INSTRUMENTATION PLAN

A. Justification of need

For almost a century, investigators have used the biophysical technique called analytical ultracentrifugation (AUC) to separate macromolecules in solution based on their hydrodynamic properties and to determine information about size, shape, and buoyant density using first-principle approaches. Application of this technique has been driven by commercially available instruments that can achieve rotor speeds of upwards of 60,000 revolutions per minute (rpm), generating upwards of 300,000 g of force at the bottom of a centrifuge sample cell. The sedimentation of macromolecules can be monitored using one of three forms of detection: UV-visible light absorbance, Rayleigh interference, and fluorescence emission. The optical signal is recorded as a function of the radial range of the sample compartment over multiple timepoints, which allows for fitting of the data with known relationships derived from the Svedberg equation to calculate experimental sedimentation and diffusion coefficients.

The Johnson Research Foundation Structural Biology and Biophysics Core (JRFSBBC) Facility is a campus-wide resource that resides within, and is supported by, the Department of Biochemistry and Biophysics at the Perelman School of Medicine of the University of Pennsylvania. The core requests a Beckman-Coulter Optima analytical ultracentrifuge to support the ongoing research of NIH-funded investigators at the University of Pennsylvania and institutions nationwide.

The Johnson Research Foundation was established in 1929 as the first endowment to support research into the physical principles fundamental to medicine and its clinical practice. The foundation has a 91-year track record of enabling instrumental invention and physical biochemical and medical applications and providing a unique environment for research and training. The JRFSBBC was established in 2016 to formalize and further promote the dissemination of biophysical technology throughout the Penn and local community. This core facility has been overseen by the PI since its founding in 2016 and exists within the already robust research environment at the University of Pennsylvania. The mission of the core is to accelerate research and advance education and training in structural biology and solution biophysics. The core provides access and training to users for a broad range of instrumentation including light scattering, calorimetry, mass spectrometry, and analytical ultracentrifugation. Since 2016, the core has provided research services and support to over 60 research groups, approximately half of which are intramural: research groups at Penn across several departments, schools, and centers. The JRFSBBC has contributed to many NIH-funded studies including discoveries in active research projects and has been an important resource for the training of graduate students and postdoctoral fellows. Additionally, the core sponsors an annual mini-symposium on analytical ultracentrifugation in partnership with Beckman-Coulter and an annual hands-on workshop on microscale thermophoresis (MST) with the company Nanotemper. Overall, the core has become a key resource for the investigation of macromolecular structure and function both at Penn and nationwide.

The analytical ultracentrifuge (AUC) is a centerpiece technology in the JRFSBBC that provides users the ability to assess many attributes of macromolecules and their assemblies in solution, including molecular weight, stoichiometry, shape, aggregation state and oligomerization, ligand binding, conjugation efficiency, and polydispersity, using first-principle methods. JRFSBBC oversees the operation of one Beckman-Coulter XL-A analytical ultracentrifuge, an instrument that is part of the Van Duyne research group within the department and was acquired in the late 1990s using institutional funds. This XL-A has single-wavelength UV/Vis absorbance optics and was refurbished with a now-antiquated Windows 7 computer with Intel i7 single processor. Compatibility issues with Beckman's proprietary data acquisition software and instrument interface preclude any further upgrade the computer operating the instrument, limiting our application of modern AUC software methods for data management and analysis on the same computer. Over its lifetime, this instrument has been extensively refurbished by the manufacturer via service contract, including lamp and photomultiplier replacements, computer board replacements, and the data acquisition computer board. Through this extensive maintenance and repair regimen, we have managed to maintain AUC service for the community. Compelling our efforts to upgrade this ~25 year-old instrument is the desire to apply to arising and cutting-edge methods in AUC such as multiwavelength analysis (described herein), alongside the considerable age of the instrument, which raises questions about the sustainability of our current approach.

The JRFSBBC is well-positioned to leverage this newest technology immediately. Via collaboration between the PI and Dr. Borries Demeler (University of Lethbridge) and Emre Brookes (University of Montana), the JRFSBBC is now a participant in the Ultrascan-III data analysis platform for the benefit of its users, which includes state-of-the-art data analysis using the XSEDE supercomputing resource (1). Furthermore, the PI is collaborating with this cohort to further develop new methods for AUC analysis leveraging this new technology (NIH R01 application submitted February 2020). The Ultrascan-III platform is has fully implemented multi-wavelength data analysis and achieves the highest rigor possible in data analysis. Via its established AUC mini-symposium held each year, the JRFSBBC is well-positioned to share this emerging knowledge to the broader AUC community.

A1. Instrument Features and Improvements

After a decades-long hiatus in any fundamental improvements in instrumentation, the Optima AUC delivers a fundamental advance in technology to the user community that will immediately accelerate research in the biomedical community. When compared to the older-generation XL-A technology (first introduced in 1991) that now resides at the JRFSBBC, the Optima AUC (introduced in 2017) is a clear advancement in all regards:

Table A1. Comparison of Beckman-Coulter XL-A with Beckman Optima

Technical Feature	Beckman XL-A	Beckman Optima
Year introduced	1991	2017
Interference Optics	~4 fringes/cell	~10 fringes/cell
Absorbance Flash Lamp Frequency (Hz)	50	300
Optical resolution in the radial dimension (microns)	30-40	10
Maximum number of wavelengths	1	20 (100 with Ultrascan platform-driven data acquisition)
Wavelength accuracy (nm)	± 3.0	± 0.5
Scan speed (sec/scan)	180	<7
Operating system	Windows	Linux
Temperature control (^{o}C)	± 0.3	± 0.1
Vacuum	Diffusion pump	Turbomolecular pump

This updated technology provides the immediate promise of higher accuracy and precision measurements for several reasons, which will immediately enhance ongoing research:

1. AUC data is a first-principle technique: sedimentation velocity data are fit with the Lamm equation. Stochastic noise within data can confound the convergence of this fitting to a unique solution. The modern electronics and technology alone will provide lower data noise. Combined with state-of-the-art optics, the instrument provides higher signal-to-noise and the lowest noise achievable. Data quality is such that far more unique solutions are obtained from the fitting of the Lamm equation, increasing rigor and reproducibility.

2. The enhanced camera (2048x1088 pixels) provides enhanced signal-to-noise compared to the older XL-A/XL-I instrument in intensity mode, and ~10 fringes per cell in interference mode. The current instrument at the JRFSBBC is not equipped with Rayleigh interference optics. Data acquisition in interference mode allows for buffers that include small non-sedimenting species like organic molecules (ligands), nucleotides, and reductants which absorb at wavelengths at or near those of protein and nucleic acid, a capacity critical for the user projects described in this proposal.

3. Finer temperature control avoids issues with convection and changes in buffer viscosity and density, both issues which confound data analysis and modelling. Temperature control is very precise, with a stability of ± 0.1^{o}C across a controlled range of 0-40°C.

4. The instrument can acquire data at a rate of less than 10 seconds per scan for a 1.4 cm cell length, with a maximum resolution of 10 microns per radial step. Faster scanning speeds allow the application of the highest rotor speeds to maximize sedimentation resolution alongside the capture of sufficient scans before samples are pelleted. Combined with the improvement in optical and radial resolution, better resolution of more complex samples with closely spaced S-value species can be achieved.

5. The Optima AUC allows for *simultaneous* collection of interference and absorbance data from the different optical systems, whereas in older XL-I instruments, data is acquired *sequentially*.

6. These improved features in scanning speed and precision yield marked improvements returns in sample throughput. With an 8-hole rotor (e.g., upwards of 8 samples when using a previously stored radial calibration from a counterbalance), data can be recorded reliably and with good data density. This makes it possible to rigorously probe concentration-dependent phenomena in solution like oligomerization and to perform titrations for binding, or comparison of series of mutants with wild-type protein, or changes in response to factors like pH, ionic strength. Multiple speeds, concentrations and wavelengths enable robust global fitting methods using available software such as ULTRASCAN-III and SEDFIT/SEDPHAT.

7. The new Optima instrument is equipped with a turbomolecular vacuum pump to facilitate rapid evacuation of the sample chamber and reliable pressure, circumventing the issues older XL-A/XL-I instruments endured with optical issues due to diffusion pump oil vapors within the instrument, and achieving vacuum in a matter of a few minutes rather than ~20-30 minutes.

8. The new instrument also employs the Linux operating system within an integrated touch-screen panel and open source software compatible with the open AUC project (2) for data acquisition, which will directly facilitate the implementation and application of the Ultrascan-III analysis software suite (3-5).

A2. Multiwavelength Analysis

The optics of the Optica AUC represent a fundamental advance in AUC technology, opening a new realm for the analysis of macromolecules in solution not previously possible. The new optics of the Optima AUC allows for multiwavelength (MWL) detection: the instrument can scan up to 20 different wavelengths per radial point with its absorbance detector (100 when implemented with Ultrascan-III) with a wavelength precision of ± 0.5 nm and using a 300 Hz Xenon flash lamp, yielding a much higher data density when compared to older instruments. This feature is a major advance when compared to the single-wavelength capacity of the XL-A/XL-I instrument from the late 1990s, which uses a 50 Hz flash lamp with a wavelength precision of ± 3.0 nm. The data density is further increased during an experiment by the <7 sec/scan capability at a radial resolution of 10 microns, compared to the 1.5 minutes needed now with the older XL-A/XL-I instrument, at a radial resolution of 30-40 microns and wavelength precision of ± 3 nm.

The arrival of MWL opens a new realm for data analysis: alongside traditional hydrodynamic separation of solutes in a mixture, unique chromophores allow for separation of molecules in a mixture by spectral deconvolution (6-17). By implementing this second dimension of analysis, the investigation of complex macromolecular interactions can be performed rigorously, providing insight into fundamental questions like stoichiometry and thermodynamic binding parameters. By exploiting different absorption spectra, it is possible to deconvolute the contribution of each component to derive molar stoichiometry and concentration for each component in the mixture and assign their status as free or bound in a complex.

Over the past five years major innovations in AUC technology were achieved by the Cölfen group in Germany in collaboration with the Demeler group at the University of Lethbridge, leading to the creation of the first multiwavelength AUC detectors (6, 8, 18), that were quickly incorporated into the latest commercial product available from Beckman-Coulter. The data created by this new instrument can be fully leveraged in the Ultrascan III data analysis framework (3, 4). With both the technology and software-driven methods now available, insights into a range of challenging biological systems are now possible by leveraging the spectral

properties of the components of the mixture, including protein-DNA and protein-RNA interactions, membrane protein analysis embedded within nanodiscs, viral vector characterization, small molecule interactions (nucleotides, drugs, co-factors) with targets, detection of fluorophores and fluorescent proteins in multicomponent systems, large macromolecular assemblies like chromatin and the ribosome, and protein-protein interactions. The user projects described herein span these new opportunities.

The Ultrascan-III software platform provides both state-of-the-art data analysis capabilities coupled with supercomputing resources (XSEDE in the US), alongside an information management system for data management. Unlike other freely available software packages, Ultrascan complies with open AUC standards (2), is open-source licensed, and supports both high-performance computing and database integration. The software is freely available to academic researchers worldwide and is compatible with any operating system. The Optima AUC would enable seamless integration with the Ultrascan framework, immediate user access to data in the core setting using the Laboratory Information Management System (LIMS, (19, 20)), and the most robust tools available for AUC data analysis, including the global modelling of multiwavelength AUC data. The software is well-supported with both documentation and workshops regularly hosted by the authors of the software. Ultrascan-III is additionally equipped with US-SOMO (21, 22), which provides a complementary tool for structural biologists to reconcile atomic models with their solution properties.

Key considerations necessary for experimental design using the multiwavelength approach are detailed in the Research section (C).

A3. Accessories Requested

Table A2. Table of Accessories Requested

Description
8-position An50Ti analytical rotor with counterbalance kit
Torque stand assembly
8 sapphire cell assemblies, usable for both forms of detection
8 six-sector epon centerpieces for sedimentation equilibrium analysis

The instrument overseen by the JRFSBBC has one An60Ti 4-position titanium rotor, which was purchased alongside its current XL-A instrument >25 years ago. This rotor has been heavily used, well beyond the 12-year lifetime recommended by the vendor. Therefore, it would be best to retire this instrument due to safety concerns ("metal fatigue") and it would be inappropriate to use this rotor in the new instrument. The An50Ti requested supports speeds up to 50,000 rpm, ideal for the optimal resolution of heterogenous macromolecular systems. Using the Ultrascan-III software framework, the full capacity of the rotor can be achieved by storing the radial calibration from a counterbalance to free-up one additional sample cell position within the rotor for subsequent runs (23).

To fully realize this enhanced sample capacity provided by the An50Ti rotor, 8 additional cell assembles are requested, with corresponding epon centerpieces for either sedimentation velocity (2-sector) or sedimentation equilibrium (6-sector) analyses (all rated for 42,000 rpm). This would provide one spare cell, given the instrument package already includes one sample cell. Currently, the JRFSBBC has access to six 2-sector cell assemblies and 3 6-sector cell assemblies (owned by the Van Duyne research group), most near end-of-life with regards to wear-and-tear. The older centerpieces are worn, and cell housings have worn ring threads which affect the ability to achieve maximum torque when tightening cells, results in periodic leaks and sample loss. The Van Duyne group only owns quartz windows to all its cell assemblies, which are best suited for the collection UV-Vis absorbance data (the only mode available in our current XL-A instrument). To assure optimal data using both optical systems in the Optima AUC in tandem, sapphire windows are necessary.

Like our current An60Ti rotor, our current cell torque stand is old and worn. It has undergone several repairs at our departmental machine shop over the years, and even with adjustment, frequently fails to properly hold via

a collar that grips the entire cell housing. A new torque stand is necessary for the optimal treatment of cell assemblies and their lifespan.

A4. Availability of equivalent AUC instrumentation elsewhere

The Optima AUC and its unique multiwavelength capabilities are not available to academic investigators anywhere else in the Greater Philadelphia region, with the next closest instrument found at Princeton University over 50 miles away, and relatively few are found nationwide in the academic setting. We have been able to collect some pilot data using the Optima AUC via the PI's collaboration with Borries Demeler at the University of Lethbridge (Canada) (User Projects Contreras, Jaffe, Daldal, Bushman, Van Duyne), which is focused on method development for multiwavelength AUC analysis.

Several older single wavelength XL-A and XL-I instruments can be found in the Greater Philadelphia region. On the Penn campus, four instruments reside, including the instrument found in the JRFSBBC, a Beckman Proteome Lab XL-I instrument in the Marmorstein research group, another Proteome Lab XL-I instrument at the Human Gene Therapy Vector Core, and an XL-A instrument at the Children's Hospital of Philadelphia. However, the instrument overseen by the JRFSBBC is the only instrument accessible in a general user core facility at Penn. At nearby institutions, similar single-wavelength instruments can also be found at Thomas Jefferson University (1), and Haverford College (1). Of all the instruments mentioned, none have the multiwavelength capabilities of the new Optima instrument.

Other commercial suppliers of analytical centrifuges, less one with multiwavelength capacity, do not exist currently. Nanolytics Instruments in Germany produces the multiwavelength absorbance optics first developed by the Cöelfen group. However, it only provides the technology within refurbished older generation Beckman preparative centrifuges, not realizing all the newest technology now available. A novel instrument designed by Spin Analytical called the Centrifugal Fluid Analyzer (CFA (24)) has been advertised on a website for several years. However, no functional instruments have been installed anywhere to our knowledge and are never mentioned among the AUC community. Therefore, the Optima AUC is the only proven and acceptable option available.

A5. Accessible User Time (AUT)

Extensive recordkeeping maintained over the past 18 years in the form of logbooks and experimental reports for our current XL-A instrument allows us to reliably estimate accessible user time for the proposed new instrument. Instruments in the JRFSBBC, like the XL-A AUC, are available 24/7 when not under repair. In the fitting of the Lamm equation, both sedimentation rates and diffusion are modelled. In each sedimentation velocity experiment designed and implemented, we select rotor speeds and run times that strike the best balance between diffusion signal and sedimentation resolution; hence, run times will vary from project to project. These experimental run times are further affected by the molar mass and size of the samples examined. In practice we only schedule one sedimentation velocity experiment per day on our instrument. Changeover to the next experiment occurs after the cells are cleaned and reloaded. Long experiments that require extensive equilibrium times like sedimentation equilibrium experiments are typically scheduled over weekends, as they may demand upwards of 60-80 hours of spin time, depending on the design of the experiment.

Dr. Gupta performs all experiments, with periodic exceptions for trained and experienced local users. In his absence, Dr. Van Duyne is also an experienced operator of the analytical ultracentrifuge. The arrival of a new Optima AUC would allow us to retire the aging XL-A currently in use while providing enhanced experimental capacity and results to our existing pool of users. We expect to be able to run upwards of five experiments a week with this new instrument comfortably, which is approximately ~250 experiments per year. Based on these considerations, we estimate the AUT at 112 hours/week (16 hours/day) across 52 weeks of the year, yielding over 5,800 instruments hours per year. Routine maintenance would be performed during changeover time and hence is not expected to affect available instrument hours.

B. Technical Expertise

B1. Staffing

The JRFSBBC is directed and operated by Dr. Gupta, Research Assistant Professor in the Department of Biochemistry and Biophysics and member of the Department's graduate group. Dr. Gupta is intimately involved in the daily operation of the core and is responsible for all aspects of the research performed, including user training for walk-up instruments, experimental design and implementation, and data analysis. He is a structural biologist with well-developed expertise in solution biophysical methods, including small-angle X-ray and neutron scattering (SAXS/SANS), light scattering, and analytical ultracentrifugation. He has performed several hundred AUC experiments over the past 15 years. This AUC work has contributed to 20 collaborative peer-reviewed publications (25-44) alongside an additional 7 manuscripts in review, revision, or preparation. He is a member of the newly formed Northwest Biophysics Consortium (https://nbc.uleth.ca/) and regularly teaches solution biophysics (including AUC) at vendor workshops, workshops on small-angle scattering at general user synchrotrons and national meetings, alongside first-year graduate school lectures (BMB 508 and BMB 509) every year at Penn and neighboring institutions. Three years ago, he established a one-day mini-symposium on analytical ultracentrifugation in collaboration with Beckman-Coulter, which is now an annual event and draws upwards of 80 investigators from across the Greater Philadelphia region from government, academia, and industry to campus. He was also a participant in the 2018 documentary "*The Instrumental Chemist*," which featured the life and contributions of entrepreneur Arnold Beckman (namesake of Beckman-Coulter), including the analytical ultracentrifuge.

B2. Experimental and User Support

The JRFSBBC has codified an end-to-end experimental workflow to provide AUC analysis to users. Since the establishment of this core in 2016, this approach has led to many positive outcomes, including AUC studies for over two dozen groups across academia and industry and the training of graduate students and postdoctoral fellows.

A. **New Users.** We average 1-3 new AUC user requests every month via our website, driven largely by periodic email distributions (a mailing list of almost-2000) and word-of-mouth. A significant portion of usage is driven by collaboration with Dr. Gupta, across a broad range of topics within the realm of biochemistry and biophysics. Also driving user access is the fact that Dr. Gupta can interact readily with faculty and other researchers across Departments in the School of Arts and Sciences, School of Engineering and Applied Sciences, the School of Medicine, the Wistar Institute, and the Children's Hospital of Philadelphia, which are all entities are on the same campus. Our annual AUC mini-symposium also serves to generate interest in our core facility and AUC services. 5% of AUT is reserved for new users and pilot experiments.

B. **Instrument Operation.** Generally, operation of the AUC under the aegis of the JRFSBBC is limited to experienced operators (primarily Drs Gupta, Van Duyne, and a few longtime users). To operate the instrument independently, extensive training and supervised activities must be completed, as there is risk of tremendous damage due to improperly balanced rotors and incorrectly assembled cells. The tremendous forces generated by the titanium rotor accelerated at 300,000 g present a significant risk to both the instrument and the user. Therefore, all routine experiments are typically performed by Dr. Gupta.

C. **Experimental Design.** With that provision, new users (most especially graduate students and postdoctoral fellows) are routinely invited and strongly encouraged to participate in all aspects of the experiment, including experimental design, cell assembly and sample loading, and data analysis and interpretation. Before any experiment is performed, the user and Dr. Gupta meet to discuss the needs of the project and design an experiment, including details of buffer selection, sample concentrations, and purification and labelling strategies. To drive hypothesis-driven research, we will commonly model theoretical data and make predictions based on available atomic models using programs like US-SOMO (a part of Ultrascan III (21, 22)) and WinHydropro (45).

D. **Sample Quality Control.** This preparation process commonly includes other quality control measures from orthogonal techniques, supported by complementary resources at the JRFSBBC, including dynamic light scattering (DLS), size-exclusion chromatography in-line with multi-angle light scattering (SEC-MALS), isothermal titration calorimetry (ITC), microscale thermophoresis (MST), and SAXS.

E. **Data Collection**. Scheduling of experiments is coordinated with Dr. Gupta using an online Google calendar made accessible to core users. Usually, the experiments begin with the delivery of properly buffer-matched samples at the correct optical absorbances discussed the morning of the experiment. Users are invited to stay and participate in the sample loading process, which includes cell assembly (under supervision) and instrument start-up. In situations where instrument demand is high, scheduling preference is given to experiments needed for manuscripts, grant submissions, and theses.

F. **Data Analysis and Management.** After data collection is completed, data can be analyzed with any of a variety of available free-to-download programs like ULTRASCAN III (19, 46), SEDFIT/SEDPHAT (47, 48), Heteroanalysis (49), and DCDT (50), and all raw data are made available to users via university-sponsored cloud services. Hand-in-hand with the data reports he provides, Dr. Gupta encourages users (especially graduate students and post-docs) to learn how to analyze their data using any of these programs, and provides tutorials on how to use the programs the first time.

With the new Optima instrument and the improved computing provided, this workflow will be vastly improved with the implementation of the Ultrascan-III framework, which will enable enhanced data management tools ideal for a general user scheme, including arising innovations that will enable standards for Good Manufacturing Practices (GMP) to be applied, including improved and more rigorous data handling and analysis, alongside automatic data acquisition, editing, and processing (personal communication, Borries Demeler). With the arrival of the Optima, Dr. Borries Demeler will consult in the implementation of this experimental framework and methodologies. A letter of collaboration is provided.

G. **Publication**. Frequently Dr. Gupta collaborates with users in drafting manuscripts and helping prepare necessary figures from the experimental results. Users will be instructed and reminded during the data analysis and publication stages about citing this S10 award. An annual survey among users for citations will be performed by the advisory committee to record this information.

H. **Education**. In-line with the mission of the JRFSBBC to both accelerate research and to advance education and training, training at the bench is provided hand-in-hand with introduction of AUC theory and application in the classroom by the PI, including first-year graduate courses (BMB 508 and BMB 509). Additionally, the Penn community benefits from the annual mini-symposium, which has included leaders in AUC methodology and application, such as Borries Demeler, Peter Schuck, and Chad Brautigam.

C. Research Projects

C1. Overview

The Optima AUC will support and immediately impact a diverse array of research projects from 8 major and 4 minor user groups that are all federally funded via the NIH (including NIGMS and NIAID) or the NSF. As a resource at the JRFSBBC, the instrument will not only serve investigators at Penn, but will also support the research of NIH and NSF investigators from other institutions in the region and from across the country. The projects presented herein reflect our typical usage patterns and are representative of the kinds of projects we expect to accelerate with this new technology. We expect that from year-to-year, accessible user time (AUT) allocated for each project will vary as studies arrive to a conclusion and new users and projects are introduced. For new users we reserve time (5% AUT) to help generate preliminary data for new grant applications.

It is expected that the many improvements in the new Optima AUC will have immediate impact on these ongoing research projects, including increased data density (via multiwavelength data collection, improved radial resolution, and faster scan time), enhanced signal-to-noise and higher wavelength and radial precision. In many of the projects presented, the 8-position An50Ti rotor alongside enhanced scan rates will make it possible to probe multiple conditions simultaneously in a single run across a range of wavelengths. This will enhance detailed study of protein oligomerization and hetero-associations (i.e., protein with DNA, protein-protein interactions, protein-ligand binding) across a large range of concentrations, leveraging different extinction coefficients at different wavelengths, leading to a dynamic range larger than is now possible with single wavelength absorption optics in the XL-A instrument. Most any project arriving at the new instrument will benefit immediately from this enhanced capacity.

A fundamental strength of the AUC method is its ability to resolve heterogenous mixtures, a strength that is enhanced by the new instrument's optical resolution. The density and quality of the data combined with state-of-the-art data analysis methods like those in ULTRASCAN-III will provide the best statistical fits to data possible, allowing for small hydrodynamic differences between individual species to be resolved. This feature will be important to projects with great heterogeneity (e.g., total ribosome profiling in User Project #6), and systems with confounding self-association of component parts. These assorted instrument features together further enable the opportunity to perform multi-wavelength experiments, a dimension to the projects presented not previously realized. In the past four years, the feasibility of these approaches has been demonstrated (9-11, 51) and now the analysis has been implemented in the ULTRASCAN-III software suite.

All the user projects described herein are well-established *in vitro* and optically heterogenous systems that will uniquely benefit from the enhanced technology and emerging multiwavelength methods. A synopsis of the types of projects presented are provided:

Table C1: Project Categories to be examined by Multiwavelength AUC.

Project Category	Project	User Status
I. Protein-Nucleic Acid complexes	Gregory Van Duyne	Major
	Ben Black	Major
	Rahul Kohli	Major
	Kristin Lynch	Major
	Fange Liu	Major
	Lydia Contreras	Major
II. Integral Membrane Proteins	Vera Moiseenkova	Minor
	Fevzi Daldal	Minor
III. Protein-Protein Interactions	Yale Goldman	Major
	Frederic Bushman and Gregory Van Duyne	Major
	Eileen Jaffe	Minor
	Elizabeth Rhoades	Minor

Key to the application of the multiwavelength approach and successful spectral deconvolution of the data are spectral absorbance profiles unique to the different species to be resolved. In the absence of specific labelling strategies or unique chromophores, it is expected to be difficult to resolve different proteins based on the ratio of their aromatic residues to the peptide bond contributions in the low UV without a very large number of collected wavelengths during the experiment. A major feature of all the user projects in this proposal are distinguishing optical features in the mixtures to be studied, including protein vs nucleic acid, heme chromophores, and labelled species with unique absorbance properties in complex mixtures. All these projects are already well positioned for immediate acceleration of ongoing research by the availability of the Optima instrument. In all cases, expression and purification schemes are well-established with quantities at the levels needed to support AUC study, and in all but one case, preliminary data has been collected using the older XL/A instrument or via pilot experiments on an Optima instrument located in Canada (the Demeler research group). And for many of the user projects described, labelling protocols have already worked out and applied.

Integral Membrane Proteins. The experimental systems described in Minor User Projects #2 and #3 (Daldal and Moiseenkova-Bell) are representative for a large and important class of systems involving integral membrane proteins (over a third of the human genome and among the most important drug targets). Membrane-bound and associated proteins (including trans-membrane proteins, receptors, and channels) are often insoluble in aqueous medium, and therefore tend to aggregate without a surrogate carrier which emulates their native hydrophobic environments, such as detergents or lipid. The application of MW-AUC to their study provides an important complement to their structural biology, including the ongoing cryo-EM efforts at Penn, as the information gathered from these analyses are paramount to project success. These measurements provide rigorous quality control and valuable information for interpretation of samples undergoing structural analysis, including mass, shape, stoichiometry, and monodispersity.

Generally, such quality control is difficult to achieve with integral membrane proteins and cognate protein-detergent complexes due to technical challenges with conventional methods such as light scattering or standard AUC methods. As needed, we will guide users to embed such proteins into nanodiscs to facilitate their study. Nanodiscs are protein-stabilized lipid rafts with are monodisperse and water-soluble. They can be used to mimic the native phospholipid bilayer to solubilize membrane-embedded targets. The size of the membrane bilayer in nanodiscs are stabilized by recombinant constructs of high-density apolipoprotein A1, which can be created in different lengths. Because nanodiscs are stable and their composition readily adjusted, this technology is a particularly powerful vehicle for the biophysical study of integral membrane proteins.

Nanodiscs will be prepared as described previously using established methods (52, 53) and are already routinely used by both user groups for structural studies by cryo-EM. In AUC experiments, the belt protein will be labeled with a fluorescent dye, providing a unique chromophore suitable for MW-AUC. We will further label membrane proteins with a different site-specific probe to create a second, distinct chromophore signature, unless the proteins already contain a unique chromophore, e.g., prosthetic heme cofactors. As a control, nanodiscs alone also will be measured to confirm their homogeneity. Reference spectra will be collected for each isolated species and will be used to aid the spectral decomposition of data from MW-AUC sedimentation experiments performed at wavelengths encompassing the regions of interest (11, 15). Experiments will be performed with increasing amounts of embedded membrane protein to monitor the change in hydrodynamic properties. Molar masses of the integral membrane protein-nanodisc assemblies will be ascertained by D_2O density matching experiments to derive partial specific volumes (54, 55).

C2. User Projects

D. Major and Minor User Summary:

Table D1 shows a summary for all major and minor user projects listed in this application. As detailed above, our calculated AUT is based on a use of 16 hours/day, 7 days a week, providing 112 hours/week, 52 weeks/year for a total of 5,824 instrument hours per year. Routine maintenance will be performed during working hours between scheduled experiments. Major user research projects (8 users) comprise 80% of the AUT, while minor user research projects (4 users) comprise 15% of the AUT. The last 5% of AUT is reserved for new core users and pilot experiments for new projects.

Table D1: Major research projects (80% AUT)

ID	Major Users:	Grant #	Institution	Title:	Period:	AUT
1	B. Black	NIH R35GM130302	Penn	Centromere Identity, Strength, and Regulation	1-Apr-2019 to 31-Mar-2024	10%
		NIH R01HD058730		Age and Molecular Mechanisms Contributing to Aneuploidy in Oocytes	1-Aug-2009 to 30-Nov-2020	
2	R. Kohli	NIH R01GM127593	Penn	The Molecular Basis for the Bacterial SOS Signal	1-May-2018 to 28-Feb-2022	10%
3	G. Van Duyne	NIH R01GM108751	Penn	Large Serine Recombinase Mechanisms	1-Feb-2014 to 31-Jul-2019[A]	10%
4	K. Lynch	NIH R01AI125524	Penn	Splicing and Nuclear Transport of Influenza Virus mRNA	25-May-2016 to 30-Apr-2021	10%
		NIH R35GM118048		Molecular Mechanisms and Signal-Induced Regulation of Alternative Splicing	9-May-2016 to 30-Apr-2021	
5	F. Liu	NIH R35GM133721	Penn	Coregulation of mRNA, tRNA, and rRNA Species Through RNA Modifications	1-Aug-2019 to 30-Jun-2024	10%
6	L. Contreras	NSF 1932780	UT-Austin	Molecular Characterization of Interacting Bacterial Regulatory Networks	1-Sep-2019 to 31-Aug-2022	10%
		NSF 1716777		Molecular Characterization of Target Scheduling in Bacterial	1-Aug-2017 to 31-July-2020	
7	Yale Goldman	NIH R35GM118139	Penn	Structural Dynamics of Molecular Motors and the Ribosome	16-Jul-2016 to 30-Jun-2021	10%

8	F. Bushman and G Van Duyne	NIH R01AI129661	Penn	Optimization HIV Inhibition by Allosteric Integrase Inhibitors	17-Jan-2017 to 31-Dec-2021	10%

Table D2: Minor research projects (20% AUT, including 5% AUT for new users and pilot experiments)

ID	Minor Users:	Grant #	Institution:	Title:	Period:	AUT
1	E. Jaffe	NIH R01NS100081	Fox Chase Cancer Center	A New View of PAH Allostery Correlation with Disease-Associated Alleles	15-Sep-2016 to 31-Jul-2021	4%
2	F. Daldal	NIH R01GM038237	Penn	Respiratory Complex III: Supercomplexes and ROS from Bacteria to Human	1-Jul-2018 to 30-Jun-2020	4%
3	V. Moiseenkova	NIH R01GM103899	Penn	Structural Insights into TRPV Channel Gating	1-Aug-2013 to 30-Jun 2023	4%
		NIH R01GM129357		Molecular Mechanisms of TRPV5 Gating	10-Sep-2018 to 31-May 2022	
4	E. Rhoades	NIH RF1AG053951	Penn	Molecular Mechanisms and Cellular Implications of Tau Dysfunction	15-Jul-2016 to 31-Mar-2021	3%

A. Project in process of renewal application in 2020.

E. Administration – Organizational Management Plan:

The Johnson Research Foundation Structural Biology and Biophysics Core (JRFSBBC, https://www.med.upenn.edu/jf/bsbcore/) is a core within the Department of Biochemistry and Biophysics at the Perelman School of Medicine of the University of Pennsylvania. The facility is directed by Dr. Gupta, who is a member of the department faculty and graduate group. Most of the facility's instrumentation is located on the 8th, 9th, and 10th floors of the Stellar-Chance Building at the School of Medicine within host laboratories. The laboratory within which the AUC will reside is approximately 2,000 sq. ft. of modern (1996) space. Additional autoclave and glass-washing equipment, plus a shared cold-room and space for freezers and incubators, are centrally located. We are very well-equipped for molecular biology, tissue culture, cell growth, fluorescence microscopy & spectroscopy, protein production, electrophoresis, chromatography, centrifugation, crystallization, radioisotope work, and many other procedures. In addition to the modern wet lab facilities needed for routine AUC sample preparation, the JRFSBBC instrumentation includes light scattering (SEC-MALS, DLS, CG-MALS), analytical ultracentrifugation, isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC), MALDI-TOF, circular dichroism (CD), and an LTQ Orbitrap mass spectrometer for hydrogen-deuterium exchange studies.

Business management is the responsibility of the department business administrator Katie Heer, who ensures that fiscal transactions follow NIH and University guidelines and corresponds with other departmental business administrators at the University. We have a strong working relationship with our local Beckman-Coulter service engineer, Mark Graber, who carries out repairs and preventative maintenance as needed on our current analytical ultracentrifuge. The JRFSBBC has one full-time research technician, who contributes to the day-to-day operation of the core, including sample preparation and handling, MST, and crystal preparation for X-ray crystallography. Dr. Gupta oversees most aspects of user training and experimentation, including instrument maintenance, experimental design, and data analysis.

An end-to-end workflow for user support and experimentation is provided in Section B2 of this proposal.

E1. Advisory Committee:

The Department of Biochemistry and Biophysics has an advisory committee comprised of senior department members and distinguished scientists to advise and oversee the operation of the core, including instrument policy and support. The committee provides input on user fees and rates, facilities issues, and makes recommendations regarding core technologies and services. This committee meets once every four months and additionally communicates via email. This committee will perform annual survey of users and request citations that make use of the new centrifuge and acknowledge the S10 award.

Committee members:

Dr. Kristen Lynch, Professor (Department Chair)
Dr. Kushol Gupta, Research Assistant Professor, Department of Biochemistry and Biophysics (Core Director)
Dr. Ronen Marmorstein, Professor (Department Vice-Chair)
Dr. Kim Sharp, Associate Professor (Director, High Performance Computing Resource)
Dr. Walter Englander, Professor
Dr. Leland Mayne, Associate Director, Johnson Research Foundation
Dr. Gregory Van Duyne, Professor

E2. Safety:

The instruments of the JRFSBBC are housed in BSL-2 level laboratories within the Department of Biochemistry and Biophysics. The current XL-A AUC and the proposed new instrument will reside within a modern 2000 sq. ft. laboratory space on the 8th floor of the Stellar-Chance Laboratory building. AUC analysis is restricted to non-infectious and non-toxic biological macromolecules from recombinant or synthetic sources in standard aqueous biological buffers. Standard PPE is always used in sample management. The Optima AUC maintains a <1-micron vacuum pressure during operation and is designed to facilitate cleaning in the event of cell leakage.

E3. Financial Plan

The new Optima AUC arrives with a one-year warranty period. After this period expires, the Johnson Research Foundation will purchase a service contract for the instrument at an annual cost of $22,945 (see attached quotation). As this instrument will be replacing our aging XL-A instrument, we will incur cost savings from the budgeted monies not spent on the XL-A service contract in year 1 ($10,503). These funds will offset the new service contract expense, and an additional $12,442/year in additional service contract costs will need to be budgeted in years 2 through 5. We expect to absorb this expense via an increase in productivity and demand for a more reliable instrument with more powerful analytical capabilities, yielding more billable experiments. In addition to basic laboratory supplies, we also budget for the purchase of sample cell consumables such as screw hold seals, window gaskets, cell replacement parts, replacement windows, and housings via user grants.

The costs of the JRFSBBC are recouped via grant and foundation support of staff salaries and user fees for instrument use and services. This support includes 30% salary support for the core director (Dr. Gupta), 100% salary support for its research technician, and parts and supplies for instruments as needed via the Johnson Research Foundation. A letter of support from the Department Chair (Dr. Kristen Lynch) is included with this application which states that this level of support will be sustained by the Johnson Foundation for least five years from installation.

Table E1. Table of Service Charges

	Intramural *(including Children's Hospital of Philadelphia and the Wistar Institute)*	**Extramural**
Experimental Design	NC	NC
Sedimentation Velocity Analysis	$270/experiment (3 sample cells)	$448/experiment (3 sample cells)
Sedimentation Equilibrium Analysis	$300/experiment (3 sample cells)	$498/experiment (3 sample cells)
Data Analysis	Call	$166/hour

Table E2. JRFSBBC Budget

Year:	2021	2022	2023	2024	2025
Expenses					
Core Director (30% Effort)	($30,280)	($31,188)	($32,124)	($33,087)	($34,080)
Research Technician (100% Effort)	($47,007)	($48,487)	($49,943)	($51,440)	($52,984)
Salary Total[A]	*($77,287)*	*($79,675)*	*($82,067)*	*($84,527)*	*($87,064)*
Service Contract[B]	$0	($22,945)	($22,945)	($22,945)	($22,945)
Revenue					
User Fees[C]	$30,000	$33,000	$36,300	$39,930	$43,923
User Grants	$0	$10,503	$10,503	$10,503	$10,503
Johnson Foundation Subvention[D]	$47,287	$59,117	$58,209	$57,039	$55,583
Surplus	$0	$0	$0	$0	$0

A. Salary calculations include fringe benefits and salary escalation year-to-year.
B. The service contract includes parts, labor, technician travel expenses, annual preventative maintenance, and online support.
C. Assuming a conservative figure of two AUC experiments provided per week through 50 weeks in FY2021, with subsequent growth of user fees increasing 10% each year.
D. Johnson Foundation subvention to ensure cost-neutral operation.

F. Institutional Commitments.

Included are strong letters of support from:

1. Dr. Kristen Lynch, the Chair of the Biochemistry and Molecular Biophysics Department, University of Pennsylvania
2. Dr. Borries Demeler, University of Lethbridge (Canada), collaborator and AUC expert who will consult on the application of the multiwavelength method for AUC and the implementation of Ultrascan-III framework for the core.
3. Dr. Dawn Bonell, Vice Provost for Research, University of Pennsylvania

A list of recent S10 acquisitions at the University of Pennsylvania with requisite data is also attached.

G. Overall Benefit

The state-of-the art Optima AUC will primarily serve NIH-funded research at the University of Pennsylvania and other educational institutions across the US. In line with the mission of the university and national biomedical research efforts, the instrument will enhance and accelerate a variety of research projects from 8

major users and 4 minor users who are funded by the NSF and RO1 grants from the NIH, including NIGMS and NIAID. And additionally, this instrument will drive the training of graduate students and postdoctoral fellows. The analyses supported by this new instrument are novel and currently not available to investigators at the University of Pennsylvania. In all cases, AUC will advance the understanding of macromolecular interactions in solution with rigor and reproducibility, towards the goal of fundament insight into basic biology of disease needed for potential medical interventions. These areas include HIV, cancer, antibiotic resistance, gene therapy, RNA splicing and epigenetics, and neurodegenerative diseases. The overall benefit includes not only the community at the University of Pennsylvania, but also other extramural institutions who collaborate with investigators at Penn and the JRFSBBC.

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Penn Medicine

Raymond and Ruth Perelman School *of* Medicine
at the University *of* Pennsylvania

**Department of Biochemistry
and Biophysics**

Kristen W. Lynch, Ph.D.
Benjamin Rush Professor and Chair

May 25, 2020

To Whom It May Concern:

This letter is to affirm my support for the S10 SIG proposal from Dr. Kushol Gupta to support the purchase of a Beckman-Coulter Optima Analytical Ultracentrifuge.

In 2016 I worked with Dr. Gupta to consolidate much of the biophysical instrumentation in the Department of Biochemistry and Biophysics into a core facility, in order to promote and facilitate the use of our equipment for the good of the entire local scientific community. This facility is subsidized by the Johnson Research Foundation, which provides ~$250K on-going annual support, thus enabling us to provide cutting-edge biophysical technology to the broader community at a competitive cost. We believe that methods such as AUC can answer scientific questions that are not accessible by other approaches, and our goal is to provide both the equipment and expertise to facilitate the broad use of such technologies to promote scientific discovery and progress.

I affirm that the support of the Johnson Research Foundation is sufficient to cover the difference between revenue and expenses for the Beckman Optima AUC. Indeed, we have been supporting the current AUC at a similar level since 2016. Importantly, while our current AUC is widely used, it is no longer state-of-the-art. Dr. Gupta is recognized as a national leader in the use of AUC, and has partnered with Beckman-Coulter to train hundreds of researchers in the local community about the capabilities of AUC and, in particular, how much more we could answer with the advances inherent to the Optima.

We feel this purchase is critical to meeting the ever-increasing demand for AUC capacity and capability amongst our faculty, and important to maintaining our position as a leader in the application of biophysics to biomedical research.

Best wishes,

Kristen W. Lynch, Ph.D.
Benjamin Rush Professor and Chair, Department of Biochemistry and Biophysics
Perelman School of Medicine, University of Pennsylvania

University of Lethbridge

NORTHWEST BIOPHYSICS CONSORTIUM
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UT Health, Dept. of Biochemistry & Structural Biology | 7703 Floyd Curl Dr. | San Antonio, TX 78229 | USA

May 26, 2020   Phone: +1 (406) 285-1935

To: Dr. Kushol Gupta, Perelman School of Medicine
         University of Pennsylvania, Philadelphia, PA

Dear Kushol,

I am writing in enthusiastic support of your NIH-S10 proposal to obtain a Beckman-Coulter Optima AUC under the Shared Instrumentation grant program. My research group in Canada makes extensive use of this exciting new technology (we have two Optima AUCs) and I am closely involved with Beckman to assure its continued improvement and development. Very few instruments of this type have been made available to academic researchers since its introduction in 2017, so I know it will be of tremendous value to your vibrant biomedical research community at the Perelman School of Medicine, and the many users of your core facility.

In our ongoing collaboration, we have been advancing the analytical ultracentrifugation approach in two important ways. First, we are working together to develop new methods to leverage the multi-wavelength capacity of the instrument to study challenging experimental systems to accelerate important basic and biomedical research (we recently submitted our first collaborative R01 proposal); I look forward to our upcoming work to develop these needed methods. Additionally, I have participated in your annual minisymposium on AUC at Penn each of the last two years, where I have shared emerging knowledge about AUC methodologies in the Ultrascan-III framework with investigators from academia, government, and industry. I look forward to continuing this partnership and the prospects of hosting UltraScan workshops at Penn in the years to come.

It has been a rewarding experience working with you and your colleagues in obtaining preliminary data on several exciting projects using the multi-wavelength capabilities of the new instrument. As you know from our collaboration, my research group is at the forefront of the development of methodologies using these newly realized capabilities. The data we collected very nicely illustrate the power of the multi-wavelength analysis with some challenging biological systems, including protein-nucleic acid complexes and integral membrane proteins.

I will be delighted to consult with you on the implementation of the new instrument in your core, and to help you with the integration of the Ultrascan-III framework at your facility for the benefit of your users. I am happy to visit Penn and provide a workshop for all of your users to provide the necessary training for data analysis, experimental design, and result interpretation, and to help you take advantage of the latest emerging innovations from our research group employing GMP reporting standards.

I am confident that the installation of the Beckman-Coulter Optima AUC in your user-friendly core will have a major impact on the ongoing research at Penn and the Northeastern US. I look forward to working with you and wish you the best of luck with this grant application.

Sincerely,

Canada 150 Research Chair for Biophysics

May 26, 2020

Dear Review Committee:

RE   Shared Instrumentation (PAR-20-113)
        https://grants.nih.gov/grants/guide/pa-files/par-20-113.html

        High End Instrumentation (PAR-20-114)
         https://grants.nih.gov/grants/guide/pa-files/PAR-20-114.html

        Shared Instrumentation for Animal Research (SIFAR) Grant Program (PAR-20-112)
        https://grants.nih.gov/grants/guide/pa-files/PAR-20-112.html

As per the Shared and High-End Instrumentation Grant program guidelines, attached please find a table that provides information about instrument performance of all previous S10 awards for instruments awarded or installed within the past five years. The information for the table was provided by our grantees.

I would like to take this opportunity to thank NIH for this critically important research infrastructure program. Expensive, specialized equipment is essential to support the quality, breadth and magnitude of the biomedical research conducted at Penn. As you will see in the attached table, the equipment awarded within the past five years has and continues to advance cutting-edge, collaborative research in our most research-intensive schools. As Vice Provost for Research, I know how important high-end, state-of-the-art equipment is to discovery and scientific advancement, and I enthusiastically support and endorse the S10 proposals submitted by our faculty in response to PAR-20-113, PAR-20-114 and PAR-20-112.

If you have any questions or need additional information please contact me.

Sincerely,

Dawn A. Bonnell, PhD
Vice Provost for Research
Henry Robinson Towne Professor of Engineering and Applied Science
University of Pennsylvania
1 College Hall, Suite 118
Philadelphia, PA 191904-6303

S10 Grant Number	Year of Award	Installation Date	PD/PI's name	Generic Name	Instrument Status	Actual Usage Time	Maintenance Agreement	Number of Publications Citing the S10 Award
1-S10-OD-020090-01	2015	1/22/2015	CHERRY, SARA	Janus MDT	Active	750	None	5
1-S10-OD-021633-01	2016	7/1/2016	FREEDMAN, BRUCE D	PVIC 2-Photon microscope	Active	35 hrs/week	Active	26
1-S10-OD-023592-01	2017	8/30/2017	SHARP, KIM A	Computational Resource for Structural Biology and Molecular Biophysics	Active	6307	Active	0; this grant was omitted in error but will be listed in future publications
1-S10-OD-023495-01	2017	4/2/2018	DAVATZIKOS, CHRISTOS	Computer Cluster	Active	8,750 Hours	Active	53
1-S10-OD-021573-01A1	2017	11/30/2018	GOLDMAN, YALE E	Multi-Parameter Fluorescence Detection Single Molecule Microscope	Active	1200 hrs	Not Available	0; recently installed no publications to date
1-S10-OD-025172-01	2018	9/18/2018	MEYER, NUALA J	High-performance electrochemiluminesce nse immunoassays	Active	1560	Active	0
1-S10-OD-023465-01A1	2018	6/25/2018	DURHAM, AMY C	Slide scanner and digital pathology platform	Active	15 hrs/week	Active	4
1-S10-OD-025098-01A1	2019	10/2019	BAUER JOSEPH A	Telemetry System	Active	4 weeks	None	None
1-S10-OD-025098-01A1	2019	2/20	Bauer, Joseph A	C 3000 BOMB CALORIMETER, C5 CRUCIBLES, SMALL SET 25, REGULATOR, OXYGEN, HI PRESS	Active	1 Day	No	None
1-S10-OD-025098-01A1	2019	12/2019	BAUER, JOSEPH A	16 MOUSE CAGE PROMETHION MULTIPLEXED SYSTEM	Active	4 Weeks	Active	None
1S10OD026860-01	2020	1/20/20	BENNETT, JEAN	Spectraslis tracking system-hra advancedquote 00027779	Active	10 Hrs/Week	None, 1yr. Warranty	0 – Newly Installed
1S10OD026860-01	2020	Pending	BENNETT, JEAN	Spectralis tracking system-hra multicolorquote 00027779	Inactive	N/A	N/A	N/A
1S10OD026860-01	2020	Pending	BENNETT, JEAN	Spectralis tracking oct system-oct plus w/oct2advancedquote 00027779	Inactive	N/A	N/A	N/A