Advanced analyses like Wyman plots/linkage, thermodynamics and deltaG, hydrodynamic modeling, nonideality considerations, etc.

HDR-MULTIFIT – Turbidity and Particle size (Johannes Walter)

Franz-Josef-Strauß-Raum

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, MWL 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 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.

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. Multiwavelength analysis of GS experiments
5. Concentration coupling experiments for polydisperse distributions
6. Simultaneous analysis of size, density and refractive index increment using density variation

Most importantly, this workshop will include a hands on training on HDR-MULTIFIT.

Monday, 22 July | Session 3: 14:30 – 16:30

Basic/Advanced ITC (Chad Brautigam)

Seminar Room 3

Isothermal titration calorimetry (ITC) is widely used to study molecular interactions. A single experiment yields an unprecedented wealth of thermodynamic information, including changes in free energy, enthalpy, and entropy. No other method can boast this informational efficiency. In this workshop, ITC theory, experimental design, and analysis will be covered. Particular focuses will be integration using NITPIC, analysis using SEDPHAT, and illustration using GUSSI. Advanced experiments that utilize the global analysis of several experiments to yield information on changes in heat capacity, cooperativity, proton involvement, and "intrinsic" enthalpy will also be covered. Students will be led through guided analysis tutorials using provided data sets.

SOMO Hydrodynamics (Emre Brookes, Saeed Mortezazadeh, Mattia Rocco)

Seminar Room 4

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.

[1] Brookes, E. and Rocco, M., 2018. European Biophysics Journal, 47(7), pp.855-864. doi:10.1007/s00249-018-1296-0
[2] Rocco, M., Brookes, E. & Byron, O. 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] Rocco M, Byron O., 2015, Methods Enzymol. 2015;562:81-108. doi: 10.1016/bs.mie.2015.04.010

GMP data processing (Borries Demeler)

Seminar Room 5

The UltraScan GMP module completely automates AUC data processing, observing 21 CFR, Part 11 GMP requirements for electronic records [1]. The GMP process includes a complete description of the experimental design, which is stored read-only in the UltraScan LIMS database, and proceeds with automated data acquisition on the Optima AUC. After data collection is complete, the program automatically imports the data in the original double precision floating point format into the UltraScan LIMS database, without the ASCII loss of precision incurred in the Beckman program. In the next step, the program automatically edits the data. Next, the data will be analyzed automatically according to an analysis and result refinement workflow that is pre-defined in the experimental design protocol in the database. The analysis is performed on a supercomputer in parallel for all datasets in the experiment. After analysis is completed, and finite element models have been refined with 2DSA, 2DSA-FMB, 2DSA-IT, 2DSA-MC, and PCSA, the program proceeds to the reporting stage, automatically generating a comprehensive GMP report that is stored read-only in the UltraScan LIMS database. The program applies a collection of validation tests and reports automatically generated graphs, spreadsheets, and report items. In this workshop the participants will learn how to design a GMP protocol and run that protocol on the Optima AUC at the Canadian Center for Hydrodynamics. Questions about reporting formats, experimental design, 21 CFR-part 11 adherence and UltraScan data acquisition will be discussed.

[1] Savelyev A, Gorbet GE, Henrickson A, Demeler B. Moving analytical ultracentrifugation software to a good manufacturing practices (GMP) environment. PLoS Comput Biol. 2020 Jun 19;16(6):e1007942. doi: 10.1371/journal.pcbi.1007942

AUC of membrane proteins (Christine Ebel, Karen Fleming, Caroline Mas)

Seminar Room 6

Integral membrane proteins (MPs) are physiologically embedded in a lipid bilayer, which provides a hydrophobic environment compatible with their nonpolar, transmembrane surfaces. Both structural and solution studies of MPs often involve their extraction, solubilization, purification, and characterization in a wide variety of detergent micelles. In this workshop, we will describe the AUC characterization and analysis of detergent solubilized membrane proteins. Parameters that can be evaluated include MP homogeneity, protein molar mass, bound detergent, and protein-protein association constants.

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 both sedimentation velocity and sedimentation equilibrium experiments and analysis of membrane protein/detergent solutions. Topics to be covered include the following:

Sedimentation Velocity

- 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.

Sedimentation Equilibrium

- How to determine parameters needed for data analysis (partial specific volumes)
- Strategies for experimental design, including how to density match and what to do if density matching using water is not feasible
- How to globally analyze the data using WinNonlin
- How to evaluate molecular weight distributions for reversible equilibrium

Using SEDANAL to analyze interacting systems (Alexander Yarawsky)

Franz-Josef-Straβ-Raum

This workshop is targeted to those familiar with the fundamentals of AUC, and who are interested in learning how to globally fit SV-AUC data. New users are encouraged to also attend the preceding workshop: "Basics of studying interacting systems by AUC." Direct boundary fitting provides many benefits over fitting Sw isotherms. SEDANAL is extremely flexible and users can build custom models to fit against. This workshop will aim to familiarize users with a workflow for using SEDANAL to analyze SV-AUC data from interacting systems. Participants are encouraged to follow along on their own laptops. A copy of SEDANAL and simulated datasets will be provided prior to the workshop. Participants are also encouraged to read Stafford & Sherwood's 2004 Biophysical Chemistry paper describing SEDANAL. This workshop may also provide a useful primer for Walter Stafford and Jack Correia's more advanced SEDANAL workshops.

Topics will include:

1. SEDANAL installation
2. Preprocessing data
3. Building a model
4. Fitting and simulating data to a model
5. Error analysis
6. Overall interpretation and revisiting experimental design

Monday, 22 July | Session 4: 17:00 – 19:00

HDR-MULTIFIT – Turbidity and Particle size (Johannes Walter; repeat)

Seminar Room 3

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, MWL 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 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.

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. Multiwavelength analysis of GS experiments
5. Concentration coupling experiments for polydisperse distributions
6. Simultaneous analysis of size, density and refractive index increment using density variation

Most importantly, this workshop will include a hands on training on HDR-MULTIFIT.

SOMO Hydrodynamics (Emre Brookes, Saeed Mortezazadeh, Mattia Rocco; repeat)

Seminar Room 4

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.

[1] Brookes, E. and Rocco, M., 2018. European Biophysics Journal, 47(7), pp.855-864. doi:10.1007/s00249-018-1296-0
[2] Rocco, M., Brookes, E. & Byron, O. 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] 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

Optima AUC data acquisition (Borries Demeler)

Seminar Room 5

In this workshop we will cover and compare the different ways in which you can use UltraScan to import and export data with UltraScan, and how to retrieve data from the Optima AUC to your desktop, and into the UltraScan LIMS database. UltraScan now includes a fully-featured data acquisition module that allows you to control the Optima AUC directly from UltraScan using a network connection with the instrument. The data acquisition module comes in two flavors: One is for R&D projects, the other for GMP projects. The R&D version allows the user to control the instrument, watch the data acquisition in real time, and import the acquired data directly into the LIMS database. The GMP version also includes completely automated data editing, analysis and reporting workflows, which are further discussed in Session 3 of the AUC2024 workshops. In addition, we will discuss how to retrieve past experimental

data from the Optima, which are stored in the PostgreSQL database on the Optima AUC's built-in Linux computer. We will also cover the openAUC data format and how to interconvert data from OpenAUC to the legacy Beckman format for export, as well as the reverse direction to import from the legacy data format to the openAUC data format.

Simulating AUC data (Chad Brautigam)

Seminar Room 6

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 with 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 Fundamentals (Tom Laue; remote)

Franz-Josef-Strauβ-Raum

Three aspects of AUC experiment design will be discussed in detail:

1. Hardware setup: selection of rotor, centerpiece, windows, cell assembly/cleaning, cell alignment
2. Protocol description: rotor speed selection, temperature control, radial spacing, interval between scans
3. Optical systems: how they work, advantages and disadvantages of each and which to choose

An AUC simulator will be used for the presentation and will be made available to the participants. In addition to walking you through setting up an experiment, the program simulates the signals for the rotor timing pulse, as well as for each optical system so that you can see how data are acquired. Sedview, a simple, but powerful, wide-distribution data analysis program is part of the simulator. This program allows you to see the consequences of various experimental design choices, provides model-independent data analysis, and overlays data from different detectors and different experiments. Students will see the effects of multiple components and self-association on the raw data and in the simple analysis.

Tuesday, 23 July | Session 5: 9:00 – 11:00

Membrane Protein and Glycoprotein AUC (Chad Brautigam)

Seminar Room 3

Membrane proteins and glycoproteins make up a large portion of the protein complement in a mammalian cell. But they offer special challenges to the hydrodynamicist. Membrane proteins are often solubilized by detergents, amphipols, or nanodiscs that cosediment with the protein. Glycoproteins have covalently attached sugar moieties that can obscure the nature of the protein component. However, with judicious use of the AUC's optical systems and proper analytic methods, these proteins can be characterized. This workshop will explore experimental design, data acquisition, and data analysis for both membrane proteins and glycoproteins. An emphasis will be hands-on instruction regarding the utility functions built into GUSSI for the analysis of these challenging proteins.

Ultrascan GMP novel features (Alexey Savelyev, Borries Demeler)

Seminar Room 4

The UltraScan GMP module completely automates AUC data processing, observing 21 CFR, Part 11 GMP requirements for electronic records. The GMP process includes a complete description of the experimental design, which is stored read-only in the UltraScan LIMS database, and proceeds with automated data acquisition on the Optima AUC. After data collection is complete, the program automatically imports the data in the original double precision floating point format into the UltraScan LIMS database, without the ASCII loss of precision incurred in the Beckman program. In the next step, the program automatically edits the data. Next, the data will be analyzed automatically according to an analysis and result refinement workflow that is pre-defined in the experimental design protocol in the database. The analysis is performed on a supercomputer in parallel for all datasets in the experiment. After analysis is completed, and finite element models have been refined with 2DSA, 2DSA-FMB, 2DSA-IT, 2DSA-MC, and PCSA, the program proceeds to the reporting stage, automatically generating a comprehensive GMP report that is stored read-only in the UltraScan LIMS database. The program applies a collection of validation tests and reports automatically generated graphs, spreadsheets, and report items. We will discuss the following novel features moving UltraScan into cGMP environment: (1) a comprehensive protocol development tool, that will empower less experienced scientists to design experiments more efficiently, and accelerate protocol development for samples whose sedimentation behavior is initially unknown; (2) a fully automated reporting module that will eliminate analysis bias, enhance data

understanding, streamline processes, and establish a consistent format for comparing various experiments of the same sample; (3) the audit trail and electronic signatures modules allowing for enhanced traceability of the GMP data processing.

Multiwavelength SV and ABDE (DGE) AUC for the characterization of AAVs (Amy Henrickson)

Seminar Room 5

Multi-wavelength analytical ultracentrifugation (MW-AUC) is a recent development made possible in the new Optima AUC. It extends the hydrodynamic information typically obtained by measuring the sample at multiple wavelengths. MW-AUC adds an orthogonal spectral dimension to the traditional hydrodynamic characterization, allowing the researchers to identify different spectral molecules in the sample. It is poised to become an essential analytical tool for studying macromolecular interactions due to its ability to exploit unique chromophores in analyte mixtures.

This workshop will take a detailed look at the ABDE method and its setup and compare the results with SV results. ABDE separates analytes in a sample based on their densities as they sediment and float to the isopycnic points in a density gradient. By allowing the sample to settle within the solution column at the isopycnic point, the amount of sample required is reduced compared to SV experiments. This method has gained significant interest, particularly in the field of gene therapy, where samples can be expensive. The workshop will focus on the analysis of adeno-associated viruses (AAVs) and will examine their analysis and method setup using multiwavelength SV and ABDE methods, analyzed using UltraScan.

AUC of Macromolecular and Colloidal Systems (Ivo Nischang)

Seminar Room 6

1. Basic relations (intrinsic) sedimentation coefficient, (intrinsic) diffusion coefficient, intrinsic viscosity, partial specific volume, translational friction properties, molar mass, hydrodynamic invariant
2. Sedimentation velocity experiments
   a. Sedimentation-diffusion analysis physically consistent values of the molar mass, alternative methods (size-exclusion chromatography / asymmetrical flow field-flow fractionation—multi-angle laser light scattering (SEC / AF4—MALLS))
   b. (Self-)assemblies in solution degree of aggregation, stability against dilution, complexation of genetic material, encapsulation of drugs, hydration
   c. Nanoscale drug delivery systems solution complexity including nanoparticles (NPs), encapsulated and free drug, targeting dye moieties, presence or absence of surfactants, colocalization studies

3. Sizing aspects (AUC, DLS, SEC / AF4—MALLS) hydrodynamic size estimates based on sedimentation analysis (AUC), sedimentation-diffusion analysis (AUC), diffusion measurements (DLS), and radii of gyration (MALLS)

Calculation of hydrodynamics with HullRad (Patrick Fleming)

Franz-Josef-Strauβ-Raum

HullRad is an algorithm and computer program to calculate hydrodynamic properties such as sedimentation and diffusion coefficients of macromolecules from structural models. Output includes Stokes radius, radius of gyration, rotational correlation time, and ellipsoidal axial ratio which are useful parameters to estimate the size and shape of molecules in solution. The algorithm is implemented in Python and is available as a web server and as freely distributed code. Calculations are fast, and with locally installed code, it is feasible to calculate the hydrodynamic properties of thousands of structures in a very short time. Such calculations are useful for validation of model ensembles of unfolded, disordered, or highly flexible molecules. An alternate version, HullRadSAS, provides additional information about molecular hydration and hydrated radius of gyration for comparison to SAXS data. This workshop on HullRad will demonstrate different ways to run HullRad. An introduction to the rationale behind the HullRad algorithm will also be presented.

Hands-on: Bring your laptops and PDB/mmCIF files of any structural models you are interested in. We will download HullRad and calculate hydrodynamic properties of your favorite molecules. If you have an ensemble collection of structural models, even better.

Tuesday, 23 July | Session 6: 11:30 – 13:30

Analytical Ultracentrifugation of Nanoparticles (Lukas Dobler)

Seminar Room 3

Analytical Ultracentrifugation (AUC) was originally invented for the analysis of nanoparticles. It is still a great tool for their analysis. However, nanoparticles pose special challenges to the researcher, which are typically not encountered in the analysis of proteins.

These are:

- Polydispersity
- Hybrid character and therefore folded particle size and particle density distribution
- Stabilization by charge, which cannot be shielded by buffers
- A size which already leads to light scattering superimposing the signal by absorption
- Shape distributions
- Size dependent optical properties

The workshop will be in form of a presentation demonstrating solutions to these challenges as well as a discussion. These strategies can also be adapted to extremely polydisperse Biopolymers and complexes, which also have some of the above problems in common with nanoparticles. Users are encouraged to bring their own examples with them for discussion.

Complementary Methods: MST/FP/BLI (Chad Brautigam)

Seminar Room 4

A wide host of methods are available to the modern biophysicist to characterize the in vitro thermodynamics of interactions. Among these are microscale thermophoresis (MST), fluorescence polarization (FP), and biolayer interferometry (BLI). All of these methods are commonly used to determine the free energy of interaction of two or more macromolecules. Less usual is the study of the binding of small molecules to protein, but this can also be accomplished in some cases. The theoretical aspects of these methods will be summarized in this workshop, along with experimental strategies and analytic practices. Hands-on analyses of these data types using the software PALMIST will demonstrate the advanced fitting and error-analysis aspects of the program.

BASIS: BioAnalysis SEDFIT Integrated Software for cGMP Analysis of SV-AUC data (Alexander Yarawsky, Lake Paul)

Seminar Room 5

This workshop is targeted to participants interested in implementing SV-AUC in a cGMP environment. There is a major interest in using SV-AUC for characterization and release testing of gene and cell therapy products. BioAnalysis, LLC has developed a program (BASIS) which allows for data loading, data analysis using SEDFIT, integrating, reviewing, and reporting of SV-AUC data. The program was developed specifically for use in a cGMP environment and is 21 CFR Part 11 compliant, has undergone computer software validations, evaluated in numerous audits, and used for over 2 years at BioAnalysis, LLC. The objective of BASIS is to be a simple and efficient tool, and it offers many features that simplify and improve the overall user experience. This workshop will be a presentation that includes a walkthrough of the BASIS workflow.

Multi-wavelength SV and BS Analysis with Sedfit and CsCI DGE, for AAV and VV (Susumu Uchiyama)

Seminar Room 6

Outline:
AAV characterizations by following three types of analytical ultracentrifugation will be covered in this workshop.

- Sedimentation Velocity (with or without multiwavelength detection) [1]
- Band Sedimentation [2]
- Equilibrium Density Gradient [3, 4]

Specific contents:
Reliable identification of full, empty, extra-filled, and partial particles.

- Accurate quantification of full and empty particles.
- Purity assessment.
- Deep characterization of full particles with different VP ratio.

[1] Maruno T et al, Comprehensive Size Distribution and Composition Analysis of Adeno-Associated Virus Vector by Multiwavelength Sedimentation Velocity Analytical Ultracentrifugation. J. Pharm. Sci. 110, 3375-3384 (2021).
[2] Maruno T et al., Size Distribution Analysis of the Adeno-Associated Virus Vector by the c(s) Analysis of Band Sedimentation Analytical Ultracentrifugation with Multiwavelength Detection. J Pharm Sci. 112, 937-946 (2023).
[3] Hirohata K et al, Applications and Limitations of Equilibrium Density Gradient Analytical Ultracentrifugation for the Quantitative Characterization of Adeno-Associated Virus Vectors. Anal. Chem. 96, 642-651 (2024).
[4] Ohnishi et al., Enhancement of recombinant adeno-associated virus activity by improved stoichiometry and homogeneity of capsid protein assembly. Molecular Therapy: Methods & Clinical Development 31, (2023).

Tuesday, 23 July | Session 7: 14:30 – 16:30

Detecting Conformational Changes with SV (Chad Brautigam)

Seminar Room 3

AUC in the sedimentation-velocity (SV) mode is excellent for the determination of molar masses of macromolecules and the stoichiometries their complexes. A less-exploited strength of the method is its very high resolution in sedimentation-coefficient space. In carefully conducted SV experiments, differences in the sedimentation coefficients on the order of 0.01 S can be detected. This extremely high resolution can be leveraged to determine the existence and extent of a ligand-induced conformational change, even in moderately sized proteins. This workshop will focus on historical exploitation of this information, followed by the precepts of experimental design for SV studies to detect ligand-induced conformational changes. Finally, data-analysis will be discussed, and students will be guided through the analysis of real SV data sets using the software DiSECT.

Multi-wavelength analysis using SEDANAL (Walter Stafford, Jack Correia)

Seminar Room 4

This workshop will cover the analysis of multiwavelength (MWL) data from both the Beckman Optima AUC, and the Colfen and Schilling type multiwavelength instruments using the SEDANAL software. We will cover how to use the Preprocessor to deconvolute the individual concentration profiles of species for which the extinction spectra are known. We will also demonstrate how to determine each individual species' extinction spectrum in unknown mixtures if the species exhibit sufficient resolution in the AUC using Wide Distribution Analysis (WDA). Direct whole boundary fitting of models to mixtures–either interacting or non-interacting–can be carried out with the SEDANAL Fitter given the extinction spectra of the various components involved. Students are encouraged to bring multiwavelength datasets to workshop for analysis. Students should prepare for this workshop by reading the following:

Johannes Walter, Peter J. Sherwood, Wei Lin, Doris Segets, Walter F. Stafford, and Wolfgang Peukert (2015) Simultaneous analysis of hydrodynamic and optical properties using analytical ultracentrifugation equipped with multiwavelength detection. Anal Chem 87:3396–3403

Sherwood, P.J. and Stafford W.F. SEDANAL: Model-Dependent and Model-Independent Analysis of Sedimentation Data" in Analytical Ultracentrifugation. Instrumentation, Software, and Applications, Eds. Uchiyama, S, Arisaka, F., Stafford, W.F., and Laue T.M., Springer Japan. Chapter 6, pp 99-101.

Please note: The current version of SEDANAL along with the User Manual can be downloaded from http://sedanal.org/latest/.

Analytical Ultracentrifugation of Nanoparticles (Lukas Dobler; repeat)

Seminar Room 5

Analytical Ultracentrifugation (AUC) was originally invented for the analysis of nanoparticles. It is still a great tool for their analysis. However, nanoparticles pose special challenges to the researcher, which are typically not encountered in the analysis of proteins. These are:

- Polydispersity
- Hybrid character and therefore folded particle size and particle density distribution
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- A size which already leads to light scattering superimposing the signal by absorption
- Shape distributions
- Size dependent optical properties

The workshop will be in form of a presentation demonstrating solutions to these challenges as well as a discussion. These strategies can also be adapted to extremely polydisperse Biopolymers and complexes, which also have some of the above problems in common with nanoparticles. Users are encouraged to bring their own examples with them for discussion.

SOMO SAXS (Emre Brookes, Saeed Mortezazadeh and Mattia Rocco)

Seminar Room 6

The UltraScan Solution Modeler (US-SOMO) [1] has, in addition to hydrodynamic parameter calculation from structure, native and wrapped tools for computation of simulated biological solution small angle scattering (BioSAS) data from structure, processing of BioSAS data and comparison of simulated data against experimental data. US-SOMO also contains powerful tools for the analysis of size exclusion coupled small angle X-ray scattering (SEC-SAXS) data [2]. In this two session workshop, we will begin with an introduction to BioSAS. This will begin with an overview of the basics of BioSAS and its importance to the researcher. Next, during the first session, we will introduce the attendees to the available BioSAS tools within US-SOMO. In the second session, the attendees will work through hands-on exercices using US-SOMO with a focus on the SEC-SAXS tools.

It is suggested that attendees attend both sessions, as earlier material will not be covered again during the 2 session course. Registered attendees will receive detailed software installation instructions in the week prior to the workshop.

This workshop will be presented on Tuesday 23 July from 14:30-16:30 and 17:00-19:00 (sessions 7 and 8).

[1] 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
[2] Brookes, E., Vachette, P., Rocco, M. and Pérez, J., 2016. US-SOMO HPLC-SAXS module: dealing with capillary fouling and extraction of pure component patterns from poorly resolved SEC-SAXS data. Journal of applied crystallography, 49(5), pp.1827-1841. doi: 10.1107/S1600576716011201

Intro to SEDNTERP, a handy tool for AUC data interpretation (John Pilo; remote)

Franz-Josef-Strauβ-Raum

Program overview and demonstration

Prerequisites: none (suitable for novice AUC users)

Description

Introduction to SEDNTERP and its use for calculating:

- solvent density, viscosity, and refractive index
- sample partial specific volume, hydration, and specific refractive increment (dn/dc)
- standardized sedimentation or diffusion coefficients (s20,w or D20,w¬) from raw experimental values
- sedimentation or diffusion coefficients extrapolated to zero concentration, and their concentration-dependence coefficients ks and kD
- hydrodynamic parameters from SV or diffusion results:
  - anhydrous sphere radius, and corresponding maximum possible sedimentation and diffusion coefficients
  - sample frictional coefficient, f/f0 ratio, and Stokes radius
  - sample overall hydrodynamic shape and hydration characteristics (max hydration, Perrin P function, actual molecular size and shape for ellipsoid or cylinder models)

Tuesday, 23 July | Session 8: 17:00 – 19:00

AUC of Macromolecular and Colloidal Systems (Ivo Nischang; repeat)

Seminar Room 3

1. Basic relations (intrinsic) sedimentation coefficient, (intrinsic) diffusion coefficient, intrinsic viscosity, partial specific volume, translational friction properties, molar mass, hydrodynamic invariant
2. Sedimentation velocity experiments
   a. Sedimentation-diffusion analysis physically consistent values of the molar mass, alternative methods (size-exclusion chromatography / asymmetrical flow field-flow fractionation—multi-angle laser light scattering (SEC / AF4—MALLS))
   b. (Self-)assemblies in solution degree of aggregation, stability against dilution, complexation of genetic material, encapsulation of drugs, hydration
   c. Nanoscale drug delivery systems solution complexity including nanoparticles (NPs), encapsulated and free drug, targeting dye moieties, presence or absence of surfactants, colocalization studies
3. Sizing aspects (AUC, DLS, SEC / AF4—MALLS) hydrodynamic size estimates based on sedimentation analysis (AUC), sedimentation-diffusion analysis (AUC), diffusion measurements (DLS), and radii of gyration (MALLS)

High-concentration AUC (Walter Stafford, Jack Correia)

Seminar Room 5

Since the early days of protein characterization, the development of AUC analysis for high concentration SV data has been a critical focus. The early Model E Schlieren optical system worked best from 5-20 mg/ml. With the advent of therapeutic antibodies delivered at up to 150 mg/ml, high concentration AUC techniques continue to be proven irreplaceable. This workshop will discuss two essential steps in FDS AUC data collection and analysis:

1. Experimental setup of FDS SV runs at high concentrations.
2. Analysis of FDS SV data with nonideal, associating models in SEDANAL.

We typically perform experiments in tracer mode with 100 nM Alexa-labeled mAbs in unlabeled mAb concentrations from 1 to 150 mg/ml. FDS optics do not need reference channels, so we run up to six samples in a four hole rotor with 3 mm centerpieces. For this reason, sample labeling and equilibration will be discussed. We process FDS data for meniscus and base regions, and globally fit with models that account for hydrodynamic k s and thermodynamic nonideality BM 1 , plus weak association. Therefore, basic SEDANAL procedures (data preprocessor and ModelEditor) will be presented. Lastly, we perform error analysis by bootstrap with

replacement methods. Experiments can also be performed in human serum, which necessitates knowing serum protein concentrations and the use of matrix methods for k s and BM 1 cross term nonideality parameters. Data sets will be provided for student testing. SEDANAL runs in Windows 10 or 11, and on a Mac, requires Parallels, Boot Camp or VWware. Students should prepare for this workshop by reading Correia, et al. Eur. Biophys. J 49, 687, 2020.

Please note:

The current version of SEDANAL along with the User Manual can be downloaded from http://sedanal.org/latest/.

SOMO SAXS (Emre Brookes, Saeed Mortezazadeh and Mattia Rocco; repeat)

Seminar Room 6

The UltraScan Solution Modeler (US-SOMO) [1] has, in addition to hydrodynamic parameter calculation from structure, native and wrapped tools for computation of simulated biological solution small angle scattering (BioSAS) data from structure, processing of BioSAS data and comparison of simulated data against experimental data. US-SOMO also contains powerful tools for the analysis of size exclusion coupled small angle X-ray scattering (SEC-SAXS) data [2]. In this two session workshop, we will begin with an introduction to BioSAS. This will begin with an overview of the basics of BioSAS and its importance to the researcher. Next, during the first session, we will introduce the attendees to the available BioSAS tools within US-SOMO. In the second session, the attendees will work through hands-on exercices using US-SOMO with a focus on the SEC-SAXS tools.

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[2] Brookes, E., Vachette, P., Rocco, M. and Pérez, J., 2016. US-SOMO HPLC-SAXS module: dealing with capillary fouling and extraction of pure component patterns from poorly resolved SEC-SAXS data. Journal of applied crystallography, 49(5), pp.1827-1841. doi: 10.1107/S1600576716011201

Finding Confidence Limits for the Mass and Areas of Peaks in c(s) Distributions Using the Program SVEDBERG (John Philo; remote)

Franz-Josef-Strauβ-Raum

A tutorial

Prerequisites: at least some familiarity with c(s) analysis in SEDFIT (or similar size-distribution methods in ULTRASCAN)

Description

Although sedimentation coefficient distributions are widely used to identify what species (peaks) are present in a sample, it is usually difficult to determine the confidence limits for the properties of those species. In particular, SEDFIT provides no information about the precision of the peak fractions, which in many cases is the property of most interest. Furthermore, although SEDFIT can use Monte-Carlo approaches to try to assess a confidence region for each point (sedimentation coefficient) in the c(s) curve, that approach can be misleading because it doesn't explicitly deal with the fact that there is uncertainty in the position of each peak. A third important point is that Monte-Carlo approaches assume the noise in the raw data is randomly distributed, which is never really true for AUC data.

This tutorial will show you how to use the user-friendly, public-domain program SVEDBERG to easily translate your c(s) distribution to a mixture model and then calculate confidence limits for the peak fractions, sedimentation coefficient, and the molar mass for each peak using any of three different statistical approaches (including the bootstrap method, which makes no assumptions about the noise in the data being random).

The workshop will also demonstrate how we can use SVEDBERG to sequentially release the built-in constraint of the c(s) method that every species has the same hydrodynamic shape (f/f_0 ratio), and thereby learn how much information about the molar mass (or shape) of each species is truly present in the raw data.

Wednesday, 24 July | Social Event

1. Departure from Banz Abbey

Depature of the bus transfer in front of Banz Abbey at 09:00.

2. Rabenstein Castle and Sophie Cave

Rabenstein Castle (German: Burg Rabenstein) is a former high medieval aristocratic castle in the municipality of Ahorntal in the Upper Franconian county of Bayreuth in the German state of Bavaria. Originally built c. 1175 to 1200, the castle was destroyed and re-built several times. The spur castle can be visited for an entrance fee (not included).

(c) www.burg-rabenstein.de

(c) www.burg-rabenstein.de

In walking distance to Rabenstein Castle, Sophie Cave can be visited (admission gratefully sponsored by Nanolytics GmbH). The stalactite with its three large sections and winding passages, is considered one of the most beautiful show caves in Germany.

After the visit of the cave, lunch is available for purchase in the tavern and beer garden of Rabenstein Castle (not included in the conference fee).

3. Bavarian Brewery Museum in Kulmbach

Our next stop brings us to Kulmbach and its Bavarian Brewery Museum. With over 3000 square metres of exhibition space, it is one of the largest specialist museums and its individual sections showcase the special significance of beer in Bavaria, Franconia and Upper Franconia in particular. The exhibition begins thematically with the art of craft beer brewing around the year 1900 and then leads through the exhibition from beer brewing in the time of the ancient Egyptians to the time of the Romans and Celts to the medieval beer brewing of the monks.

(c) Von Benreis (https://commons.wikimedia.org/w/index.php?curid=33528001)

Our tour (gratefully sponsored by Nanolytics GmbH) includes admission and a guided tour 'The Art of Brewing', moderated beer tasting, tasting sip of museum beer and taster snack of museum bread as well as a certificate and beer glass.

4. Return to Banz Abbey

Transfer back to Banz Abbey at 17:00.

Welcome Dinner

We will conclude the day and begin the conference symposium with our welcome dinner at Banz Abbey at 18:30.

MULTI-WAVELENGTH ANALYSIS WITH THE OPTIMA AUC ANALYTICAL ULTRACENTRIFUGE

The Optima AUC Analytical Ultracentrifuge with its new multiwavelength absorbance optics, resulting from decades of innovation and research at Beckman Coulter Life Sciences, has led to an entirely new generation of analytical ultracentrifuges, allowing for the in-depth and accurate characterization of viral vectors, LNPs, and interacting systems. See for yourself.

Viral vectors (e.g., AAVs)

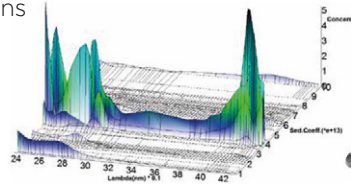
Empty, full, and partial capsid determination

Nanoparticles (e.g., LNPs and EVs)

Drug payload

Protein interactions

Enzymes and other proteins



BECKMAN COULTER Life Sciences

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com

2024-GBL-EN-105880-v1

danaher

Rediscover Centrifuges!

AUC 2024 CONTEST

Experience our Beckman centrifuge models - each consisting of over 400 individual components.

WIN ONE OF 20 CENTRIFUGE MODELS* HERE AT AUC 2024

* The winners will be determined in camera at the end of July 26, 2024. The prizes will be handed over by BECLS employees on July 27,2024

BECKMAN COULTER Life Sciences

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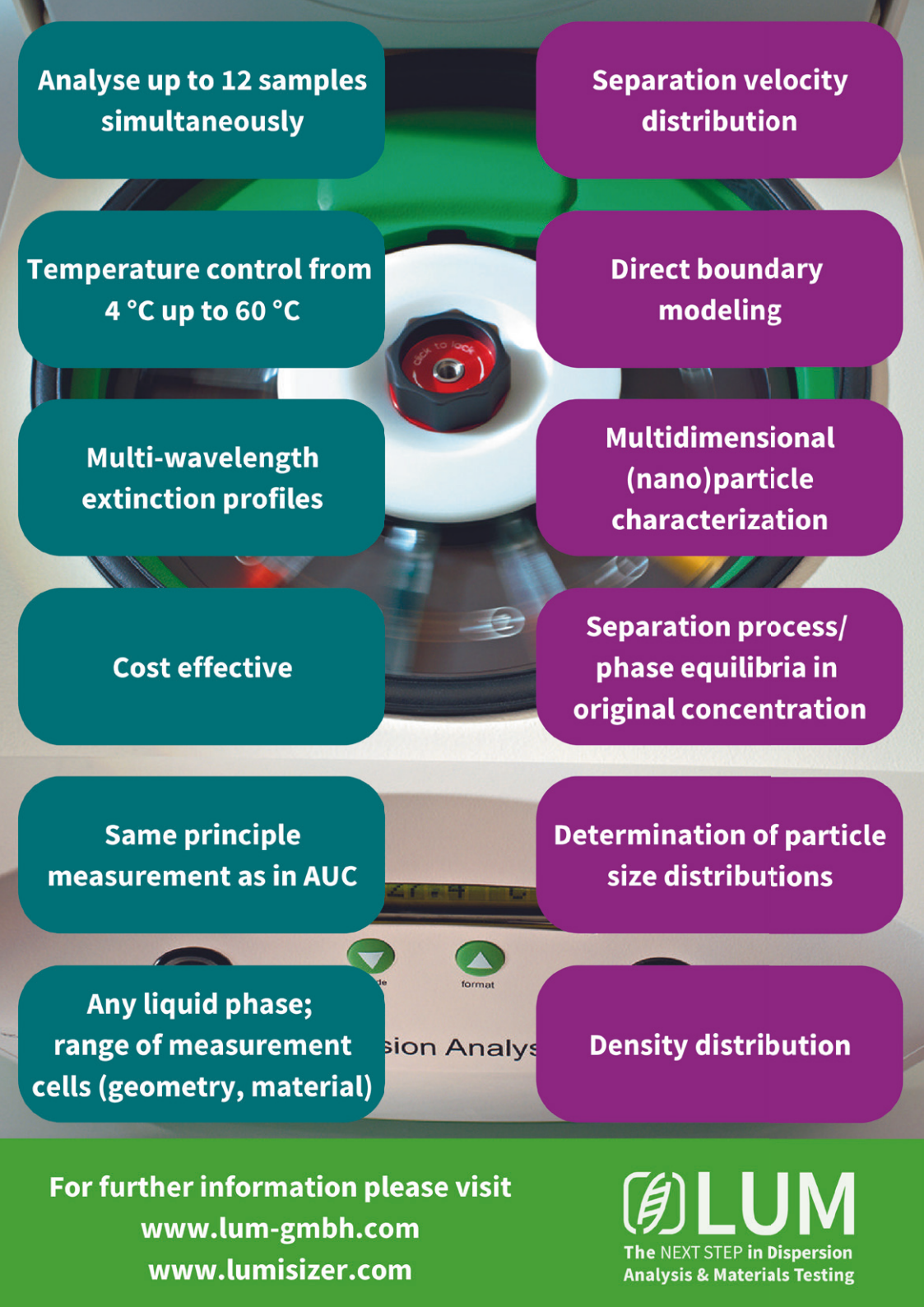
danaher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Thursday, 25 July | Session 1: New methods and developments - Part 1

Chair: Johannes Walter | FAU Erlangen-Nürnberg, Germany

The involvement of Beckman Coulter Life Sciences in analytical ultracentrifugation from 1947 and beyond – A personal perspective

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This presentation will guide you through the history of Analytical Ultracentrifugation (AUC) and Beckman Coulter's involvement in this characterization technique. It will showcase images from Beckman Coulter's archives and provide a personal perspective to inspire us to reflect on our own AUC stories and continue writing them in the future.

Additionally, the presentation will discuss Beckman Coulter's future plans and engagement in AUC. It will analyze the current state of AUC hardware and hardware support, addressing the upcoming discontinuation of the Optima XL-I/-A and ProteomeLab XL-I/-A analytical ultracentrifuges.

The Svedberg: The Nobel Lecture
The Svedberg Presentation Speech (Award ceremony speech) by Professor H-G. Söderbaum, Secretary of the Swedish Academy of Sciences on Dec 10, 1926
Arnold O. Beckman – One hundred years of Excellence, Arnold Thackray and Minor Myers, jr. Chemical heritage Foundation, Philadelphia, USA, 2000, ISBN 0-941901-23-8,
Physik die uns angeht, Walter Mächtle, 1978, Bertelsmann, Germany
AUC 101 historical video CENT-1435VID01.16
Selected Beckman Coulter advertisements and brochures from 1947 - now
Selected Beckman Coulter IFUs

What you can learn from multiwavelength AUC

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Multi-wavelength AUC (MW-AUC) offers a seconf, orthogonal characterization dimension in addition to the traditional hydrodynamic characterization in AUC experiments. The UltraScan software contains advanced algorithms to maximally exploit this approach for the characterization of complex mixtures and hetero-interactions between components that feature discrete spectral profiles. In this talk I will review the theory behind MW-AUC and present design guidelines for successful experiments, and explain the steps required to obtain useful data, and how to analyze the data using UltraScan. I will present several representative example applications that best demonstrate the additional information that can be gained from the optical separation dimension in AUC experiments.

Establishment of equilibrium density gradient analytical ultracentrifugation for the analysis of AAV

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Recombinant adeno-associated virus (rAAV) is one of the widely used for gene therapy. Currently, several rAAVs with different serotypes are marketed and more than 200 clinical trials of rAAV are ongoing. Sedimentation velocity analytical ultracentrifugation (SV-AUC) is recognized as a gold standard method for the purity assessment in terms of size distribution profile of rAAV. With multiwavelength (MW) detection, SV-AUC can identify and quantify the rAAV composition.

Preparative ultracentrifugation is still powerful method for virus purification, and in fact cesium chloride (CsCl) density gradient ultracentrifugation has been utilized for the purification of rAAV, in which CsCl generates a density gradient under the centrifugal field and macromolecule species migrate to the isopycnic point where the buoyant density of rAAV is equal to the density of CsCl solution at equilibrium. Recently, we developed a new approach to characterize rAAV by equilibrium density gradient analytical ultracentrifugation (DGE-AUC). The buoyant density-based separation has high resolution in density (~0.001 g/cm^3), thus the rAAV particles with different buoyant densities could be observed as well-separated peaks in DGE-AUC.

In the DGE-AUC profile, the observed peaks were identified from the UV absorption spectra using MW detection and fitted to Gaussian peaks after subtracting the baseline. The estimated amounts of particles in DGE-AUC were well corresponded to those from SV-AUC. Furthermore, two peaks corresponding to AAV encapsidating full-length DNA, which are both full particles were observed in several samples. Based on characterizations of these two types of full particles, we concluded that the cause of the different buoyant density is the difference in viral protein (VP) stoichiometry of the capsid. Thus, DGE-AUC is a unique technique that can separate and evaluate even full particles with different VP stoichiometry quantitatively. We developed a workflow to assign the unknown peaks as components.

In summary, DGE-AUC is a unique and effective method that evaluates the particle density heterogeneity of rAAV with limited sample amount compared to SV-AUC, without numerical analysis.

Hirohata *et al.*, Applications and Limitations of Equilibrium Density Gradient Analytical Ultracentrifugation for the Quantitative Characterization of Adeno-Associated Virus Vectors. **Anal. Chem.** 96, 642-651 (2024).

Band forming experiments in the AUC

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Nowadays, the mainly used experiment type of the Analytical Ultracentrifuge (AUC) is Sedimentation Velocity (SV). Not so popular and not often used is the band forming experiment (BFE) although it has potentially several benefits over SV. The short talk will be a review of the history of BFE, the possibilities, the current challenges, and the possible future improvements. All presented arguments will be supported by experimental examples.

Thursday, 25 July | Session 2: New methods and developments – Part 2

Chair: Borries Demeler | University of Lethbridge, Canada

Easy, interactive simulations of sedimentation velocity data using SViMULATE

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The simulation of analytical ultracentrifugation (AUC) data, particularly in the sedimentation velocity (SV) mode, is a time-honored means of experimental planning. Simulations can answer critical questions regarding timing, species resolution, rotor speed, choice of optical system, and many other practical aspects of the SV experiment. Although many means of simulation are available to the AUC practitioner, they can have limitations that require, for example, restricting the simulation to only a few species, calculation of abstruse quantities such as translational diffusion coefficients or density increments, or complying with idiosyncratic software requirements. With the goal of facilitating more straightforward SV simulations and thus encouraging rigorous experimental planning, in 2022, I introduced SViMULATE, a Python-based standalone program that is extremely flexible and allows interactive simulation using experimental parameters that even casual AUC users likely have at hand. In this presentation, I summarize advances in SViMULATE that have been added to the newest release (1.3.0). These include (1) simulation of band sedimentation velocity experiments using an adaptation to the finite-element method that allows the starting state to be very similar to the standard assumption of a step function; (2) non-ideal sedimentation, including consideration of cross-terms of the non-ideality constants; (3) two useful interacting systems; and (4) an interactive graph of the concentration profiles of all species. Examples and demonstrations of these new features will be discussed.

Multidimensional particle and liquid dispersion characterization by advanced analytical centrifugal sedimentation

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Multidimensional particle characterization is nowadays much advanced by analytical ultracentrifugation (AUC), which provides access to sizes below one nanometer. Besides the size, manifold characteristics of the particles or macromolecules can be determined today, such as shape anisotropy, density or optical properties. As the AUC measurement range is favorable for low sedimentation coefficients, micro- und submicro-particles especially of higher density are often sedimenting too fast even at the lower permitted rpm of an AUC. In this case, analytical centrifuges (AC) may be applied [1]. Here we focus on a multiwavelength-LUMiSizer running at 200-4000 rpm corresponding to centrifugal accelerations between 6-2300 x g. The rotor can be loaded with up to 12 samples cells of various materials (disposables or reusable) with a sedimentation length of 25 mm and optical paths from 1-10 mm. Sector shaped cells are available, too, similar to AUC cells [2]. A linearly shaped parallel NIR or VIS light beam passes through the cells placed horizontally onto the rotor. Transmitted light is instantaneously recorded from the cell bottom to the meniscus with a local resolution of 14 µm. The measurement temperature can be adjusted from 4-60 °C and recording time steps from one second up to hours are programmable via the browser-based software platform SEPView, which manages user and measurement datasets and provides different analysis modules [1].

After briefly presenting some results regarding particle characterization [1, 2, 3], we will focus in our contribution in more detail on the characterization of dispersion behavior especially at higher volume concentrations. This concerns the evaluation of suspension and emulsion stability and prediction of the shelf life; determination of the hindrance function for the separation behavior; centrifugal filtration; particle deformation; sediment formation and its packing concentration in dependence of applied centrifugal forces. The latter allows also the study of the deformation/packing of flocculated or soft particles. Special designed cells allow the superposition of magnetic fields.

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[2] M. Uttinger et al., *Part. Part. Syst. Charact.* **2020**, 2000108, 1-12.
[3] J. Walter et al., *Nanoscale* **2015**, 7, 6574.

Ultrascan GMP novel features

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The UltraScan GMP module completely automates AUC data processing, observing 21 CFR, Part 11 GMP requirements for electronic records [1)]. The GMP process includes a complete description of the experimental design, which is stored read-only in the UltraScan LIMS database, and proceeds with automated data acquisition on the Optima AUC. After data collection is complete, the program automatically imports the data in the original double precision floating point format into the UltraScan LIMS database, without the ASCII loss of precision incurred in the Beckman program. In the next step, the program automatically edits the data. Next, the data will be analyzed automatically according to an analysis and result refinement workflow that is pre-defined in the experimental design protocol in the database. The analysis is performed on a supercomputer in parallel for all datasets in the experiment. After analysis is completed, and finite element models have been refined with 2DSA, 2DSA-FMB, 2DSA-IT, 2DSA-MC, and PCSA, the program proceeds to the reporting stage, automatically generating a comprehensive GMP report that is stored read-only in the UltraScan LIMS database. The program applies a collection of validation tests and reports automatically generated graphs, spreadsheets, and report items. We will discuss the following novel features moving UltraScan into cGMP environment: (1) a comprehensive protocol development tool, that will empower less experienced scientists to design experiments more efficiently, and accelerate protocol development for samples whose sedimentation behavior is initially unknown; (2) a fully automated reporting module that will eliminate analysis bias, enhance data understanding, streamline processes, and establish a consistent format for comparing various experiments of the same sample; (3) the audit trail and electronic signatures modules allowing for enhanced traceability of the GMP data processing.

Deep learning based synthesis of sedimentation boundaries in centrifugation experiments

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Deep neural networks are becoming increasingly important in various fields of science and technology due to their ability to analyze large and complex datasets. Predictions or decisions can be based on patterns and trends that may not be immediately apparent. One challenge of deep learning methods is the large amount of training data required, which can often not be obtained from experiments alone. It has already been shown that images can be synthesized using neural networks [1, 2]. The sedimentation of particles during centrifugation can be mapped very well using Brownian dynamics or Lamm equation modelling and can be converted to image data, in this way generating training data.

In a feasibility study, deep neural networks are developed for simulating centrifugation experiments. On the one hand, a classifier is trained that can distinguish between four different kinematics, namely sedimentation, flotation, sedimentation and flotation as well as no recognizable kinematics. To overcome the limited amount of training data, a neural network is first trained on experimental data to synthesize realistic images to increase the amount of training data. In the next step, another network is programmed based on those training data, to decide which kinematics are present in the experiment. On the other hand, a regressor is implemented that interpolates and generates sedimentation profiles based on a dimensionless sedimentation rate. The dimensionless parameter eliminates ambiguities regarding particle properties and experimental parameters. Additional training data is generated using neural networks and evaluated using established methods [3]. The training data will also be used to implement deep neural networks that can calculate the sedimentation coefficient from experimental data. This study examines for the first time the possibilities of deep learning-based classification and synthesis of sedimentation profiles, which serves as a promising approach for the application of deep learning in particle measurement. In our contribution, we will further discuss current problems, limitations, and potential future improvements.

[1] Frei et al., *Powder Technol.* **2020**, 360, 324-336
[2] Goodfellow et al., *Advances in neural information processing systems* **2014**, 27
[3] Schuck et al., *Biopolymers: Original Research on Biomolecules* **2000**, 54, 328-341

Thursday, 25 July | Session 3: MWL-AUC – New methodologies and applications

Chair: Susumu Uchiyama | Osaka University, Japan

Size-resolved emission properties of core/shell CdSe/CdS quantum dots via multiwavelength emission analytical ultracentrifugation

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Colloids provide manifold opportunities for targeted product design due to their tunable properties with respect to size, shape, composition, surface, and spectral characteristics. However, the determination of structure-property relationships is quite challenging as most particulate samples exhibit polydispersity of their disperse properties. AUC is a fractionating technique and highly accurate method for the multidimensional analysis of nanoparticles as it permits the differentiation of spectral information linked to hydro- and thermodynamic properties of the particles [1, 2]. So far, characterization capabilities for fluorescent nanoparticles by AUC were limited, as the formerly commercially available fluorescence detector could not provide any spectral information [3]. Using a multiwavelength emission detector developed in our group, it is possible to extract spectra of fluorescent particles and biomolecules alongside their sedimentation and diffusion coefficients within a single centrifugation experiment [4, 5]. In our contribution, we will highlight that even narrowly distributed core/shell CdSe/CdS quantum dots still show size- and structure-dependent shifts of their fluorescence spectra, which can be resolved with our AUC system in an ensemble measurement. Thereby, we can link spectral changes of only a few nanometers to particle sizes retrieved with Angstrom size resolution. Single-particle measurements not only supported the results obtained with the AUC system but also provided deeper insights into the photophysical processes of individual QDs, clearly demonstrating the complementary nature of the techniques used. With our novel multiwavelength emission detector and the established extinction-based detector for AUC, a comprehensive platform for the holistic characterization of fluorescent colloids is now available.

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[2] J. Walter et al., *Anal. Chem.* **2015**, 87, 3396.
[3] I.K. MacGregor et al., *Biophys. Chem.* **2004**, 108, 165.
[4] S.E. Wawra et al., *Nanoscale Advances* **2019**, 1, 4422.
[5] V. Lautenbach et al., *Nanoscale Advances* **2024**, *6*, 2611.

New functions in UltraScan for the processing of multi-wavelength AUC SV and ABDE experiments

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Multi-wavelength Analytical Ultracentrifugation (MW-AUC) has recently gained significant importance for the analysis of a wide range of applications, from nanoparticle characterization to biomolecular complexes. Adeno-Associated Virus (AAV), a promising gene therapy vector, is an important research field studied extensively by AUC. MW-AUC is a critical new method for the characterization of empty, partially loaded, and filled AAV capsids. The Analytical Buoyant Density Equilibrium (ABDE) method, performed in MW-AUC mode, has dramatically reduced sample requirements, improved quantification accuracy, and significantly enhanced throughput. In this talk, I will present new methods implemented in the UltraScan software that aid the analysis of data from MW-AUC experiments. MW-AUC experiments, whether they are performed in sedimentation velocity (SV) or ABDE mode, require a spectral decomposition step. One of the new tools implemented in UltraScan monitors the quality of the decomposition with a 3D graphical monitor, and assesses randomness of residuals to assure appropriate spectral decomposition. Examining the errors associated with the non-negative least squares decomposition reveals information about deviations caused by hypo/hyperchromic spectral shifts induced by hetero-interaction, as well as the linear decomposition's reliability. A second tool allows time-invariant noise subtraction from equilibrium data, which, by definition, are always time invariant themselves. Conversion of intensity data from equilibrium experiments is performed with the second tool to produce noise-corrected pseudo-absorbance data from ABDE experiments. The main challenge in this approach is to identify and eliminate the time-invariant noise associated with the experimental data, contributed by the time invariant noise profile from the optical system, which differs for each wavelength of observation, and also includes refractive contributions from the buffer. Recent developments in the UltraScan software provide a variety of methods and a robust toolbox for data modeling and interpretation. UltraScan allows for accurate modeling of sedimentation velocity and pseudo-absorbance calculations, as well as a variety of utilities for analyzing and determining sedimentation coefficients, molecular weights, and hydrodynamic properties of biomolecules. This talk will go over the programs and utilities required to use the techniques mentioned above.

Insights into anisotropic silver nanoparticles growth via reaction quenching

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Silver nanoparticles (AgNPs) have a wide range of applications. Beyond their well-known antibacterial properties, they are also used in catalysis for hydrogen production, chemical and biochemical sensors and plasmon-enhanced solar cells. [1-3] Unlike isotropic AgNPs, anisotropic AgNPs have a high surface-to-volume ratio and sharp edges, resulting in highly tunable localized surface plasmon resonances (LSPRs). The specific LSPR intensity depends on the size, shape, and surrounding medium of the particles. Among the various types of anisotropic AgNPs, such as tetrahedrons, rods, filaments, pyramids and cubes, Ag nanotriangles (AgNTs) stand out due to their broad plasmon resonance range. [3] In our contribution, we investigate the growth dynamics of AgNTs using a novel quenching method during a one-pot synthesis, derived from the synthesis approach of Haber and Sokolov[2], employing 11-mercaptoundecanoic acid. This versatile approach is demonstrated in both batch and continuous flow synthesis. We utilize a comprehensive characterization strategy, combining UV/Vis spectroscopy, multi-wavelength analytical ultracentrifugation, size exclusion chromatography and transmission electron microscopy to monitor time-dependent morphological and size changes of the AgNPs. By coupling sedimentation and spectral data, we can identify and discern intermediate species within quenched samples, providing valuable insights into the evolving AgNPs population. Based on our findings, we perform boundary element method simulations for AgNPs and AgNTs with varying particle sizes, edge lengths, rounding and thicknesses. This integrated approach not only captures the evolution of the AgNPs but also offers a detailed understanding of the intermediate species present during the synthesis process. Our findings contribute to the fundamental understanding of the growth mechanism of AgNTs, offering valuable insights for further tailoring their properties in diverse applications. The presented methodology is adaptable and can be applied to other particle systems, providing a robust platform for exploring and optimizing synthesis processes.

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Thursday, 25 July | Session 4: MWL-AUC in biology

Chair: Ute Curth | Hannover Medical School, Germany

Analytical ultracentrifugation for the comprehensive characterizations of adeno-associated virus vector

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Recombinant adeno-associated virus (rAAV) is a leading platform for gene therapy, however accurate and reliable method for the biophysical characterization of rAAV needs to be developed. To assign the major peaks in C(s) distribution in sedimentation velocity analytical ultracentrifugation (SV-AUC) of “full particle preparation” and “empty particle preparation” of rAAV, partial specific volume and molecular weight of the two species were experimentally determined. Molar extinction coefficients at different wavelength of full particle and empty particles were determined by C(s) analysis of the multiwavelength detection SV-AUC, which enabled to estimate the population of partial and extra-filled particles besides the accurate quantitation of full and empty particles. Then band sedimentation AUC with C(s) analysis was developed, which we can clarify size distribution of rAAV with the sample amount of around 10^10 vg. The results of size distribution analysis from AUC were compared with other methods, such as charge detection mass spectrometry and cryo-electron microscopy. Furthermore, density gradient equilibrium AUC clarified the heterogeneity of full particle that had provided a single peak in C(s) results, due to the variation in stoichiometry of virus proteins comprising rAAV capsid. The developed AUC methods will be powerful methods for the characterizations of not only AAV vectors but also other virus and virus vectors.

[1] Nishiumi et al., Combined 100 keV cryo-electron microscopy and image analysis methods to characterize the wider adeno-associated viral products. **J Pharm Sci.** in press (2024).
[2] Hirohata et al., Applications and Limitations of Equilibrium Density Gradient Analytical Ultracentrifugation for the Quantitative Characterization of Adeno-Associated Virus Vectors. **Anal. Chem.** 96, 642-651 (2024).
[3] Ohnishi et al., Enhancement of recombinant adeno-associated virus activity by improved stoichiometry and homogeneity of capsid protein assembly. **Mol. Ther. Meth. Clin Dev.** 31, 101142 (2023).
[4] Maruno T, et al. Size Distribution Analysis of the Adeno-Associated Virus Vector by the c(s) Analysis of Band Sedimentation Analytical Ultracentrifugation with Multiwavelength Detection. **J Pharm Sci** 112, 937-946 (2023).
[5] Maruno T, et al., Comprehensive Size Distribution and Composition Analysis of Adeno-Associated Virus Vector by Multiwavelength Sedimentation Velocity Analytical Ultracentrifugation. **J. Pharm. Sci.** 110, 3375-3384 (2021).

Biophysical analysis of PlzA-nucleotide interactions with multi-wavelength analytical ultracentrifugation

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Lyme disease spirochetes are pathogens that utilize cyclic-di-GMP (c-di-GMP) to help regulate its enzootic cycle through rapid adaptation. PlzA, a PilZ domain protein, has been previously identified as a c-di-GMP receptor in many Lyme disease isolates. A biophysical analysis of the interaction between PlzA and c-di-GMP was conducted using multi-wavelength analytical ultracentrifugation (MW-AUC). This technique utilizes the Optima AUC's ability to collect experimental data at multiple wavelengths and the UltraScan software to deconvolute signal contributions from different solutes according to their unique spectral properties. The determination of the intrinsic extinction spectra of both PlzA and c-di-GMP was collected to accurately deconvolute the contribution of each component. Combined with AUC's capacity to collect sedimentation and diffusion data, a multi-wavelength approach allowed stoichiometry and binding affinity data to be determined. Our collected sedimentation data suggested two c-di-GMP binding sites on one PlzA protein with different Kd values. The first site showed complete binding in both PlzA:c-di-GMP 1:2 and 2:1 molar ratios, while the second site exhibited a weaker binding affinity with rapid association and dissociation throughout the experiment. Changes in sedimentation patterns between the protein and the complexes also complement previous studies indicating a conformational change of the complex. This study showed the power of MW-AUC to study protein-nucleic acid interactions to reveal stoichiometry, binding affinity information, and global conformational changes.

Biophysical characterization of GFP-nanobody complex formation

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Anti-GFP nanobodies have been engineered for high specificity and affinity towards GFP and its variants, making them an ideal candidate for protein purification. This research compares the affinities between GFP variants and nanobodies by multiple biophysical characterization methods, including Microscale Thermophoresis (MST), multiwavelength analytical ultracentrifugation (MW-AUC), and Isothermal titration calorimetry (ITC). GFP offers a unique fluorophore that can be used by MST and MW-AUC to track the binding of GFP to nanobodies. MST can determine the binding affinity by measuring molecular diffusion in a temperature gradient. In MW-AUC, the excitation wavelength of GFP offers a unique chromophore to follow in this protein-protein interaction. Spectral data enable the identification of both free and complexed GFP and nanobodies in mixtures, offering insights into stoichiometry of association, binding strengths, and rate constants for large molecules or slow interactions. Isothermal titration calorimetry (ITC) is used to determine binding affinity as well as stoichiometry by measuring the heat released or absorbed during molecular interaction. All methods characterize these interactions under solution-phase conditions that mimic physiological environments. The integration of multiwavelength detection in AUC leverages the unique spectral properties of biopolymers. By using all three methods, we are able to conclusively determine a single-digit nanomolar kD and a 1:1 stoichiometry of interaction.

Friday, 26 July | Session 5: Biopharma applications

Chair: Alexander Bepperling | Sandoz, Germany

Analytical ultracentrifugation: A platform technology for Proteolysis Targeting Chimeras (PROTACs)

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PROTACs (Proteolysis Targeting Chimeras) offer a therapeutic approach to degrading a specific target protein. PROTACs are small molecules that specifically bind to and bridge a target protein to an E3 ligase that ubiquitinates the target protein, ultimately leading to degradation via the proteasome. Currently, high-throughput screening of PROTAC-mediated ternary complex formation is often performed using fluorescence-based assays. Once initial PROTAC candidates are observed, surface plasmon resonance (SPR) is often utilized to determine binding constants, kinetics, and to confirm complex formation. Thus far, analytical ultracentrifugation (AUC) has been overlooked as an ideal biophysical technique to characterize multiple attributes of a PROTAC candidate. AUC was evaluated for its ability to act as a "medium-throughput" platform technology to screen PROTACs. Further, advanced fitting approaches allowed for complete characterization of PROTAC systems - including purity, stoichiometry, equilibrium constants, kinetics, and hydrodynamics. Overall, AUC is shown to be an extremely powerful platform technology for characterizing PROTACs.

Ionic strength and pH dependence of antibody reversible self-association probed by SV-AUC

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Reversible self-association of monoclonal antibodies in response to changes in solution ionic strength and pH was characterized using sedimentation velocity AUC, dynamic light scattering and other biophysical techniques. Two antibodies were shown to have opposing responses to solution conditions, indicating different mechanisms of self-association. Under conditions that mimic physiological conditions (i.e. in PBS, pH 7.4) mAb 1 is a soluble, monomeric protein but shows increasing self-association as solution ionic strength and pH are reduced, leading to increased viscosity and eventually gelation at high concentration. mAb 2 shows increased self-association with increasing ionic strength and increasing pH. Under physiological conditions (i.e. in PBS, pH 7.4), increasing protein concentration eventually leads to aggregation and precipitation. Analytical ultracentrifugation, along with other biophysical tools, under a variety of solution conditions were used to study these phenomena in an attempt to characterize the soluble species in each case that lead to aggregation vs. gelation and to try to determine if these soluble species that lead to one phenomenon are also on the pathway that leads to the other. Analytical and computational results were used to propose mechanisms of self-association for each case. Mutational studies were used to validate the proposed mechanisms.

A new twist on drug design: modifying metabolism to inhibit virulence

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Our work is focused on the development of alternative strategies to the use of antibiotics to treat enterohemorrhagic Escherichia coli (EHEC) infections. A family of anti-virulence compounds, the salicylidene acylhydrazides (SA), cause an attenuation of virulence and a reduction in expression of the major system used by EHEC to attach to host cells. One target of these compounds is the bacterial bidirectional enzyme AdhE that catalyses the conversion of acetyl-CoA to ethanol. Deletion of the adhE gene in EHEC generates a phenotype similar to wild type EHEC in the presence of SA compounds. Unusually, AdhE oligomerises *in vivo* and *in vitro* to form filaments heterogenous in length called spirosomes.

Previously we studied the solution properties of unfractionated, and notoriously heterogeneous, AdhE but the resultant data were difficult to fully interpret. Here, using super-resolution single-molecule and FRET microscopy, we show for the first time that unfractionated AdhE spirosomes are dynamic. However, it was possible to partially fractionate AdhE spirosomes using size exclusion chromatography (SEC) and to characterise the spirosome oligomers present in each fraction with biophysical techniques such as sedimentation velocity analytical ultracentrifugation (SV-AUC) and small angle X-ray scattering (SAXS). Enzymatic assays with representative fractions helped us to understand why AdhE spirosomes are formed. The results suggest that AdhE spirosome formation is necessary to balance the two directions of the enzymatic reaction. Then, AdhE was incubated with one of the SA compounds called ME0054, to elucidate its effect on AdhE spirosomes. SV-AUC, in addition to transmission electron microscopy (TEM), showed that ME0054 disrupts the spirosomes, thereby enhancing the conversion of ethanol in acetyl-CoA. Importantly, SV-AUC data show that ME0054 binds to the AdhE filaments. Finally, time-resolved (TR) SAXS allowed us to follow the kinetics of spirosome disruption produced by ME0054, confirming its effectiveness at biologically relevant temperatures and timescales.

Development of molecular standards to validate analytical ultracentrifugation instruments for GMP qualification

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The accurate characterization of pharmaceutical formulations is critical for ensuring quality, efficacy, and patient safety. Analytical ultracentrifugation (AUC) is widely considered as the gold standard for the characterization of such formulations. However, variability in instrument calibration between laboratories can result in statistically significant discrepancies in results. The current lack of validated molecular standards for AUC poses significant challenges as the technique seeks to transition into current Good Manufacturing Practices (cGMP) environments. This project aims to address this need by developing double-stranded DNA-based molecular standards to validate AUC instruments in cGMP environments. The suitability of double-stranded DNA molecules as molecular standards was recently explored by Ranasinghe *et al.* [1]. Official validation of these standards as standard reference materials will be pursued based upon guidelines set forth by the National Institute of Standards and Technology (NIST). The standards, comprising of two different-sized linear DNA molecules will be characterized at varying rotor speeds and temperatures to reflect the operational range of the instrument. Characterization data will be primarily collected at the Canadian Center for Hydrodynamics as part of a broader multi-lab study in collaboration with NIST to investigate the reproducibility of the data. To ensure that results from different instruments are accurate and comparable, a radial calibration profile correction is being developed. This correction will address radial position errors using a radial calibration disk that can be validated orthogonally [2]. The standards will also aid the determination of instrument limits of detection (LoD) and quantification (LoQ). As well as assist in testing and validating data fitting methods in the UltraScan GMP analysis software, as required in the performance qualification (PQ) of cGMP methods.

[1] Ranasinghe M, Fogg J, Catanese D, Zechiedrich L, Demeler B (2023). Suitability of double-stranded DNA as a molecular standard for the validation of analytical ultracentrifugation instruments. European Biophysics Journal. 52. 10.1007/s00249-023-01671-y.

[2] Stoutjesdyk M, Henrickson A, Minors G, Demeler B. A calibration disk for the correction of radial errors from chromatic aberration and rotor stretch in the Optima AUC™ analytical ultracentrifuge. Eur Biophys J. 2020 Dec;49(8):701-709. doi: 10.1007/s00249-020-01434-z. Epub 2020 May 9. PMID: 32388675.

SV-AUC during the development of LNPs and ATMPs

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Analytical ultracentrifugation is an established method during the development of therapeutic proteins and antibodies and contributes supporting information regarding size, oligomerization state and impurity contents of oligomeric forms and aggregates. While precision of orthogonal methods, such as high performance size exclusion chromatography (HP-SEC) may be higher, the ability of sedimentation velocity analytical ultracentrifugation (SV-AUC) to perform the analysis in the absence of any potentially interfering matrices leads to high confidence into the accuracy of the results, as information on the solutes will not be affected by potentially specific matrix interactions. This is particularly important during characterization of lipid nanoparticles (LNPs) as well as viral vectors, where the hydrophobic chemical nature of LNPs and the size of both particulate API-types may lead to solute-specific matrix interactions. We present case studies during the development of LNPs, adeno associated virus (AAV)-based therapeutics and lentiviral vectors, all of which are strongly supporting SV-AUC as a standard method during the development and GMP-compliant batch release of novel vaccines as well as viral vector-based advanced therapy medicinal products (ATMPs).

Friday, 26 July | Session 6: Protein applications – Biological interactions

Chair: Olwyn Byron | University of Glasgow, UK

TRAPped in an elevator: amino-sugar uptake and utilisation by bacteria

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Sialic acids are a group of nine-carbon amino sugars that are widely distributed among mammals. Commensal and pathogenic bacteria that colonise heavily sialylated niches (e.g., the mammalian respiratory tract and gut) 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. Sialic acid utilization is essential in a range of human bacterial pathogens. In this talk I will present our work that defines how bacteria import sialic acids using TRipartite ATP-independent Periplasmic (TRAP) transporters. I will report the cryo-EM structures of several sialic acid TRAP transporters (HiSiaQM) at 3 Å resolution or better, revealing a broad framework to understand how they work. Rather than a monomer, we find that some TRAP transporter are homodimers. We observe lipids at the dimer interface, as well as a lipid trapped within the fusion that links the SiaQ and SiaM subunits. We show that the affinity (KD) for the complex between the soluble HiSiaP protein and HiSiaQM is in the micromolar range and that a related SiaP can bind HiSiaQM, highlighting features of the complex interface. This work provides key data that enhances our understanding of the 'elevator-with-an-operator' mechanism of TRAP transporters.

Davies et al. 2024. TRAPs: the "elevator-with-an-operator" mechanism. Trends Biochem Sci 49(2): 134
Currie et al. 2023. Structural and biophysical analysis of the Haemophilus influenzae tripartite ATP-independent periplasmic (TRAP) transporter. eLife 13:RP92307
Davies et al. 2023. Structure and mechanism of a tripartite ATP-independent periplasmic (TRAP) transporter. Nat Commun 14:1120

The impact of von Willebrand factor on immune complexes in hemophilia A

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Congenital hemophilia A is an X-linked hereditary bleeding disorder characterized by reduced or absent activity of coagulation factor VIII (FVIII). The infusion of therapeutic FVIII protein is used to treat or prevent bleeding, but results in the formation of neutralizing antibodies in up to 40% of patients. In plasma FVIII is bound to von Willebrand factor (VWF) in a tight, non-covalent complex involving mainly FVIII's C1 domain but also the C2 domain and a short acidic peptide (a3) at its light-chain N-terminus. The interaction with VWF is important for FVIII stability, but also determines how it is presented to the immune system.

In this study, we wanted to investigate the effect of VWF on the secondary immune response to FVIII, such as in immune tolerance induction when anti-FVIII antibodies are already abundant and FVIII is administered at high doses. Using different monoclonal antibodies and a mixture of them, we were able to show that VWF prevents the binding of FVIII immune complexes to complement component 1q, immobilized Fc-γ receptors and bone marrow-derived dendritic cells. Interestingly, this was the case whether or not the antibodies targeted a VWF binding site on FVIII. Therefore, we used analytical ultracentrifugation with fluorescence detection to investigate the influence of VWF on FVIII immune complex formation. When using a mixture of antibodies, pre-incubation of FVIII with VWF resulted in both the appearance of free antibodies and the formation of larger complexes. The order in which FVIII, VWF and antibodies were mixed did not play a major role. Testing of individual antibodies revealed that those that targeted one of the VWF binding sites on FVIII were no longer able to bind to FVIII, whereas VWF was incorporated into immune complexes formed with antibodies directed against other epitopes on FVIII. In the latter case, it is reasonable to assume that VWF, a large multimeric glycoprotein, is able to occlude the Fc regions of the antibodies, thereby preventing at least some of their effector functions.

Characterization of Rabies virus phosphoprotein thermoresponsive liquid-liquid phase separation

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Biomolecular liquid-liquid phase separation (LLPS) is the spontaneous condensation of multivalent molecules (proteins and/or nucleic acids) leading to the formation of droplets of a high concentrated “dense” phase into a dilute “light” phase. Recently, LLPS has received increasing attention, as it appeared to be a key mechanism in the formation of normal and pathological membrane-less organelles (MLOs). Thus, the phosphoprotein (P) of rabies virus (RABV) is an essential component of the RNA synthesizing machine and is involved in the formation of MLOs in the cytoplasm of infected cells, where transcription, replication and encapsidation of the viral genome occur. RABV P is an intrinsically disordered protein forming star-shaped dimers with two long, flexible and negatively charged N-terminal arms and two shorter C-terminal arms. Using various biophysical methods, including static light scattering, analytical ultracentrifugation (AUC), small-angle X-ray scattering (SAXS), and capillary flow experiments, we have characterized RABV P thermoresponsive phase separation. We found that phase separation is only possible in a narrow range of NaCl concentration and established a protein concentration versus temperature phase map. Interestingly, we found that this minimal system does not follow a simple binary model (protein/solvent) and we are investigating the mechanisms of assembly of the protein in both one-phase and two-phase regions of the phase map.

Investigation of the complexation between glycinin and block copolymers through analytical ultracentrifugation

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Diblock copolymers of PEG-b-PSS with varied lengths of polystyrene sulfonate (PSS) blocks have been synthesized, and the impact of ionic strength and PSS degree of polymerization on the interaction between PEG-b-PSS and glycinin, as well as the structural change that occur during this interaction, has been explored using analytical ultracentrifugation (AUC). The findings suggest that the PEG block effectively impedes the formation of insoluble complexes, with its efficacy being contingent upon the PSS degree of polymerization. A lower ionic strength can diminish the electrostatic repulsion's screening effect, leading to an enhanced binding affinity between PEG-b-PSS and glycinin and the development and resolution of soluble complexes. At an ionic strength of 0.025 M, PEG-b-PSS can encourage the disintegration of glycinin into subunits and peptides. Diblock copolymers with a higher PSS degree of polymerization facilitate the creation of insoluble 11S complexes via soluble intermediates. This research demonstrates that by manipulating the ionic strength and the PSS degree of polymerization, the binding affinity between glycinin and the polyelectrolyte, as well as the structural change during binding, can be modulated.

Friday, 26 July | Session 7: Nanoparticles and polymers – Part 1

Chair: Jeffrey Fagan | NIST, USA

Leveraging analytical ultracentrifugation for comprehensive characterization and quantification of lipid nanoparticles

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In recent years, lipid nanoparticles (LNPs) have gained significant attention as a promising drug delivery system due to their ability to encapsulate and deliver therapeutic molecules. However, understanding their physical properties and stability is crucial for their successful development and application.

Analytical ultracentrifugation (AUC) offers several advantages for LNP characterization. It provides detailed information about the hydrodynamic radius distribution and polydispersity of LNPs, which plays an important role in assessing their uniformity and stability, directly impacting their drug delivery efficiency. AUC also enables the determination of the formulation's density distribution, allowing for differentiation between formulations with similar size distributions but different density profiles. It can also be used to determine the copy number distributions of the drug loaded in the LNPs. Moreover, AUC can be used to understand the stability and aggregation behavior of LNPs, by monitoring changes in the sedimentation profile over time and under different stressors, which could have implications for their efficacy and safety. This presentation will highlight the pivotal role of AUC in enabling researchers to develop an in-depth understanding of their LNP formulations. By leveraging AUC's capabilities, researchers can gain valuable insights into the size distribution, density, stability, and aggregation behavior of LNPs, facilitating the optimization of these drug delivery systems for enhanced therapeutic outcomes.

Hydrodynamic tools for assessing the molecular integrity of archaeological consolidants. The Saving Oseberg project

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There is an urgent demand for the design of a new generation of polymeric archaeological consolidants that are (i) bioinspired and not petrochemical based (ii) that can be administered as either aqueous or non-aqueous formulations and then subsequently cured inside the object (iii) whose size distribution, conformation and hydrodynamic properties are accurately understood (iv) lead to long lasting stability.

Building on earlier studies we review the hydrodynamic tools available with the analytical ultracentrifuge and its huge dynamic range at the cornerstone (see, e.g., ref [1]) - then describe their current and future application to potential candidate materials for the Saving Oseberg project

[1] Harding SE (2018) The Svedberg Lecture 2017. From nano to micro: the huge dynamic range of the analytical ultracentrifuge for characterising the sizes, shapes and interactions of molecules and assemblies in Biochemistry and Polymer Science. *European Biophysics Journal* 47, 697-707 https://doi.org/10.1007/s00249-018-1321-3

Characterization of gold nanoparticles by cryogenic electron tomography and analytical ultracentrifugation

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We have developed a method to extract a number of thermodynamic quantities from a dispersion of generic nanoscale particles utilizing cryogenic electron tomography (cryoET) of vitrified samples [1,2]. Initially demonstrated for gold nanoparticles, the technique is capable of providing a pair distribution function and the potential of mean force without any assumption. Additionally, the method allows the extraction of Kirkwood–Buff integrals based on spatial fluctuation of the particles in the tomograms. The structure factor and the agglomeration states of the particles are also evaluated directly. To validate our results, we use small angle X-ray scattering and sedimentation velocity analytical ultracentrifugation (AUC-SV). Furthermore, we have used sedimentation equilibrium analytical ultracentrifugation (SE-AUC) to study thermodynamic properties of nanoparticles [3,4], and have designed a capillary cell that is versatile and reusable for a concentrated solution including that of nanoparticles, proteins, nucleic acids, and polymers [4].

[1] Petretto, E.; Ong, Q. K.; Olgiati, F.; Mao, T.; Campomanes, P.; Stellacci, F.; Vanni, S. Monovalent Ion-Mediated Charge–Charge Interactions Drive Aggregation of Surface-Functionalized Gold Nanoparticles. Nanoscale 2022, 14 (40), 15181–15192.
[2] Ong, Q.; Mao, T.; Anaraki, N. I.; Richter, Ł.; Malinverni, C.; Xu, X.; Olgiati, F.; Silva, P. H. J.; Murello, A.; Neels, A. Cryogenic Electron Tomography to Determine Thermodynamic Quantities for Nanoparticle Dispersions. Materials Horizons 2022, 9 (1), 303–311.
[3] Xu, X.; Ong, Q.; Mao, T.; Silva, P. J.; Shimizu, S.; Rebecchi, L.; Kriegel, I.; Stellacci, F. Experimental Method to Distinguish between a Solution and a Suspension. Advanced materials interfaces 2022, 9 (19), 2200600
[4] Ong, Q.; Xufeng, X.; Stellacci, F. Versatile Capillary Cells for Handling Concentrated Samples in Analytical Ultracentrifugation. Analytical Chemistry 2024, 96(6), 2567–2573.

Quantifying the number of total and accessible functional groups on nanomaterials

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Inorganic and organic functional nanomaterials (NM) of different size, shape, chemical composition, and surface chemistry are relevant for many key technologies of the 21st century. Decisive for most applications of NM are their specific surface properties, which are largely controlled by the chemical nature and number of ligands and functional groups (FG) on the NM surface. The surface chemistry can strongly affect the physicochemical properties of NM, their charge, hydrophilicity/hydrophobicity, reactivity, function, stability, and processability and thereby their impact on the environment and biological species as well as their possible risk for human health. Thus, reliable, validated, and eventually standardized analytical methods for the characterization of NM surface chemistry, i.e., the chemical identification, quantification, and accessibility of FG and surface ligands,[1,2] flanked by interlaboratory comparisons, control samples, and reference materials,[2,3] are of considerable importance for process and quality control of NM production and function. This is also important for the safe use of NM, the design of novel NM, and sustainable concepts for NM fabrication.

Here, we provide an overview of analytical methods for FG analysis and quantification and highlight method- and material-related challenges for selected NM. [1,2] Analytical techniques addressed include electrochemical titration methods, optical assays, nuclear magnetic resonance (NMR) and vibrational (IR) spectroscopy, and X-ray based and thermal analysis methods. Criteria for method classification and evaluation include the need for a signal-generating label, provision of either the total or derivatizable number of FG, and suitability for process and production control.

[1] D. Geißler et al., *Microchim. Acta* **2021**, 188, 321-348.
[2] F. Kunc et al., *Anal. Chem.* **2021**, 93, 15271−15278.
[3] J. Labuda et al., *Pure Appl. Chem.* **2023**, 95, 133-163.

Friday, 26 July | Session 8: Protein applications – Protein hydrodynamics

Chair: Christine Ebel | Université Grenoble Alpes, France

The molecular basis for hydrodynamic properties of PEGylated proteins

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Polyethylene glycol conjugation provides a protective modification that enhances the pharmacokinetics and solubility of proteins for therapeutic use. A knowledge of the structural ensemble of these PEGylated proteins is necessary to understand the molecular details that contribute to their hydrodynamic and colligative properties. Because of the large size and dynamic flexibility of pharmaceutically important PEGylated proteins, the determination of structure is challenging. In addition, the hydration of these conjugates that contain large polymers, is difficult to determine with traditional methods that identify only first shell hydration water which does not account for the complete hydrodynamic volume of a macromolecule. Structural ensembles, generated by coarse-grained simulations, can be analyzed with HullRad and used to accurately predict sedimentation coefficients and concentration dependent hydrodynamic and diffusion nonideality coefficients of PEGylated proteins. A knowledge of these concentration dependent properties enhances the ability to design and analyze new modified protein therapeutics. HullRad accomplishes this analysis by effectively accounting for the complete hydration of a macromolecule, including that of flexible polymers and protein-polymer conjugates.

Hydrodynamic and thermodynamic analysis of PEGylated human serum albumin

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Covalent labeling of therapeutic drugs and proteins with polyethylene glycol (PEGylation) is an important modification for improving stability, solubility, and half-life. PEGylation alters protein solution behavior through its impact on thermodynamic nonideality by increasing the excluded volume and hydrodynamic nonideality by increasing the frictional drag. To understand PEGylation's impact, we investigated the thermodynamic and hydrodynamic properties of a model system consisting of PEGylated human serum albumin (PEG-HSA) derivatives using analytical ultracentrifugation (AUC) and dynamic light scattering (DLS). We constructed PEG-HSA derivatives of single, linear 5K, 10K, 20K, 40K PEG chains and a single branched-chain PEG of 40K (2x20K). Sedimentation velocity (SV) experiments were analyzed using SEDANAL direct boundary fitting to extract ideal sedimentation coefficients so, hydrodynamic nonideality k_s, and thermodynamic nonideality BM_1^{SV} terms. These quantities allow the determination of the Stokes radius R_s, the frictional ratio f/f_o, and the swollen or entrained volume V_s/v, which measure size, shape, and solvent interaction. We performed sedimentation equilibrium experiments to obtain independent measurements of thermodynamic nonideality BM_1^{SE}. From DLS measurements we determined the interaction parameter, kD, the concentration dependence of the apparent diffusion coefficient, D, and from extrapolation of D to c = 0 a second estimate of R_S. R_s values derived from SV and DLS measurements and ensemble model calculations (see Fleming et al) are then used to show that $k_s + k_D$ = theoretical $2B_{22}M_1$. In contrast, experimental BM_1 values from SV and SE data collectively allow for similar analysis for protein-PEG conjugates and show that $k_s + k_D = 1.02\text{-}1.07*BM_1$, rather than the widely used $k_s + k_D = 2BM_1$ developed for hard spheres. The random coil behavior of PEG dominates the colloidal properties of PEG-protein conjugates and exceeds the sum of a random coil and hard-sphere volume due to excess entrained water.

Hydrodynamic comparison of the structure and interactions of the last line of defence antibiotics vancomycin and teicoplanin

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We review and compare vancomycin and teicoplanin in physiologically relevant solvents. Vancomycin reversibly dimerizes at higher concentration, although in its biologically active range it is still essentially monomer [1]. By contrast teicoplanin forms 18-19mers [2]. In addition, vancomycin appears to cause its target membrane receptor VanS to undergo a significant conformation change [3]. Both formulations are normally administered intravenously although also orally or topically. Of relevance to non-intravenous formulations, both vancomycin and teicoplanin cause significant complexation of mucins from the oral-gastrointestinal tract [4,5].

[1] Phillips-Jones MK, Lithgo R, Dinu V, Gillis RB, Harding JE, Adams GG, Harding SE. Full hydrodynamic reversibility of the weak dimerization of vancomycin and elucidation of its interaction with VanS monomers at clinical concentration. Sci. Rep. 2017, 7, 12697. https://doi.org/10.1038/s41598-017-12620-z

[2] Chun T, Pattem J, Gillis RB, Dinu VT, Yakubov GE, Corfield AP, Harding SE. Self-association of the glycopeptide antibiotic teicoplanin A2 in aqueous solution studied by molecular hydrodynamics. Sci Rep. 2023, 13, 1969. https://doi.org/10.1038/s41598-023-28740-8

[3] Phillips-Jones MK, Channell G, Kelsall CJ, Hughes CS, Ashcroft AE, Patching SG, Dinu V, Gillis RB, Adams GG, Harding SE. Hydrodynamics of the VanA-type VanS histidine kinase: An extended solution conformation and first evidence for interactions with vancomycin. Sci. Rep. 2017, 7, 46180. https://doi.org/10.1038/srep46180

[4] Dinu V, Lu Y, Weston N, Lithgo R, Coupe H, Channell G, Adams GG, Torcello Gómez A, Sabater C, Mackie A, Parmenter C, Fisk I, Phillips-Jones MK, Harding SE. The antibiotic vancomycin induces complexation and aggregation of gastrointestinal and submaxillary mucins. Sci. Rep. 2020, 10, 960. https://doi.org/10.1038/s41598-020-57776-3

[5] Chun T, Pattem J, Gillis RB, Dinu VT, Yakubov GE, Corfield AP, Harding SE. Comparative hydrodynamic and nanoscale imaging study on the interactions of teicoplanin-A2 and bovine submaxillary mucin as a model ocular mucin. Sci Rep. 2023, 13, 11367. https://doi.org/10.1038/s41598-023-38036-6

Poly acidic amino acids sequence drives variation of protein in hydrodynamic properties

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Here several repeats of Asp/Glu were added to the C-terminal of SnRK2.6(1-332), PDI (1-440) and PYL10, which SnRK2.6 and PDI had a natural acidic amino acids tail on their C-terminal, and PYL10 performed like a globular protein. Recombinant protein were produced by *E.coli*BL21 followed by purification with affinity chromatography, ion-exchange column chromatography and size exclusion chromatography. With analytical ultracentrifugation, static light scattering analysis and size exclusion chromatography, we demonstrated that the addition of acidic amino acids on the C-terminal resulted in early elution on size exclusion chromatography without impacting oligomerization, while hydrodynamic properties such as stokes radius and friction ration increased clearly

Saturday, 27 July | Session 9: Nanoparticles and polymers – Part 2

Chair: Amy Henrickson | Beckman Coulter Life Sciences, Canada

Molecular hydrodynamic characterization of PEG-lipid conjugates

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The molecular understanding of lipids and their polymorphism is key to understanding modern delivery systems. Very recently, relatively small molar mass poly(ethylene glycol)-lipid conjugates (PEG-lipid)s have been used as a component of the lipid nanoparticles (LNPs), utilized in the vaccines against SARS-CoV-2. They were approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) as the first, widely used mRNA vaccines. This is partly due to their stealth behavior toward the immune system and protection of the encapsulated genetic material. Here, we present a quantitative in-depth molecular hydrodynamic analysis of the PEG-Lipids used in the FDA and EMA approved vaccines against SARS-CoV-2. [1] Next to sedimentation velocity (SV) AUC experiments, we also performed viscometric measurements in solvents ethanol and water. The determined partial specific volume of the polymer-lipid system in ethanol and water by molecular densimetry then allowed for sedimentation-diffusion analysis. The interrelation of the macromolecular hydrodynamic characteristics in ethanol indicates random coil polymers with typical values of the hydrodynamic invariant and a molar mass representing unimeric polymeric species of a narrow, unimodal molar mass distribution. At the same time, experiments in water indicate the formation of larger molar mass species. The interrelation of the hydrodynamic characteristics allowed the calculation of the number of individual PEG-lipid polymer chains that must form those species. The calculated hydrodynamic diameter of those highly defined species was in quantitative agreement with direct imaging by cryo-transmission electron microscopy (cryo-TEM) experiments and dynamic light scattering (DLS). Knowledge of the hydrodynamic characteristics of translational and rotational origin allowed the quantitative determination of hydration, i.e., water associated to individual micellar species. Hydration values calculated based on the translational frictional ratio and intrinsic viscosity are in close agreement and amount to as much as 4-5 g water per g of micelle. The interrelation of all characteristics leads to the experimentally derived conclusion that the micelles can be considered solvent-permeable, hydrated spheres.

[1] Anufriev, I.; Hoeppener, S.; Nischang, I. *Anal Chem* **2023**, 95, 10795-10802.

Fractionation of colloidal particles: From analytical ultracentrifugation to preparative ultracentrifugation

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Effective fractionation is not always easy to obtain, as preparative ultracentrifugation (PUC) are mostly conducted in an empirical way. In principle, one can apply the quantitative results from analytical ultracentrifuge (AUC) to establish suitable centrifugal conditions in preparative centrifuge experiments. Following this principle, we have demonstrated that the fractionation of a binary colloidal systems can be tuned to different degree. Among the different degrees of fractionations, a gradient structure could be obtained. However, the difference between the sedimentation process in an AUC cell and a preparative centrifuge tube has not been well characterized, prohibiting high-resolution fractionation. In this contribution, I will mainly talk about how image analysis was utilized to map the particle concentration distribution throughout the centrifuge tube, revealing the evolution of nanoparticle flows in a preparative centrifuge tube.

Using analytical ultracentrifugation to explore macroionic solutions by determining critical parameters

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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 have solution behavior fundamentally different from the above two categories. The best model macroions are structurally well-defined molecular clusters with accurately tunable charges. Such 1-5-nm-size macroions can selectively attract counterions around them, create counterion-mediated attraction and therefore tend to strongly attract the like-charged macroions with each other. This leads to interesting solution behaviors – for both microphase (self-assembly) and macrophase separations. In dilute solutions, they tend to reversibly self-assemble into single-layered 2-D nanosheets, which eventually form stable, hollow, spherical "blackberry" structures with their sizes accurately tunable by solvent content, macroionic charge density or pH. The blackberry structure represents a universal, free energy favored state of soluble macroions with moderate charge, and mimics some biological processes, such as the virus capsid shell formation. The macrophase separations result in hydrogel and coacervate phases, both are common for charged polymers but very unique for inorganic molecules.

The macroions are perfect models to understand some fundamental biological behaviors such as the self-recognition and chiral selection of biological assemblies. Inorganic macroions can achieve the level of self-recognition similar to biomolecules in dilute solution, even among those with identical size, shape and charge, or enantiomers. The metal-organic cage-based macroions containing amino acid components can be used for clarifying the chiral discrimination phenomenon among amino acids, which is connected to the homochirality feature of our lives.

My group has been the leading team for studying macroionic solutions in the past 20 years, by using techniques such as SLS, DLS, ITC, Zeta-potemtial analysis, TEM and SEM. We rely on our newly purchased AUC to determine some critical parameters in such systems, such as intermolecular distance between macroions for their dimers and in blackberry type structures, as well as the reason for the chiral discrimination.

Saturday, 27 July | Session 10: Beadmodeling and hydrodynamic simulations

Chair: Karen Fleming | Johns Hopkins University, USA

Some recent modelling/computation advances in hydrodynamics, analytical ultracentrifugation and scattering techniques

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This talk is intended to communicate works developed or under active development by the author over the past seven years, after the 23rd International AUC Workshop and Symposium (Glasgow, 2017). The widely employed bead modelling methodology, implemented in the HYDRO suite of computer programs, has been revised in aspects regarding further efficiency and simplicity. The suite now includes new programs, intended for either geometrically simple models, like ellipsoids, rods and disks [1], or complex shapes like those of 2D nanoparticles [2].

In the field of analytical ultracentrifugation (AUC), our group originally proposed [3] a procedure for predicting the outcome, and analyzing the results of sedimentation velocity or equilibrium techniques by means of Brownian dynamics simulation. The computer programs in the SimuSed suite -- Predised for simulation and AnaSed for analysis -- have been thoroughly revised in order to provide these program in public domain.

Among the techniques usually associated to AUC are those based on scattering of light, x-rays or neutrons. The possibility of predicting static scattering using bead models, proposed years ago [5] is being implemented in the HYDRO suite. Also, dynamic scattering is (beyond its use for measuring diffusion coefficient and hydrodynamic radius) a powerful technique to study macromolecular shape and flexibility.

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[2] J.G. Hernández-Cifre, R. Rodríguez-Schmidt, C.M. Almagro-Gómez, J. García de la Torre, "Calculation of the friction, diffusion and sedimentation coefficients of nanoplatelets of arbitrary shape", *Polymer*, 262, 125467, 9 pages (2022).

[3] A. Diez, A.Ortega, J. García de la Torre, "Brownian dynamics simulation of analytical ultracentrifugation experiments", *BMC Biophys.*, 4:6, 7 pages (2011).

[4] J. Garcia de la Torre, J.G. Hernández Cifre, A.I. Díez Peña, "Prediction and analysis of analytical ultracentrifugation experiments for heterogeneous macromolecules and nanoparticles based on Brownian dynamics simulation", *Eur. Biophys. J.*, 47, 845-854 (2018).

[5] J. García de la Torre, B. Carrasco, S.E. Harding. "Calculation of NMR relaxation, covolume and scattering-related properties of bead models". *Eur. Biophys. J.* 28, 119-132 (1999).

Atomic level hydration and bead models hydrodynamics: Parallel GRPY for all and an adjusted ZENO method in US-SOMO

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The determination of (bio)-macromolecular structures hydrodynamic properties is a staple of the UltraScan SOlution MOdeler (US-SOMO) suite [1]. Utilizing the Generalized Rotne-Prager-Yamakawa (GRPY) formalism allowing bead models of variable size with overlapping regions [2], representing atomic-level details of proteins, nucleic acids, and carbohydrates is now possible. We address the challenges of accounting for hydration by calculating pH-dependent "bound" water molecules for specific atomic groups such as hydroxyl, amides, and oxygen anions, and adding their volume to the corresponding bead volume. This improved “van der Waals” (vdW) modeling method includes an Accessible Surface Area (ASA) screening to hydrate only exposed atomic groups, and was assessed by calculating the hydrodynamic properties of 26 well-characterized proteins. The translational diffusion coefficient $D_{t(20,w)}$ was primarily used for comparison, along with the intrinsic viscosity [η] and the rotational correlation time $\tau_{h(20,w)}$. A 10 Å2 ASA cut-off effectively included only hydrodynamically relevant waters, constituting ~40% of those theoretically available. For $D_{t(20,w)}$, excluding clear outliers, an average deviation of 0.04 ± 1.76% (range -3.14 - +3.10%) was observed across 21 proteins (MW 12.4 - 157.2 kDa). An outward translation of hydrated beads by roughly half of a water radius produced a match within 10% of available experimental [η] values for about half of those structures, and of $\tau_{h(20,w)}$ values measured by NMR on another set of 10 proteins. This outward translation still produced excellent matches for $D_{t(20,w)}$ values. The GRPY method is now implemented in all US-SOMO releases in parallel mode, accommodating routine computations for structures up to ~100 kD on standard multi-core PCs. For larger structures and intensive jobs, we revisited the ZENO [3] method, which showed structure-dependent minor deviations from GRPY results. Adjusting the "skin" parameter in ZENO based on a linear correlation with the gyration radius/surface fractal dimension ratio aligned the results. This comprehensive analysis not only advances our understanding of biomolecular hydrodynamics but also refines computational techniques to better reflect experimental observations.

[1] Brookes et al., *Eur. Biophys.* J. 47, 855-864, **2018**.
[2] Zuk et al., *Biophys.* J. 115, 782–800, **2018**.
[3] Juba et al. *J. Res. Natl. Inst. Stand. Technol*, 122(1), **2017**.

Saturday, 27 July | Session 11: Cross-disciplinary research

Chair: Klaus Richter | Coriolis Pharma, Germany

Advances in the US-SOMO Small-Angle Scattering module: UV-Vis spectral data processing, Multi-Angle Light Scattering (MALS) coupled to SAXS data analysis, and improved SEC Gaussian decomposition of not resolved species

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UltraScan SOlution MOdeler (US-SOMO) is a suite of software modules for the analysis and simulation of hydrodynamic and solution scattering data [1]. We present recent important advances in the Small-Angle Scattering (SAS) module, now including UV-Visible spectral analysis from Diode Array Detector (DAD) or Charged-Coupled Device (CCD) data, and Multi-Angle Light Scattering (MALS) data processing and analysis. The UV-Vis module can process absorbance data in the time and wavelength domains, allowing a better determination of the concentration of solutes either eluting or in batch mode. MALS data can be analyzed independently or joined with SAXS data concurrently collected using in-line size-exclusion chromatography (SEC) or in batch/kinetic mode. MALS and SAXS intensity data are first put on an absolute scale [g/mol] using known and calculated physico-chemical parameters such as the dn/dc, the partial specific volume, and the extinction coefficient of the (bio)-macromolecules under study. They are subsequently joined in the intensity vs. scattering vector q domain at common or interpolated acquisition times, using a global scaling fitting procedure to improve their alignment. In the SEC mode, SAXS data Gaussian decomposition for not-baseline resolved peaks has been significantly improved, and is now provided also for MALS data. Under aqueous solvent conditions, the integrated MALS+SAXS data analysis over an extended q range of almost three decades (~6×10 4 to ~0.3 Å 1) allows for the recovering of time-resolved important parameters such as the molecular weight and overall/cross-sectional radii of gyration. Examples with SEC-MALS-SAXS data of fibrinogen and stopped-flow-MALS-SAXS kinetic data of fibrin monomer re-polymerization experiments performed at the SWING beamline of the synchrotron SOLEIL (Gif-sur-Yvette, FR) are presented. These new US-SOMO capabilities represent a significant step forward in the analysis of solution scattering data complementing higher resolution information, such as the improved refinement of AI-derived macromolecular models [2] or the modeling of the formation of supramolecular structures [3].

[1] Brookes et al., *Eur. Biophys. J.* 47, 855-864, **2018**. https://doi.org/10.1007/s00249-018-1296-0
[2] Brookes et al., *J. Appl. Cryst.* 56, 910-926, **2023**. https://doi.org/10.1107/S1600576723005344
[3] Rocco et al., *J. Am. Chem. Soc.* 136, 5376–5384, **2014**. https://doi.org/10.1021/ja5002955

Molecular hydrodynamic characterization of synthetic polymers for life science and energy applications

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The molecular understanding of synthetic polymers and macromolecular systems is key to their technological advancement in a variety of areas of modern science. Those contemporary areas are the field of energy and life science. The origin of modern polymer science and the beginning of the "polymer age" is commonly associated with Hermann Staudinger and his seminal article "Über Polymerisation" from 1920. Based on the discovery of polymers and macromolecular systems, commonly understood as disperse systems, an experimental framework for their understanding has grown and matured. The framework includes key hydrodynamic properties, which are complemented with data by modern light scattering approaches.

Here, we present a compact framework of such hydrodynamic characteristics based on the historical evolution of this research field. Modern examples, of how the synthetic polymer properties determine physical estimates, are highlighted. Those examples concern life science polymers, also known as stealth polymers, such as linear poly(ethylene glycol)s, linear poly(2-alkyl-2-oxazolines) as well as linear and hyperbranched poly(glycerol)s. In contrast to those hydrophilic, non-charged polymers, polyelectrolytes, utilized for reference gene transfer applications such as the linear poly(ethylene imine), represent particular challenges. The latter is due to the polyelectrolyte effects and, in addition, their very high dispersity originating from the preparation pathways.

Besides life science applications, synthetically tailored polymers play an increasing role in the modern field of energy research. A class of polymers gaining renewed interest are the so called redox-active polymers. Those polymers can accept, store, and release electrons. In case of, e.g., polymer redox flow batteries, they are at the core of overall battery performance. Their molecular structure determines the global polymer electrolyte properties. Initial hydrodynamic studies of redoxactive polymers, that are not well-defined, impose challenges in the framework of hydrodynamics and light scattering. Strategies to assess the quantitative structure-property relationships and optimize polymer electrolyte properties are provided.

Insights into the stability and agglomeration of metal oxide nanoparticles by analytical (ultra)centrifugation

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In our contribution, we will show by AUC studies how the degree of functionalization with a single type of surface-active ligand, namely cetyltrimethylammonium bromide (CTAB), influences the colloidal stability of supraparamagnetic iron oxide nanoparticles (SPIONs). [1]

Furthermore, we will demonstrate how the addition of CaCl2 salt within a mixture consisting of SPIONs and silica nanoparticles (NPs), induces agglomeration and precipitation of each NP species at different salt concentrations and how this effect can be utilized for the targeted formation of different morphologies of supraparticles produced by spray drying. [2]

Concerning the SPIONs functionalized initially with citric acid, we show by AUC how the amount of additionally added CTAB influences the colloidal stability. The colloidal stability is related to the formation of a second and third ligand shell of CTAB on top of the first shell. This leads to a changing polarity of the ligand-solvent interface depending on the added amount of CTAB, which allows to control either agglomeration or deagglomeration of the NPs.

In a NP mixture composed of hydroxylated silica NPs and citric acid stabilized SPIONs, different agglomeration states can be matched to different critical coagulation concentrations (CCCs) related to the addition of $CaCl_2$. Due to their different surface functionalities, SPIONs absorb Ca^{2+} ions more selectively than silica and agglomerate at lower $CaCl_2$ concentrations compared to the silica NPs. Subsequently, by spray drying of the dispersions, the different agglomeration states are the decisive criterion for the formed morphology of the supraparticles. It has been demonstrated that smaller NPs move to the outer region of the supraparticles while bigger ones or agglomerates migrate to the core region, which can be explained by the thermophoretic effect. [3]

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[2] H. Zhou, R. Pujales-Paradela, P. Groppe, S. Wintzheimer and K. Mandel, *Part. Part. Syst. Charact.* **2022**, 39, 2200127.
[3] K. Jabłczyńska, J. M. Gac and T. R. Sosnowski, *Adv. Powder Technol.* **2018**, 29, 3542-3551.

The tail of DNA: Polymers in a density gradient in the AUC still tell new genome stories

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Key properties of genomes were deduced by density gradient AUC already decades before the first genomes were sequenced. The more we learn about genomes, the more additional properties we find that were already visible and quantifiable in the earliest CsCl AUC absorbance profiles at sedimentation equilibrium obtained beginning in 1957, and could have been properly recognized and interpreted if we had known how. Inverse problems (AUC profile → genome sequence) were being extensively used already well before whole genome sequencing began. They are tractable because of a remarkably linear relationship, still not fully understood, between the GC (percentage of G-C base pairs) of a genomic fragment and its buoyant density (i.e., position) in a CsCl density gradient. The relation between position and GC appears to be mirrored in the nucleus of eukaryotic cells, where chromosome territory positioning correlates with GC of the chromosomal region; the existence of a maintained concentration gradient in the nucleus might be posited as a cause, but is not yet clear. This presentation will review inverse problems that have met with success in advancing our understanding of eukaryotic genomes in the last 60 years, involving deductions such as (a) total genomes' GC distributions, (b) how gene density varies along chromosomes, (c) gene and protein prediction, (d) chromosome territory mapping, (e) characterizing long-range autocorrelations, (f) quality checks of short-read NGS genome assemblies, and (g) separating out the gene space in genomes with large gene deserts. At the end, we return to the fundamental linear relation GC/100 =·(rho - 1.660 g cm^{-3})/0.098, and consider how we could make progress in quantatively explaining it.

Abstracts for Poster Presentations

A new design of capillary cells for studying concentrated samples by analytical ultracentrifugation

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We present a novel versatile capillary-cell design for analytical ultracentrifugation-sedimentation equilibrium (AUC-SE) for studying samples at high concentrations [1]. The design is easy to use, robust, and reusable for samples in both aqueous and organic solvents. We use the new design to study the equation of state and second virial coefficients, the gelation phase transition, and liquid–liquid phase separation.

[1] Ong, Q.; Xufeng, X.; Stellacci, F. Versatile Capillary Cells for Handling Concentrated Samples in Analytical Ultracentrifugation. *Analytical Chemistry* **2024**, *96(6)*, 2567–2573.

New Functions in UltraScan for the processing of Multi-wavelength AUC SV and ABDE experiments

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Multi-wavelength Analytical Ultracentrifugation (MW-AUC) has recently gained significant importance for the analysis of a wide range of applications, from nanoparticle characterization to biomolecular complexes. Adeno-Associated Virus (AAV), a promising gene therapy vector, is an important research field studied extensively by AUC. MW-AUC is a critical new method for the characterization of empty, partially loaded, and filled AAV capsids. The Analytical Buoyant Density Equilibrium (ABDE) method, performed in MW-AUC mode, has dramatically reduced sample requirements, improved quantification accuracy, and significantly enhanced throughput. In this talk, I will present new methods implemented in the UltraScan software that aid the analysis of data from MW-AUC experiments. MW-AUC experiments, whether they are performed in sedimentation velocity (SV) or ABDE mode, require a spectral decomposition step. One of the new tools implemented in UltraScan monitors the quality of the decomposition with a 3D graphical monitor, and assesses randomness of residuals to assure appropriate spectral decomposition. Examining the errors associated with the non-negative least squares decomposition reveals information about deviations caused by hypo/hyperchromic spectral shifts induced by hetero-interaction, as well as the linear decomposition's reliability. A second tool allows time-invariant noise subtraction from equilibrium data, which, by definition, are always time invariant themselves. Conversion of intensity data from equilibrium experiments is performed with the second tool to produce noise-corrected pseudo-absorbance data from ABDE experiments. The main challenge in this approach is to identify and eliminate the time-invariant noise associated with the experimental data, contributed by the time invariant noise profile from the optical system, which differs for each wavelength of observation, and also includes refractive contributions from the buffer. Recent developments in the UltraScan software provide a variety of methods and a robust toolbox for data modeling and interpretation. UltraScan allows for accurate modeling of sedimentation velocity and pseudo-absorbance calculations, as well as a variety of utilities for analyzing and determining sedimentation coefficients, molecular weights, and hydrodynamic properties of biomolecules. This talk will go over the programs and utilities required to use the techniques mentioned above.

Reliable particle sizing in vaccine formulations using advanced dynamic light scattering

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Understanding the impact of lipid nanoparticle (LNP) size on immunogenicity represents an important step for enabling the rapid development of novel vaccines against known or emergent diseases. Dynamic light scattering (DLS) is the required analytical method for quality control (QC) to determine the correct particle sizes of such medication.

Multiple scattering effects, caused by large particle sizes and high concentrations, prevents measurements of unaltered formulations and requires dilution, otherwise erroneous sizes are obtained. This forces QC laboratories to increase efforts due to additional dilution steps and to modify the original formulation which can potentially complicate the delivery of correct quality statements.

In our contribution, we show how advanced DLS technology overcomes the multiple scattering issue and allows to measure unmodified original LNP formulations, e.g. modern vaccines. For this, we compare the results from advanced DLS (modulated 3D cross-correlation) and industry standard DLS. Polystyrene beads serve as model compounds and Addavax™ as typical vaccine adjuvant [1].

We complement the results with a systematic study on polystyrene beads of particle sizes between 40 nm and 400 nm. The chosen range illustrates the onset of multiple scattering as a function of particle diameter and concentration. It provides a measure for QC laboratories to decide in which size regime extra caution is required if advanced DLS technology is not available (e.g. due to GMP requirements that new technologies do not always comply with). The offset of multiple scattering, and thus falsification of results, falls in a size range that is typical for modern lipid nanoparticle and vaccine formulations and can not be predicted *a priori*. Therefore, we hope that the results are well suited for an analytical audience as found on the AUC2024.

Density gradients revisited: A systematic study of excipient gradients formed in SV-AUC experiments

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Biopharmaceutical products such as peptides, proteins and viral vectors are nowadays used to treat cancer and other diseases. To be approved by the authorities, critical quality attributes (CQA) must be defined and tested. One crucial CQA for proteins is the aggregate content that we can now measure with the required quality standards for market release (good manufacturing practice, GMP) using SV-AUC.

However, there is one limitation that hampers the quantification of aggregates in formulations with large concentrations of excipients as used in the pharmaceutical industry. The co-sedimentation of excipients can cause density and viscosity gradients that depend on the concentration and type of applied excipients. These gradients are usually not accounted for in the models used to extract the aggregate characteristics from the SV-AUC raw data, leading to errors in the aggregate quantification [1]. Although, more complex analytical models can be used, industry and quality control require easy identification of gradients and simple mitigation actions. It is yet still unclear what type and concentrations are critical. We herein attempt to add to the limited collection by systematically investigating excipient concentration gradients in SV-AUC experiments which is a major source of error and data misinterpretation in characterization of biopharmaceuticals.

The experimental factors required to predict whether excipients have a negative impact on the correctness of the SV-AUC analysis, are sample class, excipient type and excipient concentration. To cover the entire mass/size range of biopharmaceuticals, we complemented the experimental results of NIST mAb with in silico simulations of representative examples of the sample classes AAV (adeno-associated virus) and peptides.

Besides our interest in presenting these results, we would like to participate in the conference for an exchange with our AUC-peers, in particular for discussing the impact of Mass Photometry on the usage of AUC under GMP for quality control in the pharmaceutical industry in the future.

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Development of Molecular Standards to validate Analytical Ultracentrifugation Instruments for GMP qualification

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The accurate characterization of pharmaceutical formulations is critical for ensuring quality, efficacy, and patient safety. Analytical ultracentrifugation (AUC) is widely considered as the gold standard for the characterization of such formulations. However, variability in instrument calibration between laboratories can result in statistically significant discrepancies in results. The current lack of validated molecular standards for AUC poses significant challenges as the technique seeks to transition into current Good Manufacturing Practices (cGMP) environments. This project aims to address this need by developing double-stranded DNA-based molecular standards to validate AUC instruments in cGMP environments. The suitability of double-stranded DNA molecules as molecular standards was recently explored by Ranasinghe *et al.* [1]. Official validation of these standards as standard reference materials will be pursued based upon guidelines set forth by the National Institute of Standards and Technology (NIST). The standards, comprising of two different-sized linear DNA molecules will be characterized at varying rotor speeds and temperatures to reflect the operational range of the instrument. Characterization data will be primarily collected at the Canadian Center for Hydrodynamics as part of a broader multi-lab study in collaboration with NIST to investigate the reproducibility of the data. To ensure that results from different instruments are accurate and comparable, a radial calibration profile correction is being developed. This correction will address radial position errors using a radial calibration disk that can be validated orthogonally [2]. The standards will also aid the determination of instrument limits of detection (LoD) and quantification (LoQ). As well as assist in testing and validating data fitting methods in the UltraScan GMP analysis software, as required in the performance qualification (PQ) of cGMP methods.

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What do unfolded Outer Membrane Protein ensembles look like?

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Outer membrane protein (OMP) biogenesis is biologically conserved to aid in virulence, nutrient and protein transport, and cellular communication. In Gram-negative bacteria, these hydrophobic proteins are synthesized in the cytosol, translocate across the inner membrane, traverse the aqueous periplasm, where they are notably unfolded, and are inserted, and subsequently folded, into the outer membrane. OMPs consist of hydrophobic stretches that form transmembrane β-sheets. Unlike soluble proteins, these hydrophobic stretches in an aqueous environment render them prone to aggregation and misfolding. In order to ensure efficient trafficking to the outer membrane, periplasmic chaperones dynamically bind OMPs, in various conformations, to prevent misfolding and aggregation. We aim to understand whether uOMPs collapse or expand in an aqueous environment, contributing to development of chaperone-uOMP models and a deeper understanding of their interactions. Analytical Ultracentrifugation (AUC) is used to measure the sedimentation coefficient as a function of urea concentration, where the overall shapes and sizes of the uOMPs can be described in the absence of denaturant. Furthermore, Small Angle X-ray Scattering (SAXS) is intended to directly measures the radius of gyration (R_g) and the maximum determination of protein radius (D_{max}), which inform on the conformational properties of the uOMPs prior to binding chaperones. These data are then used to parameterize a coarse-grained simulation and model unfolded conformational ensembles. Building denatured state ensembles enhances current understanding of OMP biogenesis and further contributes to the development of chaperone-uOMP structural models.

Structural insights into interaction of potato Kunitz inhibitors with serine proteas

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Plant protease inhibitors of the Kunitz family (I3 in MEROPS) are 20-25 kDa proteins widely distributed in the plant kingdom. They share a conserved β-trefoil fold in which variable loops form reactive centers for protease interaction. Kunitz inhibitors target serine proteases using the canonical (Laskowski) mechanism based on a single binding loop with a conserved structure.

Here we present three different Kunitz inhibitors from potato (*Solanum tuberosum*) targeting various serine proteases with trypsin and chymotrypsin specificities. The structures of the inhibitor-protease complexes were determined using X-ray crystallography, revealing a novel non-canonical two-loop mechanism of serine protease inhibition. This mechanism was investigated by site-directed mutagenesis. In addition, the stoichiometry of the inhibitor-protease complexes was investigated by analytical ultracentrifugation. Our data suggest that canonical and non-canonical binding loops have different inhibitory specificities towards serine proteases.

Provisional SRM 5003: An Artifact for Validating Radial Calibration

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A first-principles based method, analytical ultracentrifugation (AUC) relies on calibration of time, temperature and radial position to achieve accurate quantitative values. Previous multilaboratory studies have found that significant interlab variation in sedimentation coefficient values of a common analyte is traceable to undetected or uncalibrated variation in these parameters. Extending on efforts published in LeBrun *et al. PLoS ONE* **2018**, 13(7), e0201529, a provisional standard (certified) reference material (SRM) of a lithographically patterned window is now in production for use in absolute calibration of the radial magnification in AUC instruments. Similar to prior results, this artifact is anticipated to identify radial calibration and functional performance of all optical detection systems, reducing uncertainty and interlab variation and aiding precise and accurate conversion of hydrodynamic and particle size measurands from AUC data. Information and examples of utilization of protype artifacts will be presented.

Evaluating Depletion-Based Length Separation of Nanotubes by Analytical Ultracentrifugation

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Particle size is a dominant, material property affecting, characteristic of nanoparticles. For nanotube particles, such as single-wall carbon nanotubes (SWCNTs), transport properties, material properties, application effectiveness, and biological activity are all strongly affected by the absolute length of the nanotube structure. While fractionation methods exist for separating different lengths of nanotubes, development of such techniques is limited by the characterization difficulty and poor capability of many common instruments to accurate measure the length distributions of the separated populations.

Analytical ultracentrifugation (AUC), despite some technical challenges, provides a relatively rapid, powerful and broadly applicable measurement of nanotube length distributions compared to other methods. In this effort, we utilize AUC in conjunction with atomic force microscopy (AFM) to measure the length distributions of several distinct SWCNT populations as fractionated by a polymer depletion-based separation method. The results and complications of length measurement using AUC for dispersed SWCNT populations of various diameter ranges will be presented.

Characterisation of AAV-based Gene Therapy Product-related Impurities Using SV-AUC and Mass Photometry

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Pharmaron Biologics (UK) is a commercial cell and gene therapy development and manufacturing organisation (CDMO) which, using state-of-the-art cGMP biomanufacturing facilities, provide a range of innovative end-to-end laboratory services for the development, production and testing of recombinant adeno-associated virus (rAAV) vectors. Since the first approved AAV product, Glybera in 2012, interest in AAV products has rapidly increased. One challenge for AAV has been product quality, as presence of product-related impurities, such as empty, partially filled, and overfilled vector capsids can impact safety and efficacy of AAV-based therapeutics. Characterisation of AAVs includes reliable quantification of these impurities and plays a crucial role in the AAV manufacturing process, enabling product quality monitoring. Sedimentation Velocity Analytical Ultracentrifugation (SV-AUC) is a gold standard method for characterisation of AAV species with different vector payloads due to its high resolution, robustness, accuracy, and precision. Its utilisation for the accurate identification of multiple species may require optimisation, typically due to differences in product impurity profiles. Sedfit and UltraScan software packages are used to support testing across process development and product characterisation, with UltraScan software, and a custom GMP workflow, used to support good manufacturing practices (GMP release testing) at Pharmaron. Challenges inherent to AAV material production may lead to limited material availability for analytical testing, which combined with the short turnaround time requirements for results, raise needs for an alternative approach for product-related impurity characterisation. During in-process product testing, the use of Mass Photometry (MP) has shown to be an advantageous orthogonal technique to SV-AUC. By utilising MP, sample requirements can be reduced significantly with sufficient resolution achieved between empty and full capsid species, to offer an alternative approach to SV-AUC when sample volume is limited. Nevertheless, due to its superior resolution, SV-AUC continues to be a preferable approach for product characterisation. Here, Pharmaron present a comparison of orthogonal approaches taken to determine product-related impurities using Pharmaron's wealth of experience in MP and SV-AUC showing different features from Sedfit and UltraScan. Both software packages are currently deployed by Pharmaron during process development, characterisation, and GMP product release testing within the AAV product development lifecycle.

Direct measurement of pair interaction potential of proteins

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This work proposes a cryogenic electron tomography (cryo-ET) approach for directly measuring protein pair interaction potential. In contrast to small-angle X-ray scattering (SAXS), in our method, there is no need to make any assumptions on the shape of interaction potential or perform any fitting procedure. The interaction potentials were measured for proteins of different structures and molecular weights: ovalbumin, hemoglobin, bovine, and human serum albumins. The results obtained were successfully cross-validated using the SAXS technique and analytical ultracentrifugation.

Everything falls apart: Analytical Ultracentrifugation contributes to the assessment of lifecycle degradation of nanocomposites and microplastics.

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We recently reviewed how solid materials, especially plastics, coatings, and cements, degrade and release embedded nanomaterials, composite fragments, or micro- and nanoplastics [1]. The methodologies applied were indeed similar for these different target analytes, because reliable release assessment requires the same three steps: 1. Aging by a combination of stresses that is representative of the scenario to be assessed, using standards of durability testing; 2. Sampling of releases, and separation of released entities from the remaining solids; and 3. Analysis and characterization of particulate fragments, including nanomaterials, transformed composite fragments, micro- and nanoplastics, and other degradation products such as soluble organics. The differentiation of these different releases still needs to be improved, e.g. by analytical tools that assess multidimensional distributions of size, chemical composition, molar mass, and further properties. Analytical Ultracentrifugation with interference optics contributes size distributions in mass metrics in the size range of few nm to few µm. By spiking controls, the limit of detection was determined on the order of 10 ppm, and the limit of quantification on the order of 50 ppm. Specifically to microplastics, the developed protocols are suitable for various types of polymers [2], and the measured rates can serve to parameterize mechanistic fragmentation models [3]. We also found that primary microplastics matched the same ranking of weathering stability as their corresponding macroplastics and that dissolved organics constitute a major rate of microplastic mass loss. The results imply that previously formed micro- and nanoplastic fragments can further degrade into water-soluble organics with measurable rates that enable modeling approaches for all environmental compartments accessible to UV light.

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Insights into the Stability and Agglomeration of NPs by Analytical (Ultra)Centrifugation

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In our contribution, we will show by analytical ultracentrifugation (AUC) studies how the degree of functionalization with a single type of surface-active ligand, namely cetyltrimethylammonium bromide (CTAB), influences the colloidal stability of supraparamagnetic iron oxide nanoparticles (SPIONs). [1]

We will also demonstrate how the addition of $CaCl_2$ salt within a mixture consisting of SPIONs and silica nanoparticles (NPs), induces agglomeration and precipitation of each NP species at different salt concentrations, depending on their critical coagulation concentration (CCC). [2]

Furthermore, we will give an overview about the *Shell-by-Shell* (*SbS*) functionalization method based on TiO_2 NPs, phosphonic acids of different chain lengths and SDBS. [3] In particular, we would like to address the dependence of the phosphonic acid chain length on the agglomeration state.

Lastly, we would like to show which methods work best to individualize Carbon Nano Onion (CNO) agglomerates which were formed directly during their synthesis. [4]

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Analytical ultracentrifugation to investigate COSAN-protein interactions

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COSAN anion ($[Co(C_2B_9H_{11})_2]^-$) is composed of two bulky dicarbollide semicages, each of them bearing two negative charges that "sandwich" a metal cation Co^{3+}. COSAN derivatives are considered for applications in various fields, for example, in the medicine field, as potent inhibitors toward HIV protease, or as a source of boron for boron neutron capture therapy treatment of cancer tumors. COSAN derivatives spontaneously cross biological membranes to accumulate in living cells, halting their proliferation and growth.

COSAN is a "nanoion", with one charge delocalized over the nanometric hydrophobic structure (11 Å long and 6 Å large). The low charge density (≈2.5 charge /nm) 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 di-H bonds (B-H…H-C), and electrostatic interactions, provide multiple ways for intermolecular interactions.

COSAN have most surfactant properties in water: it is surface active, forms vesicles and micellar-like aggregates at moderate concentration. COSAN specifically binds to the hydrophobic cavity of BSA at low stoichiometry, and binds non-specifically with BSA surface at high stoichiometry. COSAN interacts with Octyl Glucoside (OG) monomer. Above OG micellar concentration, COSAN at low concentration adsorbs on the micelle surface; at higher concentration COSAN disrupts OG micelles. COSAN is able to solubilize membrane protein from E. Coli cells (unpublished).

We aim to describe the interactions of COSAN with proteins. We will present our first Analytical Ultracentrifugation results obtained in complementarity to SAXS and DLS and biochemical analysis; to characterize the interactions with COSAN and the soluble Myoglobin, and the detergent-solubilized membrane protein FhuA.

Hydrodynamic characterization of a vesicular stomatitis virus-based oncolytic virus using analytical ultracentrifugation

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Determination of the size, density, and mass of viral particles can provide valuable information to support process and formulation studies in clinical development. Analytical ultracentrifugation (AUC), as a first principal method, has been shown to be a beneficial tool for the characterization of the non-enveloped adeno associated virus (AAV). We demonstrate the suitability of AUC for the challenging characterization of a representative for enveloped viruses, which usually are expected to exhibit higher dispersity than non-enveloped viruses. Specifically, the vesicular stomatitis virus (VSV)-based oncolytic virus VSV-GP was used to evaluate potential occurrence of non-ideal sedimentation by testing different rotor speeds and loading concentrations. The partial specific volume was determined via density gradients and density contrast experiments. Additionally, analytical band centrifugation was compared to standard sedimentation velocity AUC. Overall, this study demonstrates the applicability of AUC for the characterization of size, density, and molar mass of an enveloped virus, namely VSV-GP.

Characterization of polydopamine shells on polystyrene particles by analytical buoyant density equilibrium experiments

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Polystyrene-polydopamine core-shell particles are used as novel building blocks for colloidal photonic crystals [1]. To advance the hierarchical complexity of this system, understanding the particles' properties in solution, especially their surface morphology and composition, is crucial. In our contribution, we implement analytical buoyant density equilibrium (ABDE) experiments within an analytical ultracentrifuge (AUC), complemented by H_2O/D_2O-AUC methods, to investigate the porosity and hydration of the polydopamine shell. ABDE-AUC experiments involve the in-situ formation of a density gradient within an AUC cell, achieved by introducing a gradient forming material into the dispersed system [2]. In the gradient, particles migrate to their isopycnic position, i.e., a position where the particle's density matches that of the surrounding medium. Moreover, H_2O/D_2O-AUC methods enable determining the anhydrous density of particles by transferring them into various H_2O/D_2O composition media. In our methodology, we first identify suitable gradient media and simulate the resulting gradients with the Hermans-Ende model [3] and Ultrascan III ABDE module [4]. Subsequently, we determine the anhydrous and buoyant density of bare polystyrene particles, validating our findings through a comprehensive approach that includes scanning electron microscopy for particle size analysis and sedimentation velocity measurements. Building on these foundations, we move to polystyrene-polydopamine particles, synthesized under varying reaction parameters, namely the concentrations of core particles and of the dopamine monomer. Following a similar approach, we retrieve buoyant densities and validate our results with additional techniques. Next, we developed an algorithm to extract the optical spectra of the particles in the formed gradient, which permits optical differentiation of the evaluated species. With the latter implementation, the application of ABDE-AUC, beyond simply determining buoyant density, allows for the assessment of critical quality attributes of the functional particles. For instance, the shell porosity, cross-linking density and water adsorption of the dopamine shell as well as effects on the optical properties of the particles can be retrieved.

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Luminescence Efficiency of Molecular and Nanoscale Luminophores and Luminescent Particles in the UV/vis/NIR/SWIR in Dispersion and in the Solid State – How to Get It Right

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The rational design of the molecular and nanoscale reporters and luminescent particles for applications in the life and material sciences, the comparison of different emitter classes, and photophysical and mechanistic studies require quantitative photoluminescence measurements and the reliable determination of their luminescence efficiency and brightness. A key performance parameter presents the photoluminescence quantum yield (*QY*), i.e., the number of emitted per absorbed photons.

To improve the reliability and comparability of photoluminescence and *QY* measurements across laboratories, pitfalls, achievable uncertainties, and material-specific effects related to certain emitter classes must be explored. Also, suitable protocols and reference materials are needed which have been validated in interlaboratory comparisons for different wavelength regions and transparent and scattering luminophores. [1]

Based on absolute and relative photoluminescence measurements of functional dyes and nanomaterials like semiconductor quantum dots and rods, spectrally shifting lanthanide upconversion nanocrystals, perovskites, and YAG:Cer converter materials, reliable methods for determining *QY* of transparent and scattering luminophores, nonlinear emitters, and solid luminescent nanomaterials have been developed. [2,3] Thereby, material- and method-related uncertainties of relative and absolute *QY* measurements and achievable uncertainties could be quantified for linear and nonlinear UV/vis/NIR/SWIR emitters and lately for solid phoshors. [4] Also, a first set of UV/vis/NIR quantum yield standards has been certified with complete uncertainty budgets to provide the basis for comparable relative QY measurements. [4]

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Biophysical analysis of PlzA-nucleotide interactions with Multi-wavelength AUC

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Lyme disease spirochetes are pathogens that utilize cyclic-di-GMP (c-di-GMP) to help regulate its enzootic cycle through rapid adaptation. PlzA, a PilZ domain protein, has been previously identified as a c-di-GMP receptor in many Lyme disease isolates. A preliminary biophysical analysis of the interaction between PlzA and c-di-GMP was conducted using multi-wavelength analytical ultracentrifugation (MW-AUC). This technique utilizes the Optima AUC's ability to collect experimental data at multiple wavelengths and UltraScan's software to deconvolute signal contributions from different solutes according to their unique spectral properties. The determination of the intrinsic extinction spectrums of both PlzA and c-di-GMP was collected to accurately deconvolute the contribution of each component. Combined with AUC's capacity to collect sedimentation and diffusion data, a multi-wavelength approach allowed stoichiometry and binding affinity data to be determined. Our collected sedimentation data suggested two c-di-GMP binding sites on one PlzA protein with different Kd values. The first site showed complete binding in both PlzA-c-di-GMP 1:2 and 2:1 molar ratios, while the second site exhibited a weaker binding affinity with rapid association and dissociation throughout the experiment. Changes in sedimentation patterns between the protein and the complexes also complement previous studies indicating a conformational change of the complex. This study showed the power of MW-AUC to study protein-DNA interactions to reveal stoichiometry, binding affinity information, and global conformational changes.

A Biophysical Characterization of Poly(2-ethyl-2-oxazoline) with Density-Matching

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Samples of poly(2-ethyl-2oxazoline) synthetic polymers, synthesized through cationic ring-opening polymerization at the University of Wuppertal, were characterized by analytical ultracentrifugation (AUC) at the University of Lethbridge. The aim of this project was to determine the molar mass and sedimentation coefficient distributions, as well as mass-action-dependent self-association properties. Density-matching experiments using multiple concentrations of heavy water were employed to determine the partial specific volumes of samples. Using the partial specific volume, a more accurate molar mass can be calculated using the Svedberg equation, facilitating the identification of later syntheses and blends. AUC measurements were used as an orthogonal approach to gel permeation chromatography (GPC) measurements, the standard method of resolving molar mass distributions, resulting in excellent characterization aligning with GPC results.

Sedimenting Light Chains: Tracking molecular Changes in Monomer Dimer Distributions of pathological FLCs by SV-AUC

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A set of nine free immunoglobulin light chain (FLC) samples purified from urine of multiple myeloma patients was subjected to sedimentation velocity analysis. Aim of the study was to characterize the oligomerization state of each FLC and their ability to aggregate into larger structures. Information was acquired to unravel mechanisms underlying the detrimental action of overexpressed FLCs in patients with multiple myeloma or other monoclonal gammopathies.Sedimentation velocity experiments were performed to determine sedimentation coefficient distributions for each patient sample under different experimental conditions. FLCs are stabilized by two intramolecular disulfide bonds, while covalent dimerization occurs through an unpaired C-terminal cysteine residue. Incubation with the reducing agent TCEP cleaves intra- and intermolecular disulfide bonds, destabilizing both FLC monomers and dimers. Remarkably, different incubation times revealed that destabilized dimers do not dissociate into monomers but instead accumulate directly into oligomers and higher-order aggregates. In addition to larger aggregates, fragments with sizes around 1 S were detected with increasing TCEP incubation time. This fragmentation pattern was consistent among FLCs originating from the immunoglobulin kappa variable 1-33 gene (IGKV1-33). Sedimentation velocity-based characterization of FLCs can provide insights into the relationship between their stability and aggregation capacity. An understanding of this relationship is crucial for the development of therapeutic strategies to prevent renal complications associated with monoclonal gammopathies such as multiple myeloma.

High resolution single particle light scattering - a high-quality orthogonal method for protein aggregation studies

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Protein aggregation plays an essential role in many applications. Fields of application range from medical diagnostics, pharmaceutical, chemical and biotechnological applications to the food industry and beyond.

Light scattering methods have proven to be a useful complement to AUC for many years. They provide valuable information on complex biological sample systems, such as proteins, including particle size distributions and special hydrodynamic parameters. Our innovative light scattering method presented in this article uses the basic principle of a flow cytometer. The new analysis system simultaneously records the scattered light signal of each individual particle in the forward and sideward direction in a volume-calibrated sample stream. As a result, the method allows a highly precise determination of particles number concentration. Measured scattered light intensities of each particle are classified in up to 3 x 10^6 bins and the particle sizes are calculated from the nanometer to the micrometer range on the basis of Mie theory. Recorded signals also allow a (qualitative) assessment of the optical and geometric properties of the detected particles.

The use of the new analysis system for investigating the temperature-controlled aggregation kinetics of protein samples will be demonstrated using the BSA system as an example. Bovine serum albumin has a high structural similarity to human serum albumin, which is highly relevant as a transport vehicle and carrier particle in pharmaceutical applications. Results of the investigations show that changes in aggregate size and aggregate number concentration can be sensitively detected and the temperature and time dependency of protein aggregation can be shown in detail. The method is therefore a valuable tool for the development and quality control of innovative drug delivery systems and polymer-drug conjugates, to name just two examples.

Classification and Synthesis of Analytical Centrifuge Experiments using Deep Learning Models

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The state of suspensions and emulsions plays a crucial role at various stages in the process chain leading to the production of high-quality products. In recent years, significant progress has been made in product design, including control over surface properties, size, shape, and density, enabling the creation of highly specialized products. Liquid-based dispersions can be analyzed using an analytical photo-centrifuge, which quantifies the state of the disperse phase. This method allows for the photometric detection of concentration kinetics under centrifugation with high temporal and spatial resolution [1].

However, further analysis based on separation mechanisms, including deriving particle properties from experimental data, generally requires specialized knowledge [2-3]. Experienced experts can classify and identify properties of the state by visually inspecting experimental sedimentation kinetics based on extinction data. This prior knowledge is often essential for further quantitative analysis, such as stability analysis, sedimentation velocity determination, or particle/droplet size distribution calculations.

This study presents a fair comparison of deep learning-based approaches for the classification and synthesis of various separation kinetics of dispersions. We analyze and compare different families of state-of-the-art deep learning models. The proposed approach was evaluated using an in-house dataset containing 463 experimental datasets of various separation mechanisms obtained via analytical photo-centrifugation. Each instance in the dataset includes the corresponding ground truth class ID, encompassing a total of four classes.

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Renaissance of the Schlieren Centrifuge

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In today's detection landscape for ultracentrifugation, absorbance and Rayleigh interference optics dominate the field. The formerly common, nowadays niche detection method of schlieren detection is to be revived and further developed. Such a schlieren detection system could aid the detection of high concentration samples or samples that form high refractive index gradients, where Rayleigh interference systems have limitations (Cölfen and Harding 1995).

Schlieren detection is a refractive index based detection technique. In contrast to Rayleigh interference optics, which uses the refractive index difference between the sample and a reference, the schlieren optical system measures the refractive index gradient in the sample cell.

The refractive index change in the sample causes a change in the path of light through the cell, which can be detected using a schlieren detection system (Toepler 1864).

Former ultracentrifuge models, such as the Model E, employed this technique. But due to the spacial requirement of the optics, with smaller centrifuge footprints, the schlieren technique disappeared and Rayleigh interference optics were used for refractive index based sample detection.

An optical setup that makes it possible to fit a schlieren detection system in the setup of a modern Rayleigh interference setup (Laue, Anderson, and Demaine 1994) as well as a standalone modification of a Optima XL centrifuge for schlieren detection (Mittal and Lechner 2012) have been made.

Further, optical setups have been proposed to enhance the sensitivity of schlieren optics (Cölfen and Borchard 1994).

An effort is made to implement these setups and bring schlieren detection systems back to the detector landscape for ultracentrifugation.

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Spider silk proteins. Analytical ultracentrifugation in application to biomolecular materials.

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Bio-based materials are functional, sustainable, renewable and have a good mechanical properties. All these features make them in demand in the modern world. One of the most promising representatives is spider silk, the fibers of which are formed on the basis of triblock proteins. Spider silk stands out for its excellent mechanical properties. However, to achieve the properties similar to native spider silk using artificial analogues, a deep understanding of processes and interactions at the molecular level is required. One of the features of spider silk protein solution is ability to undergo liquid-liquid phase separation at high concentrations, that considered as a intermediate step to fiber formation. In this research we studied the effect of terminal domains on process of liquid-liquid phase separation and demonstrated metastability and time/path dependence of spider silk protein solution. AUC was used to characterize strength of terminal domains interactions and spider silk protein solution composition at different stages of the solution aging.

Weak but deadly: (super)natural killer cell receptor:ligand recognition unravelled

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Natural killer (NK) cells, a subset of effector lymphocytes, are an essential component of non-specific immunity, whose primary function is to recognise and spontaneously destroy damaged, infected, or malignant cells. The cytotoxicity of NK cells is regulated by their surface receptors through which they examine ligands on target cells. Some receptors enhance cytotoxicity (activating receptors), while others suppress it (inhibitory receptors). Which of the signals prevails then determines the action of the NK cell. NK cell cytotoxicity is further modulated by various other stimuli, such as cytokines, and it is executed upon direct cell-to-cell contact with the target cell by a deathly cocktail of enzymes released from lytic granules into the immune synapse. Thus, NK cells promise great therapeutic potential, which is currently being explored using various protein immunotherapeutics and genetically engineered CAR NK cells [1].

Over the last fifteen years, we have contributed to the structural description of various activating and inhibitory NK cell receptors of mice, rats, and man, as well as of their cognate protein ligands and their mutual complexes. Using a combination of protein X-ray crystallography, small-angle X-ray scattering, and in-solution biophysical methods utilizing individual soluble recombinant proteins, with single-cell localization microscopy techniques observing the proteins expressed on the cell surface, we have become unravelling the almost supernatural nature of NK cell ligand recognition where the usually rather low affinity of single receptor-ligand interaction is overcome by their oligomerization and/or cross-linking/clustering within the immune synapse, thereby deploying avidity instead of affinity [2-4].

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Dimerization of a ~7-kDa domain determines architecture and stoichiometry of a 5-MDa multi-enzyme complex

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Pyruvate dehydrogenase from *Escherichia coli* is a ~5-MDa multi-enzyme complex composed of the catalytic subunits pyruvate dehydrogenase (E1), dihydrolipoamide acetyltransferase (E2), and dihydrolipoamide dehydrogenase (E3). In the complex, eight E2 homotrimers assemble to a cubic core to which homodimers of the peripheral subunits E1 and E3 associate via binding to E2's peripheral subunit binding domain (PSBD). Previous reports indicated that 12 E1 dimers and 6 E3 dimers bind to the 24-meric E2 core. Here, we used an assembly-arrested homotrimeric E2 variant ($E2_3$) to show that two of the three PSBDs in $E2_3$ dimerize, that the PSBD dimer cooperatively binds two E1 dimers, and that E3 dimers only bind to the unpaired PSBD in $E2_3$. We further show that this mechanism is preserved in wild-type PDHc, resulting in an E1 dimer:E2 monomer:E3 dimer stoichiometry of 16:24:8. In addition, sequence conservation analysis of the PSBD dimer interface indicates that PSBD dimerization is the previously unrecognized architectural determinant of gammaproteobacterial pyruvate dehydrogenase megacomplexes.

Functional Particles of Different Size, Shape, and Surface Chemistry – An Overview

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In this contribution, we present the particle platform of division *Biophotonics* synthesized, surface-functionalized, and explored by us. This particle platform constitutes of differently sized and shaped inorganic, organic, and hybrid nanoparticles and microparticles with hydrophobic and hydrophilic surface chemistries and different functionalities such as different luminescence or magnetic properties. These nanoparticles and microparticles include lanthanide-based upconversion particles like $NaYF_4$ or$LiYF_4$ doped with the sensitizers and activators Yb, Nd, Er, and Tm, unstained, dye-stained, and dye-labeled silica particles, and unstained, dye-stained, and dye-labeled polymer nanoparticles. [1-3] In addition, magnetic nanoparticles such as Fe_2O_3, noble metal nanoparticles, and different types of semiconductor quantum dots like InP, AgInS, and Ag_2S are studied. [4,5] Typical applications of these particles, which are also available from us, range from nano- and microparticle sensors and reporters to test and reference materials with well characterized or even certified properties such as size, shape, and surface chemistry. [5,6]

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